MENTAL STRESS, SOCIOECONOMIC STATUS, AND CARDIOVASCULAR DISEASE: INTEGRATING SOCIOECONOMIC CIRCUMSTANCES INTO THE PARADIGM OF PSYCHO-NEURO-ENDOCRINO-IMMUNOLOGY.

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This thesis is submitted for the degree of Doctor of Philosophy

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Dedicated to my parents

Contents

Abstract

Background

Mental stress is a recognized risk factor and trigger for heart disease. Socioeconomic status (SES) is associated with morbidity and mortality, with low SES people having poorer health compared to their counterparts. I hypothesised that those two factors may interact with each other, so that when they are present simultaneously the total harmful effect is more than the sum of the two risk factors alone.

Research aim

My aim was to test whether lower SES interacts with mental stress and amplifies its effect on heart disease, so that the effect of mental stress on heart disease would be more pronounced in people from low SES backgrounds.

Studies

I carried out three studies. For my first study, I analysed data from a cross-sectional study involving about 500 disease-free middle/old-aged men and women drawn from the Whitehall II epidemiological cohort. I evaluated their salivary cortisol responses to standardized mental stress tests (exposure variable) and their cardiac troponin T plasma concentration (a marker of heart damage) using a high-sensitivity assay (HS-CTnT, outcome variable). I also used measures of coronary calcification levels using electron-beam dual-source computed tomography and Agatston scores. After adjustment for demographic and clinical variables associated with heart disease as well as for inflammatory factors, I found a robust association between cortisol

response to mental stress and detectable troponin T (odds ratio [OR] =3.8, 95% confidence interval [CI] =1.5-9.4, P =0.005). The association remained when I restricted the analysis to participants without coronary calcification (n =222, OR =4.8; 95% CI =1.2-18.3; P =0.023) or when I further adjusted for coronary calcification in participants with positive Agatston scores (n =286, OR =6.2, 95% CI =1.9-20.6; P =0.003). In analyses stratified by SES, there was a trend showing that the lower the SES was, the higher the OR, although this trend was not significant (P >0.05).

In my second study, I selected about 67,000 male and female participants from the Health Survey for England who were 35 years or older, free of cancer and cardiovascular disease at baseline, and living in private households in England from 1994 to 2004. Selection used stratified random sampling (hence representative of the nation), and participants were linked prospectively to mortality records from the Office of National Statistics (mean follow-up, 8.2 years). Mental stress was measured using the 12-item General Health Questionnaire, and SES was indexed by occupational class. The crude incidence rates for heart disease and all-cause mortality of the cohort were 1.9 (95%CI =1.7-2.0) and 14.5 (95%CI =14.2-14.8) per 1,000 person-years. After adjustment for age and sex, mental stress was associated with increased mortality rates. In a stratified analysis, the association of mental stress with the outcomes differed with SES, with the strongest associations being observed in the lowest SES categories (the adjusted P values for interaction were 0.012 for all-cause mortality and 0.047 for heart disease mortality).

My third study involved about 80,000 post-menopausal women selected from the United Kingdom Collaborative Trial of Ovarian Cancer Screening study, who were followed up for about three years on average. Mental stress was measured using the

hopelessness/helplessness index and incident heart disease was assessed using hospital electronic records. The overall incidence rate of hospitalisation for acute heart disease event was 2.7 per 1,000 person years (95% CI=2.5-3.0). The augmented incidence for people who experienced mental stress was higher in people of low SES, medium in people of medium SES, and lower in people of high SES (adjusted P value for interaction =0.013).

Conclusion

These studies suggest that the interaction between socioeconomic status and mental stress is associated with ischemic heart disease, in such a way that people in low socioeconomic circumstances are more vulnerable to the negative effects of mental stress. In other words, the harmful effect of mental stress for human cardiac health may be modified by socioeconomic position and rendered more deleterious for people from disadvantaged backgrounds. Further research is needed to disentangle the dynamics of this effect amplification.

Abbreviations and acronyms

95%CI	95% confidence interval
ACTH	adrenocorticotropic hormone
AMI	acute myocardial infarction
ANS	autonomic nervous system
AVP	arginine-vasopressin
BMI	body mass index
CABG	coronary artery bypass grafting
CAC	coronary artery calcification
CAPI	computer-assisted personal interviewing
CI	confidence interval
CNS	central nervous system
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CTnT	cardiac troponin T
CVD	cardiovascular disease
ECG	electrocardiography
EO	executive officer
GABA	γ-aminobutyric acid
GH	growth hormone
GHQ	general health questionnaire
GnRH	gonadotropin-releasing hormone
HbA1C	glycated haemoglobin
HDL	high-density lipoprotein
HEO	higher exectivie officer
HH	hopellness/helplessness index
HPA	hypothalamic-pituitary-adrenal
HR	hazard ratio
HS-CTnT	high-sensitivity cardiac troponin T
HSE	health survey for England
ICD	international classification of diseases

IDL	intermediate density lipoprotein
IHD	ischaemic heart disease
IL-1	interleukin-1
IL-6	interleukin-6
IMD	index of multiple deprivation
L2	second lumbar vertebra
LDL	low-density lipoprotein
LH	luteinizing hormone
LRT	likelihood ratio test
MCP-1	monocyte chemoattractant protein-1
MINAP	Myocardial ischaemia national audit project
mmHg	millimetres of mercury
NK	natural killer
NNT	number needed to treat
NSTEMI	non st-elevated miocardial infarction
ONS	office for national statistics
OR	odds ratio
PAF	population attributable fraction
PAVIX	psychosocial adversity and vulnerability index
PCI	percutaneous coronary intervention
PNEI	psycho-neuro-endocrino-immunology
PNS	peripheral nervous system
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RCT	randomised controlled trial
SD	standard deviation
SEO	senior executive officer
SES	socioeconomic status
STEMI	st-elevated miocardial infarcion
T1	first thoracic vertebra
T3	triiodothyronine
T4	thyroxine
TNF	tumour necrosis factor

TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
UK	United Kingdom
UKCTOCS	United Kingdom Collaborative Trial of Ovarian Cancer Screening
USA	United States of America
VCAM-1	vascular cell adhesion molecule-1
VLDL	very low density lipoprotein
vWF	von Willebrand factor

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Chapter 1 - Introduction

This PhD dissertation is based on original medical research developed within University College London, department of Epidemiology and Public Health, Psychobiology group. The main domain that this dissertation refers to is psycho-neuro-endocrino-immunology (PNEI), which is a relatively new branch of medicine that takes an interdisciplinary approach and tends to integrate human body systems, organs, tissues, and cells into a whole dynamic system, and that has its focus on the communication and interaction between the different parts of the human body rather than on the structures and functions of the individual components. For this research I have therefore used concepts and instruments that are pertinent to different disciplines such as psychology, sociology, economics, immunology, endocrinology, and cardiology, and hence the description of this work cannot follow the ordered and hierarchical structure that is typical of research presentations that are centred on single and clear domains. Consequently this introduction will only describe scientific areas of interest that are relevant to my research aims, without the intent of being systematic and exhaustive.

I will initially give a brief description of the cardiovascular system and introduce cardiovascular disease (CVD) with a focus on acute myocardial infarction (AMI). I will then bring in two factors that are pertinent to disciplines other than medicine and that are relevant to AMI: mental stress (psychology and psychiatry) and socio-economic status (sociology and economics). Afterwards, I will present literature relevant to my research hypothesis. Finally, I will show the results of a systematic literature review that has been carried out to check whether my research hypothesis has already been addressed, in order not to duplicate existing evidence.

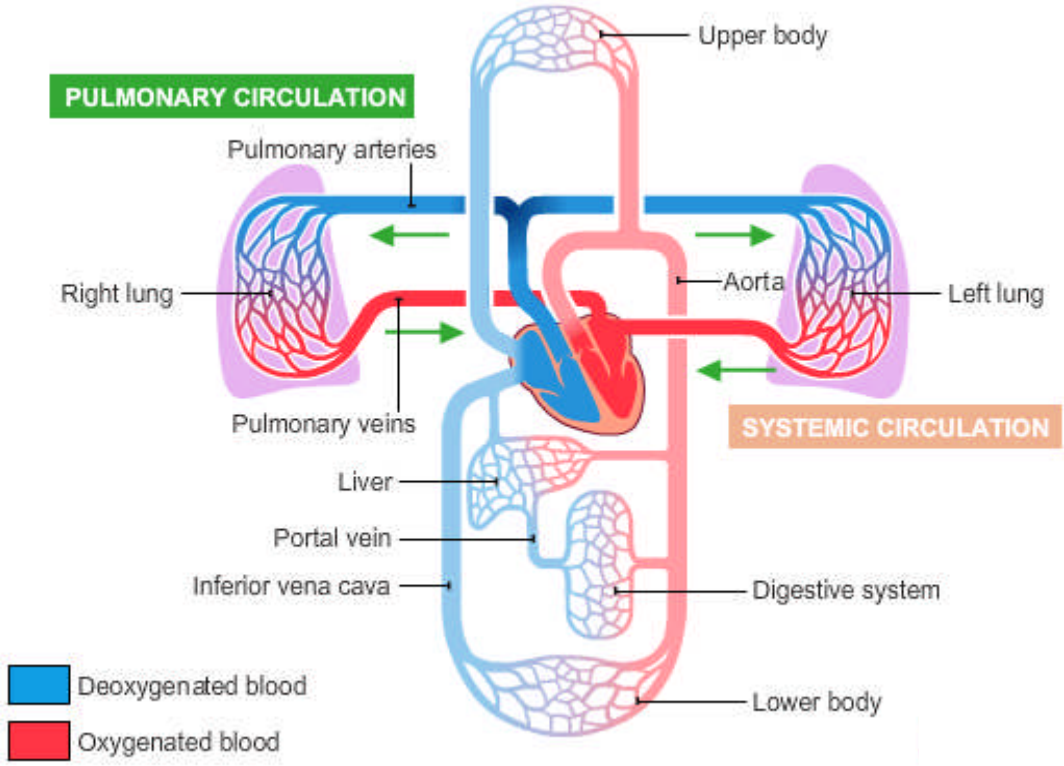
1.1 The cardiovascular system

Within the human body, blood flows continuously. Its perpetual and cyclical movement is necessary to supply body organs, tissues, and cells with oxygen and nutrients, and to remove carbon dioxide and other waste substances from them at the same time. Blood circulation occurs within a close network of organs and vessels called the cardiovascular system. The heart, the arteries, the capillaries, and the veins are the main components of the cardiovascular system, and the automatic pumping activity of the heart is the principal determinant of blood circulation. The heart is a hollow muscular organ divided into four chambers: the right atrium, the right ventricle, the left atrium, and the left ventricle.[1]

A complete cardiac cycle within the cardiovascular system can be described as follows. The venous blood, coming from all districts of the organism, reaches the right atrium through two veins called venae cavae and then flows into the right ventricle. From this chamber venous blood is pumped into the pulmonary artery that goes inside the lungs and then splits into smaller and smaller arteries, similarly to the trunk of a tree that splits into smaller and smaller branches. When, after a number of subdivisions, those arteries reach the smallest calibre, they become capillaries, their walls acquire new structural and functional characteristics so that the inside-outside exchange becomes possible, and the blood acquires oxygen and eliminates carbon dioxide. The capillaries then merge into larger and larger veins that finally form the pulmonary vein that is connected to the left atrium, which receives oxygenated blood. From the left atrium the blood moves into the left ventricle from which it is pumped into an artery called the aorta. Smaller and smaller branches of the aorta reach all peripheral organs (excluding the lungs) where they form nets of capillaries,

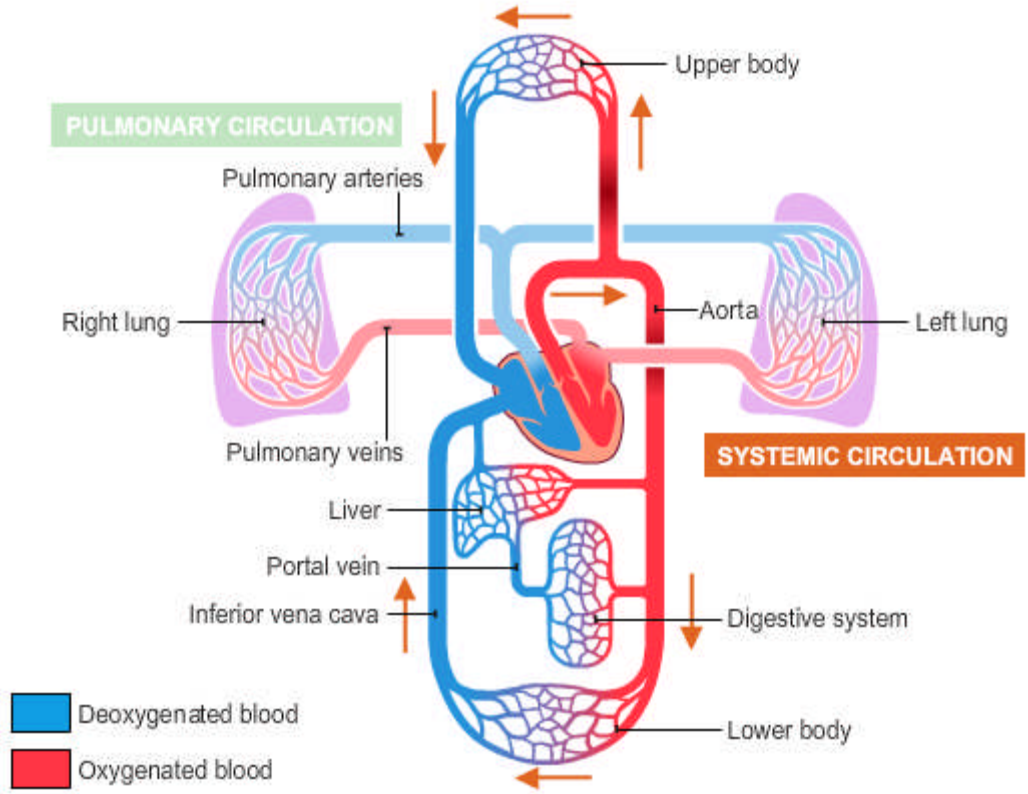
from which oxygen and nutrients diffuse into tissues and cells, and into which carbon dioxide and catabolites are disposed. Capillaries then merge together into larger and larger veins that form the venae cavae, which are connected to the right atrium. The superior vena cava carries venous blood coming from the upper body (mainly the brain), whereas the inferior vena cava carries venous blood coming from the rest of the body (see figures 1.1. and 1.2).[1]

Figure 1.1. Schematic diagram of the pulmonary circulation



Adapted from http://www.bbc.co.uk/

Figure 1.2. Schematic diagram of the systemic circulation.



Adapted from http://www.bbc.co.uk/

The above describes one complete cycle of the cardiovascular system in a schematic way and - for a matter of clarity - I have excluded many aspects of it. In fact many other organs and sub-systems interact with that cycle and can determine cardiovascular dysfunctions if they work in abnormal conditions. For example, liver disease can have repercussions for the whole dynamics of the cardiovascular systems because the liver is connected to the heart through the inferior vena cava.[1,2] The description of all indirect conditions that can affect the cardiovascular systems goes beyond the aims of this thesis.

One additional aspect of the cardiovascular system that has to be noted because it is relevant to my research is the following: as soon as the aorta emerges from the left

ventricle, some small branches split off to form the coronary circulation that serves the heart muscle itself.[1]

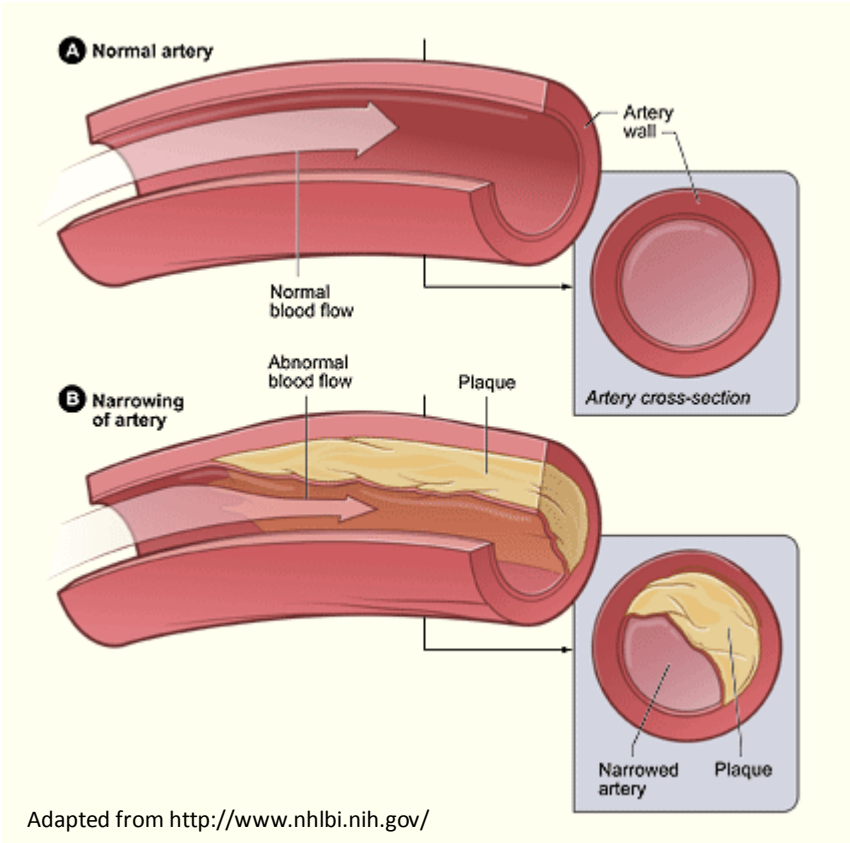
The most important haemodynamic alterations are those that impair the inside-outside exchange of gas and metabolites that I have described, because the availability of oxygen and nutrients for the tissues can never be inferior to their need in order to avoid cellular death. There are two main alterations of that can occur: ischaemia and congestion. Ischaemia is a restriction of blood supply (i.e. a lack of blood within the capillaries) to organs, tissues, or even small groups of cells, whereas congestion is a stagnation of blood (i.e. a defect in blood replacement in the capillaries after the inside-outside exchange). The terms ischaemia and congestion are sometimes used interchangeably and often ischaemia acquires the more general meaning of imbalance between oxygen demand and supply. Ischaemia can be caused by a variety of conditions such as vessel compressions from the outside (e.g. neoplasms), vessels ruptures (e.g. haemorrhagic stroke), vessel obstructions from the inside, i.e. stenosis (e.g. atherosclerosis and thrombus), vessel spasms, arrhythmia (irregular heart beat), as well as other conditions.[2]

1.2 Atherosclerosis, thrombus, and inflammation.

Arteriosclerosis can be generically defined as the thickening and hardening of the arterial walls such that the lumen of the arteries is reduced (stenosis) and the blood flow is impaired. Atherosclerosis is a form of arteriosclerosis, where the thickening is due to the deposition of lipids within the wall of the arteries (particularly in the stratum of the wall called the tunica intima), that typically occurs to medium and large size arteries and has a defined localisation. The sites that are mostly damaged

are the aorta and its major branches, the cerebral vessels, the vessels of the lower limbs, and the coronaries (see figure 1.3).[2]

Figure 1.3. Diagram of atherosclerosis



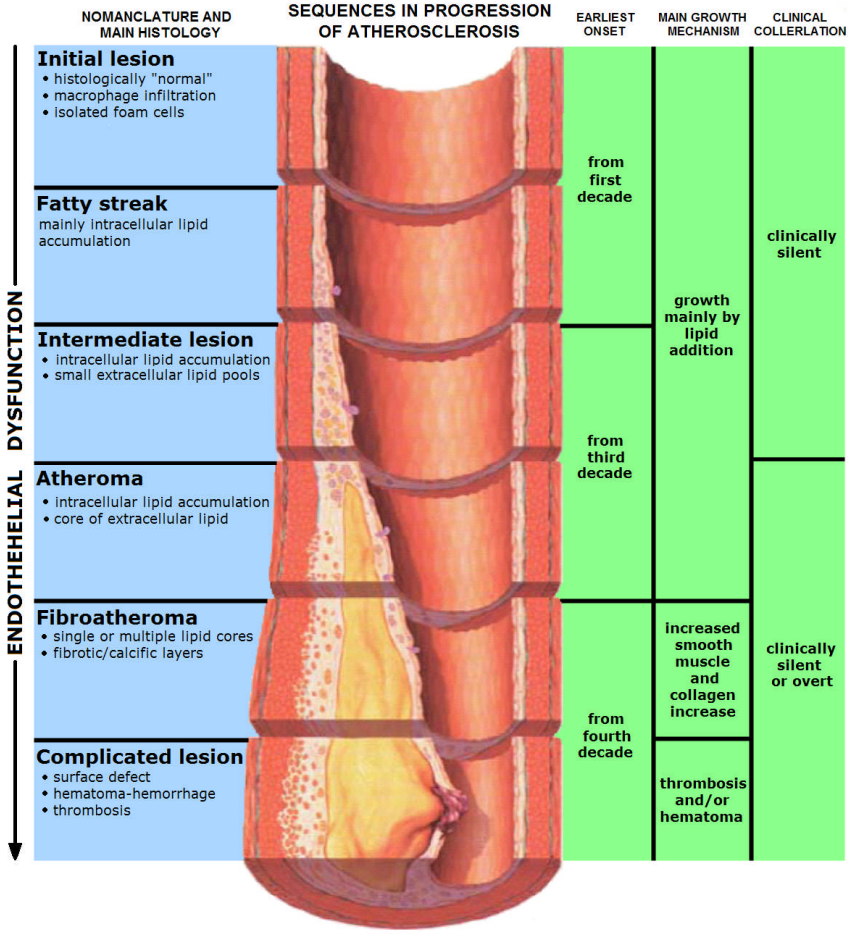
Adapted from http://www.nhlbi.nih.gov/

There are two main phases for the formation of atherosclerosis.

1. The first phase is potentially reversible and is called a fatty streak. Macrophages are cells produced by the differentiation of monocytes in tissues, and monocytes are a type of white blood cells (leukocytes). A fatty streak consists of the accumulation of aggregates of lipoprotein-loaded macrophages, also called foam cells, in the tunica intima of the artery.

2. The second phase is not reversible and is called an atheroma or an atherosclerotic plaque. This is an additional deposit of degenerative material consisting mostly of calcium and fibrous connective tissue, supported by immune system cells other than macrophages, such as T lymphocytes amongst others (see figure 1.4).[2]

Figure 1.4. Stages of atherosclerosis.



Adapted from http://en.wikipedia.org/wiki/Atherosclerosis

Atherosclerosis was traditionally considered as a passive process of lipid accumulation, a sort of passive trapping and retention of cholesterol-rich, apolipoprotein B–rich lipoproteins within the arterial wall. This was due to a variety of observations. For example, it was noted that, even in the presence of other risk

factors, atherosclerosis does not develop when serum cholesterol concentrations are below 80 mg per decilitre (2.1 mmol per litre), a concentration found in many animals. Genetic, dietary, and other interventions that raise serum concentrations of atherogenic lipoproteins in animals provoke atherosclerosis, i.e. focal increases in arterial LDL concentration precede development of fatty streak lesions. In animals with newly induced hypercholesterolemia, intramural lipoprotein retention and aggregation occur within hours in lesion-prone arterial sites. Interventions that remove arterial lipids in animals reduce the size of the lesion and correct endothelial dysfunction, and similar effects occur in humans treated aggressively with lipid-lowering drugs. Finally, animals expressing mutations of human apolipoprotein B engineered so that it no longer binds to arterial matrix have high serum LDL concentrations but few atherosclerotic lesions; their LDL is no longer atherogenic, because it cannot be retained within the prelesional vessel wall.[3–5]

While we now acknowledge the role of inflammation in the pathophysiology of atherosclerosis, inflammation was previously viewed as a consequence rather than a concurrent cause of atherosclerosis, mainly because it was observed that the arterial wall contains matrix and enzymes that retain and modify lipoproteins, thereby generating biologically active products that induce key components of the inflammatory response, and because, once trapped, lipoproteins become enzymatically and oxidatively modified, they accumulate, and then they stimulate, among other responses, inflammation.[3–5]

Although it has been recently found that elevated low-density lipoprotein cholesterol can cause heart disease even without inflammation,[6] the medical community has

now accepted the notion that inflammation plays a central role in the development of atherosclerosis.[7–9]

Atherosclerosis and inflammation

Several conditions promote the atherosclerotic process:

- **Lipoprotein blood levels.** The augmented concentration of lipoproteins in the blood plasma, and particularly of low-density lipoproteins carrying cholesterol (LDL), facilitates atherosclerosis by increasing the rate of lipoproteins being captured by macrophages.
- **The alteration of LDL.** Macrophages are one of the specialised cells that form the immune system and their role is to engulf and digest cellular debris and pathogens. Therefore if LDL molecules are altered through the phenomenon of oxidation for example, the rate of their capture by macrophages is increased.
- **Endothelial lesions.** Endothelial cells are cells that form the thin layer that lines the interior surface of the artery and that are in direct contact with the blood. Macrophages have to migrate from the blood to the tunica intima of the arteries in order to start atherosclerosis and therefore their penetration through the endothelium is necessary. It follows that lesions to the endothelium promote atherosclerosis. Lesions to endothelium that contribute to the atherosclerotic process can be of a mechanical nature, such as the effect of high blood pressure as well as of a chemical nature, such as the toxic chemicals inhaled through tobacco smoking for example, or the direct cytotoxic activity that altered LDL has.

- **Macrophage adherence to endothelial cells.** Macrophages have to adhere to the endothelium to then be able to infiltrate the artery wall and move to the tunica intima. The adherence is enhanced by chemical stimuli produced by endothelial, macrophage, and other cells in response to inflammatory and immunological processes of a diverse nature.[2]

The communication, the interaction, and the aggregation of the cell types that promote atherosclerosis are crucial for the determination of the disease. That communication is made possible by chemical signals called cytokines. These are small, nonstructural proteins with molecular weights ranging from 8 to 40,000 dalton. Nearly all nucleated cells are capable of synthesizing these proteins and, in turn, of responding to them. There is no amino acid sequence motif or three-dimensional structure that groups cytokines; rather, their biological activities allow us to classify them into different categories. Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma, and promote the communication and movement of cells (the term cytokine comes from the Greek words *cytos* [cell] and *kinesis* [movement]). Some cytokines act to promote inflammation (proinflammatory), whereas others serve to reduce inflammation (anti-inflammatory).[10]

Due to the key role of proinflammatory cytokines in enhancing the cell's activation and interaction that trigger and maintain atherosclerosis, chronic inflammation has been recently emphasised as a major determinant of that disease.[7,11,12] For example, immune cells, mediators of host defence and inflammation, are known to concentrate in the earliest lesions of atherosclerosis. Moreover, normal endothelium does not in general support the binding of white blood cells. However some specific

molecules produced in response to inflammation such as vascular cell adhesion molecule-1 (VCAM-1) bind to some types of leukocytes such as monocytes and T-lymphocytes that are found in early atheroma. Additionally, VCAM-1 expression increases on endothelial cells overlying emerging atheroma. Augmented wall stresses due to hypertension, for example, may promote the production of proteoglycans (by arterial smooth muscle cells) that bind and retain lipoprotein particles, facilitating their oxidative modification and thus promoting an inflammatory response at sites of lesion formation. Once the leukocytes adhere to the endothelium, they penetrate into the intima and some molecules produced in response to inflammation, such as monocyte chemoattractant protein-1 (MCP-1), are responsible for this transmigration. Moreover, once the blood-derived inflammatory cells become resident in the tunica intima they promote a local inflammatory response. For instance, T cells release inflammatory cytokines such as gamma-interferon and lymphotoxin (tumour necrosis factor [TNF]–beta) that in turn can stimulate macrophages as well as vascular endothelial cells and arterial smooth muscle cells.[7,11,12]

When the volume of an atherosclerotic plaque increases, the plaque tends to protrude into the lumen of the artery and to reduce blood flow progressively, eventually causing ischaemia.[2] Furthermore, a plaque can encounter several complications that are relevant to acute myocardial infarction:

- There can be ulceration and consequent rupture of the plaque surface that determines the emptying of the necrotic residuals into the artery lumen, with a resulting microembolism that blocks the terminal parts of the arteries and therefore produces ischaemia.

- The ulceration can start a coagulation process with platelet aggregation and fibrin deposition that can result in the formation of a thrombus, i.e. a blood clot that can suddenly block the blood flow of large vessels and consequently cause ischaemia in large parts of organs.[2]

Inflammatory processes not only promote initiation and evolution of atheroma, but also contribute decisively to the complications described above. For example, the macrophages that become activated in response to inflammation that are abundant in the atheroma can produce proteolytic enzymes capable of degrading the collagen that forms the plaque's protective fibrous cap, rendering the cap thin, weak, and susceptible to rupture. Moreover, gamma-interferon arising from the activated T lymphocytes in the plaque can halt collagen synthesis by arterial smooth muscle cells, limiting their capacity to renew the collagen that reinforces the plaque. Macrophages also produce tissue factor, the major procoagulant and trigger for thrombosis that is found in plaques. Furthermore, inflammatory mediators regulate tissue factor expression by plaque macrophages, demonstrating an essential link between arterial inflammation and thrombosis.[7,11,12]

Therefore inflammation participates in all stages of atherosclerosis:

1. **Initial phase: fatty streak formation.** Blood leukocytes adhere poorly to normal endothelium and when the endothelial monolayer becomes inflamed, it expresses adhesion molecules for leukocytes. Also, proinflammatory cytokines expressed within the atherosclerotic plaque provide a chemotactic stimulus to the adherent leukocytes, directing their migration into the tunica intima. Moreover, inflammatory mediators enhance

the expression of macrophage receptors leading to the uptake of modified lipoproteins and the formation of foam cells and other inflammatory mediators produced in plaques which can also promote the replication of macrophages within the intima.

2. **Second phase: atherosclerotic plaque formation.** T lymphocytes join macrophages in the intima during lesion evolution. These leukocytes secrete cytokines and growth factors that can promote the migration and proliferation of smooth muscle cells, which express specialised enzymes that can degrade the elastin and collagen in response to inflammatory stimulation. This degradation of the arterial extracellular matrix permits the penetration of the smooth muscle cells through the elastic laminae and collagenous matrix of the growing plaque.
3. **Last phase: ulceration and thrombus formation.** Inflammatory mediators can inhibit collagen synthesis and evoke the expression of collagenases by foam cells within the intimal lesion. These alterations thin the fibrous cap and render it weak and susceptible to rupture. Cross-talk between T lymphocytes and macrophages heightens the expression of the tissue factor, which is a strong procoagulant. Thus, when the plaque ruptures, the tissue factor induced by the inflammatory signalling triggers the thrombus that causes the most acute complications of atherosclerosis. [7,11,12]

1.3 Ischaemic heart disease

The term ischaemic heart disease (IHD) refers to a spectrum of disease that have different aetiology but common pathophysiology, which is represented by the imbalance between the metabolic demand of the heart and oxygen support. IHD is

predominantly secondary to atherosclerosis of the coronary arteries but there can be IHD without atherosclerosis and atherosclerosis without IHD. Apart from "fixed" stenosis of an atherosclerotic nature, the myocardium can become ischaemic due to "dynamic" stenosis of the coronaries: the most documented phenomenon of such kind is coronary spasm, which is a temporary, sudden narrowing of the coronary lumen that is due to a squeezing of muscles in the artery wall. The imbalance between metabolic demand and oxygen support can happen even with normal coronaries for a disproportionate increase in the demand. However, this happens only when the metabolic demands become particularly elevated, for example, in the presence of a marked myocardial hypertrophy especially if it is associated with aortic valvulopathy.[2]

It is not infrequent that two or more pathophysiologic dynamics happen simultaneously. For example, coronary spasm tends to happen more often and to have more ischaemic effect in arterial segments where atherosclerosis accumulates. Thrombus and spasm can also coexist; platelets can produce vasoactive substances such as thromboxane A2, which has a strong vasoconstrictive effect.[2] It is interesting to note that inflammation is also a component of coronary spasm.[13]

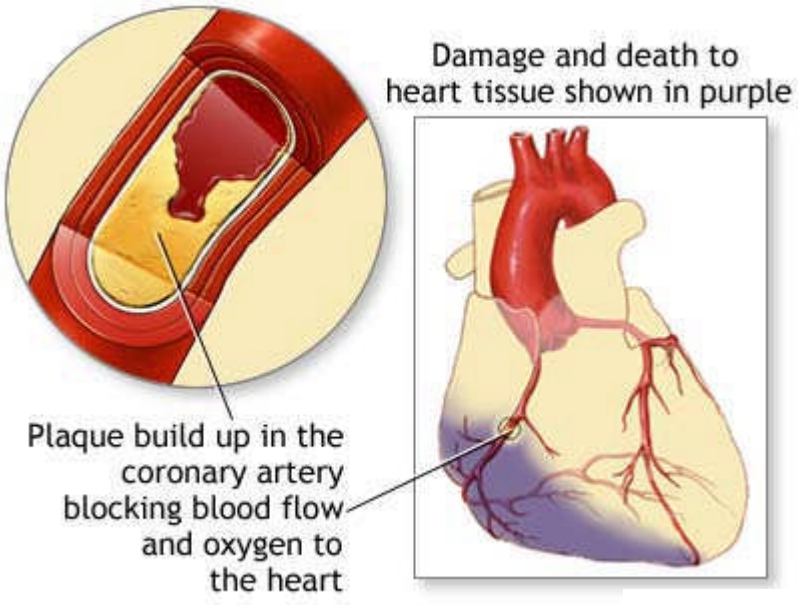
The clinical manifestations of IHD are:

1. Primary cardiac arrest
2. Angina pectoris
3. Cardiac failure
4. Arrhythmia
5. Acute myocardial infarction

Acute myocardial infarction

Acute myocardial infarction (AMI) is caused by an ischaemia in one or more coronaries that persists for a period of time that is sufficiently long to induce the cellular necrosis (death) of myocytes. AMI is therefore characterised by an irreversible anatomical alteration of the myocardium (see figure 1.5).[2]

Figure 1.5. A diagram of a typical AMI.



Adapted from http://nursingcrib.com/.

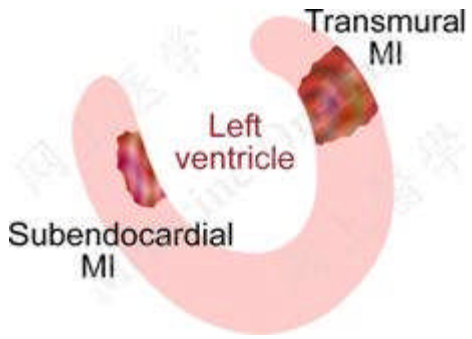
There are two main types of acute myocardial infarction:

1. **Transmural AMI** is associated with atherosclerosis involving a major coronary artery and it extends through the whole thickness of the heart muscle and is usually a result of complete occlusion of the area's blood supply, often by a

thrombus. The spastic component is less important for the pathophysiology of this type of AMI.

2. **Intramural AMI** does not extend through the whole thickness of the heart muscle but only involves the subepicardial or the subendocardial wall, which is particularly susceptible to ischemia. Therefore intramural AMI is usually a result of incomplete occlusion for causes other than thrombus (see figure 1.6).[2]

Figure 1.6. Subendocardial vs transmural AMI



Adapted from http://www.medicine-on-line.com/.

Coronary occlusion is therefore the most important factor of myocardial necrosis. However it is not the only one. In the presence of myocardial ischaemia caused by an acute blood flow reduction in a coronary region, catecholamine production is often elicited and this has negative repercussions to the heart. In fact, the increase in circulating catecholamines determines an increase in heart rate, an increase in peripheral vessel resistance (smaller vascular calibre), and an increase in myocardial contractility, which all entail an increase of oxygen demand which can start a vicious circle that aggravates the ischaemia.[2]

The anatomical pathology of an AMI follows a typical pattern: soon after the heart attack a pale and dry area appears on surface of the tissue that encountered the

necrosis; after two-four days it becomes yellow due to the occurrence of infiltration by neutrophil granulocytes; after eight-ten days the area turns dark red due to the formation of granulation tissue; after about four weeks cicatrisation begins and the area tends to becomes grey; within three-six months scar formation is completed. Therefore, when not fatal, an AMI implies the loss of muscular mass by the heart.[2]

The location, the extent, and the fatality of an AMI depend on which coronary artery is affected, on its size, on the extent of the stenosis, on the duration of the ischaemia, treatment processes, and other factors. A complete categorisation of the different AMIs according to those parameters goes beyond the aim of this dissertation.

The typical symptoms of an AMI are:

1. Pain in the chest or in the abdomen that tends to irradiate, often to the arms.
2. Intense asthenia
3. Pale skin and algid sweating
4. Nausea and vomiting
5. Anxiety
6. Arrhythmia
7. Shock and sudden death in severe cases
8. Others

However, the typical symptoms do not always occur. For example the incidence of AMI without the typical associated pain is relatively frequent in diabetic and older patients. Moreover symptoms such as pale skin and algid sweating do not co-exist with nausea and vomiting because the former symptoms are due to catecholamine

activity whereas the latter symptoms are due to augmented vagal stimulation. There are also gender differences in AMI presentations: nausea, back pain, dizziness, and palpitations are more common in women, who usually also display a greater number of symptoms than men.[14]

The diagnosis of AMI must be therefore confirmed using blood tests, electrocardiography (ECG), and imaging test. Acute myocardial infarctions are schematically divided into two types on the basis of ECG results: ST-elevated and non-ST-elevated. An occlusive thrombus complicating a high-grade stenosis would arrest the blood flow and cause ST-segment elevation myocardial infarction (STEMI). Acute coronary syndromes without ST-segment elevation (NSTEMI) would result from an incomplete or transient obstruction of flow in the culprit coronary artery at a site of critical stenosis.[2,12]

The latest universal definition of AMI for diagnostic purposes (2007) is as follows: troponin T elevation ≥0.035 ng/ml (i.e. above the 99th percentile of the upper reference limit with a coefficient of variation <10%) with at least one of the following: symptoms of ischaemia; ECG evidence of AMI (acute ischaemic changes or development of pathological Q waves); imaging evidence of new loss of viable myocardium.[15,16]

Cardiac troponins are regulatory proteins that control the calcium-mediated contraction from within the myocyte (heart muscle cell). The troponin complex consists of three subunits: troponin T, troponin I, and troponin C. Troponins are not normally present in the plasma of healthy individuals. The role of Troponin T is to bind to a filament called tropomyosin in order to enhance its interaction with

another filament called actin that is responsible for contraction. Contraction happens when the two filaments can slide reciprocally levering on bridges that link them. When cellular necrosis happens, the contents of the myocyte, including Troponin T, are released into the capillaries and from here they reach the main blood stream. There is a rapid early release of plasma Troponin T after ischaemic injury, which peaks at 12–24 hours, and may remain raised for more than two weeks. The detection of Troponin T in the plasma inevitably indicates cardiac cell damage.[17]

Increased blood concentrations of troponin T can be seen in a variety of diseases other than AMI, such as sepsis, hypovolemia, atrial fibrillation, heart failure, pulmonary embolism, myocarditis, myocardial contusion, renal failure, stroke, toxic injury, severe cardiac overload, and others. Thus, while negative troponin T may be useful to "rule out" an AMI, there are limitations of using troponin T to "rule in" an AMI, especially in patients with low clinical likelihood of IHD, because troponin T is highly indicative of myocardial injury, which nonetheless can be the result of AMI but also of other conditions. In epidemiological terms, troponin T has great sensitivity but medium/low specificity for AMI when the other diagnostic criteria for AMI are not met. As a consequence, if troponin T testing is applied indiscriminately in broad populations with a low pre-test probability of AMI, the positive predictive value for AMI (i.e. the probability of having an AMI if the test is positive) is greatly diminished.

In clinical settings, troponin T is measured using standard assays that have a lower detection limit of 10 ng/L,[18] and a diagnostic threshold of 35 ng/L.[15,16] However, high-sensitivity assays have now been developed with a lower detection limit of 3 ng/L.[19–21] With the advent of high-sensitivity assays, causes of troponin T elevation not related to heart disease have become common findings in patients with chest pain

and in those with acute or chronic systemic disorders. However, troponin T elevation in the absence of heart disease still retains significant prognostic value, and screening may be justified on this basis. Indeed, troponin T elevations in a variety of settings predict cardiac events, and worse short- and long-term survival, independently of the underlying disease, and also in disease-free people. The reasons for this increase in morbidity and mortality are currently poorly understood, and the clinical conditions leading to Troponin T release in patients who do not have any chronic or acute disease, and the appropriate diagnostic and therapeutic strategies for these individuals, are largely unknown.[22]

Although Troponin I is not currently used for the diagnosis of AMI, this other component of the troponin complex, measured with a high-sensitivity assay, is also an independent predictor of cardiovascular events and might support selection of at risk individuals.[23]

Treatment

In STEMIs nearly half of the potentially salvageable myocardium is lost within 1 hour of the coronary artery being occluded, and two-thirds are lost within 3 hours. Apart from resuscitation from any cardiac arrest, the highest priority in managing STEMI is to restore an adequate coronary blood flow as quickly as possible. In the 1980s and 1990s, the best way to restore flow was to administer a fibrinolytic drug (i.e. a drug that is able to dissolve the fibrin clot occurred following plaque rupture causing ischaemia). This treatment is called thrombolysis (i.e. the breakdown of a thrombus). However, fibrinolysis is not suitable for use in all people because of bleeding complications. In around 20-30% of individuals, fibrinolysis failed to result in

coronary reperfusion, and in a minority (1.0%) its administration caused haemorrhagic stroke. To improve outcomes, attention turned to mechanical techniques to restore coronary flow (for example, coronary angioplasty, thrombus extraction catheters and stenting). These techniques are grouped under the umbrella term of primary percutaneous coronary intervention (primary PCI). Primary PCI is both feasible and cost effective and is the treatment of choice for STEMI, providing it can be delivered in a timely fashion. PCI is one of the two coronary revascularisation techniques currently used in the treatment of ischaemic heart disease, the other being coronary artery bypass grafting (CABG). PCI involves non-surgical widening of the coronary artery, using a balloon catheter to dilate the artery from within. A metallic stent is usually placed in the artery after dilatation. Antiplatelet agents are also used. Stents may be either bare metal or drug-eluting. The coronary artery is accessed with a method called angiography: a long, thin, flexible tube called a catheter is threaded into an artery in the arm or leg, and the tip is advanced through the arterial system into the coronaries. The catheter is firstly used to administer an X-ray contrast agent that enables the X-ray imaging on the coronaries in order to diagnose and locate stenosis. The catheter is then used to mechanically remove the obstruction and insert the stent.

Alternatively, CABG is a type of open-heart surgery that is usually performed with the heart stopped, necessitating the usage of cardiopulmonary bypass, although techniques are available to perform CABG on a beating heart, which is called "off-pump" surgery. In CABG the stenosis in not resolved but by-passed: a blood vessel is taken from the chest, leg, or arm of the patient and used to bypass a narrowed or

blocked coronary artery. PCI is preferred to CABG, although CABG is superior to PCI in some conditions, for example for some patients with multivessel IHD.[24–27]

Although NSTEMIs result from incomplete and often undetectable stenosis and their consequences on the vitality of the myocardium are often less severe than for STEMIs, patients presenting with NSTEMI are also tested with angiography and eventually treated with primary PCI.[25,28]

It has to be noted that gender and SES have been studied independently as factors influencing the care and outcomes after IHD, and it was found that they are influential, partly through barriers in timely access to PCI, with women and low-income people being more likely to be excluded from appropriate care. Moreover a recent study evaluated the combined effect of gender and SES, and demonstrated that the association between SES and both care and outcomes in IHD differs in men versus women: low-income women seem to be particularly vulnerable in terms of access to PCI and short-term mortality after IHD.[29]

Epidemiology and risk factors

According to 2010 data, IHD is the leading cause of death worldwide resulting in more than seven million deaths per year, with an increasing trend over the last twenty years.[30] The incidence of AMI has decreased in the United Kingdom over the past three decades and currently there are about 100,000 heart attacks on average in the UK every year.[31] In England around 11% of men and 15% of women who were admitted to hospital with an AMI in 2010 died within 30 days. In Scotland, case fatality rates were higher, with 12% of men and 19% of women admitted with an AMI

dying within 30 days.[32] Prevalence studies indicate that around 1 million men and nearly 500,000 women have had an AMI in the UK.[33]

The conventional risk factors for AMI are older age, male gender, hypertension, smoking, hypercholesterolaemia, and diabetes.[34]

- **Hypertension**. Hypertension is defined as a consistently elevated blood pressure over a certain threshold. The British Hypertension Society has set the threshold as 140 mmHg for systolic blood pressure and 90 mmHg for diastolic blood pressure.[35] According to the Health Survey for England, the prevalence of hypertension in 2012 was 31% among men and 27% among women, remaining at a similar level over the last few years.[36]
- **Smoking.** Regarding cigarette smoking, among men there has been an increase in the proportion of who have never regularly smoked cigarettes (from 39% in 1993 to 51% in 2012). Correspondingly, the proportion of men who are current smokers declined overall from 28% 1993 to 22% in 2012. The proportion of women who have never regularly smoked increased from 52% in 1993 to 61% in 2012, while the proportion of current smokers decreased overall in the same period, falling from 26% to 18%. The proportion of men and women who smoked 20 or more cigarettes per day has fallen: from 11% of men in 1993 to 5% in 2012 and from 8% women to 3% over the same time period.[36]
- **Hypercholesterolaemia.** Hypercholesterolaemia is the presence of high levels of cholesterol in the blood. Since cholesterol is insoluble in water, it is transported in the blood plasma within protein particles (lipoproteins). Lipoproteins are classified by their density: very low density lipoprotein

(VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL). All the lipoproteins carry cholesterol, but elevated levels of the lipoproteins other than HDL, particularly LDL-cholesterol, are associated with an increased risk of atherosclerosis and IHD coronary heart disease. In contrast higher levels of HDL cholesterol are protective. Elevated levels of non-HDL cholesterol and LDL in the blood may be a consequence of diet, obesity, genetic diseases (such as LDL receptor mutations in familial hypercholesterolemia), or the presence of other diseases such as diabetes and an underactive thyroid. The term hypercholesterolaemia has been recently replaced by the term dyslipidaemia, meaning that the ratio between offensive and protective plasma lipids is more important that absolute values of the offensive ones.[37–39]

- **Diabetes.** Diabetes mellitus is a condition characterised by an elevated concentration of glucose, a type of sugar, in the blood (hyperglycaemia). There are two types of diabetes. Type 1 diabetes is particularly prevalent in younger non-obese individuals and is mainly due to autoimmune mechanisms that destroy the islets of Langerhans, which are the regions of the pancreas that contain insulin-producing cells. The hormone insulin has an antidiabetic effect because it removes blood sugar by promoting its entrance into the cells and its conversion into storage lipids. Type 2 diabetes is more prevalent in older and obese individuals and has a more complex pathophysiology: it results from insulin resistance, a condition in which cells fail to use insulin properly. In order to try to maintain glucose tolerance, this reduction in insulin sensitivity causes an increased release of insulin. The

resulting hyperinsulinaemia and hyperglycaemia contribute to the development of atherosclerosis through hypertension, endothelial dysfunction, dyslipidaemia, and inflammation. At its final stages, type 2 diabetes may also manifest an absolute insulin deficiency. Type 2 diabetes is a direct risk factor for IHD because the excess amount of glucose in the blood can lead to non-enzymatic glycosylation of plasma proteins that form high-volume molecules that can produce a mechanical damage to the arteries.[1,2] Not only can diabetes produce inflammation but inflammation can also be a promoting factor for the genesis of diabetes.[40] In England, the prevalence of doctor-diagnosed diabetes increased between 1994 and 2012 from 2.9% to 6.7% among men and from 1.9% to 4.9% among women.[36]

- **Gender**. It has been recognised over the past years that women form a distinct subpopulation within patients with IHD, which is markedly more common in men than in women. Cardiovascular disease develops 7 to 10 years later in women than in men on average, but after midlife it has a sharper increase with age among women. Many reasons have been proposed for this phenomenon, although there is no definitive explanation. There are some risk factors that are unique to women, such as preeclampsia and menopause for example (oestrogen may have a protective role against IHD). Furthermore, non-gender-specific risk factors also play a role for the gender differences in IHD incidence: many risk factors are more prevalent in one gender, and the significance and the relative weighting of these risk factors are different between men and women. For instance, at younger ages (<50 years) smoking is more deleterious in women than in men; women with diabetes are at greater risk for cardiovascular complications than their male

counterparts; and low HDL cholesterol implicates a higher IHD risk in women than in men.[41–44] As a consequence, widely-used IHD risk scores, such as the Framingham, the Joint British Societies & British National Formulary, the Assign, and the Q-Risk 2 for example, are indeed gender specific.[34,45–47]

Regarding non-conventional risk factors, growing evidence indicates that in heart disease, elevated circulating inflammatory markers, in particular C-reactive protein (CRP) and Interleukin-6 (IL-6), predict an unfavourable course, independent of the severity of the atherosclerotic or ischemic burden. Thus, inflammation represents one potential novel pathophysiological mechanism of heart disease that may furnish a new target for therapy.[7,11,12]

The next three sections (1.4-1.6) I will describe some other non-conventional risk factors for heart disease that are relevant to my research aims. I will go through the different types of mental adversity and then illustrate the mechanisms that link them with heart disease. As the reader will note, inflammation plays a central role. Afterwards, I will discuss the effect of socioeconomic status on human health.

1.4 Mental stress

The term stress comes from the Latin term strictus which means strained, compressed. Even before there was an understanding of the biological process of stress, researchers have been interested in how it might influence health and therefore the study of stress and disease has a long history.[48]

Under the expression of mental stress I have included several types of psychosocial conditions that can be schematically divided into three main themes, i.e. chronic stressors, mood factors, and social relationships:[49]

1. Chronic psychological distress
    a. Work stress
        i. High-demand/low-control
        ii. Effort-reward imbalance
    b. Family conflicts and care giving
    c. Low emotional support
    d. Financial strain
2. Mood and behavioural factors
    a. Depression
    b. Anxiety
    c. Hostility and anger
    d. Optimism
    e. Positive thinking
    f. Hopelessness/Helplessness
3. Social relationships
    a. Social cohesion
    b. Social support
    c. Social network
    d. Neighbourhood problems

The above conditions do not have well-defined boundaries and hence each condition can be pertinent to more than one theme.

Factors such as job strain, social network, and others can be measured objectively, even though they are often measured by self-report, and can therefore mark someone's objective exposure to stress, whereas some other factors such as depression, anxiety, and others are rather subjective mental representations, and can be thus considered as markers of individual response and adaptation to stress. A description of all the different elements of mental stress goes beyond the aims of this dissertation.

Mental stress is therefore a multi-dimensional concept and can be considered as a subjective state as well as a biological phenomenon. There are several strategies for studying mental stress as a risk factor for cardiovascular disease:[50]

- **Animal research**. Research on animals has provided many important insights into the role of mental stress. There are many advantages of using this approach: animals can be experimentally subjected to stronger stressors than humans; the period of time that goes from the inducement of stress and the development of cardiovascular disease is shorter than in humans; information and selection bias that are typical of human studies can be avoided (e.g. no losses to follow-up); histological, anatomical, and autoptical methods make disease ascertainment very accurate as well as describe complete aetiological processes; genetic and environmental factors that may confound the results can be standardised. The disadvantages of animal research are the following: experimental animals must be employed with care and utilised under ethically rigorous conditions; direct inferences to humans cannot be made; some kinds of stressor are difficult to investigate

(e.g. depression); important cofactors such as socio-economic status cannot be included into the models.[50]

- **Laboratory studies in humans**. As a biological phenomenon, mental stress can be studied in laboratory-based experiments where nonspecific biological markers of activation that are influenced by stress are evoked in human participants in response to behavioural simulation.[49] The standardised stimuli can assume various forms, e.g. problem-solving tasks or emotionally demanding social interactions, the crucial element being that conditions are perceived as stressful, challenging, and involving. These tests elicit consistent physiological responses with good test-retest reliability in many biological measures including blood pressure, heart rate, and inflammatory and haemostatic variables.[50] This approach has several compelling advantages: the experimental design can be used with randomisation to different conditions, eliminating the effect of known and unknown confounding factors; given the very short period of time between exposure and monitored outcomes (markers of activation) and the environmentally controlled conditions, issues such as of information bias (e.g. changes in stress exposure and losses to follow-up) become irrelevant; stratification or restriction for specific groups of people (e.g. people with or without certain diseases) is efficient and accurate. On the other hand, mental stress testing in humans has some limitations: although these biological responses to stress are associated with future cardiovascular events, real clinical outcomes are not measured; although the stimuli always precede the recorded biological outcomes, these studies have cross-sectional nature and in some cases (e.g. if there is no random allocation to stimuli) the possibility of reverse causality

cannot be ruled out; only short-term biological responses can be recorded, whereas chronic stressors may elicit different response patterns because of habituation, adaptation, and chronic disturbances in autonomic or neuroendocrine regulation. In order to address this issue, laboratory stress testing has been increasingly complemented by naturalistic and ambulatory methods.[50]

- **Naturalistic and ambulatory methods**. These studies involve the measurement of biological factors continuously or intermittently during everyday life and take several forms, such as Holter monitors for cardiac function and ambulatory blood pressure monitors for example. These methods have the advantage that the biological activity is assessed under natural conditions. The dynamic covariation between everyday activities, emotions, and biology can also be evaluated. The disadvantages are: the range of biological markers that can be assessed is relatively narrow (blood pressure, heart rate variability, physical activity, and salivary cortisol are amongst the commonest measures); the measurement techniques need to be relatively unobtrusive, so as not to interfere with ongoing activities, and this typically rules out blood sampling; there are factors that influence biological function in everyday life that need to be taken into account such as time of the day, cigarette smoking, food and caffeine intake, sleep, and physical activity; participants may change their behaviour because they are aware that they are being monitored.[50]
- **Observational epidemiological studies**. As a subjective state, mental stress can be studied in large-scale population-based surveys using validated questionnaires.[51,52] These often take the form of longitudinal observational

cohort or population studies, in which large, preferably representative, populations are screened to ensure they do not already suffer from the outcome condition under investigation (e.g. IHD), the measurement of the exposure variable (e.g. anxiety, depression, etc.) is then carried out along with other variables that are known to influence the outcome (possible confounders) or interact with the exposure, and then the population is followed up in time (usually many years) and the occurrence of the outcome is monitored. The main advantages of these kind of studies are: they can have actual clinical events as main outcome variables; they are usually large in sample size and therefore they are able to capture relatively small effect sizes (provided that some other conditions are met, e.g. limited information bias, sufficient number of events, etc.); the issue of reverse causality can usually be addressed (although not completely) by excluding participants who experienced the outcome event soon after their study recruitment and who may had been already in the initial phases of the disease at the time. These types of studies have of course limitations: longitudinal studies usually take many years before sufficient number of events occur to obtain statistical evidence (unless one would opt for a case-control design, in which case the retrospective nature of the study would pose major limitations such as recall bias, selection bias, and reverse causality for example); they are usually very expensive in terms of human and financial resources; the exposure variable in usually measured only at baseline (i.e. there are no repeated observations) and one cannot be sure that the initial condition is stable throughout the follow-up period (information bias, misclassification); possible confounding factors are also measured only at baseline and their quantification can be

inaccurate (usually many cofactors are measured in these questionnaires, which often take a long time to be completed, diminishing participants' compliance) or they may be even unknown, rendering that statistical adjustment at the data analysis stage inefficient when not impossible (residual confounding).[50]

- **Prevention and treatment studies**. There are psychological and behavioural methods aimed at decreasing mental stress. These methods can be experimented on participants, even with randomisation against placebo or against each other, to ascertain their effect on disease incidence. However there are limitations for these kinds of trials. To study the association between mental stress reduction and IHD prevention for example, one would firstly implement an efficacy trial (explanatory trial) to get a precise and robust quantification of the risk reduction determined under ideal, experimentally-controlled conditions. Afterwards, an effectiveness trial (pragmatic trial) should complement the results, by testing the stress reduction method in everyday, real life circumstances. Efficacy trials are typically focused on restricted categories of patients (and therefore have good internal validity but poor generalisability) whereas effectiveness trials are focused on wide categories, for the general public. There are several steps that must occur for an efficacious intervention to be effective in clinical practice, and therefore an efficacy trial can often overestimate an intervention's effect when implemented in clinical practice. In other words, efficacy trials answer the question "can it work?", whereas effectiveness trials answer the question "does it work?". The combination of those two

type of trials that form a continuum is rarely applicable in psychological and behavioural interventions, especially in the cardiovascular domain.[50,53]

As part of the present thesis I have designed and conducted studies on human participants using two of the above-mentioned approaches: laboratory-based experiments and epidemiological cohorts.

In the next sections, we will see how mental stress is increasingly becoming recognised as a risk factor for IHD in acute conditions, when sudden stressful events trigger acute IHD events, as well as in chronic conditions, when poor adaptation to recurrent daily stressors produces a pathophysiological substratum for IHD. Although the mechanisms underlying these associations have not been completely clarified, inflammation plays a central role and therefore proinflammatory factors, cortisol levels, haemostatic processes and endothelial function factors have been identified as key mediators.[54–58]

The neuroendocrine system

By endocrine system we refer to the group of glands that secrete hormones directly into the main blood stream of the circulatory system, through which they reach distant target organs. The major endocrine glands include the hypothalamus, the hypophysis (pituitary gland), the thyroid and parathyroid glands, the adrenal gland, the pineal gland, the ovaries and the testes, and the pancreas. However many other organs have endocrine activity, such as the gastrointestinal tract, the skin, the heart, etc. The metabolic activities of the target organs or cells depend on the stimulus they receive from the hormones. Glands not only influence effector cells but also influence each other. For example, the hypothalamus produces the thyrotropin-

releasing hormone (TRH), which stimulates the release of thyroid-stimulating hormone (TSH) from the hypophysis, which stimulates thyroxine (T4) and triiodothyronine (T3) synthesis and release from the thyroid. Similarly, the corticotropin-releasing hormone (CRH) released by the hypothalamus stimulates the release of the adrenocorticotropic hormone (ACTH) from the hypophysis, which stimulates corticosteroid (glucocorticoid and mineralcorticoid) and androgen synthesis and release from the adrenal gland.

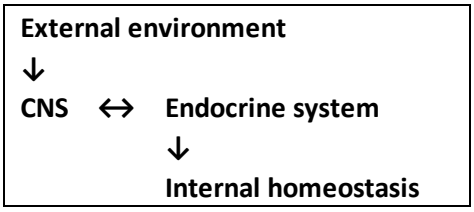
The secretory activity of the endocrine glands is constantly controlled by complex regulatory systems that adjust hormonal circulating levels to the metabolic needs of the effector cells. This regulation can happen through negative feedback mechanisms, whereby parts of the body that function as stimuli for the determination of certain conditions are inhibited by the status of the conditions they promote, achieving an automatic self-regulation. For example, the hormones that are produced by the adrenal cortex (cortisol), by the thyroid (T3 and T4), and by the gonads (testosterone, oestrogen, and progesterone) in response to hypophyseal stimulus, regulate their own production by acting at the hypothalamic-pituitary level on the excretion of their releasing factors (ACTH, TSH, and LH [Luteinizing Hormone]), i.e. the negative feedback works in such a way that the response (e.g. cortisol) inhibits its releasing factor (e.g. ACTH). Therefore, the increase in circulating cortisol inhibits ACTH secretion and, vice versa, the decrease in circulating levels of cortisol augments ACTH secretion. However, the regulatory mechanisms are more complex than it may appear from the example above, since usually many factors concur to the control of each hormone. Moreover, the hormones produced by peripheral glands give negative feedback not only to the hypophysis but also to hypothalamic and even

supra-hypothalamic structures (e.g. endorphinergic neurones in the hippocampus, as well as in the frontal and parietal lobes of the brain). Further complexity is given by the fact that in some cases there is a positive feedback, and that sometimes it is not hormone concentration that works as controller but a function regulated by the hormone (e.g. parathyroid hormone for circulating ionised calcium concentration).[1,59]

The secretory activity of each endocrine gland is therefore integrated in a system or axis (e.g. the CRH-ACTH-cortisol axis, the THR-TSH-thyroid hormones axis, etc.), whereby an axis has to be considered as a functional unit and not as a mechanic cascade with hierarchical ordering. Therefore, for each axis, the central nervous system (CNS) and the effector cells have to be seen as two terminals, and the hypothalamus as their fulcrum.[1,59]

The CNS regulates endocrine activity in response to stimuli that can be external (e.g. mental stress) and internal (e.g. metabolic variation in osmolarity) through neurotransmitters (e.g. central monoamines, GABA [γ-aminobutyric acid], etc.) and neuromodulators (e.g. cerebral neuropeptides) that act at the hypothalamic-pituitary level. Peripherally, some substances that are derived from cellular metabolic activity intervene in hormonal regulation with several direct or indirect mechanisms that aim at keeping internal conditions stable and constant (homeostasis) in response to different types of stimuli that tend to modify them. The relationship between the CNS and endocrine system can be therefore framed within the more complex interaction between the external and internal environment (Figure 1.7).

Figure 1.7. A diagram for the interaction between the external and internal environment of the human body.



The hypothalamic-pituitary system is the core of the connections between the CNS and the endocrine glands and can be regarded as the most important and complex regulating system for homeostasis. In fact, monoaminergic and peptidergic cells come in contact just in the hypothalamus. Monoaminergic cells are nerve cells that produce neurotransmitters (noradrenalin, dopamine, serotonin, etc.) that act locally. Peptidergic cells are instead real transducers between the nervous and the endocrine system, because they can be activated by neurotransmitters just like the standard nervous cells are, but in response to the nervous stimulus they produce peptides that are biologically active and that are secreted in the hypothalamic-hypophyseal portal vessels. These are the so-called hypothalamic-hypophyseal peptidic hormones (TRH, somatostatin, GnRH [gonadotropin-releasing hormone], etc.), that reach the adenohypophysis (anterior pituitary gland) through the portal vessels and bind to specific receptors belonging to secreting cells (thyrotrophic, gonadotrophic, somatotrophic cells, etc.). Some hypothalamic hormones stimulate ("releasing hormones" or "releasing factors") and some others inhibit ("inhibiting hormones" or "inhibiting factors") the release of their respective hormones from adenohypophyseal cells. The hormones that are pertinent to the posterior pituitary gland (neurohypophysis), such as arginine-vasopressin (AVP) and oxytocin, are instead produced in the neurons of the supraoptic and paraventricular nuclei of the

hypothalamus and reach the neurohypophysis through the axons of the cells that have produced them. They are accumulated in the neurohypophysis and then released in the main blood stream. However, hypophyseal responses to releasing or inhibiting factors cannot be seen as simple monophasic triggers because they are modulated by additional neuropeptides and hormones.[1,59]

We have seen how complex the relationship between hypothalamus and hypophysis is, but the relationship between the CNS and hypothalamus is even more complex. The CNS interacts with the hypothalamus through the activation or inhibition of several systems based on neurotransmitters (noradrenergic, dopaminergic, serotoninergic, etc.), but, while the single peptidergic cells of the hypothalamus synthetize only one releasing or inhibiting hormone and are grouped in well-defined areas on the basis of their secretory activity, each neurotransmitter produced by the monoaminergic cells can stimulate different parts of the hypothalamus for the production of different neurohormones. For example, the dopaminergic system inhibits the release of prolactin but stimulates the release of growth hormone (GH). Furthermore, the activity of the hypothalamic cells producing hypophysiotrophic hormones is controlled by more than one kind of neurotransmitter. For example, the CRH-ACTH-cortisol axis is inhibited by the noradrenergic system and stimulated by the serotoninergic and dopaminergic systems. Therefore, the complexity of the connection between CNS and hypothalamus, between mind and body, is given by this many-to-many system of relationships, where one stimulus can have many responses and one response can be evoked by many stimuli.[1,59]

I have focused on the CNS because the hypothalamus is located inside the brain. However, the parts of the nervous system that consists of the nerves and ganglia

outside of the brain and the spinal cord are also very important. These form the peripheral nervous system (PNS), which can be divided into the somatic nervous system and the autonomic nervous system (ANS). The main function of the PNS is to connect the CNS to the limbs and organs, essentially serving as a communication relay going back and forth between the brain and the extremities. The ANS is particularly relevant in the study of mental stress because it acts as a control system that functions largely below the level of consciousness to control visceral functions, including heart rate, vasoconstriction, digestion, respiratory rate, etc. Most autonomous functions are involuntary but they can often work in conjunction with the somatic nervous system which provides voluntary control. The ANS is divided into three main sub-systems: the parasympathetic nervous system and the sympathetic nervous system, and the enteric nervous system. Depending on the circumstances, these sub-systems may operate independently of each other or interact co-operatively. The sympathetic nervous system is often considered the "fight or flight" system (increased metabolism, heart rate, vascular tone, respiration rate, etc.), while the parasympathetic nervous system is often considered the "rest and digest" or "feed and breed" system. In many cases, but not always, these two subsystems have "opposite" actions where one system activates a physiological response and the other inhibits it. There are two kinds of neurons involved in the transmission of any signal through the sympathetic system: pre- and post-ganglionic. The shorter preganglionic neurons originate from the thoracolumbar region of the spinal cord (levels T1–L2, specifically) and travel to a ganglion where they synapse with a postganglionic neuron. From there, the long postganglionic neurons extend across most of the body and can reach any tissue where they release noradrenaline, which activates adrenergic receptors on the peripheral target tissues. The activation of

target tissue receptors causes the effects associated with the sympathetic system. Moreover, the neurons can reach and activate the adrenal gland itself, which develops in tandem with the sympathetic nervous system and can be seen as a particular type of sympathetic ganglion. Within the medulla (inner part) of the adrenal gland, pre-ganglionic neurons synapse with chromaffin cells, stimulating the release of noradrenaline and adrenaline directly into the blood (endocrine effect). Therefore the CNS, the PNS, and the endocrine system can work as a whole integrated system.[1,59]

I have described how the neuroendocrine system regulates the basal or tonic activities of the various axes in relation to the metabolic needs on the body (homeostasis). Nonetheless, neuroendocrine connections are also indispensable for the maintenance of the secretory rhythms that characterise the activity of the body, the so-called circadian rhythms. For instance, cortisol shows a strong diurnal pattern, GH and PRL can influence the sleep–wake rhythm, etc. When the frequency of the peaks, or the amplitude of the pulses, goes beyond certain limits the normal activity of an axis stops. So there is another form of negative feedback: in many endocrine systems the pattern of hormonal secretion is as important as the absolute concentration. Therefore the integrity of the nervous system is important even to control hormonal variations, for example when ACTH is suddenly released and, consequently, cortisol circulating levels are increased in response to mental stress.[1,59]

Stress response can be considered as one of the biological phenomena that are most indicative of the connections between the nervous and the endocrine systems. Acute mental stressors can induce hypertension, tachycardia, the release of cortisol, catecholamines, GH, and PRL that are typical of behaviours such as escape, defence,

anger, etc. These responses are considered as forms of adaptation, because they are meant to nullify the effect of the stressor.[1,59]

CRH induces the release of ACTH and therefore cortisol, but also of catecholamines with the interplay of the ANS, and can also produce hyperglycaemia and haemodynamic variations that are typical of acute stress stimulation. CRH-secreting neurons have been identified not only in the hypothalamus, but also in areas correlated with sympathetic and behavioural activity such as the limbic system and the mesencephalon (midbrain). CRH can be therefore considered one of the most important mediators of stress that is able to activate metabolic, circulatory, endocrine, and behavioural responses.[1,59]

Neuroendocrine-immune interactions

While the endocrine system is responsible for internal homeostasis, the role of the immune system is to counteract external agents such as bacteria, viruses, other germs, and proteins. However, some neuropeptides, peptide hormones, and hormonal receptors that are typical of the endocrine system are expressed also by immune cells. These cells not only respond to non-self agents with immunoglobulins but also with several peptides including cytokines (lymphokines and monokines) and some hormones and peptides that are usually associated with endocrine activity.[1,59] Furthermore, the hypophyseal hormones that are produced by the lymphocytes are regulated by the same factors that regulate the activity of the hypophysis. For example, ACTH production by the lymphocytes is suppressed by glucocorticoids and stimulated by CRH, whereas the production of GH is stimulated by GHRH (growth-hormone-releasing hormone) and suppressed by somatostatin. Cytokines regulate

the differentiation, the proliferation, and the function of other immune cells, but also influence other tissues and systems such as fever, protein and lipid metabolism in muscles, and neuroendocrine function. For example, Interleukin 1 (IL-1) stimulates the release of CRH at the hypothalamus.[1,59]

Importantly, during inflammation cytokines are not only released locally but also in the main blood stream and can influence neuroendocrine function by acting on the encephalon and/or on the hypothalamus and/or directly on the pituitary gland. Some endocrine cells are also able to synthetize cytokines.[1,59]

Therefore, the nervous system can modulate immune function through the hypothalamic-pituitary-peripheral gland system and the autonomic nervous system. The hypothalamic-pituitary-adrenal (HPA) system is one of the most important because the production of glucocorticoids by the adrenal gland has a considerable suppressive effect on immune function by decreasing the rate of lymphocyte proliferation, as well as the production of immunoglobulin, cytokines, and mediators of inflammation such as leukotrienes. The anti-inflammatory action of the glucocorticoids that are used in clinical practice is based on the inhibitory effects that I have described. Therefore the HPA response to stress can modulate immune function and its inflammatory components, including those that influence vessel permeability. The fact that some products of the inflammation such as interleukin 6 (IL-6) can activate the HPA axis is indicative of the existence a negative feedback that controls inflammation. Since the HPA axis is controlled by the nervous system we have to think about a psycho-neuro-endocrine-immune system.[1,59]

In summary, mental stress is able to alter the HPA axis, which regulates many body processes, including the immune system and inflammation.[54–57] Mental stress initiates the release of cortisol by activating corticotrophin releasing factor and arginine vasopressin neurons in the paraventricular nucleus of the hypothalamus,[60] and this leads to the release of adrenocorticotrophic hormone from the pituitary gland, which triggers the release of glucocorticoids from the adrenal glands. Moreover, known cross-talk between the brain and the immune system includes the sympathetic-adrenal-medullary axis, which controls stress-induced catecholamine release in support of the fight-or-flight reflex.[1,59,61,62]

Other dynamics

Other dynamics may operate for the association between mental stress and IHD.[63]

Abnormalities in serotonergic function may influence atherogenesis. Serotonin is a neurotransmitter found in the CNS and is critical in the regulation of mood, emotions, and behavior. Serotonin is also found in the platelets which release serotonin when they bind to a clot, where the neurotransmitter serves as a vasoconstrictor and helps to regulate haemostasis and blood clotting. Therefore, serotonin has vasoactive properties and is involved in thrombogenesis and platelet activation, and hence both central and peripheral serotonergic mechanisms influence thrombovascular processes.[64–66] It is interesting to note that the serotonin that is found in the platelets is primarily secreted by the enterochromaffin cells of the gastrointestinal tract, which is innervated by the PNS, where the neurotransmitter is used to regulate intestinal movements, and then it moves into the platelets. Preclinical investigations and clinical studies indicate that depression is associated

with serotonergic dysfunction in the central nervous system and in peripheral circulating platelets.[67–69] Depressed patients, especially those with high levels of anxiety, showed serotonin-stimulated increases in platelet intracellular calcium, which is involved in platelet activation and maintenance of blood pressure.[70] Other chronic stressors such as anxiety can also produce alterations in serotonin levels and function, and serotonergic dysregulation plays a critical role in aggressive behavior and impulsivity and may be associated with high levels of anger and hostility.[71,72] Current hostility and lifetime history of aggressive behavior in adults with major depression have been associated with high levels of platelet serotonin,[73] and increasing levels of hopelessness have been associated with high whole blood serotonin levels in a population-based sample of older adults.[63]

A study on mice has recently ascertained the role of mental stress on hematopoietic stem cells. Apparently stress increases proliferation of hematopoietic stem cells, i.e. the most primitive hematopoietic progenitors, giving rise to higher levels of disease-promoting inflammatory leukocytes. It has also been found that chronic stress induces monocytosis and neutrophilia in humans. While investigating the source of leukocytosis in mice, it was discovered that stress activates upstream hematopoietic stem cells. Under conditions of chronic variable stress in mice, sympathetic nerve fibres released surplus noradrenaline, which signalled bone marrow niche cells to decrease CXCL12 levels through the β3-adrenergic receptor. Consequently, hematopoietic stem cell proliferation was elevated, leading to an increased output of neutrophils and inflammatory monocytes. When atherosclerosis-prone Apoe−/− mice were subjected to chronic stress, accelerated haematopoiesis promoted plaque

features associated with vulnerable lesions that cause myocardial infarction in humans.[74]

Biological markers of mental stress

The key markers of dysregulation related to mental stress that are relevant to cardiovascular disease are: blood pressure, heart rate variability, cortisol, C-reactive protein (CRP), IL-6, von Willebrand factor (vWF), MCP-1, and fibrinogen:[54–57,75,76]

- **Blood pressure**. The physical pressure that the blood mass exerts on the walls of the arteries is measured in millimetres of mercury (mmHg) and is recorded as two figures: systolic pressure, i.e. the pressure of the blood when the heart beats to pump blood out, and diastolic pressure, i.e. the pressure of the blood when the heart rests in between beats, which reflects how strongly the arteries are resisting blood flow. A blood pressure reading below 140/90mmHg is considered to be normal.[1]
- **Heart rate variability** is the physiological phenomenon of variation in the time interval between heartbeats. It is measured by the variation in the beat-to-beat interval, usually within electrocardiographic sessions.[1]
- **Cortisol** is a glucocorticoid hormone that is part of the steroid family. It is produced by the adrenal cortex, specifically in the zona fasciculata. Its primary functions are to increase blood sugar through gluconeogenesis, suppress the immune system, and enhance the metabolism of lipids, proteins, and carbohydrates.[1]
- **CRP** is an acute phase protein (i.e. it is released into the plasma in response to inflammation) that is synthesised by the liver in response to factors

released by macrophages and adipocytes (fat cells). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system (a part of the immune system). CRP secretion is stimulated by proinflammatory cytokines, in particular IL-6. CRP is one of the most widely studied inflammatory markers.[1]

- **IL-6** is secreted by T cells and macrophages to stimulate immune response during infections and traumas, leading to inflammation. It is an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. More recent studies show that adipocytes are also able to produce IL-6.[77]
- **vWF** is produced by endothelial cells and bone marrow cells, and is a blood glycoprotein involved in haemostasis, which is the process which causes haemorrhage (bleeding) to stop. Its primary function is binding to other proteins and specific cells during the formation of a blood clot. vWF is important in platelet adhesion.[78]
- **Chemokines** (chemotactic cytokines) are small heparin-binding proteins that constitute a large family of peptides structurally related to cytokines, whose main function is to regulate cell trafficking. Chemokines play a major role in selectively recruiting monocytes, neutrophils, and lymphocytes. MCP-1 is a chemokine that regulates the migration and infiltration of monocytes, memory T lymphocytes, and natural killer (NK) cells. MCP-1 is produced by many cell types, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and microglial cells. These cells are important for antiviral immune responses in the peripheral circulation and in

tissues. However, monocyte/macrophages are found to be the major source of MCP-1, which is responsible for their migration from the blood stream across the vascular endothelium.[79]

- **Fibrinogen** is a plasma protein produced by the liver and is a major coagulation factor. It is a positive acute-phase reactant protein, i.e. its concentration increases with inflammation, and it is traditionally considered as a risk factor for IHD because it promotes formation of thrombus.[80,81]

1.5 Epidemiological studies of mental stress and ischaemic heart disease

Epidemiological studies about the relationship between mental stress and IHD have been conducted with regards to specific aspects of stress and specific cardiovascular diseases.

- **Work stress.** The positive association between high work stress and IHD incidence has been recently clarified in an individual-level meta-analysis from 13 European cohort studies (1985-2006) of men and women who did not have any IHD and were employed at the time of baseline assessment, with a total sample size of about 200,000. The authors screened published and unpublished data to avoid publication bias. Job strain was measured with questions from a validated job-content questionnaire and demand-control questionnaire, which were included in the baseline self-report questionnaire of all studies. The authors defined the binary exposure variable as job strain (high demands and low control) versus no strain (all other combinations, e.g. high demands but also high control, low demands and low control, etc.). The

authors obtained information about incident coronary heart disease during the follow-up time from national hospital admission and death registries in all studies. Individuals were defined as having incident IHD according to the type and time of diagnosis of their first disease event. The hazard ratio (HR) for job strain versus no job strain was found to be 1.23 (95% confidence interval [CI] 1.10-1.37), which means that the incidence rate of IHD was on average the 23% higher on those who had reported job strain .[82]

- **Anxiety**. The link between anxiety IHD has a long history and it has been now accepted by the scientific community.[83–87] For example, a recent meta-analysis assessed the association between a comprehensive set of common anxiety constructs (including anxiety, panic, phobia, post-traumatic stress, and worry) and incident IHD. The included studies were prospective in nature, following a nonpsychiatric cohort of initially healthy persons over time. Studies had to include at least one self-report or interview-based assessment of anxiety symptoms or anxiety disorder at baseline (e.g., post-traumatic stress disorder, tension, worry, phobic anxiety, panic, or generalized anxiety disorder), and had to monitor the incidence of IHD. The mean follow-up period was about eleven years. Anxious persons were at higher risk of IHD (HR =1.26; 95%CI =1.15-1.38; P <0.001).[88] Moreover, a prospective cohort study evaluated the effect of generalised anxiety disorder on cardiovascular prognosis, i.e. in subsequent cardiovascular events after the first one. After having followed up about a thousand outpatients with stable IHD for six years on average (patients were interviewed on an annual basis), it was found that patients with anxiety were more likely to experience

a relapse or worsening of the disease (HR =1.74; 95%CI= 1.13-2.67; P =0.01).[89]

- **Social relationships**. Although some studies show no associations with disease,[90] the overwhelming consensus is that positive social factors do protect against heart disease to some extent.[63,91–93] For example, in a recent meta-analysis of prospective studies on loneliness and social isolation, the pooled relative risk for social isolation or loneliness and first IHD event across the 9 studies identified was 1.5 (95%CI =1.2-1.9).[57]
- **Anger and hostility.** Investigations into the effects of anger and hostility on risk for CVD have a long history. Early psychoanalytic and psychodynamic literature described episodes of anger, hostility, or other strong emotions or personality characteristics, such as aggressiveness and a need to be hard-driving and tough-minded, in patients with heart disease or hypertension. These observations, together with the need to provide a clearer definition and assessment of what was deemed "coronary-prone behavior," motivated work in the 1950s and 1960s on what came to be called the Type A behavior pattern. On the basis of observations of their own cardiac patients, Rosenman & Friedman described the Type A individual as one who was exceedingly hard-driving and ambitious, competitive, time-urgent, and unusually quick-tempered and tightly wound. Their initial work suggested that Type A men and women had higher cholesterol levels and greater evidence of IHD, compared with those who were "Type B".[94] In the past two decades, numerous studies have investigated hostility and anger, measured with various instruments, in relation to risk of hypertension, stroke, and IHD morbidity and mortality, with both positive and null findings. However, a

meta-analytic review of 45 studies published in 1996 concluded that hostility is an independent risk factor for IHD and all-cause mortality.[95] A number of studies investigating hostility and/or anger and incident CVD have been published since then; the majority reported positive associations.[63]

- **Depression and other mood factors**. Over the past 40 years more than 60 prospective studies have examined the link between established indices of depression and IHD. Despite differences in samples, duration of follow-up, and assessment of depression and depressive symptoms, these studies have demonstrated relatively consistent results and the literature continues to support both an etiologic and a prognostic role for depression.[96] For example, a recent systematic literature review on depression and adverse medical outcomes after IHD (all-cause mortality, cardiac mortality, and nonfatal events) that included 57 studies, concluded that despite the heterogeneity of published studies, the preponderance of evidence supports the recommendation to elevate depression to the status of a risk factor for adverse medical outcomes in patients with IHD.[97] Hopelessness is one symptom of depression that appears to have particularly adverse effects on health. A single item on hopelessness extrapolated from a multi-item measure of depressed affect predicted a more than two fold risk of fatal and nonfatal IHD and was a stronger predictor than the complete measure.[98] In the San Antonio Heart Study, high levels of hopelessness predicted all-cause and CVD mortality in Mexican Americans and Caucasians.[99] Everson and colleagues found that hopelessness predicted a two fold increase in cardiovascular mortality, AMI, and all-cause mortality over six years of follow-up in a population sample of middle-aged Finnish men from the

Kuopio Ischemic Heart Disease study, after controlling for demographic characteristics, cardiovascular risk factors, and overall depressive symptoms.[100] Furthermore hopelessness was related to accelerated progression of intimal-medial thickening in the carotid arteries and threefold greater risk of incident hypertension over four years in the KIHD study.[101,102]

Combination of stressors. Stress sources can be studied in combination. For example, a systematic review and meta-analysis of the association between perceived stress and incident IHD at >6 months was carried out combining observational cohort studies that measured self-reported perceived stress from different sources, i.e. work stress, marital conflicts, financial strain, and emotional stress. The meta-analysis yielded an aggregate risk ratio of 1.27 (95% CI =1.12-1.45) for the magnitude of the relation between high perceived stress and incident IHD.[103]

There are however limitations to the studies that I have reported. Often mental stress measures are not objectively assessed but are self-reported, with issues of information bias. They are observational studies, and therefore there may be unknown factors confounding the results. Confounding happens when an unknown and therefore unmeasured risk factor for the outcome under study is associated with the exposure under investigation, and therefore it can be an alternative explanation for the supposed exposure-outcome association. Some residual confounding can also come from known factors, if they have been measured without sufficient accuracy and precision. Furthermore, although these observational studies had prospective nature, the issue of reverse causality cannot be ruled out (early stages of the disease causing mental stress). These issues could be addressed by implementing randomised controlled trials (RCTs), in which the exposure variable is randomly

allocated to study participants (RCT have many other features, such as blindness for example, but the description of those features goes beyond the aims of this dissertation). Well-designed RCTs can eliminate the issues of known and unknown confounding factors as well as of reverse causality.

There have been some RCTs carried out to study the association between mental stress and IHD. The disadvantage of RCTs is that they are more expensive than observational studies and therefore tend to have smaller sample sizes, and thus they are not able to detect small effect sizes and therefore are not very effective for relatively rare events such as AMI and cardiovascular mortality. For example, in a double-blind, placebo-controlled randomised trial conducted in 40 outpatient cardiology centres and psychiatry clinics in the United States, Europe, Canada, and Australia, 369 patients with major depressive disorder and who had recent AMI or unstable angina were randomly assigned to receive sertraline (a drug used to treat depression) with the aim of assessing drug safety by measuring the change in left ventricular ejection fraction. The drug resulted to be safe and effective for the treatment of recurrent depression in patients with IHD and in secondary analyses, it was also found that the incidence of severe cardiovascular adverse events was 14.5% with sertraline and 22.4% with placebo, but this results was not statistically significant ($P > 0.05$).[104] Furthermore, a RCT with about 2,400 AMI patients, evaluated the effect of cognitive behavior therapy that was designed to tackle depression and low-perceived social support towards mortality or recurrent AMI, and it was found that the intervention did not increase event-free survival.[105]

A meta-analysis of 21 aetiological and 34 prognostic cohort studies on the effect of depression on IHD was explicitly set out to estimate the contribution of confounding,

reverse causality and other biases.[106] The authors argued that several biases are likely to lead to an overestimation of the depression–IHD association. For example they found some evidence of publication bias, with smaller negative aetiological studies missing. Furthermore, no adjustment for coronary risk factors could be included for nearly half of the aetiological studies, and in these studies, the adjusted effect was systematically lower than the unadjusted effects in studies which also reported adjusted differences. This suggests that adjustment for IHD risk factors was selectively reported in studies which had stronger effects, and therefore, had adjustment been available in all aetiological studies, the overall adjusted depression effect would have been weaker. When adjustment was carried out, it seldom included all the major IHD risk factors. Many studies omitted adjustments for IHD risk factors known to be associated with depression such as smoking, exercise, BMI, and alcohol. Time-dependent covariates, to allow for change in health behaviours during follow-up, were very rarely used. The authors also noted that the healthy population studies tended to remove patients with prevalent IHD at baseline, but this does not preclude the possibility of reverse causality. Coronary disease commonly presents with chronic angina, or non-specific chest pain (which were seldom explicitly excluded) and this may lead to depression, but many studies made limited or no attempt to remove such patients from analyses. Among those without symptoms of chest pain, depression might initiate atherosclerosis de novo or accelerate the progression of underlying atherosclerosis. Consistent with the latter possibility, the authors found that the strongest effect of depression on IHD incidence was found in early periods of follow-up. In prognosis studies, it was found no evidence that more severe depression (as indicated by either lower prevalence of depression or clinical assessment) had stronger associations with prognosis than less severe depression.

This is consistent with depression being a consequence of ill-heath rather than an adverse prognostic risk factor. Selection bias and information bias (in particular differential misclassification, i.e. a type of measurement error that distorts the results) were also considered by the authors with the following arguments. Higher effects were found when depression was assessed clinically rather than defined by symptom scales in aetiological studies. Studies with clinical assessment are likely to have a higher proportion of more severely depressed patients in their exposed group than studies with detection by symptom scale, suggesting that more severe depression carries a higher risk of IHD. Moreover, studies with a lower prevalence of depression at baseline also reported higher effect estimates. Although true underlying prevalence of depression will vary between study populations, it is plausible that a lower prevalence of depression also denotes more severe depression, supporting the findings on the mode of assessment. In other words, if study participants with non-severe forms of depression and who were at lower risk of developing IHD were wrongly categorised as not having depression or even excluded from the study, the effect of depression on IHD would be overestimated.[106] Some of the mood and behavioural conditions that are linked with IHD appear to be different or sometimes opposite from each other. Anxiety and depression are an example of that. Anxiety is an unpleasant state that is often accompanied by nervous behaviour and a feeling of worry that usually lead to overreaction to normal situations, whereas depression is a persistent sadness or low mood with associated loss of interest in activities and often failure to react to situations. In spite of those dissimilarities, both conditions are positively associated with higher chances of IHD. It has been argued that affective dispositions can overlap and that a general disposition toward negative

affectivity may be more important for disease risk than any specific negative affect.[107]

Furthermore, it is highly likely that the psychosocial risk factors described above do not work in isolation and that there is a resultant clustering of psychosocial risk factors, which produces an interactive pathophysiological effect, potentially greater than the effect of the factors added together. For example, people experiencing severe work stress may also become anxious or depressed; or, slightly abnormal levels of work stress may be particularly harmful for depressed or anxious people, for whom the deleterious effect of stress may be amplified. Moreover, psychosocial variables converge with conventional CVD risk factors (such as smoking for example) which compound the effect further.[63,108]

Mental stress may not only converge with classic heart disease risk factors, but also with low socioeconomic status (SES). For example, Steptoe and Marmot (2003) devised a scale aggregating chronic stress exposure and available social resources to investigate the cumulative effect of psychosocial adversity on quality of life. Using a Whitehall II sample of men and women, the PAVIX (Psychosocial Adversity and Vulnerability Index) was associated with depression and hostility and correlated with SES.[109]

1.6 Socioeconomic status

The study of the relationships between SES and health has a long scientific history. Since the time of rapid industrialisation and migration of the population into urban areas in Europe, observers have noted links between poor health and adverse living conditions associated with poverty. In the past few centuries, carefully-conducted

studies documented disease associations with poverty using early statistical approaches, e.g. mapping cases within geographical areas, comparing diseased and healthy groups on living conditions. Medicine, epidemiology, sociology, demography, and economics are only some of the disciplines that participated in establishing the strong and consistent gradients between SES and health. Across diverse health outcomes, individuals who are less educated, have lower-status jobs, who earn less or no income are at greater risk for poor health than their higher-SES counterparts. The associations extend from relatively minor illnesses, e.g. headaches, to serious and life-threatening diseases, e.g. coronary heart disease, to early mortality, and are apparent across the life course. Individually and in aggregate, across the life course, time, and space, a vast number of studies have shown how socioeconomic disadvantage is related to poorer health.[110]

SES is an umbrella term for a range of indicators and interconnected concepts that are key to understanding inequalities in health. SES is therefore related to numerous exposures, resources, and susceptibilities that may affect health. There is no single best indicator of SES suitable for all study aims and applicable at all time-points in all settings. Each indicator measures different, often related aspects of socioeconomic stratification and may be more or less relevant to different health outcomes and at different stages in the life course. The choice of SES measure(s) should ideally be informed by consideration of the specific research question and the proposed mechanisms linking SES to the outcome. This is the case when SES is the exposure of interest as well as when it is being considered as a confounding/mediating/modifying factor. If the central interest is to show the existence of a socioeconomic gradient in a particular health outcome then the choice of indicator may not be crucial.

However, even in a case such as this, using different indicators of SES may result in gradients of varying slopes. Furthermore, while a single measure of SES may show an association with a health outcome, it will not encompass the entirety of the effect of SES on health. This issue is of particular importance when SES is a potential confounding/mediating/modifying factor. Multiple SES indicators, preferably measured across the life course, will be needed to avoid residual confounding, or mediation, or effect modification, by unmeasured socioeconomic circumstances.[111–116]

A description of the theoretical basis, measurement, interpretation, strengths, and limitations of each SES indicator goes beyond the aims of this dissertation, and I will just note that the markers of SES that are typically used in medical research are:[111–116]

1. Occupational status
2. Level of education
3. Individual or household income
4. Social deprivation
5. Neighbourhood deprivation

Socioeconomic status has therefore different components: it can be an indicator for an individual's rank or prestige in relation to others (i.e. the "social" component of SES) and/or can describe access to material and social resources and goods (i.e. the economic component), and/or can be correlated to an individual's education level and consequently to his/her psychological skills. Therefore, many factors may contribute to generate SES disparities in health, and among the most prominent

candidates are limited access to appropriate health care, poor health-related behaviours, and mental stress.[111–116]

Health care systems are vital determinant of health. Their dedicated resources are often too scarce and/or misused in developing and developed countries.[117] In 2008 the general director of the World Health Organisation declared: "no one should be denied access to life-saving or health-promoting interventions for unfair reasons, including those with economic, social, or political causes."[118] Health care is inequitably distributed around the world and the pattern of inequity in utilisation is pronounced in low- and middle-income countries but is also present in high-income countries. The so-called 'inverse care law', in which the poor consistently gain less from health services than the better off, is visible in every country across the globe.[119]

In the United States of America (USA), ethnic minorities are more likely to be diagnosed with late-stage breast cancer and colorectal cancer than whites and patients in lower socioeconomic strata are less likely to receive recommended diabetic services and more likely to be hospitalised for diabetes and its complications. Inequalities in health care are related to a host of socioeconomic and cultural factors, including income, ethnicity, gender, and rural/urban residency in the USA.[120] In China, low socioeconomic status is closely associated with poor secondary prevention in patients at high risk of IHD: among patients affected by IHD who are very likely to experience a new IHD event, secondary prevention was prescribed less in low SES patients.[121] In a systematic comparison of hospital quality indicators in the USA and United Kingdom (UK) with regards to IHD patient in need of surgical or minimally-invasive treatment procedures such as CABG and PCI, low SES strata were

less likely to be treated in a timely fashion and were assigned to longer waiting lists.[122] Despite differences between the USA third-party payer health system and the UK socialised one, each country faces the same SES inequalities regarding waiting times.[122] Similar results have been found in other countries such as Sweden,[123] Finland,[124] and Denmark.[125] Moreover, in a historical cohort study with ecological analysis comprising about 8,000 patients with new-onset stable chest pain but no known cardiac history, it was found that those from low SES were less likely to be referred to chest pain clinics.[126] Furthermore, using hospital administrative data for all patients admitted to any English public hospital, a survey of about 200,000 stroke patients found that least socio-economically deprived patients appeared to have more chance of being selected for appropriate brain scan.[127] Nevertheless there are some contrasting results. Using the data from the Whitehall II prospective cohort study, about 10,000 civil servants were followed up for over 15 years. Service employment grade was used as a measure of individual socioeconomic position, and the need for cardiac care was determined by the presence of angina, myocardial infarction, and coronary risk factors. The use of exercise electrocardiography, coronary angiography, and coronary revascularisation procedures and secondary prevention drugs were the main outcome measures, and there was no evidence that low SES was associated with lower use of cardiac procedures or drugs, independently of clinical need.[128]

As we have seen, the treatment for IHD is one of the most studied indicators of health inequality. This is mainly because IHD is the most common cause of death in the developed world and there are internationally-recognised guidelines for its appropriate treatment.[129]

Behaviours such as smoking, food choice, physical inactivity, and alcohol consumption are well recognised determinants of health.[130]

There is a SES gradient in health-related behaviours - with low SES groups being more likely to make unhealthy choices - an effect that has been consistently described in population surveys.[131–133] Moreover, Wardle & Steptoe (2003), while confirming that high SES sectors of the population are less likely to make unhealthy choices, demonstrated that low SES is associated with less health consciousness (being able to distinguish between the healthy and the unhealthy choices), stronger beliefs in the influence of chance on health, less thinking about the future, and lower life expectancies. These attitudinal factors are in turn associated with unhealthy behavioural choices.[134]

As a consequence, health-related behaviours may be considered as important mediators between SES and health (cardiovascular health in particular): low SES people are more likely to make unhealthy choices which in turn put them at higher risk of CVD and mortality.[135,136]

Nonetheless, the socioeconomic gradient in healthy behaviours may be different between developed and developing countries. In an analysis that I carried out in collaboration with colleagues in Thailand and Australia of an international comparison of representative samples from Thailand and England with about 40,000 participants, I found that, while mental stress was consistently associated with low SES in both countries, in Thailand there was a flat slope (in men), or even a negative slope (in women) for the association between SES and healthy behaviour, with Thai females being less likely to have unhealthy behaviours when they were in low SES.[137]

The next section will describe how mental stress can interact with SES for the determination of IHD.

1.7 The reserve capacity model

We have seen how mental stress is becoming increasingly recognised as a risk factor and trigger for IHD events[54,55,57] and how SES is also a recognised determinant of health status: the lower a person's SES the worse his or her health.[113,138,139] In particular, low SES is associated with high risk of IHD in developed countries such as England.[140,141]

I have mentioned that mental stress may be one of the mediators between SES and IHD. However there are inconsistent findings in the literature on this matter with consequent limited support for a mediating role of stress. For example, some studies report either no relationship between SES and stress[142,143] or, even more surprisingly, an opposite association such that higher stress was found to be present in more advantaged SES groups.[144] While it has been proposed that different aspects of chronic work stress contribute to the relationship between SES and health outcomes such as IHD,[145,146] some studies investigating whether various forms of stress contribute to SES-health associations have produced null findings.[147,148] Moreover, whereas some studies have identified support for an intermediate role of negative emotions, including depression and anxiety, in connecting SES with health outcomes,[147,149] other studies have failed to provide evidence for these pathways.[150,151]

The relatively weak support for an intermediary role of stress stimulated the researchers Gallo and Matthews to hypothesise their reserve capacity model.[110,152]

This model focuses on interpersonal and intrapersonal resources as modifiers of the effect of environmental demands and stresses on emotional and physiological responses. From this perspective, on the one hand low-SES environments foster greater exposure to frequent and intense harmful or threatening situations and fewer rewarding or potentially beneficial situations, which in turn, are believed to have direct negative effect on emotional experiences. On the other hand, low-SES individuals maintain a smaller bank of resources (tangible, interpersonal, and intrapersonal) to deal with stressful events when compared to their higher-SES counterparts.

Resources banks, termed reserve capacity, may be low in low-SES circumstances for two reasons:

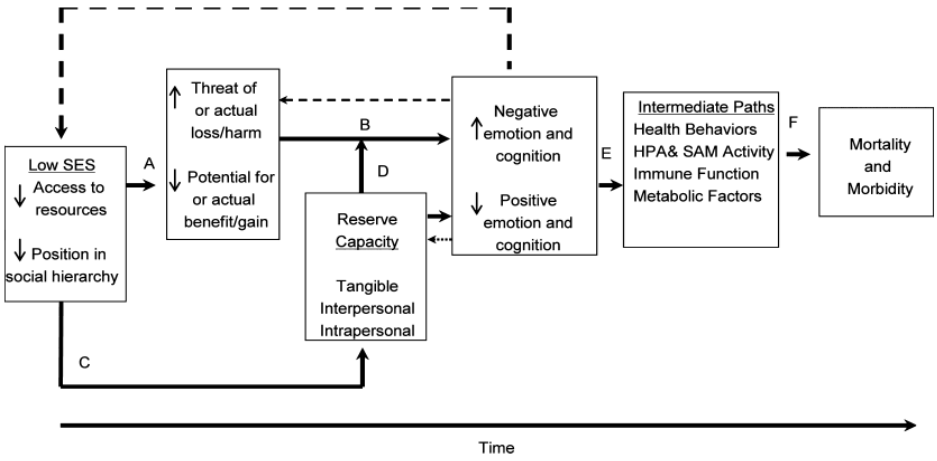
1. Low-SES individuals are exposed over the life course to more situations that require the use of resources.
2. The environment of low-SES people prevents the development and replenishment of resources to be kept in reserve.

Hence, because individuals of low SES have fewer stress-dampening resources, which are further reduced by enduring or repeated stress exposures, they are likely to show increased responsiveness when faced with challenges and demands.[110,152]

Figure 1.8 shows a diagram for the reserve capacity model for the dynamic associations among environments of low SES, stressful experiences, psychosocial resources, emotion and cognition, and biological and behavioral pathways predicting morbidity and mortality over time. Dashed lines depict possible reciprocal influences. Arrow A depicts the direct influence of SES on exposure to stressful experiences.

Arrow B indicates the direct impact of stressful experiences on emotion and cognition. Arrow E shows the effects of stress on intermediate pathways hypothesized to affect health outcomes. Arrow C shows that socioeconomic environments condition and shape the bank of resources (i.e., the reserve capacity) available to manage stress. Arrow D shows that the reserve capacity represents a potential moderator of the association between stress and cognitive–emotional factors. Arrow E indicates the direct impact of cognitive–emotional factors on intermediate pathways and Arrow F on intermediate pathways to illness and death.

Figure 1.8. A diagram for the reserve capacity model.



HPA: hypothalamic-pituitary-adrenocortical axis.
SAM: sympathetic adrenal-medullary axis.
Adapted from Matthews KA, Gallo LC, Taylor SE. Ann N Y Acad Sci. 2010 Feb;1186:146-73.

In synthesis, people in higher SES categories may have greater economic, social, and psychological resources and better coping strategies for dealing with adversity, and therefore mental stress may not be considered as a mediator between SES and health but it may interact with SES, such that the effect of mental stress on health is modified by SES, so that measures of association between mental stress and health

may differ according to categories of SES. In other words, low-SES may be an amplifier of the detrimental effect that mental stress exerts on human health.

Chapter 2 - Aim of the study

The aim of this thesis was to analyse the combined association of mental stress and low SES with the risk of acute myocardial infarction, with an emphasis on the interaction between the two risk factors. I hypothesized that SES can operate as an amplifier of mental stress, such that when both risk factors are present the resulting effect on the risk of AMI is not the mere sum of the two (additive effect) but that some extra risk may appear (multiplicative effect). In other words, I hypothesized that the effect of mental stress on AMI would be greater in lower than higher SES groups.

Chapter 3 - Systematic literature review

The present systematic literature review has been carried out to check whether my research hypothesis had been already addressed, in order not to duplicate existing evidence, and hence it was not designed to produce a synthesis of knowledge. Therefore I aimed at retrieving works that had explicitly hypothesised the existence of an interaction between mental stress and SES and a priori, and I deliberately neglected secondary results from subgroup analyses. In fact, it has been shown that post hoc subgroup analysis can lead to false positive results simply due to chance.[153]

3.1 Methods

I systematically searched the databases Medline and Embase for relevant articles. I used the following MeSH terms (for Medline) or Emtree terms (for Embase) to identify articles that were relevant to my research hypothesis (the search was restricted to major topics):

1. Behavior and Behavior Mechanisms
2. Socioeconomic Factors
3. Cardiovascular Diseases

The three groups were combined using the Boolean logic term “AND”.

The Mesh/Emtree term Behavior and Behavior Mechanisms includes behavioural and affective concepts such as depression and psychological distress, emotions such as anxiety and hostility, and psychosocial concepts such as family conflict, psychosocial deprivation, and social distance, and many other psychosocial conditions. The

Mesh/Emtree term Cardiovascular Diseases can include all cardiovascular diseases that may share common pathophysiology with AMI. I have therefore deliberately chosen broad entry terms to make my search as sensitive as possible. In addition, I have performed a free-text search on the same databases using the entry terms described in Table 3.1. Entry terms within each group were combined using the Boolean logic term "OR", whereas the groups were combined together using "AND". This search was restricted to titles and abstracts.

Table 3.1. Free-text entry terms for the systematic literature review.

Mental stress	SES	CVD	Interaction
• (mental OR psychological OR psychosocial OR perceived OR work OR job) AND (stress OR distress OR stressor* OR strain)	• (socioeconomic OR social OR economic OR occupational OR employment) AND (status OR class* OR position* OR deprivation OR grade* OR type*)	• (cardiovascular OR cardiac OR heart) AND disease*	• interact*
• ("high demand") OR ("low control") OR ("effort reward")	• income	• "coronary artery disease"	• modif*
• (family OR marital) AND (conflict OR status)	• education OR "educational level"	• "coronary heart disease"	• buffer*
• "care giving"	• neighbourhood OR area	• "ischaemic heart disease"	• amplif*
• depression		• infarction	• synerg*
• anxiety		• angina	• multipli*
• hostility		• stroke	• additi*
• optimism			• subgroup*
• hopelessness			• strat*
• helplessness			• attenuat*
• "positive thinking"			• moderat*
• "social support"			
• "social network"			

The asterisk (*) represent truncation, i.e. the search considers all words starting with the text before the asterisk. For example class* = class, classes, classification, etc. Double quotes indicate exact phrase searching. Hyphenation gives the same results of non-hyphenation; e.g. high demand = high-demand.

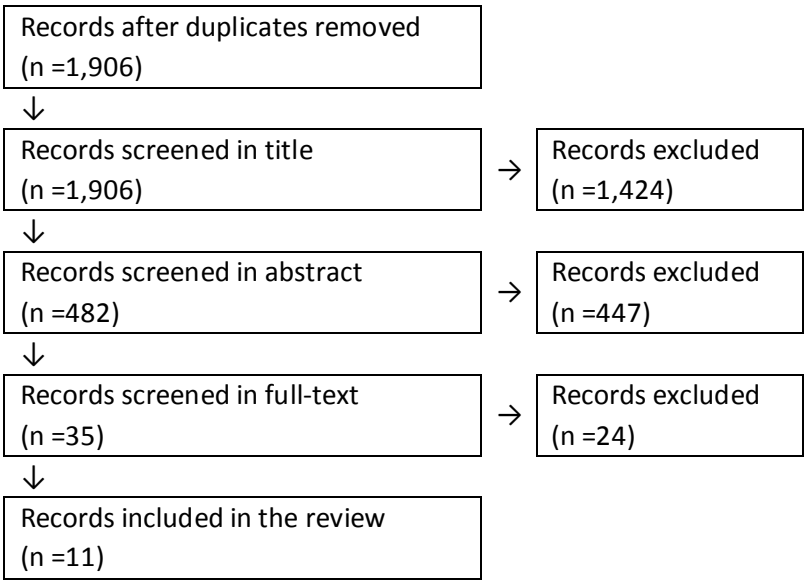
The identified articles were then screened for eligibility by reading their titles and abstracts. Among the articles that passed this screening, a further screening was applied by reading their full text. The present systematic review was conducted and

reported according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidance.[154]

3.2 Results

1,906 hits were obtained after combining all searches and after excluding duplicate articles, although only 35 passed the title/abstract screening and only eleven articles were included in the review (Figure 3.1).

Figure 3.1. Adapted PRISMA flowchart



The description of each study is presented in chronological order below.

Kuper and Marmot in 2003 conducted a cohort study on around 10,000 British civil servants followed up for about eleven years (Whitehall II study). They have considered job strain as the main exposure variable, which was defined as the combination between low control and high demand and was measured using questionnaires. Incident IHD was the main outcome variable and it was ascertained

using either the National Health Service Central Registry for fatal events or questionnaires and clinical records for non-fatal events. SES was considered as a potential effect modifier and indexed using occupational grades grouped into three categories: clerical, professional, and administrative. While confirming the role of job strain for the determination of IHD, the authors concluded that there was no effect modification by employment grade.[155]

Toivanen et al. (2008) developed a register-based cohort study of nearly 3.5 million Swedish workers, and studied the effect of job control on fatal stroke, taking into account socioeconomic status. They used population registry data from the 1990 Swedish Census that were linked to the national Cause-of-Death Registry (1990 to 1995). Occupational holders 25 to 64 years of age in 1990 who did not emigrate during the follow-up period were included. The exposure variable was not measured at the individual level but imputed using external data, i.e. mean job control exposures stratified by gender and age groups have been estimated based on the Swedish Surveys of Working Conditions (nearly 50,000 respondents) for the period 1989 to 1997 for 320 occupational families (3-digit Nordic Occupational Classification Codes), and information from this job exposure matrix was used to impute exposures to each occupational code in the census. It was found that the association between job control and fatal stroke was significant only for lower non-manual jobs.[156]

Janszky et al. (2010) studied the association between depression or anxiety and heart disease within a cohort study of about 50,000 men recruited in their adolescence who were followed up for an average time period of 37 years. All the conscripts were seen by a psychologist for a structured interview and depression and anxiety was diagnosed according to International Classification of Diseases. SES was measured at

baseline and was indexed using self-reported information about the type of occupation that the head of the household had during participants' childhood. Hospitalization and mortality for IHD were identified by the Swedish health registers. The authors considered SES as a potential effect modifier and did not find any positive results in stratified analyses.[157]

Toivanen (2011) further evaluated the interplay between work stress and socioeconomic position in relation to health. Data from the Swedish Level of Living Survey provided a representative sample of the working population in Sweden including women and men in a variety of occupations. The surveys were carried out through personal interviews and the questions covered a wide spectrum of living and working conditions. At each measurement point, the surveys comprised a random sample of roughly 2,600 persons from the Swedish population. Job strain was defined as having high psychological demands and low job control and self-reported health was the main outcome. Occupational class was used as the indicator of socioeconomic position and was categorised into five groups: unskilled manuals, skilled manuals, lower non-manuals, intermediate non-manuals, and higher non-manuals. The variable was further categorised into lower socioeconomic position (manual classes) and higher socioeconomic position (non-manuals). Interaction was studied by comparing odds ratios for work stress across strata of socioeconomic position, and assessed by calculating the synergy index.[158] A synergy index with a value greater than one implies synergism, whereas a value below one indicates antagonism concerning the influence of work stress and low SES on health. Following this method, a composite variable with four categories was computed for each work stress model combining work stress and SES: those exposed to work stress and low

SES, work stress but not low SES, low SES but not work stress, and neither work stress nor SES. The authors concluded that the joint influence of work stress and low SES on health was additive rather than multiplicative.[159]

Tsutsumi et al (2011) studied the effect of occupational stress on stroke across occupational classes for about 6400 Japanese workers, with an average follow-up of 11 years, and found an interaction between stress and work class. Data were acquired from routine mass screening examinations for cardiovascular diseases in the aged. Two categories of occupation (white-collar and blue-collar) and two categories of position (manager and non-manager) were used to reflect the occupational classes related to SES. Occupational stress was evaluated at baseline using a Japanese version of the Demand-Control Questionnaire from the WHO-MONICA Psychosocial Study Questionnaire. During the follow-up participants were contacted annually by direct interview or telephone/letter to determine their health status. Participants were asked if they had suffered a stroke or cardiovascular disease after enrolling. They were asked which hospital they attended and when, to ascertain the incidence of these diseases. When an incident case was suspected, all the medical records were reviewed and duplicate computer tomography films or magnetic resonance imaging films for these patients were obtained. The outcome was also measured using mortality records and specific causes of mortality were determined for all participants using the Cause-of-Death Register found at the public health centre located in each community. The findings show a significantly higher risk of incident stroke in men with high job strain among blue-collar workers and those in non-managerial jobs, but not among white-collar workers or those in managerial positions. The opposite trends were observed in women, i.e. significant elevated risks

among white-collar and managerial workers, but not among blue-collar workers or those in non-managerial positions.[160]

Suadicani et al. (2011) used data from the Copenhagen Male Study that was established in 1970–1971. At 14 companies in Copenhagen, covering the railway, public road construction, military, post, telephone, customs, national bank, and the medical industry, all men aged 40 to 59 years were asked to participate in the study. The final sample size comprised about 4,900 men. The examination consisted of a questionnaire, a short interview, and a clinical examination. The following questions were used to mark psychological pressure at work and leisure: "Are you under psychological pressure when performing your work? Do you take sedatives or sleep medicine? Are you under psychological pressure in your leisure time?" Answer options were: "Rarely", "Regularly", or "Never". The men were divided into five SES classes according to a system based on education level and job position in terms of number of subordinates. Information on death diagnoses within the period 1970-71 to end of 2001 was obtained from official national registers. Also hospitalisation due to stroke identified in the National Hospital Register between 1977 and 2001 was used. The result was that regular psychological work pressure is a highly prevalent and independent risk factor for stroke among men in higher SES classes, while no association to stroke risk was found among low SES men.[161]

Mittag et al. (2012) evaluated whether the association between depressive symptoms and ischemic heart disease in older adults is moderated by education, using data from a health survey commissioned by the U.S. government. The baseline cohort included a sample of about 64,000 older adults who had not reported IHD, who were then followed up for around two years. Depression was assessed by three

items: “In the past year, have you had two weeks or more during which you felt sad, blue or depressed; or when you lost interest or pleasure in things that you usually cared about or enjoyed? In the past year, have you felt depressed or sad much of the time? Have you ever had two years or more in your life when you felt depressed or sad most days, even if you felt ok sometimes?’’ Positive responses to any of these items were taken as an indication of depressive symptoms or episodes in the past. Education was assessed by this item: “What is the highest grade or level of school that you have completed?” Possible answers were: “less than high school education; high school education; greater than high school education.” IHD was assessed by two questions: “Has a doctor ever told you that you had angina pectoris or coronary artery disease? Has a doctor ever told you that you had a myocardial infarction or heart attack?” If any of these items were answered positively the existence of IHD was assumed. The authors concluded that the association of depressive symptoms and IHD in older adults is not moderated by education.[162]

Wiernik et al. (2013) tested whether occupational status moderates the association between current perceived stress and high blood pressure with a cross-sectional design. Resting blood pressure was measured in about 120,000 adults aged ≥30 years without history of cardiovascular and renal disease and not on either psychotropic or antihypertensive treatment that attended a medical centre for a medical check-up in Paris, France. Perceived stress in the past month was measured with the French version of the 4-item Perceived Stress Scale. Occupational status was categorized in 6 classes: (1) high (e.g. managers); (2) medium (e.g. clerks or first-line supervisors); (3) low (e.g. blue collar workers); (4) unemployed participants (i.e. seeking employment); (5) participants without a paid occupation (e.g. housewives); and (6) others (e.g.

artisans). The interaction between occupational status and perceived stress was significant (P <0.001). In analyses stratified by occupational categories, perceived stress was negatively associated with high BP among participants of high occupational status, but positively associated among those of low occupational status and among the unemployed.[163]

Redmond et al. (2013) recruited about 24,000 community dwelling participants residing in the continental United States. Baseline data collection was completed using computer-assisted telephone interviews to collect medical history, functional status, health behaviours, and psychosocial measures. In-home examinations were conducted by trained health care professionals using standardised, quality-controlled protocols to collect physiologic measures. The primary independent variable was baseline perceived stress, as assessed by a 4-item version of the Perceived Stress Scale. SES variables included education (less than high school, high school graduate, some college, college graduate) and household annual income (<$20 000; $20 000 to $34 000; $35 000 to $74 000; ≥$75 000). During the a mean follow-up of 4.2 years, living participants or their proxies were contacted every 6 months by telephone with retrieval of medical records for reported hospitalisations. Deaths were detected by report of next-of-kin or through online sources (e.g. Social Security Death Index) or the National Death Index. Proxies or next-of-kin were interviewed about the circumstances surrounding death including the presence of chest pain. Death certificates, medical records, and autopsy reports were obtained to adjudicate cause of death and cardiovascular outcomes. The results showed that high stress was associated with greater risks of IHD and death for individuals with low but not high income.[164]

Kermott et al (2013) studied 325 high-income patients undergoing comprehensive health assessment in a cross-sectional manner. Stress was assessed using the validated Self-Rated Stress instrument. Coronary artery calcification served to assess the degree of atherosclerosis. Although in the study there was no comparison group (low SES), it was concluded that stress did not play a role in early IHD when focusing on a population in higher socioeconomic strata.[165]

In a cross-sectional study, Schreier et al. (2014) tested whether family chaos influences adolescents' inflammatory profiles and whether adolescents from low SES environments are at higher risk for experiencing adverse inflammatory profiles from living in chaotic family environments. A total of 244 families with an adolescent aged 13 to 16 years participated. Parents completed measures of family SES and family chaos. Both systemic inflammation and stimulated proinflammatory cytokine production in response to bacterial challenge were assessed in adolescents. The results suggest that SES moderates the detrimental effect of family chaos on systemic inflammation and on stimulated proinflammatory cytokine production in adolescents, such that a chaotic family environment is positively associated with greater systemic inflammation and greater stimulated proinflammatory cytokine production in adolescents as family SES declines.[166]

3.3 Discussion

I have shown that there is a gap in medical knowledge with regards to the interaction between mental stress and SES for the determination of cardiovascular disease outcomes such as AMI, and this research hypothesis has no definitive answer. Out of the eleven studies included in the review, five found an interaction, four found no

interaction, one study on male population found an inverse interaction, with high-SES participants being more vulnerable to stress, and one study found positive interaction in men and inverse in women. Moreover, there was considerable variation in study design and quality among the articles. For example, some studies did not have IHD as an outcome although they had diseases or conditions that are linked with or have common pathophysiology compared to IHD; some did not have prospective nature and one did not have a comparison group; in many cases a formal statistical test for interaction was not performed; and sometimes variables were measured using non-validated methods or with low precision.

The methods that I have used for this literature review are not as exhaustive as those that are usually used for the purpose of knowledge synthesis and meta-analysis. For example I did not search the grey literature, I did not perform any sort of hand searches, and I did not deal with the issue of publication bias. I have however already specified that this literature review was produced only to check whether my research hypothesis had already been fully addressed, in order not to duplicate existing evidence. Given the lack of definitive evidence that I have found, the rest of this dissertation will describe the three studies that I have implemented to test my research hypothesis.

Chapter 4 - Structure of the thesis

My thesis is based on original medical research carried out since January 2011 at University College London, department of Epidemiology and Public Health, Psychobiology group. The research that I have carried out to test my research hypothesis resulted in the publication of the following studies in peer-reviewed international medical journals:

- Lazzarino AI, Hamer M, Gaze D, Collinson P, Steptoe A. The association between cortisol response to mental stress and high-sensitivity cardiac troponin T plasma concentration in healthy adults. Journal of American College of Cardiology (JACC). 2013 Oct 29; 62 (18): 1694-701.
- Lazzarino AI, Hamer M, Stamatakis E, Steptoe A. Low socioeconomic status and psychological distress as synergistic predictors of mortality from stroke and coronary heart disease. Psychosomatic Medicine. 2013 Apr; 75 (3): 311-6.
- Lazzarino AI, Hamer M, Stamatakis E, Steptoe A. The combined association of psychological distress and socioeconomic status with all-cause mortality: a national cohort study. Journal of American Medical Association (JAMA) Internal Medicine. 2013 Jan 14; 173 (1): 22-7.

Moreover the following journal article is in preparation:

- Lazzarino AI, Fourkala EO, Steptoe A, Menon U. The interaction between psychosocial and socioeconomic factors for the incidence of cardiovascular disease in women.

In Chapter 5 I will describe my first study, which is about the association between cortisol response to mental stress and high-sensitivity cardiac troponin T plasma concentration in healthy adults, which I have published in the Journal of American College of Cardiology (JACC). Although it was completed more recently than others, I have decided to present it first because it is a laboratory study carried out on selected participants and gives an insight into the pathophysiology of heart disease. In Chapter 6 I will show the methods and the results from my other two published studies, which are observational longitudinal studies that were set out to test my research hypothesis on a wider population. In Chapter 7 I will describe another observational longitudinal study carried out to test the same research hypothesis while changing key study design features as well as the reference population.

Chapter 5 - Study 1: The association between cortisol response to mental stress and high-sensitivity cardiac troponin T plasma concentration in healthy adults

5.1 Introduction

Mental stress is becoming increasingly recognised as a risk factor and trigger for cardiovascular disease (CVD) events.[54,55,167] Stress can be studied in several ways including epidemiological studies, laboratory based psychophysiological testing, and animal research. Psychophysiological testing allows mechanisms to be studied by measuring biological responses to standardized behavioural challenge. Stress markers relevant to CVD include pro-inflammatory factors, cortisol, heart rate variability, and haemostatic processes.[56,168] Mental stress initiates the release of cortisol by activating corticotrophin releasing factor and arginine vasopressin neurons in the paraventricular nucleus of the hypothalamus.[60] This leads to the release of adrenocorticotrophic hormone from the pituitary gland, which triggers release of glucocorticoids from adrenal glands. Cortisol has attracted relatively little attention as a mechanism linking stress and CVD. However, several population studies have demonstrated associations between diurnal cortisol patterns and subclinical atherosclerosis.[169,170]

Atherosclerosis, a fundamental precursor of CVD, can be defined as the thickening and hardening of the arterial walls due to the deposition of lipids within the walls. After the initial stage, atherosclerosis becomes irreversible and atherosclerotic plaques can become calcified. At advanced stage, atherosclerotic plaques can cause

progressive stenosis and ischaemia by blocking the blood flow in the arteries, or they can determine sudden and often more dangerous ischemia if they break and form a thrombus. Atherosclerosis was traditionally considered as a passive process of lipid accumulation, a sort of passive trapping and retention of cholesterol-rich lipoproteins within the arterial wall, while we now acknowledge that inflammation plays and active and central role in the pathophysiology of this condition. Inflammation participates in all stages of atherosclerosis, from the initial fatty streak formation, to atherosclerotic plaque formation, and to ulceration and thrombus formation. For that reasons, studies about the etiology of heart disease must include measurements of markers of atherosclerosis, such as coronary artery calcification for example, and indexes of inflammation such as C-reactive protein (CRP), Inteleukin-6 (IL-6), together with other more conventional risk factors for CVD such as blood pressure, serum lipids, diabetes, etc.[7–9]

A flatter slope in the decline of cortisol levels across the day (thought to be a marker of chronic stress) is associated with an increased risk of CVD mortality in British civil servants,[171] while 24hr urinary cortisol was associated with CVD death in the InCHIANTI prospective cohort study of older people,[172] and serum cortisol level were found to be a cardiac event risk predictor in patients with chronic heart failure and that cardiac event prediction based on cortisol levels was influenced by oxidative stress.[173] Recent data from our laboratory has shown that heightened increases in salivary cortisol following standardized mental stress tests in healthy older individuals are associated with greater coronary artery calcification (CAC), and with CAC progression over 3 years.[174,175]

Cardiac troponin T (CTnT) is a plasma protein routinely used for the diagnosis of acute myocardial infarction (AMI).[15,16] In clinical settings cTnT is measured using standard assays that have a lower detection limit of 10 ng/L,[18] and a diagnostic threshold of 35 ng/L.[15,16] However, high-sensitivity assays have now been developed (HS-CTnT) with an even lower detection limit of 3 ng/L.[19–21]

Cardiac troponins are regulatory proteins that control the calcium-mediated contraction from within the myocyte (heart muscle cell). The troponin complex consists of three subunits: troponin T, troponin I, and troponin C. Troponins are not normally present in the plasma of healthy individuals. The role of Troponin T is to bind to a filament called tropomyosin in order to enhance its interaction with another filament called actin that is responsible for contraction. Contraction happens when the two filaments can slide reciprocally levering on bridges that link them. When cellular necrosis happens, the contents of the myocyte, including Troponin T, are released into the capillaries and from here they reach the main blood stream. There is a rapid early release of plasma Troponin T after ischaemic injury, which peaks at 12–24 hours, and may remain raised for more than two weeks. The detection of Troponin T in the plasma inevitably indicates cardiac cell damage.[17]

In healthy people not fulfilling any diagnostic criterion for AMI, greater HS-CTnT is associated with a greater incidence of structural and functional heart disease, cardiovascular mortality, and all-cause mortality.[176,177] Among patients undergoing noncardiac surgery, the post-operative increase in HS-CTnT plasma concentration was associated with increased 30-day mortality.[178] In a study of community-derived perimenopausal women, HS-CTnT was associated with long-term mortality,

independently of amino-terminal pro-B-type natriuretic peptide and other risk factors.[179]

Research Hypothesis

Cortisol is associated with coronary atherosclerosis, although whether this hormone plays a role in structural and functional cardiac disease remains unclear. The aim our study was therefore to provide further insight into the role of cortisol in CVD by examining the association between cortisol responses to mental stress and HS-CTnT concentrations in healthy older individuals without history of CVD, taking into account underlying coronary atherosclerosis (CAC) as well as inflammatory factors relevant to CVD . We hypothesized that high cortisol responders are individuals who are hyper-reactive to mental stress. If these responses are elicited on a regular basis over many years, they might lead to chronic elevation in HS-CTnT concentration in the circulating blood. In addition, we attempted to determine whether the association between cortisol response to mental stress and HS-CTnT concentration is homogeneous across SES groups or not.

5.2 Methods

Study Design

Our study involved participants drawn from the Whitehall II epidemiological cohort[180] for psychophysiological testing between 2006 and 2008. Whitehall II is a prospective epidemiological study of 10,308 London-based civil servants recruited in 1985-1988 when aged 35-55 years to investigate demographic, psychosocial, and biological risk factors for IHD.[180] The criteria for entry into our study included no history or

objective signs of clinical or subclinical CVD, no previous diagnosis or treatment for hypertension, inflammatory diseases, allergies, or kidney disease. CVD was defined as prior myocardial infarction, stable or unstable angina, revascularization procedure, heart failure, transitory ischaemic attack, stroke, or electrocardiographic abnormalities (resting 12-lead electrocardiograms were taken). This information was confirmed by a telephone interview and verified from clinical data collected from the previous seven phases of the Whitehall II study. Volunteers were of white European origin, aged 53–76 years, and 56.5% were in full-time employment. Selection was stratified by grade of employment (current or most recent) to include higher and lower socioeconomic status participants. From the initially invited participants (n =1,169), 27.6% were not eligible (mainly because of prescribed medications) and 25.9% declined to take part. Participants were prohibited from using any anti-histamine or anti-inflammatory medication 7 days before testing and were rescheduled if they reported colds or other infections on the day of testing. Participants gave full informed consent to participate in the study and ethical approval was obtained from the UCLH committee on the Ethics of Human Research.

Sample size calculation

This study is based on secondary analysis of data gathered for another study having a slightly different research hypothesis (it concerned SES differences in stress responsivity[174]). The original aim was to select 100 men and 100 women from each of three grades of employment (lower, intermediate, and higher) reaching a total sample size of 600. The invited people were chosen from the Whitehall II database using stratified random sampling.

Data Collection

Psychophysiological Testing

We carried out psychophysiological stress testing in either the morning or afternoon in a light temperature-controlled laboratory. This procedure was based on a protocol previously used in Psychobiology Group laboratory at UCL.[181] Participants were instructed to refrain from drinking caffeinated beverages or smoking for at least 2 h before the study and not to have performed vigorous physical activity or consumed alcohol the previous evening. After a 30 min rest period, baseline blood pressure (using an automated UA-779 digital monitor) and a saliva sample were taken. Two behavioural tasks, designed to induce mental stress, were then administered in random order. The tasks were a computerized version of the Stroop task and mirror tracing, both of which have been used extensively in psychophysiological research.[182] The tasks each lasted for 5 min. Saliva samples were collected immediately before and after the tasks and then at 20, 45, and 75 min post-stress for the assessment of salivary cortisol, and the present analysis concerns the measures taken immediately before and after the tests. The samples were collected using Salivettes (Sarsted, Leicester, UK), which were stored at -30^{o}C until analysis. Levels of cortisol were assessed using a time resolved immunoassay with fluorescence detection, at the University of Dresden. The intra- and inter-assay coefficients of variation were less than 8%.

Cardiac Troponin T

Non-fasting blood samples were collected in EDTA tubes and centrifuged immediately at 2500 rpm for 10 min at room temperature. Plasma was removed

from the tube and aliquoted into 0.5 ml portions and stored at 80 ^{o}C until analysis. We measured cardiac troponin T concentrations 75 min after the end of the mental stress test using a highly sensitive assay on an automated platform (Elecsys-2010 Troponin T hs STAT, Roche Diagnostics), with a lower detection limit of 3 ng/L and a reported 99th percentile value in apparently healthy individuals of 13.5 ng/L, at which the CV is 9%, confirmed by in house studies.[19–21]

Covariates

We assayed baseline plasma IL-6 using a Quantikine® high sensitivity two-site enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Oxford, UK). The sensitivity of the assay ranged from 0.016 to 0.110 pg/ml and the intra and inter assay CVs of 7.3% and 7.7% respectively. Baseline C-reactive protein (CRP) was measured using high-sensitivity ELISA (R&D Systems, Oxford, UK).

The assessment of coronary artery calcification (CAC) was performed using electron beam computed tomography (GE Imatron C-150, San Francisco, CA, USA).[183] In brief, 40 contiguous 3 mm slices were obtained during a single breath-hold starting at the carina and proceeding to the level of the diaphragm. Scan time was 100 ms/slice, synchronized to 40% of the R-R interval. Agatston and volumetric calcium scores were calculated to quantify the extent of CAC by a single experienced investigator blinded to the psychophysiological and clinical data on an Aquarius workstation (TeraRecon Inc., San Mateo, CA, USA). Since calcified volume was very highly correlated with Agatston score (Spearman's rho =0.99), we present data for Agatston score only.

Participants reported current smoking levels, weekly alcohol intake (units per week), and hours of moderate or vigorous physical activity per week. We measured height and weight in light clothing for the calculation of body mass index (BMI). Fasting blood samples were taken during a separate clinical assessment. Total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured within 72 h in serum stored at 4^{o}C using enzymatic colorimetric methods.[184] Low-density lipoprotein (LDL) cholesterol was derived using the Friedewald equation.[185] Glucose homeostasis was assessed from glycated haemoglobin (HbA1C) concentration, assayed using boronate affinity chromatography, a combination of boronate affinity and liquid chromatography.

SES was indexed according to the latest employment grade of the individual. We obtained information on grade of employment by asking all participants to give their civil service grade title. On the basis of salary the civil service identifies twelve non-industrial grades which, in order of decreasing salary, comprise seven "unified grades", and senior executive officer (SEO), higher executive officer (HEO), executive officer (EO), clerical officer, and clerical assistant. Other professional and technical staff are assigned by the civil service to one of these grades on the basis of salary. For analysis, we have combined unified grades 1-7 into one group and the bottom two clerical grades into another, thus producing three categories: higher (administrative), intermediate (professional and executive), and lower (clerical).[180]

Data Analysis

Data were cleaned before the analysis: inconsistent, duplicate, outlier, and missing values were checked, as well as digit preferences. Normality of each continuous

variable was checked using histograms and Shapiro–Wilk tests. We quantified the cortisol response to stress by subtracting the values of salivary cortisol concentration measured immediately after the behavioural tasks from the baseline. The resulting measure was normally distributed and for the main analyses it was transformed into a binary variable using a cut point at the value of 4 nmol/L, which corresponds to the mean value (0.54 nmol/L) plus 1 s.d. (3.47 nmol/L). HS-CTnT was highly right-skewed and for 83.3% of the sample it was undetectable (below the lower detection limit of 3 ng/L) and so it was transformed into a binary variable (detectable vs undetectable). Agatston score (CAC) had a right-skewed distribution and for some analyses was transformed into a binary variable by cutting at the value of zero (0 vs >0) or 100 (<100 vs ≥100). This threshold was based on the St Francis Heart Study that demonstrated maximum sensitivity and specificity for detecting cardiovascular events at a threshold calcium score ≥ 100.[186]

Analytic strategy

We described the study sample according to the exposure (salivary cortisol response to stress tasks) and the outcome (plasma detectable HS-CTnT) variables. Triglycerides and CRP had right-skewed distributions and were described using geometric means. Afterwards, we used multiple logistic regression to model the association between cortisol stress response and odds of detectable HS-CTnT. Cortisol responses may differ according to baseline cortisol levels and time of testing, so these parameters were included as covariates. We also adjusted for age, gender, and smoking because they are related to CVD and may confound the association between cortisol reactivity and CVD. For example, people who experience mental stress may be more likely to be smokers and/or may tend to smoke more; smoking is a risk factor for CVD and

therefore it may be an alternative explanation for a supposed positive association between stress and CVD. A similar argument can be applied for age and gender: males or the elderly may experience more stress and we know that they are at greater risk of CVD then their counterparts. Additionally, we took into account clinical variables that are known to be linked with CVD such as systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin (HbA1c). These are well known risk factors for CVD and they may mediate the association between mental stress and CVD; for example, mental stress may produce an increase in blood pressure, which would then increase CVD risk, or mental stress may negatively influence people's eating habits, which would then produce dysregulated metabolic profiles leading to CVD. From this perspective, the adjustment for mediating factors would not be appropriate because it would nullify a true effect of mental stress on HS-CTnT. On the other hand, should the estimates remain unchanged after these adjustments, it would be an interesting finding, plausibly meaning that mental stress operates on different pathways. Moreover, we adjusted for CRP and IL-6 to account for vascular inflammation, which is also a possible confounder (inflamed people may overreact to normal stress and may also be at higher risk of CVD) or a mediator. Finally, we examined whether the association between cortisol reactivity and HS-CTnT was independent of underlying coronary atherosclerosis using several approaches. We firstly adjusted for CAC score as a binary variable using both cut-points separately (0 and 100). However, adjusting for a continuous factor using a binary variable can lead to residual confounding and therefore we carried out a sub-analysis of participants with no detectable coronary calcification. This approach reduces the power of the analysis (smaller sample size) but is more efficient for the purpose of eliminating the confounding effect. Similarly, we then restricted the

analysis to participants with positive CAC scores, and in this case we further adjusted for CAC score as a log-linear variable, because the variable showed a right-skewed distribution. It is theoretically possible that atherosclerosis operates as confounding factor, if for example people who know they have atherosclerosis may be more worried about their health status and have an overreaction to our stress tests. But this is not likely to have happened because all our participants were asymptomatic and were blind to the test results. However, we included CAC into our analysis to assess the mediating role of atherosclerosis, given that there are many cases of myocardial infarctions with normal coronary arteries[187] and that there may exist non-atherosclerotic pathophysiologic components even in infarctions with damaged arteries; therefore we were interested in testing the effect of mental stress on HS-CTnT independently of coronary atherosclerosis.

The interaction between stress response and SES was assessed using the Likelihood Ratio Test (LRT) in two separate models, one without any adjustment and one with full adjustment. The analysis strategy was as follows: we ran a model for HS-CTnT detection with stress response and SES as exposure variables, and eventually the other covariates; the model was then repeated adding in an interaction parameter between stress and SES; the estimates from this second model were then compared with the estimates from the initial model using the LRT. In order for this test to be valid, the comparison has to be made on the same group of individuals (missing values can distort the results) and this assumption was always satisfied.

Departure from linearity of each continuous variable in the final model was tested using the following procedure: 1) cut-offs were chosen to subdivide the sample equally in four or five categories; 2) the presence of an adequate number of

outcomes in each category was checked; 3) the final model was run assuming the variable to be categorical (three or four odds ratios were generated); 4) the final model was run assuming the variable to be ordered categorical (one odds ratio was generated); 5) the two models were compared using the LRT. Furthermore, the odds ratios for categorical variables were plotted to have a visual impression of the trend.[188]

We carried out several sensitivity analyses. We tested other two different methods of quantification: in order to test of dose-response associations, we analysed the cortisol response as a continuous variable and we also calculated cortisol responses as the ratio between the post-stress and baseline values and tested the resulting variable as a linear variable. We checked the association separately for man and women. As for the multiple adjustments, we further included additional variables such as physical activity, BMI, alcohol consumption, diastolic blood pressure, triglycerides, and LDL, which were not included in the principal analyses. We did not carry out any additional analysis on missing values (e.g. multiple imputations) because they were minimal.

5.3 Results

A total of 543 people participated in the study, but 34 (6.3%) had missing information for HS-CTnT and one had a HS-CTnT value >35 ng/L (limit for AMI diagnosis) and were therefore excluded. The final analytic sample comprised 508 participants (mean age of 62.9 years [s.d. =5.7] and 55.1% males). The excluded participants did not differ significantly from the main sample in any covariates. The sample is described according to cortisol stress response in Table 5.1 and according to HS-CTnT

categories in Table 5.2. The prevalence of detectable HS-CTnT was 16.7%. Older and male participants were more likely to have detectable HS-CTnT and higher salivary cortisol responses to stress tasks. HS-CTnT, cortisol response, and CAC appeared to be associated with each other: participants with detectable levels of HS-CTnT were more likely to have higher cortisol responses and CAC (Table 5.2), and participants having high cortisol responses were more likely to have CAC ≥100 (Table 5.1). Participants with cortisol responses <4 nmol/L were more likely to be smokers and those with detectable levels of HS-CTnT had higher IL-6 plasma concentrations (Tables 5.1 and 5.2). Baseline cortisol was not associated with HS-CTnT either in bivariate or multivariate models.

Table 5.1. Study 1 - Characteristics of the sample by categories of cortisol response to laboratory standard mental stress tasks.

Factor and category	Salivary cortisol response				P value
	< 4 nmol/L (n =459)		≥ 4 nmol/L (n =35)		
Age (mean years ± s.d.)	62.8	±5.6	64.7	±6.4	0.055
Male	54.5%	n=250	71.4%	n=25	0.056
Lab session in the morning	39.2%	n=180	41.2%	n=14	0.823
Current smoker	5.5%	n=25	0.0%	n=0	<0.001
Latest grade of employment					
Higher	38.1%	n=175	51.4%	n=18	0.071
Intermediate	39.4%	n=181	37.1%	n=13	
Lower	22.4%	n=103	11.4%	n=4	
Alcohol consumption					
No alcohol	15.5%	n=71	17.1%	n=6	0.943
Below recommended levels	71.2%	n=327	68.6%	n=24	
Above recommended levels	13.3%	n=61	14.3%	n=5	
Hours of physical activity per week					
<1 hour	23.3%	n=107	17.1%	n=6	
1-4 hours	32.4%	n=149	42.9%	n=15	0.727
5-7 hours	23.3%	n=107	14.3%	n=5	
>7 hours	21.0%	n=97	25.7%	n=9	
Body Mass Index (mean Kg/m^2 ± s.d.)	25.8	±3.8	25.8	±4.4	0.931
Systolic blood pressure (mean mmHg ± s.d.)	128.9	±15.6	130.2	±15.5	0.628
Diastolic blood pressure (mean mmHg ± s.d.)	69.7	±8.6	70.6	±9.8	0.534
Glycated haemoglobin (mean % ± s.d.)	5.4	±0.4	5.4	±0.4	0.984
Triglycerides (geometric mean g/L ± s.d.)	1.2	±1.6	1.1	±1.7	0.431
HDL (mean mmol/L ± s.d.)	1.7	±0.5	1.6	±0.4	0.527
LDL (mean mmol/L ± s.d.)	3.0	±0.9	3.1	±0.9	0.628
Total cholesterol (mean mmol/L ± s.d.)	5.3	±0.9	5.3	±0.9	0.977
C-reactive protein at baseline (geometric mean mg/L ± s.d.)	1.0	±2.7	1.2	±3.2	0.610
IL-6 at baseline (mean pg/ml ± s.d.)	1.3	±0.8	1.6	±0.9	0.716
Agatston coronary calcium score					
None	44.7%	n=205	31.4%	n=11	
<100	32.5%	n=149	28.6%	n=10	0.037
<400	14.2%	n=65	25.7%	n=9	
400+	8.7%	n=40	14.3%	n=5	
Agatston coronary calcium score >100	22.9%	n=105	40.0%	n=14	0.025
HS-cTnT detectable, i.e. >3 ng/L	14.8%	n=68	40.0%	n=14	<0.001

P values were computed using univariate logistic regression (Wald test). Ordered categorical variables such as latest grade of employment, alcohol consumption, hours of physical activity per week, and Agatston coronary calcium score were treated as linear (test for trend).

Table 5.2. Study 1 - Characteristics of the study sample by categories of high-sensitivity cardiac troponin T plasma concentration.

Factor and Category	HS-cTnT				P value
	Undetectable (N = 423)		Detectable (N = 85)		
HS-cTnT (geometric mean ng/L ± s.d.)	-	-	6.3	±1.7	
Age (mean years ± s.d.)	62.1	±5.1	67.2	±6.4	<0.001
Male	51.1%	n=216	75.3%	n=64	<0.001
Lab session in the morning	39.8%	n=168	35.9%	n=31	0.522
Current smoker	5.4%	n=23	3.5%	n=3	0.470
Latest grade of employment					
Higher	40.2%	n=170	31.8%	n=27	0.190
Intermediate	38.5%	n=163	43.5%	n=37	
Lower	21.3%	n=90	24.7%	n=21	
Alcohol consumption					
No alcohol	15.1%	n=64	20.0%	n=17	0.201
Below recommended levels	70.9%	n=300	69.4%	n=59	
Above recommended levels	14.0%	n=59	10.6%	n=9	
Hours of physical activity per week					
<1 hour	23.1%	n=98	22.6%	n=19	
1-4 hours	33.0%	n=140	32.1%	n=27	0.481
5-7 hours	23.8%	n=101	17.9%	n=15	
>7 hours	20.2%	n=85	27.4%	n=23	
Body Mass Index (mean Kg/m^2 ± s.d.)	25.8	±3.9	26.2	±3.8	0.436
Systolic blood pressure (mean mmHg ± s.d.)	128.5	±15.3	131.8	±17.4	0.082
Diastolic blood pressure (mean mmHg ± s.d.)	69.5	±8.5	70.7	±9.7	0.262
Glycated haemoglobin (mean % ± s.d.)	5.5	±0.4	5.5	±0.8	0.516
Triglycerides (geometric mean g/L ± s.d.)	1.2	±1.6	1.1	±1.6	0.047
HDL (mean mmol/L ± s.d.)	1.7	±0.5	1.6	±0.4	0.293
LDL (mean mmol/L ± s.d.)	3	±0.8	2.9	±0.9	0.243
Total cholesterol (mean mmol/L ± s.d.)	5.3	±0.9	5.1	±1.0	0.024
Salivary cortisol at baseline (mean nmol/L ± s.d.)	6.5	±4.4	7.0	±4.2	0.321
Salivary cortisol response (mean nmol/L ± s.d.)	0.3	±2.9	1.7	±5.5	0.004
Salivary cortisol response ≥ 4 nmol/L	5.1%	n=22	17.1%	n=15	<0.001
C-reactive protein at baseline (geometric mean mg/L ± s.d.)	1.0	±2.7	1.2	±3.2	0.088
IL-6 at baseline (mean pg/ml ± s.d.)	1.3	±0.8	1.6	±0.9	0.003
Agatston coronary calcium score					
None	47.3%	n=200	25.9%	n=22	
<100	32.9%	n=139	28.2%	n=24	<0.001
<400	13.2%	n=56	22.4%	n=19	
400+	6.6%	n=28	23.5%	n=20	
Agatston coronary calcium score >100	19.9%	n=84	45.9%	n=39	<0.001

P values were computed using univariate logistic regression (Wald test). Ordered categorical variables such as latest grade of employment, alcohol consumption, hours of physical activity per week, and Agatston coronary calcium score were treated as linear (test for trend).

Table 5.3 shows the results of multiple logistic regression models. We found a robust association between cortisol response and detectable HS-CTnT (OR =3.83; 95%CI =1.86-7.90; P <0.001). After adjustment for demographic variables and cardiovascular risk factors the association between cortisol response and detectable HS-CTnT did not differ from the unadjusted analysis. After further adjustments for CRP and IL-6 the association remained (OR =3.75; 95%CI =1.50-9.37; P =0.005). The adjustment for CAC score as a binary variable did not change the effect estimates. When we restricted the analysis to participants without detectable coronary calcification, the evidence of association remained in spite of the 40% drop in sample size (N =222; OR =4.83.; 95%CI =1.24-18.83; P =0.023). When the analysis was restricted to participants with coronary calcification and adjusted for Agatston CAC score as a log-linear variable, the association between cortisol stress responses and troponin T concentration remained (N =286; OR =6.19; 95%CI =1.87-20.55; P =0.003) (Table 5.3).

Table 5.4 shows the results of a fully-adjusted model. The odds of detectable plasma concentration of HS-CTnT were increased in men, in the elderly, and in people with high levels of CRP. Coronary calcification and IL-6 lost the significance that they had shown at the univariate analysis.

Table 5.3. Study 1 - Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks and plasma detectable high-sensitivity cardiac troponin T.

Model for detectable HS-cTnT (binary outcome)	OR for cortisol response (binary exposure)	P	(95% CI)	
1. Crude association	3.83	<0.001	(1.86	7.90)
2. Adjusted for baseline salivary cortisol (pre-task) and time of the session (am or pm)	3.68	0.001	(1.75	7.76)
3. With further adjustment for age and gender	2.75	0.021	(1.16	6.49)
4. With further adjustment for smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin	3.38	0.007	(1.39	8.22)
5. With further adjustment for CRP and IL-6	3.75	0.005	(1.50	9.37)
6a. With further adjustment for coronary calcification score, treated as a binary variable with cut-off at 0	3.74	0.005	(1.49	9.36)
6b. Same as model 7a but the cut-off was set at 100	3.74	0.005	(1.49	9.35)
6c. Model 6 was restricted to participants without coronary calcification (CAC score = 0, N=222)	4.83	0.023	(1.24	18.83)
6d. Model 6 was restricted to participants with coronary calcification (CAC score > 0, N=286) for which score it was further adjusted using a log-linear variable*	6.19	0.003	(1.87	20.55)

*Positive CAC score values had a right-skewed log-linear distribution.

Table 5.4. Study 1 - Multivariate logistic regression model for the odds of plasma detectable high-sensitivity cardiac troponin T (full output).

Factor	OR	(95%CI)		P
Salivary cortisol response to standard laboratory mental stress tasks (binary exposure)	3.74	(1.49	9.36)	0.005
Baseline salivary cortisol	1.03	(0.96	1.10)	0.386
Testing session in the morning	1.10	(0.58	2.10)	0.768
Age	1.19	(1.12	1.25)	<0.001
Gender male	4.85	(2.33	10.10)	<0.001
Smoker	1.30	(0.32	5.32)	0.712
Systolic blood pressure	1.00	(0.98	1.01)	0.637
Total cholesterol / HDL ratio	0.80	(0.58	1.09)	0.153
Glycated haemoglobin	0.93	(0.41	2.12)	0.870
IL-6	1.11	(0.76	1.63)	0.585
CRP	1.18	(1.03	1.36)	0.020
Coronary calcification score, treated as a binary variable with cut-off at 0	1.29	(0.66	2.50)	0.455

This is the full output for model 6a in Table 5.3.

In sensitivity analyses the results remained unchanged after further adjustment for physical activity, BMI, alcohol consumption, diastolic blood pressure, triglycerides, and LDL cholesterol. We also performed sensitivity analyses using different approaches to quantify the cortisol stress response although the same pattern of results emerged. For example, after treating the stress response as a linear proportional change in cortisol, the fully-adjusted OR for HS-CTnT was 1.38 (95%CI =1.10-1.73; P =0.005) for each unit increase in change. The results were similar

between men and women. The results from the sensitivity analyses are presented in Appendix 1.

Table 5.5 shows the results from the interaction analysis. In the crude model, the stratification by SES showed a trend such that the OR of stress response for HS-CTnT was very low in people from higher-SES categories (OR =1.03; 95%CI 0.11-9.68), higher in people from intermediate positions (OR =5.22; 95%CI =1.81-15.09), and much higher in people from lower SES circumstances (OR =6.11; 95%CI =1.92-19.52). A similar trend was found for the adjusted model: the OR in higher SES people was 1.75 (95%CI =0.13-23.74); in intermediate SES people was 3.86 (95%CI =0.94-15.79); and in lower SES people was 13.00 (95%CI =2.72-62.17). Nevertheless the Likelihood Ratio test P value for interaction was not significant for either model (0.562 and 0.698).

Table 5.5. Study 1 - Interaction analysis.

Strata of SES	**Odds ratio (95%CI) of high vs low (referent) mental stress and detectable HS-cTnT**					
	Crude			**Adjusted***		
In participants where SES = Higher	1.03	(0.11	9.68)	1.75	(0.13	23.74)
In participants where SES = Intermediate	5.22	(1.81	15.09)	3.86	(0.94	15.79)
In participants where SES = Lower	6.11	(1.92	19.52)	13.00	(2.72	62.17)
Likelihood Ratio Test P value		0.562			0.698	

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, glycated haemoglobin, CRP, IL-6 and coronary calcification score, treated as a binary variable with cut-off at 0. This model is parallel to model 6a in Table 5.3.

5.4 Discussion

Our results suggest that in healthy participants with no history of CVD, a heightened cortisol response to mental stress is associated with detectable concentrations of circulating cardiac troponin T when measured using a high-sensitivity assay. These findings add to previous data from our laboratory that demonstrate an association between cortisol reactivity and CAC.[174,175] However, importantly, while this study confirms the findings from other work that stress responsivity is associated with increased CAC,[174] the association between cortisol stress responses and circulating cardiac troponin T was independent of CAC, a reliable indicator of sub-clinical coronary atherosclerosis. Therefore, cortisol may be involved in structural and functional adaptations to the heart as well as in the atherosclerotic process.

The prevalence of detectable HS-CTnT in our British sample was 16.7%, which is similar to levels reported (15.7%) in a nationally-representative CVD-free population sample in USA .[176]

To our knowledge, this is the first study to examine the association between mental stress and cardiac troponin T in humans. Our data are consistent with research on stress-induced Takotsubo cardiomyopathy, in which emotional stress increases troponin and cortisol levels in the absence of coronary artery disease.[189,190] Our data also agree with that of Caligiuri et al. who showed that behavioural stress in laboratory animals increases troponin.[191] Although stress responses seem to be positively correlated with troponin, evidence for a direct or indirect glucocorticoid modulation of troponin is still scarce. The troponin gene contains a transcription factor binding site[192,193] previously shown to bind the activated glucocorticoid

receptor.[194] Despite the presence of this binding site, the effect of glucocorticoids on troponin levels does not seem to be via direct modulation of gene expression.[195–199]

Cortisol has a strong diurnal pattern that can cause difficulties in the interpretation of the data; we dealt with this issue by considering stress response as the difference between the post-test and pre-test measurement, and by adjusting for cortisol baseline level and daytime of testing at the data analysis stage. We also performed sensitivity analyses using different approaches to quantify the cortisol stress response although the same pattern of results emerged. It is possible that the cortisol response to stress contributes to the increased levels of troponin T observed here by several indirect mechanisms. First, oxidative stress could play a role in cortisol induced troponin release.[200,201] Supporting this hypothesis, the temporal change in serum troponin matches the increase in the concentration of myocardial malondialhehyde, a marker of free radical lipid peroxidation.[202] Second, stress induced cortisol levels could increase troponin levels by modulating ion channels. For example, dexamethasone, a synthetic glucocorticoid, significantly increases the L-type Ca2+ currents in neonatal rat cardiomyocytes[203] and accelerates myocyte spontaneous contractions.[204] Finally, cortisol responses to stress could induce troponin levels by potentiation of adrenergic signalling. Corticosteroids potentiate adrenergic signalling and increase muscle contraction and cardiomyocyte hypertrophy.[205] Corticosteroid-potentiated adrenergic signalling increases mineralocorticoid and glucocorticoid receptor expression and function in cardiomyocytes.[205]

Detectable HS-CTnT is associated with noncardiac conditions such as severe renal disease[206,207] and, theoretically, our results could be due to confounding if patients

with renal disease are more likely to test positive at mental stress tests. However, it is unlikely that this mechanism underlies our results since the study participants were free from any chronic conditions at the time of testing, as explained in the Methods section.

The effect of stress-related cortisol release on cardiomyocytes may be mediated by atherosclerosis and consequent ischaemia, although the adjustments for CAC, which is a recognised index of atherosclerosis, did not attenuate the findings. CAC and HS-CTnT do not appear to lie on the same causal pathway since their effect was not diminished by mutual adjustment, in spite of being correlated with each other. Thus cortisol might have acted through indirect effects as described above.

Non-calcified coronary plaques are less detectable using cardiac computerized tomography and that may partly explain why CAC did not attenuate the association between cortisol and HS-CTnT. However, there is a direct relationship between the number of calcified plaques present and total plaque burden, and CAC correlates highly with the severity of coronary artery disease, so the absence of calcification implies that there is probably little significant coronary artery disease.[208] On the other hand, it has been argued that raised troponin T may be due to occult or undetected plaque rupture[209] and it is known that plaque rupture is a relatively common event that is usually not followed by an acute cardiac event.[210] This process may have operated in our patients with minimal CAC score levels and detectable HS-CTnT.

A single measure of plasma HS-CTnT concentration cannot be regarded as a robust test if it is not stable in time, i.e. if it shows remarkable intra-individual short-term

variation. However, the results from the ARIC study showed that HS-CTnT intra-individual variability over 6 weeks is almost null, with a correlation coefficient of 0.94.[211] Thus, although our study collected HS-CTnT after a brief and moderately stressful behavioral challenge, it is improbable that troponin T was released in response to this task. To the contrary, we hypothesized that high cortisol responders are individuals who are hyper-reactive to mental stress in everyday life. If these responses are elicited on a regular basis over extended periods of time, they might lead to chronic elevation in HS-CTnT concentration.

This is a cross-sectional study and therefore we cannot determine the causal sequence. Heightened cortisol stress responsivity may contribute to early signs of CVD, or people at an early stage of cardiac disease may be more prone to disturbed stress responses. In fact, cardiac troponins are the most sensitive and specific biochemical markers of myocardial damage,[212] but their elevation can be due to a variety of reasons such as pericarditis, myocarditis, pulmonary embolism, and other.[213] However, it is unlikely that the undetected presence of those conditions can explain our findings since no participants reported any symptoms or signs of cardiac disease, had any previous diagnosis or treatment for hypertension, inflammatory disease or allergies, and did not show any electrocardiographic indications of CHD on tests carried out over more than 20 years in the Whitehall II study. Moreover, we found a strong association both in people with and without coronary calcification, which is consistent with another study showing that HS-CTnT is predictive of CVD in healthy people and also of secondary events in CVD-patients.[214] It is interesting to note that detectable levels of HS-CTnT were associated with salivary cortisol

response to stress test but not with its baseline levels (pre-test), corroborating its relationship with stress-induced neuroendocrine dysregulation.

The analysis of interaction did not give significant results in terms of P value, and therefore we cannot reject the null hypothesis of no interaction between SES and mental stress for the determination of IHD. However, this study may be underpowered for such analysis, since the sample size was calculated on the basis of another research question that did not consider any specific interaction analysis, while it is known that statistical tests for interaction are generally low in power.[215] Moreover, both the crude and the adjusted analysis showed a trend such that the amount of increased risk of HS-CTnT positivity for people with higher stress responses was very low in people from higher-SES categories, higher in people from intermediate positions, and much higher in people from lower SES circumstances. This clear trend comes out in favour of an existing interaction, which is consistent with my main research hypothesis.

In conclusion, heightened cortisol responses to mental stress were associated with detectable levels of cardiac troponin T using high-sensitivity assays in the plasma of healthy people. Heightened stress-induced cortisol release may increase the risk of CHD through several pathways, including atherosclerotic processes, or other indirect effects on the cardiomyocytes. Further research is needed to understand the role of psychosocial stress in the pathophysiology of cardiac cell damage. Further research is also necessary to ascertain the role of socioeconomic status as an effect modifier of the association between mental stress and CHD.

Chapter 6 - Study 2: The combined association of mental stress and socioeconomic status with all-cause mortality and ischaemic heart disease mortality: a national cohort study

6.1 Introduction

Mental stress is a term that incorporates a number of psychological risk factors including depressive symptoms, anxiety, and social dysfunction. These psychological constructs are becoming increasingly recognised as risk factors for mortality and trigger for ischemic heart disease (IHD) events.[54,55,167] Socio-economic status (SES) is also a recognised determinant of health status: in developed countries, the lower a person's SES the worse his or her health. Even in the most affluent countries, people who are in lower SES levels have considerable shorter life expectancies and more disease than people who are in higher SES levels,[112,113,139] and low SES is associated with high risk of IHD and death in developed countries such as England.[141]

In Chapter 1.7, we hypothesized that when both risk factors are present (high levels of mental stress and low levels of SES), the resulting effect on health outcomes is not additive, but that there is a multiplicative effect. This hypothesis was stimulated by concepts such as the reserve capacity model of Gallo and Matthews, which postulates that lower-SES individuals have fewer interpersonal and intrapersonal resources to manage stressful events than do more affluent individuals.[110] In other words, people in higher SES categories may have greater economic, social, and

psychological resources and better coping strategies for dealing with adversity.[110] These assets may be acquired through learning or better access to resources. Consequently, it can be argued that when both risk factors are present (high levels of mental stress and low levels of SES) the resulting effect on mortality is not the mere sum of the two (additive effect) but that some extra risk may appear (multiplicative effect). We therefore hypothesized that SES can operate as an amplifier of mental stress and that the effect of mental stress on mortality would be greater in lower than higher SES groups. As a consequence, there may be vulnerable populations of adults that are more susceptible to the detrimental effects of mental stress and have unmet care needs.

Identifying people who are more vulnerable to the health consequences of mental stress may have clinical and public health implications. For example, questionnaires such as the GHQ-12 could be of value in systematic screening at the family doctor level, aimed at improving the recognition rate of common mental disorders for reducing the risk of cardiovascular disease and other fatal conditions.

Research Hypothesis

The aim of this study was to analyse the association of mental stress and low SES on the incidence of all-cause mortality and ischaemic heart disease (IHD) mortality with an emphasis on the interaction between the two risk factors.

6.2 Methods

Study design and variable selection

The analysis was based on the Health Survey for England (HSE), a nationally representative, general population-based study, recruiting individuals living in private households in England using stratified random sampling.[216] The HSE comprises a series of annual surveys beginning in 1991, and is designed to provide regular information on various aspects of the nation's health. It has a set of core measurements that are included every year: general health; SES; height; weight; blood pressure; health behaviours such as smoking, alcohol consumption, and physical activity; blood and saliva parameters. Psychosocial factors such as mental stress and social relationships are also assessed during the household visits, during which information is collected using Computer-Assisted Personal Interviewing (CAPI). Trained interviewers collect information about physician-diagnosed cardiovascular disease and diabetes, and measure height and weight. In a separate household visit, trained nurses collect blood samples, and measure resting blood pressure using a digital monitor (Omron HEM-907, Omron Healthcare Inc).[216] Diabetes was defined from a self-reported clinician's diagnosis. Hypertension was defined from the clinical blood pressure reading using the conventional criteria (above 140/90 mmHg) or from a self-reported clinician's diagnosis, or prescribed anti-hypertensive medication. Smoking was self-reported as well as physical activity (defined as the number of sessions of moderate or vigorous physical activity per week excluding domestic activity). Body Mass Index (BMI) was defined from height and weight as Kg/m^2.

We pooled HSE years 1994 to 2004 and used all participants aged 35 or more to constitute a baseline sample for a cohort study. We have chosen to exclude younger people because they are at much lower risk of heart disease, which typically requires decades from the establishment of risk factors to the incidence of hard events. So, if on the one hand we have produced a decrease in statistical power by reducing the sample size, on the other hand we have increased the precision of the estimates by including only people who are at plausible risk of cardiac events. Consenting study members were linked to Office for National Statistics (ONS) mortality data, which record and certify all deaths in the UK. Information about their status was obtained up to 28th February 2008 (non-informative censoring date). Our main outcomes were mortality from any cause and from IHD. Classification of the underlying cause of death was based on information collected from the death certificate together with any additional information provided subsequently by the certifying doctor (e.g., secondary death cause). Diagnoses for primary cause of death was recorded using the International Classification of Diseases, Ninth (ICD-9) and Tenth (ICD-10) Revisions. Cardiovascular disease codes were 390-459 for ICD-9 and I01-I99 for ICD-10, which were further categorised into coronary heart disease (410-414 [ICD-9] and I20-I25 [ICD-10]) and cerebrovascular diseases (430-438 [ICD-9] and I60-I69 [ICD-10]). Patients with history of stroke (including Transitory Ischaemic Attack), IHD (including angina), and any other cardiovascular disease or cancer at baseline were excluded from the analysis on the basis of electronic records and of individual nurse visits trained ad hoc. The variables hypertension and physical activity were planned to be recorded only in years 1994, 1997 (physical activity only), 1998, 1999, 2003, and 2004. We have therefore excluded those variables from the main multivariate analyses.

We used the profession of the individual as an indicator of SES. The Registrar General's Social class is a social classification system that attempts to classify groups on the basis of employment, based on characteristics such as career prospects, autonomy, and mode of payment and period of notice.[217] The Health Survey for England uses the six category system in which informants are classified as managerial and professional, intermediate, small employers and own account workers, lower supervisory and technical, and semi-routine and routine occupations. For some analyses we further collapsed the six categories into three (1 = professional or managerial position; 2 = skilled manual or non-manual workers; 3 = semi-routine or unskilled workers). The classification is based on data from the head of the household. If this person was unemployed at the time of the survey the classification was based on their most recent employment.[216]

We used the General Health Questionnaire (GHQ-12) to measure mental stress.[218] The GHQ-12 is generally considered to be a unidimensional scale,[219] and it consists of twelve items relating to anxiety, depression, social dysfunction, and loss of confidence. Interpretation of the answers is based on a four point response scale scored using a bimodal method (symptom present: 'not at all' = 0, 'same as usual' = 0, 'more than usual' = 1 and 'much more than usual' = 1). The questionnaire therefore gives a score for mental stress that goes from zero to twelve. At the analysis stage, the variable GHQ-12 can be used as ordered categorical (0 = low stress; 1-3 = medium stress; 4+ = high stress) or as binary (0-3 = low stress; 4+ = high stress).[218] A full GHQ-12 questionnaire form is shown in Appendix 2.

Study participants gave full informed consent, and ethical approval was obtained from the London Research Ethics Committee.

Data analysis

Data were cleaned before the analysis: inconsistent, duplicate, outlier, and missing values were checked, as well as digit preferences. Normality of each continuous variable was checked using histograms and Shapiro–Wilk tests. The proportion of participants who died within the follow-up period was calculated as well as the crude incidence rate for the cohort.

Data were analysed using Cox-Regression with follow-up time (in months) as the time scale. We constructed a multiple Cox-Regression model for the association of GHQ-12 (ordered categorical with three categories), age (per one-year linear increase), gender (binary), current smoking (binary), BMI (linear for IHD mortality and categorical for all-cause mortality with cut-offs at <18.5; 18.5-29.9[reference]; 30+), and diabetes (binary), with the outcome using the forward step-wise approach, i.e. the variables were sequentially added to an 'empty' (intercept only) model one at a time giving priority to those that had shown the strongest evidence of association at the univariate stage (smallest P value). At each round the importance of the added variable was assessed according to changes in the rate ratios, Wald tests and Likelihood Ratio tests (LRT) P values (cut off = 0.05) of all variables in the model. If a variable lost significance we removed it from the model. After having fitted the final model, we checked the proportional hazards assumption and the departure from linearity.

The proportional hazards assumption has been assessed as follows: data has been split with the Lexis Expansion method according to follow-up time (cut-offs at 2, 4, and 6 years), and tests for departure from proportional hazards assumption were

carried out assessing the presence of interaction between follow-up time and each variable. Furthermore, Nelson-Aalen plots were displayed to have a visual impression of the proportionality.[188] An example of a Nelson-Aalen plot is shown in Appendix 4.

Departure from linearity of each continuous variable in the final model was tested using the following procedure: 1) cut-offs were chosen to subdivide the sample equally in four or five categories; 2) the presence of an adequate number of outcomes in each category was checked; 3) the final model was run assuming the variable to be categorical (three or four hazard ratios were generated); 4) the final model was run assuming the variable to be ordered categorical (one hazard ratio was generated); 5) the two models were compared using the Likelihood Ratio Test (LRT). Furthermore, the hazard ratios for categorical variables were plotted to have a visual impression of the trend.[188] The variable GHQ-12 showed a better linear trend when in the form of ordered categorical than when in the form of continuous, and therefore the categorisation was preferred. BMI showed a J-shape association with all-cause mortality and a linear association with IHD-mortality.

Finally, interaction between SES and GHQ-12 was assessed using the Likelihood Ratio Test (LRT) in three separate models, one without any adjustment, one with adjustment for age and gender, and one with further adjustment for smoking, BMI, physical activity, diabetes, and hypertension. The analysis strategy was as follows: we ran a model with SES, GHQ-12 (binary scores 0-3 vs 4+), and eventually the other covariates; the model was then repeated adding in an interaction parameter between SES and GHQ-12; the estimates from this second model were then compared with the estimates from the initial model using the LRT. In order for this test to be valid, the comparison has to be made on the same group of individuals

(missing values can distort the results) and this assumption was always satisfied. We tested for the interaction using another strategy: a new variable was calculated as the multiplication between SES (three categories) and GHQ-12 (three categories). The variable therefore had possible values ranging from zero to nine and was included as a covariate in the adjusted model.

We have carried out several sensitivity analyses: we restricted the multivariate analyses to those years that included data collection for the variables hypertension and physical activity (n =35,090) and added those variables into the models; the entire analysis was repeated separately for men and women; we excluded participants who experienced an outcome within one year from recruitment to minimize the chances of reverse causality; participants who had missing values for GHQ-12 were slightly older compared to responders, and it is arguable that they may tend to have higher levels of mental stress, and therefore we have rerun the main interaction analysis after recoding those missing values with valid values scoring 4 (cut-off for high stress).

6.3 Results

The initial study sample consisted of 96,605 adults, although 10.4% (n=10,065) did not consent to mortality follow up and were therefore removed from any analysis. Non-consenting adults had similar characteristics compared to consenting adults. 5,864 (6.8%) participants had history of stroke or IHD, or had another prevalent cardiovascular disease or cancer at baseline and were therefore excluded. Out of the resulting 80,676 participants, 15.4% had missing values for mental stress and 2.6% for SES. Participants with GHQ-12 missing values were slightly older compared with

those who completed the GHQ-12 questionnaire (56.4 vs 55.1 years; P < 0.001), whereas the gender structures of the two subgroups were similar (males = 45.4% vs 44.8%; P = 0.234). Six participants were lost during the follow-up and 27 were excluded from the analysis because they experienced an outcome within one month from recruitment. The final analytic sample comprised 66,518 participants. The measures of hypertension and physical activity had about 40% missing values.

The participants were followed up for a mean of 8.2 years (s.d. =3.4; median =7.9). During this period, 1,007 (1.5%) died of an IHD event, and 7,875 (11.8%) died for any cause. The crude incidence rates for IHD and all-cause mortality were 1.85 (95%CI = 1.74-1.97) and 14.49 (95%CI = 14.17-14.81) per 1,000 person-years. The two outcomes have shown very similar patterns in all analyses.

Tables 6.1 and 6.2 show the baseline characteristics of the sample. On average, 14.4% of the sample reported mental stress based on the established cut point of GHQ-12 score ≥ 4. Participants from lower occupational classes were older, less likely to be male, had higher GHQ-12 score, and were more likely to be smokers. Both mental stress and low SES were associated with increased mortality rates, as were diabetes, hypertension, and smoking. Physical activity was associated with lower risk of mortality. Both groups of people with BMI values of less than 18.5 or more than 30 had higher mortality rates than people with BMI values between 18.5 and 30 (reference category), although this non-linear pattern was not evident for IHD mortality. Therefore I have used a categorical BMI variable for the analysis of all-cause mortality and the native linear BMI variable for IHD mortality.

Table 6.1. Study 2 - Sample description and unadjusted hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor and category at the beginning of the follow-up	All-cause mortality within the follow-up time				HR	(95%CI)		P
	Yes (n =7,875)		No (n =58,643)					
Male	49.7%	n=3,912	44.9%	n=26,326	1.19	(1.14	1.24)	<0.001
Age (mean ± s.d.)	72.1	±12.1	52.5	±12.4	1.11	(1.11	1.11)	<0.001
Age								
35-49	5.8%	n=460	46.5%	n=27,287	5.88[2]	(5.64	6.12)	<0.001
50-64	16.9%	n=1,328	34.2%	n=20,044				
65+	77.3%	n=6,087	19.3%	n=11,318				
Socio-Economic Status based on professional category								
1 - High	24.5%	n=1,932	35.1%	n=20,566	1.20[2]	(1.18	1.22)	<0.001
2 - Medium	45.8%	n=3,605	42.9%	n=25,158				
3 - Low	29.7%	n=2,338	22.0%	n=12,919				
GHQ-12 Score (mean ± s.d.)	1.7	±2.8	1.4	±2.6	1.04	(1.03	1.05)	<0.001
GHQ-12 Score								
0	54.3%	n=4,275	62.0%	n=36,341	1.30[4]	(1.25	1.36)	<0.001
1-3	27.6%	n=2,172	24.1%	n=14,133				
4+	18.1%	n=1,428	13.9%	n=8,169				
Smoking								
20+	8.6%	n=679	8.9%	n=5,237				
1-19	15.0%	n=1,180	13.8%	n=8,116	1.54[2]	(1.47	1.61)	<0.001
Ex	44.3%	n=3,489	34.6%	n=20,290				
Never	32.1%	n=2,526	42.6%	n=24,994				
Body Mass Index Kg/m^2 ± s.d.)								
<18.5	1.9%	n=150	0.6%	n=352	3.56	(2.97	4.27)	<0.001
18.5-30	56.0%	n=4,410	66.4%	n=38,939	1[5]			
30+	42.1%	n=3,315	33.0%	n=19,352	1.13	(1.08	1.19)	<0.001
Physical activity[1] (mean ± s.d.)	0.6	±1.9	1.6	±2.8	0.80	(0.78	0.81)	<0.001
Diabetes	6.7%	n=531	2.6%	n=1,530	2.78	(2.54	3.03)	<0.001
Hypertension[6]	29.4%	n=2,313	17.5%	n=10,259	1.85	(1.73	1.98)	<0.001

Hazard ratios, 95% confidence intervals, and P values were computed using unadjusted Cox regression. (1) Number of sessions of moderate or vigorous physical activity per week excluding domestic activity. N=39,610. (2) Per one-category increase. (3) Treated as binary: 4+ versus 0-3. (4) Treated as binary: current smokers versus ex- or never-smokers. (5) Reference category. (6) N=35,090

Table 6.2. Study 2 - Sample description and unadjusted hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor and category at the beginning of the follow-up	IHD mortality within the follow-up time				HR	(95%CI)		P
	Yes		No					
	(n =1,007)		(n =65,511)					
Male	57.8%	n=582	45.3%	n=29,656	1.65	(1.45	1.87)	<0.001
Age (mean years ± s.d.)	71.9	±11.6	54.6	±13.8	1.11	(1.11	1.12)	<0.001
Age in years								
35-49	5.8%	n=58	42.3%	n=27,685	6.36[(2)]	(5.66	7.15)	<0.001
50-64	15.5%	n=156	32.4%	n=21,212				
65+	78.8%	n=793	25.4%	n=16,614				
Socio-Economic Status based on professional category								
1 - High	24.6%	n=248	34.0%	n=22,254	1.28[(2)]	(1.18	1.39)	<0.001
2 - Medium	47.8%	n=481	43.2%	n=28,281				
3 - Low	27.6%	n=278	22.9%	n=14,976				
GHQ-12 score (mean ± s.d.)	1.6	±2.6	1.4	±2.6	1.03	(1.01	1.05)	0.009
GHQ-12 score								
0	53.4%	n=538	61.2%	n=40,080	1.19[(3)]	(1.10	1.28)	<0.001
1-3	29.4%	n=296	24.4%	n=16,011				
4+	17.2%	n=173	14.4%	n=9,420				
Smoking								
20+	8.6%	n=87	8.9%	n=5,830				
1-19	14.2%	n=143	14.0%	n=9,158	1.65[(4)]	(1.45	1.89)	<0.001
Ex	46.5%	n=468	35.6%	n=23,315				
Never	30.7%	n=309	41.5%	n=27,213				
Body Mass Index (mean Kg/m^2 ± s.d.)	27.5	±4.6	27.1	±4.7	1.03	(1.01	1.04)	0.001
Physical Activity[(1)] (mean ± s.d.)	0.6	±1.9	1.5	±2.8	0.79	(0.75	0.84)	<0.001
Diabetes	10.8%	n=109	3.0%	n=1,952	4.86	(3.98	5.93)	<0.001
Hypertension[(5)]	29.8%	n=300	18.8%	n=12,293	1.89	(1.57	2.28)	<0.001

Hazard ratios, 95% confidence intervals, and P values were computed using unadjusted Cox regression. (1) Number of sessions of moderate or vigorous physical activity per week excluding domestic activity. N=39,610. (2) Per one-category increase. (3) Treated as binary: 4+ versus 0-3. (4) Treated as binary: current smokers versus ex- or never-smokers. (5) N=35,090

Tables 6.3 and 6.4 show the results from the multivariate analysis performed using Cox-Regression. GHQ-12 was associated with higher mortality rates (HR for one category increase out of three total categories=1.23; 95%CI =1.19-1.27; P <0.001) after having adjusted for age, gender, smoking, BMI, and diabetes, all of which also were independent predictors of all-cause and IHD mortality.

Table 6.3. Study 2 - Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
One category increase in GHQ-12 (3 categories in total)	1.23	(1.19	1.27)	<0.001
Gender male	1.44	(1.37	1.51)	<0.001
One-year increase in age	1.11	(1.11	1.11)	<0.001
Current smoking	1.51	(1.43	1.59)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.66	(2.27	3.12)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.07	(1.02	1.15)	0.008
Diabetes	1.60	(1.44	1.76)	<0.001

Table 6.4. Study 2 - Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	(95%CI)		P
One category increase in GHQ-12 (3 categories in total)	1.25	(1.14	1.38)	<0.001
Gender male	2.02	(1.75	2.34)	<0.001
One-year increase in age	1.12	(1.11	1.12)	<0.001
Current smoking	1.50	(1.29	1.75)	<0.001
Unit increase in BMI	1.03	(1.01	1.04)	0.002
Diabetes	2.55	(2.03	3.21)	<0.001

Tables 6.5 and 6.6 present the results of an analysis focused on the interaction between SES and GHQ-12, i.e. it shows crude and adjusted hazard ratios of GHQ-12 on mortality, stratified by SES. After adjusting for age, gender, smoking, BMI, and diabetes, there was a significant interaction showing that mental stress

demonstrated stronger associations with mortality in lower SES participants (LRT P for all-cause mortality= 0.012, for IHD mortality =0.048).

Table 6.5. Study 2 - Multivariate Cox regression models showing crude and adjusted hazard ratios and 95% confidence intervals for the association between psychological distress and all-cause mortality stratified by socioeconomic status.

Strata of SES	Hazard ratio (95%CI) of high vs low (referent) psychological distress for all-cause mortality								
	Crude			Adjusted for age and gender			Multi-adjusted*		
In participants where SES = High	1.18	(1.07	1.29)	1.26	(1.18	1.34)	1.22	(1.13	1.31)
In participants where SES = Medium	1.32	(1.23	1.41)	1.37	(1.27	1.49)	1.33	(1.21	1.45)
In participants where SES = Low	1.36	(1.25	1.47)	1.46	(1.33	1.59)	1.36	(1.23	1.51)
Likelihood Ratio Test P value		0.004			0.001			0.012	

* Adjusted for age, gender, smoking, BMI, and diabetes. Low Psychological Distress = GHQ-12 score < 4; High Psychological Distress = GHQ-12 score ≥ 4. High SES = professional/managerial positions; medium SES = skilled manual/non-manual workers; low SES = semi-routine/unskilled workers.

Table 6.6. Study 2 - Multivariate Cox regression models showing crude and adjusted hazard ratios and 95% confidence intervals for the association between psychological distress and IHD mortality stratified by socioeconomic status.

Strata of SES	Hazard ratio (95%CI) of high vs low (referent) psychological distress for IHD mortality								
	Crude			Adjusted for age and gender			Multi-adjusted*		
In participants where SES = High	0.88	(0.64	1.21)	1.43	(1.04	1.98)	1.22	(0.83	1.81)
In participants where SES = Medium	1.29	(1.02	1.63)	1.52	(1.20	1.92)	1.38	(1.05	1.83)
In participants where SES = Low	1.48	(1.13	1.94)	1.63	(1.24	2.14)	1.47	(1.06	2.02)
Likelihood Ratio Test P value		0.009			0.023			0.048	

* Adjusted for age, gender, smoking, BMI, and diabetes. Low Psychological Distress = GHQ-12 score < 4; High Psychological Distress = GHQ-12 score ≥ 4. High SES = Professional/managerial positions; Medium SES = Skilled manual/non-manual workers; Low SES = Semi-routine/unskilled workers.

Table 6.7 and 6.8 describe the results from multivariate Cox regression models in which the interaction between SES and GHQ-12 was included as a covariate that was

previously created by multiplying SES (3 categories) and GHQ-12 (3 categories) together. After adjusting for all variables in the model, the incidence of all-cause mortality was on average increased by six percent when participants experienced medium exposure from both factors and twelve percent when they experienced high exposure (HR =1.06, 95%CI =1.01-1.10, P =0.021). Similarly, the incidence of IHD mortality was on average increased by three percent when participants experienced medium exposure from both factors and six percent when they experienced high exposure (HR =1.03, 95%CI =1.01-1.05, P =0.047). These augmented risks have to be summed to the risks that the two factors exert by themselves.

Table 6.7. Study 2 - Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.38	(1.26	1.50)	<0.001
Unit increase in SES going from high to low (3 categories)	1.21	(1.16	1.26)	<0.001
Interaction SESxGHQ-12 (3x3)	1.06	(1.01	1.10)	0.021
Gender male	1.47	(1.40	1.54)	<0.001
One-year increase in age	1.11	(1.11	1.11)	<0.001
Current smoking	1.42	(1.35	1.49)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.39	(2.04	2.80)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.08	(1.02	1.15)	0.008
Diabetes	1.62	(1.48	1.77)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

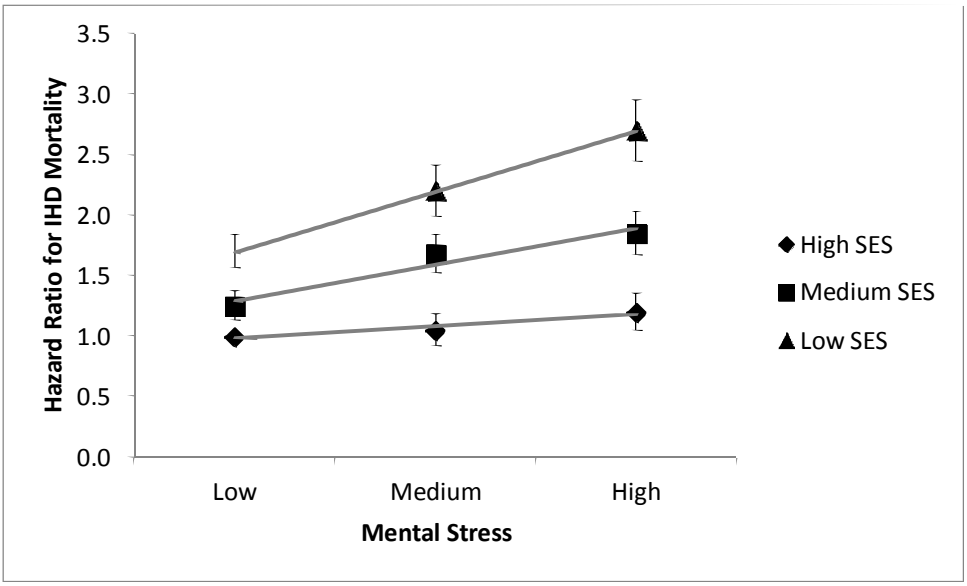
Table 6.8. Study 2 - Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.19	(1.14	1.38)	<0.001
Unit increase in SES going from high to low (3 categories)	1.15	(1.14	1.16)	<0.001
Interaction SESxGHQ-12 (3x3)	1.03	(1.01	1.05)	0.047
Gender male	2.13	(1.84	2.47)	<0.001
One-year increase in age	1.12	(1.11	1.12)	<0.001
Current smoking	1.49	(1.27	1.74)	<0.001
Unit increase in BMI	1.02	(1.01	1.04)	0.003
Diabetes	2.53	(2.01	3.18)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Figure 6.1 shows a graph that gives a visual representation of the concept of interaction. In that example, study participants have been divided according to three categories of mental stress levels (X-axis), and incidence of IHD-mortality (Y-axis), and were stratified by three categories of socioeconomic status (diagonal lines). The slopes of the lines represent the effect of mental stress on IHD-mortality; the steeper the line is, the stronger the effect. The slopes of the three lines (the three strata of socioeconomic status) are different from each other. The likelihood ratio test challenges the null hypothesis of no difference in the slope between any lines. Since the test gave a very significant result (P <0.001), we can reject the null hypothesis and acknowledge the existence of an interaction between mental stress and socioeconomic status. It has to be noted that the 95% confidence intervals for each point in the graph do not overlap with each other, strengthening the findings further. However, an interaction can exist and can be detected by the LRT even when the confidence intervals overlap.

Figure 6.1. Study 2 - Age- and sex-adjusted hazard ratios for IHD-cause mortality as a function of mental stress for each stratum of socioeconomic status.



The reference category (hazard ratio =1) included participants with low mental stress and a high SES level. Whiskers represent 95% confidence intervals. Likelihood ratio test P value <0.001. Low mental stress = GHQ-12 score 0; Medium mental stress = GHQ-12 scores 1-3; High mental stress = GHQ-12 score 4+. High SES = Professional/managerial positions; Medium SES = Skilled manual/non-manual workers; Low SES = Semi-routine/unskilled workers.

The sensitivity analyses showed a pattern of results that was similar to that found in the main analyses, with no differences in the key interactions (Appendix 3).

6.4 Discussion

We have shown that the association between mental stress and either IHD or all-cause mortality differs according to socio-economic status. Low SES operates as an amplifier of the detrimental effect of mental stress on either IHD or all-cause mortality.

The differential impact of psychological stress on health outcomes across SES groups has not been directly investigated before in a large prospective observational study,

but there is limited evidence concerning exposure to specific sources of stress. For example, in a study of Japanese workers, job strain was associated with a higher risk of stroke in men from lower occupational classes but not in higher status white-collar and managerial workers.[160] Similarly, in a register-based cohort study of nearly 3.5 million Swedish men and women, low job control was a risk factor for stroke in women working in lower manual jobs but not in higher status non-manual occupations.[156] Given that lower SES groups are more likely to be exposed to greater adversity and stress, several studies have also examined if occupational stress might explain the social gradient in IHD risk. However, in a cohort of Finnish public sector workers, job demands alone or in combination with job control suppressed rather than explained socioeconomic differences in cerebrovascular disease.[220]

The explanations of why people from disadvantaged backgrounds are more vulnerable to stress than those from higher SES groups are poorly understood. However, people from higher SES might have better coping strategies and larger support networks together with greater biobehavioral and economic resources for dealing with adversity.[221] In addition, higher SES groups demonstrate more effective recovery in cardiovascular and biological parameters following acute stress, which might over time contribute to IHD pathology.[222,223]

Smoking, BMI, hypertension, diabetes, and physical inactivity are also known risk factors for IHD or all-cause mortality. We took these factors into account but we cannot rule out the possibility of residual confounding by the measured or by other unmeasured variables. Nevertheless, these factors may be on the causal pathway between SES or mental stress and the outcomes, so adjusting for them could diminish the effect of the two main exposure variables and make their interaction

less detectable. Under this perspective, the more appropriate analysis to look at would be the age- and gender-adjusted one.

BMI showed a J-shape association with all-cause mortality, with both under-weight and obese people having higher mortality rates than normal-weight people, but had a linear trend of association with IHD mortality. Our results are compatible with the existing literature on this topic.[224]

One limitation of the present study is a lack of follow up data on mental stress, so we were unable to account for the effects of changes in distress over time. The GHQ-12 is not designed to assess specific aspects of mental health such as anxiety and depression. However, measuring symptoms of anxiety, depression, and dysfunction as a unidimensional construct of mental stress is particularly relevant in community-based samples, such as ours, as mental health problems in the community are frequently characterized by shifting patterns of symptoms that resist precise clinical classification.[225] It has also been argued that the different manifestations of mental stress are not distinctive in their associations with cardiovascular disease outcomes.[107]

Other indicators of SES might have been used, such as educational level or gross annual income. Occupational class was preferred since it is an indicator of current socio-economic circumstances, whereas education is typically completed early in life and partly dictates life-course trajectories.[226] As for annual household income, the HSE has relatively low response rate, like many other population surveys (about 50% of households had no valid data).

The HSE does not unfortunately include measures of specific sources of life stress such as work stress, domestic strain, caregiver burden, or social isolation consistently over the different years. We do not therefore know whether the elevations in mental stress were responses to adverse life conditions or enduring traits.

Since we have used mortality records, our results are not generalizable to non-fatal cardiac events. Our cohort was made of people who were free from any cardiovascular disease, including angina, at baseline. Therefore, our results are applicable only to those types of heart attacks that are fatal at their first manifestation, which may have different pathophysiology compared to those events that begin with less severe symptoms.

It has been argued that mortality records may underestimate the incidence of acute myocardial infarction, unless combined with other sources (e.g. Hospital Episode Statistics).[227] This may account for our findings if mortality records were more accurate for people with higher susceptibility to mental stress, increasing the proportion of such people in our dataset in an artificial way, and therefore distorting the results. This is very unlikely because any inaccuracies in the mortality records probably occurred independently from the interactions that we have focused on. These types of biases are called non-differential or non-informative because they do not distort the results but they just "dilute" them, i.e. they decrease the statistical power. In light of this, our results acquire even more robustness.

Another issue comes from the fact that some of our participants may have died in a foreign country and therefore the ONS may have not recorded their death or may have done so with a misleading delay. Again, it is unlikely that these missing data

happened less frequently for people with higher susceptibility to mental stress, rendering these people more present in our dataset and therefore explaining for our results.

In conclusion, the effect of mental stress on all-cause mortality is more pronounced in people from lower than higher socio-economic status groups, but further research is required to confirm our results.

Chapter 7 - Study 3: The interaction between psychosocial and socioeconomic factors for the incidence of ischemic heart disease in women

7.1 Introduction

Psychosocial factors are thought to have an influence on physical health status and in particular on the genesis of cardiovascular disease (CVD).[54,55,167] The psychosocial factors that are relevant include exposure to different forms of life stress (such as work stress and caregiver burden), aspects of impoverished social relationships including low social support and social isolation, and psychological features such as depression and hostility. We have recently argued that the magnitude of the effect that stress exerts on IHD incidence may vary with socio-economic status (SES), in such a way that people from disadvantaged backgrounds are more vulnerable to stress, which consequently has larger effects on their physical health.[228,229] This hypothesis was stimulated by concepts such as the reserve capacity model of Matthews and Gallo, which postulates that lower-SES individuals have fewer interpersonal and intrapersonal resources to manage stressful events that do more affluent individuals.[110]

Our previous cohort studies (described in Chapter 6) that found a robust interaction between stress and SES had the following design features: participants were drawn from the Health Survey for England database, which comprises a series of annual nationally-representative surveys on the health status of men and women leaving in

private households in England; baseline sample recruitment spanned years 1994-2004; stress was measured in terms of psychological distress using the 12-item General Health Questionnaire; SES was indexed using the occupation of the individual (or of the head of the household); the study outcomes were fatal IHD events and overall mortality and were assessed using mortality records from the Office of National Statistics; and follow-up times were relatively long (8 years on average).[228,229]

In the present study we wanted to challenge our previous findings by testing the same research hypothesis after changing the study design in some key factors including the reference population, the dataset analysed, the time period during which data were collected, the index of SES, the measure of psychosocial adversity, the data source from which clinical outcomes were extracted, the severity of outcome events (non-fatal IHD events instead of fatal), and the follow-up time resolution and duration (shorter follow-up times reduce the possibility of information bias due to change in psychosocial adversities over time). Because of the strong evidence that depression is a risk factor for future IHD,[106,230,231] we tested exposure to the related construct of hopelessness/helplessness. Hopeless/helplessness has more recently attracted attention as it has been argued that this specific psychosocial trait may play a more direct role in the process of IHD development than overall depressive symptoms.[75,101,102,232] Attempts to assess SES-specific prevalence of depressive disorders have been made but no studies have focused on the differential effect that depression has on IHD by categories of SES.[233] Research carried out in this field has predominantly involved men, so we deliberately tested our hypotheses in a large cohort study of middle-aged and older women.

7.2 Methods

Participants' recruitment

This cohort study involved participants from the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). The UKCTOCS is a multicentre randomised controlled trial of ovarian cancer screening in the general population. The trial invited 1.2 million women of whom 202,638 were randomised through 13 trial centres located in NHS Hospitals in England, Wales and Northern Ireland. Eligibility criteria included (a) age between 50 and 74 years and (b) postmenopausal status defined as >12 months amenorrhea, following a natural or surgical menopause or >12 months of hormone replacement therapy commenced for menopausal symptoms. The exclusion criteria were: (a) history of bilateral oophorectomy, (b) active non-ovarian malignancy (women with a past history of malignancy were eligible if they had no documented persistent or recurrent disease), (c) increased risk of ovarian cancer because of familial predisposition and (d) previous history of ovarian cancer. At recruitment, women completed a baseline questionnaire and written consent was obtained which included use of their data in secondary studies. Women were then randomised in a 2:1:1 ratio to: (1) a control group with no intervention (101,359) (2) a multimodal group with annual screening with CA125 (50,640) (3) an ultrasound group with annual screening with ultrasound (50,639).[234] Women who had been on the trial for at least 3.5 years following randomisation were sent a follow-up questionnaire and constitute the baseline sample of the present study. Therefore, for the purpose of the present study, the cohort follow-up started at the time of questionnaire completion, which happened from July 2005 to December 2012. The data for these analyses were made available through a collaboration with Professor

Usha Menon, Professor of Gynaecological Cancer at the Institute of Women's Heath, University College London.

Measurement of hopelessness/helplessness

The baseline questionnaire included questions on demographics and current health since randomisation. In addition, participants were asked to complete a 2-item measure of hopelessness and helplessness (HH): (1) "The future seems to me to be hopeful and I believe that things are changing for the better." (2) "I feel that it is possible to reach the goals I would like to strive for." These items followed the 2-item scale of Everson et al.[100] with the only change being that they were phrased positively rather than negatively ("The future seems to me to be hopeless…"), to avoid reactions of distress observed with the negatively phrased HH scale.[235] There were 5 categories of response ratings ranging from 1-absolutely agree, 2-somewhat agree, 3-cannot say, 4-somewhat disagree, to 5-absolutely disagree. The scores of both items were added together to give a HH total score ranging from two to ten, where high scores indicate higher levels of hopelessness/helplessness. This measure has been recently validated in a cohort study on 2,413 postmenopausal women. The HH index correlated 0.87 with the Beck Hopelessness Scale after correction for attenuation and its association with the Centre for Epidemiological Studies Depression Scale mirrored that seen with the Beck Hopelessness Scale. There was no change in mean scores on the HH measures with repeat testing, and the test–retest reliability was good (intraclass correlation =0.72).[235]

Measurement of socio-economic status

We applied the Index of Multiple Deprivation v.2010 (IMD) as a marker of socio-economic status using participants' home postcode. The IMD is a geographically-based measure of multiple deprivation built on the principle of distinct domains of deprivation which can be recognised and measured separately in small communities and then combined, using appropriate weights, into a single overall index of multiple deprivation. This can then be used to rank every small area in England according to the deprivation experienced by the people living there (one area includes around 1,500 people). Each domain represents a specific form of deprivation and is in turn measured using a number of indicators. Seven distinct domains form the IMD: income; employment; health; education; barriers to housing and services; living environment; crime.[236] The score is unidimensional and values range from less than one to more than eighty, with higher scores indicating higher deprivation and deciles being typically used as units of measurement.[237]

Covariates

The questionnaire included demographic and clinical measures such as education, smoking, body height and weight, blood pressure, blood lipid profile, diabetes, rheumatoid arthritis, osteoarthritis, and osteoporosis. The body mass index (BMI) was calculated at the data analysis stage from self-reported height and weight as Kg/m^2.

Outcome measurement

The study outcome was non-fatal acute ischemic heart disease event (IHD) and we used Hospital Episode Statistics records (HES) to measure their incidence with a resolution of one day. The HES is a data warehouse containing details of all admissions, outpatient appointments, and A&E attendances at public NHS hospitals in England.[238] The primary cause of hospitalisation was ascertained using the International Classification of Diseases produced by the World Health Organization, versions ten (ICD10) or nine (ICD9), as for some years the older version was used. The codes for IHD were I20 to I25 for ICD10, or 410 to 414 for ICD9. The codes for stroke were I60 to I69 for ICD10, or 430 to 438 ICD9. The end of the follow-up was set at 31st March 2010 (non-informative censoring date).

Data analysis

Out of the 202,638 British post-menopausal women who have been recruited in the UKCTOCS trial, we excluded those who have not stayed in the trial for at least 3.5 years following randomisation and were therefore not sent the questionnaire containing the HH index, our main exposure variable. We have also excluded individuals who had already experienced heart disease or cerebrovascular disease. This information was derived by inspecting primary and secondary diagnostic fields of HES hospital for admissions that occurred before baseline questionnaire completion using ICD codes indicating IHD or stroke (same codes as above), and by using the self-reported health status information included in the questionnaire. We have also excluded participants with prevalent cancer using the same procedure (ICD10 codes from C00 to D48 and ICD9 codes from 140 to 239). Moreover, since the IMD only

considers England and not the whole United Kingdom, the analysis was restricted to English residents. Our final analytical baseline sample therefore comprised 80,197 individuals. Unfortunately we are not aware of the number of people excluded for each unmet condition.

The final dataset was cleaned before the main analyses with respect to inconsistent and missing values, duplicates, outliers, digit preference, and normality of continuous variables. Normality of each continuous variable was checked using histograms and Shapiro–Wilk tests. The proportion of participants who died within the follow-up period was calculated as well as the crude incidence rate for the cohort.

For the purpose of the interaction analysis, we transformed the hopelessness/helplessness values (subtracted two points from each value) so that they could range from zero (no HH) to eight (maximum HH) and measured IMD in deciles ranging from zero (no socio-economic deprivation) to nine (maximum deprivation). For data presentation purpose, we cut HH values into a binary variable: low HH (scores 0 to 2) versus medium/high HH (scores 3 to 8). Since the HH index is a relatively novel measure in medical research, a histogram describing its frequency distribution is reported in Appendix 6. The index follows a normal distribution.

We used Cox proportional hazards regression with time-scale in days to model the hazard ratios (HR) of developing IHD as a function of HH adjusting for all other available covariates such as age, education, smoking, high blood pressure, osteoarthritis, osteoporosis, diabetes, and BMI, which all showed potential confounding effects by being associated with both the main exposure variable and the outcome variable. The only covariate that did not show potential confounding

effect was rheumatoid arthritis, which was excluded from the multivariate analysis. Diabetes and education level were not associated with the outcome after the multiple adjustments, and they were therefore removed from the model. Estimates for the other covariates did not change after their removal.

The proportional hazards assumption has been assessed as follows: data has been split with the Lexis Expansion method according to follow-up time (cut-offs at 1, 1.5, and 2 years), and tests for departure from proportional hazards assumption were carried out assessing the presence of interaction between follow-up time and each variable. Furthermore, Nelson-Aalen plots were displayed to have a visual impression of the proportionality, as in Chapter 6.[188] An example of a Nelson-Aalen plot is shown in Appendix 4.

Departure from linearity of each continuous variable in the final model was tested using the following procedure: 1) cut-offs were chosen to subdivide the sample equally in four or five categories; 2) the presence of an adequate number of outcomes in each category was checked; 3) the final model was run assuming the variable to be categorical (three or four hazard ratios were generated); 4) the final model was run assuming the variable to be ordered categorical (one hazard ratio was generated); 5) the two models were compared using the likelihood ratio test (LRT). Furthermore, the hazard ratios for categorical variables were plotted to have a visual impression of the trend.[188]

The interaction between IMD and HH was evaluated using the following approach: the raw values of IMD were firstly divided into three tertiles; we then run a model with HH and tertiles of IMD as main covariates; the model was then repeated after

adding in an interaction term between the main covariates; finally the estimates from the second model were compared with the estimates from the first one using the LRT. In order for this test to be valid, the comparison has to be made on the same group of individuals (missing values can distort the results) and this assumption was always satisfied. This approach was used in four models using different adjustments: none; age; age and education; fully adjusted. Afterwards, we tested for the interaction using another strategy: the multivariate model included an additional variable calculated by multiplying the two main exposure variables together (continuous by continuous interaction).

Regarding sensitivity analysis, we excluded participants who experienced an outcome within six months from recruitment to diminish the chances of reverse causality. We did not carry out any sensitivity analysis on missing values because they were minimal.

7.3 Results

We recorded 569 IHD events during the average 2.9 year follow-up (median= 3.0). The overall incidence rate of hospitalisation for acute IHD event was 2.7 per 1,000 person years (95% confidence interval [CI]= 2.5-3.0).

Table 7.1 and 7.2 describe the sample according to categories of helplessness/hopelessness (HH) and incidence of IHD events. HH, IMD, and IHD appeared to be associated with each other, with higher-HH and lower-IMD women having higher incidence of IHD, and lower-IMD women having higher level of HH. All other variables were also associated with both HH and IHD, with the exception of rheumatoid arthritis that was associated with HH only: women with high HH scores

were typically older and less educated, had higher BMI, blood pressure, and blood cholesterol, had higher prevalence of diabetes, rheumatoid arthritis, osteoarthritis, and osteoporosis, were more likely to be smokers and to live in deprived areas, and showed higher incidence of IHD events; similarly, women with higher incidence of IHD events were typically older and less educated, had higher BMI, blood pressure, and blood cholesterol, had higher prevalence of diabetes, osteoarthritis, and osteoporosis, were more likely to be smokers and to live in deprived areas, and had higher levels of hopelessness/helplessness (Table 7.1 and 7.2).

Table 7.1. Study 3 - Sample description by categories of hopelessness/helplessness index.

Variable at the beginning of the follow-up	HH Index at the beginning of the follow-up				P
	Low (scores 2-4) (n =44,184)		Medium/High (scores 5-10) (n =36,013)		
Age (mean years ± s.d.)	63.7	±5.9	65.0	±6.2	<0.001
BMI (mean kg/m^2 ± s.d.)	26.1	±4.4	26.3	±4.5	<0.001
IMD Index (mean ± s.d.)	17.9	±13.6	18.7	±14.0	<0.001
IMD Index (geom. mean ± s.d.)	13.6	±2.1	14.3	±2.1	<0.001
IMD Index = 20 or more	32.3%	n=14,267	34.6%	n=12,450	<0.001
Level of Education*					
None	24.6%	n=10,847	33.8%	n=12,180	<0.001
Basic	51.2%	n=22,635	48.8%	n=17,556	
Higher	24.2%	n=10,701	17.4%	n=6,277	
Current Smoker	42.9%	n=18,959	45.2%	n=16,271	<0.001
High Blood Pressure	27.6%	n=12,195	30.9%	n=11,128	<0.001
High Blood Cholesterol	19.5%	n=8,625	23.1%	n=8,301	<0.001
Diabetes	4.0%	n=1,759	4.7%	n=1,700	<0.001
Rheumatoid Arthritis	3.9%	n=1,701	5.1%	n=1,847	<0.001
Osteoarthritis	14.4%	n=6,345	17.5%	n=6,309	<0.001
Osteoporosis	5.9%	n=2,585	7.0%	n=2,514	<0.001
Incident IHD event during the follow-up	0.6%	n=283	1.0%	n=342	<0.001

P values were computed using univariate logistic regression. *The ordered categorical variable education was treated as linear (P for trend). Basic level includes O-level, A-level, nursing, teaching, clerical or commercial qualification. Higher level indicates university degree.

Table 7.2. Study 3 - Sample description by incidence of IHD, with unadjusted hazard ratios, 95% confidence intervals, and P values, from univariate Cox regression.

Variable at the beginning of the follow-up	Incident IHD event within the follow-up time				HR	(95% CI)		P
	Yes (n =569)		No (n =79,628)					
Age (mean years ± s.d.)	66.5	±6.0	64.3	±6.1	1.07	(1.06	1.08)	<0.001
BMI (mean kg/m^2)	27.1	±4.8	26.2	±4.5	1.04	(1.02	1.06)	<0.001
IMD Index (mean ± s.d.)	20.9	±14.7	18.3	±13.8	1.01	(1.01	1.02)	<0.001
IMD Index (geom. mean ± s.d.)	16.0	±2.2	13.9	±2.1	1.25	(1.12	1.39)	<0.001
IMD Index = 20 or more	40.8%	n=232	33.3%	n=26,492	1.33	(1.12	1.57)	0.001
Level of Education*								
None	35.9%	n=204	28.7%	n=22,837	0.78	(0.69	0.88)	<0.001
Basic	48.9%	n=278	50.1%	n=39,910				
Higher	15.2%	n=86	21.2%	n=16,881				
Current Smoker	49.9%	n=284	43.9%	n=34,949	1.25	(1.06	1.47)	0.008
High Blood Pressure	45.2%	n=257	29.0%	n=23,068	2.05	(1.74	2.41)	<0.001
High Blood Cholesterol	32.0%	n=182	21.0%	n=16,746	1.81	(1.52	2.16)	<0.001
Diabetes	5.8%	n=33	4.3%	n=3,424	1.38	(0.97	1.97)	0.070
Rheumatoid Arthritis	4.9%	n=28	4.4%	n=3,520	1.09	(0.75	1.60)	0.650
Osteoarthritis	22.7%	n=129	15.7%	n=12,533	1.57	(1.29	1.91)	<0.001
Osteoporosis	9.1%	n=52	6.3%	n=5,048	1.50	(1.13	2.00)	0.005
HH Index (mean ± s.d.)	5.0	±1.7	4.7	±1.7	1.11	(1.06	1.16)	<0.001
Category of HH Index								
Low (scores 2-4)	44.8%	n=255	54.8%	n=43,668	1.33	(1.17	1.50)	<0.001
Medium (scores 5-7)	47.1%	n=268	38.2%	n=30,426				
High (scores 8-10)	8.1%	n=46	7.0%	n=5,542				

Ordered categorical variables such as education and HH were treated as linear (P for trend). *Basic level includes O-level, A-level, nursing, teaching, clerical or commercial qualification. Higher level indicates university degree.

Table 7.3 shows the results for the multivariate analysis. After adjusting for age, smoking, high blood pressure, osteoarthritis, osteoporosis, BMI, and high blood cholesterol, HH was associated with greater incidence of IHD. For each one point increase in HH score the incidence rate of IHD increased by seven percent on average (HR =1.07, 95%CI =1.02-1.12, P =0.006). The other independent predictors of IHD were older age (P <0.001), smoking (P =0.001), high blood pressure (P <0.001), osteoarthritis (P =0.004), osteoporosis (P =0.042), BMI (P =0.006), and high blood cholesterol (P =0.002).

Table 7.3. Study 3 - Multivariate Cox regression model showing adjusted hazard ratios, 95% confidence intervals, and P values for IHD event incidence.

Variable at the beginning of the follow-up	Mutually-adjusted HR for IHD event	(95%CI)		P
HH Index (one point increase)	1.07	(1.02	1.12)	0.006
Age (one year increase)	1.05	(1.04	1.07)	<0.001
Current smoker	1.32	(1.12	1.56)	0.001
High blood pressure	1.53	(1.28	1.84)	<0.001
Osteoarthritis	1.34	(1.10	1.64)	0.004
Osteoporosis	1.35	(1.01	1.81)	0.042
BMI (unit increase)	1.03	(1.01	1.04)	0.006
High blood cholesterol	1.35	(1.12	1.63)	0.002

Table 7.4 show the results for the interaction analysis. For each level of adjustment there was a trend such that the augmented incidence of IHD for people who experienced HH were higher in people of low SES, medium in people of medium SES, and lower in people of high SES (P values <0.03).

Table 7.4. Study 3 - Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for hopelessness/helplessness index towards IHD event incidence, stratified by tertiles of socioeconomic status (IMD).

Stratum of IMD	Effect of each one-point increase in HH on IHD event incidence rate, HR (95%CI)											
	Crude			Adjusted for age			Adjusted for age and education			Multi-adjusted*		
Least deprived	1.05	(1.01	1.10)	1.02	(0.98	1.06)	1.02	(0.98	1.07)	1.02	(0.97	1.06)
Medium tertile	1.08	(1.04	1.13)	1.05	(1.01	1.10)	1.05	(1.01	1.09)	1.04	(1.00	1.09)
Most deprived	1.12	(1.07	1.16)	1.09	(1.05	1.13)	1.07	(1.03	1.12)	1.06	(1.02	1.10)
Likelihood Ratio test P value		0.015			0.027			0.021			0.018	

*Adjusted for age, education, smoking, blood pressure, osteoarthritis, osteoporosis, BMI, and high blood cholesterol.

Table 7.5 also shows results from the interaction analysis, but instead of presenting the results for the association between HH and IHD in three separate groups of women to highlight the difference between IMD categories, this time the analysis was not stratified and included an extra covariate calculated as an interaction parameter between HH and IMD. After having adjusted for demographic and clinical variables, HH was associated with higher rates of incident IHD, such that people had an average 11% increase in incidence rates per each point increase in HH score, when they had no socio-economic deprivation (HR =1.11, 95%CI =1.01-1.23, P =0.033). Socio-economic status was also associated with IHD, with an average 7% increase per each decile increase in IMD, when people had no HH (HR =1.07, 95%CI =1.01-1.13, P =0.023). When both risk factors deviated from their zero values, some extra increased risk appeared, evaluated to be 2% per each combined unit increase (HR =1.02, 95%CI =1.01-1.03, P =0.013), meaning that the effects of hopelessness/helplessness and socio-economic deprivation are amplified by each other. The other independent predictors of IHD remained significant; these were older age (P<0.001), smoking (P <0.005), high blood pressure (P <0.001), osteoarthritis (P =0.005), osteoporosis (P 0.043), BMI (P =0.014), and high blood cholesterol (P =0.002).

Table 7.5. Study 3 - Multivariate Cox regression model showing adjusted hazard ratios, 95% confidence intervals, and P values for IHD event incidence.

Variable at the beginning of the follow-up	Mutually-adjusted HR for IHD event	(95%CI)		P
HH Index (one point increase)	1.11	(1.01	1.23)	0.033
IMD Index (one decile increase)	1.07	(1.01	1.13)	0.023
Continuous interaction between IMD and HH	1.02	(1.01	1.03)	0.013
Age (one year increase)	1.06	(1.04	1.07)	0.000
Current Smoker	1.28	(1.08	1.51)	0.005
High Blood Pressure	1.53	(1.27	1.84)	0.000
Osteoarthritis	1.34	(1.09	1.64)	0.005
Osteoporosis	1.35	(1.01	1.81)	0.043
BMI (one unit increase)	1.02	(1.00	1.04)	0.014
High blood cholesterol	1.34	(1.11	1.62)	0.002

The interaction between HH and IMD was calculated manually.

The sensitivity analysis carried out by excluding participants who experienced the outcome during the first six months of follow-up showed a pattern of results that was similar to that found in the main analysis, with no differences in the key interaction (Appendix 5).

7.4 Discussion

This study confirms our previous findings that stress-related psychological factors and socio-economic status interact with each other for the pathogenesis of IHD. We modified several important design features adopted in previous studies and consistently found that people in lower-SES conditions are more vulnerable to the detrimental effect of psychosocial adversities on IHD. The magnitude of the synergistic effect found in this study is similar compared to that found in the other study (Chapter 6). Comparing Table 6.8 with table 7.5, the HR for the interaction

parameter was 1.03 for the previous study and 1.02 for this one, with confidence intervals abundantly overlapping. Moreover, the sensitivity analysis carried out in the previous study by restricting the analysis to female participants - rendering that study more comparable to this one - gave very similar results (HR =1.01). Although these hazard ratios appear to be very small and close to the null value of 1, their effect should not be underestimated. This is a consequence of the fact that they result from analyses performed on continuous interaction parameters, i.e. on scales that measure each combined increase in the two exposure variables, and therefore a HR of 1.01 for example reflects a one percent increase in the event rate for each one unit increase in the parameter, and the parameter can range over several units.

It may be argued that the estimates from this study are overall smaller than the estimates from the previous study (Chapter 6). For example, the HR for the mental stress variable was 1.27 in the previous study (females only) and 1.11 in the present study, and the HR for the SES variable was 1.21 whereas it is now 1.07. However it has to be noted that the two measures were taken on different scales. In the previous study mental stress and SES had three possible values, while in the present study mental stress has eight possible values and SES has ten. It follows that a HRs for one unit increase calculated on longer scales tend to be smaller than a HRs for one unit increase calculated on smaller scales, and this accounts for the apparently different results.

Our findings can have important implications for health policy makers, because they can constitute the foundations for large scale programmes aiming at identifying persons who are particularly vulnerable to psychosocial adversities. Such forms of screening may have high cost-effectiveness in preventing cardiovascular disease.

We may have had missing information for those events that were treated in private hospitals or that were asymptomatic (e.g. silent AMIs in diabetes patients), or if hospitals recorded their administrative records with some inaccuracies, as Herrett at al. suggested.[227] This may account for our findings if people with higher vulnerability to mental stress tend to go to public rather the private hospitals, or tend to have silent rather than manifest heart attacks, or if hospital records were more accurate for people with higher susceptibility to mental stress. These instances would make stress-vulnerable people more present in our dataset in an unnatural way, therefore altering the results. This is very unlikely. Rather, if these missing values exist in our dataset, they have probably occurred independently from the patterns of interactions that we have focused on. As I have described in my second study, these non-differential (non-informative) biases do not distort the results, but they actually provide more robustness once the results are found to be statistically significant.

Our previous measurement of stress was based on the GHQ-12 which is not designed to assess specific aspects of mental health such as anxiety and depression, which we think are assessed by the hopelessness/helplessness score. The two scores gave consistent results.

The IMD score is an area-based score and we had to assume that the amount of deprivation recorded in each area was an accurate measurement of residents' deprivation, while people living in an area might vary in their affluence and socioeconomic status. The argument that was put forward in the discussion of missing values is applicable for this potential limitation too: it is very unlikely that the occurrence of inaccuracies in the SES measurements was dependent on individuals' vulnerability to mental stress.

The adjustment for clinical covariates may have been inefficient because they were self-reported in self-administered questionnaires. Nonetheless, those factors may be on the causal pathway between IMD or HH and IHD and therefore adjusting for them would obscure a real interaction. From this perspective, the more appropriate analysis to consider would be the one adjusted for age and education.

There is much less literature on psychosocial factors and heart disease in women than men, so the focus of the present study on women is particularly valuable. We know that women are at lower risk of IHD then men and it is therefore plausible to hypothesise that they may also have different patterns of SES-stress interaction. However, our analyses found similar interaction patterns between the two genders.

Our study design did not allow for changes in HH over time, i.e. participants' conditions were assessed only at baseline with no repeated measurements during the follow-up. However, the average follow-up time was relatively short and this diminished the occurrence of the changes, which, if ever happened, would have not distorted the interaction analysis (non-differential misclassification).

The relatively short-term nature of the study has also other implications. We have seen that atherosclerosis and IHD take several years to develop, and therefore it is probable that the pathophysiological dynamics that lead to IHD had already initiated before the study recruitment in most of the women who have then suffered a cardiac event during our follow-up. Therefore it is likely that the phenomenon that we have detected, i.e. the interaction between SES and mental stress, has a role in the trigging of acute events as well as in the chronic production of a substratum for the events. Rather than leading to slowly progressive atherosclerosis, triggers represent the final

step in the pathophysiological process leading to cardiovascular outcomes among susceptible individuals, such as those with vulnerable atherosclerotic plaque, chronic atherosclerotic disease, disorders of the cardiac conduction system, and microvascular disease. In the presence of a vulnerable atherosclerotic plaque, chemical, physical, and psychological stressors may trigger transient vasoconstrictive and prothrombotic effects that ultimately cause plaque disruption and thrombosis. Even in the absence of an occlusive thrombus, triggers may lower the threshold for cardiac electric instability and increase cardiac sympathetic activation via centrally mediated release of catecholamines.[239–241] From a public health perspective, it has been argued that mental triggers have similar or even higher population attributable fractions (PAF) for the incidence of heart attacks compared to respiratory infections, physical exertion, alcohol abuse, pollution, sexual activity, and cocaine use.[242]

In conclusion, the hypothesis that low socio-economic status amplifies the harmful effect of psychosocial adversities for the development of IHD is strongly supported by the present findings.

Chapter 8 – Final discussion

Summary of findings

I have shown that the interaction between socioeconomic status and mental stress is associated with ischemic heart disease, in such a way that people in low socioeconomic circumstances are more vulnerable to the negative effects of mental stress. In other words, the harmful effect of mental stress for human cardiac health appears to be modified by socioeconomic position and rendered more deleterious for people from disadvantaged backgrounds. This is different to saying that socioeconomic status is a mediator or a confounder between stress and ischemic heart disease and that mental stress is a mediator or a confounder between socioeconomic status and ischemic heart disease.

In addition, I found evidence of interaction between mental stress and socioeconomic status even when the outcome was all-cause mortality. There are four main possible explanations for this observation:

- Ischemic heart disease is the leading cause of death both in the UK and worldwide. It is responsible for more than 73,000 deaths in the UK each year. About 1 in 6 men and 1 in 10 women die from ischemic heart disease. In the UK, there are an estimated 2.3 million people living with ischemic heart disease and around 2 million people affected by angina (the most common symptom of ischemic heart disease).[243] Since my three studies were all based on British samples, all-cause mortality may have been mainly driven by IHD deaths.

- Diseases such as cancer may share a common pathophysiology with ischaemic heart disease. The extent to which psychosocial factors can promote cancer incidence has been highly controversial. In contrast, there is much stronger evidence supporting a role of psychosocial factors in cancer progression and mortality.[244] Converging evidence from clinical, animal, and in vitro models supports the presence of both direct and indirect links between psychosocial risk factors, related neuroendocrine hormones, and a number of dynamic biological processes that promote cancer growth modulate immunocompetence, or both. Stress-related factors have been associated with impairment of the cellular immune response and upregulation of pathways supporting inflammation, angiogenesis, invasion, and metastases.[244] Since cancer deaths will have been another major driver for all-cause mortality in my sample, along with ischaemic heart disease, common pathophysiological processes may explain the association.
- The combination of high mental stress and low socioeconomic status may operate as a determinant of general susceptibility to chronic disease. Indeed, the concept of general susceptibility to disease has developed as a unifying explanation for the finding that a variety of health outcomes are associated with certain social and cultural situations.[245] Also, from a more clinical and less sociological perspective, it has to be considered that the fight against infectious disease advanced dramatically with the consolidation of the germ theory in the 19th century. This focus on a predominant cause of infections (i.e., microbial pathogens) ultimately led to medical and public health advances (e.g., immunization, pasteurization, antibiotics). However, the resulting declines in infections in the 20th century were matched by a rise in

chronic, non-communicable diseases, for which there is no single underlying etiology. The discovery of a form of low-grade systemic and chronic inflammation ("metaflammation"), linked to inducers (broadly termed "anthropogens") associated with modern man-made environments and lifestyles, suggests an underlying basis for chronic disease that could provide a 21st-century equivalent of the germ theory.[246] However, these postulates have not yet been scientifically validated.

- Other mechanisms may have operated in determining all-cause mortality or even in causing ischaemic heart disease incidence and mortality. For example, a prospective cohort study on depressive disorder defined using the GHQ-30, ischaemic heart disease, and stroke found evidence of a dose-response effect of depressive symptoms on risk of ischaemic heart disease, but not on stroke.[247] This finding contrasts with the widely-accepted idea that primarily somatic aspects of depression are linked with ischaemic heart disease.[248] The GHQ-30 was developed from the GHQ-60 and involved removing most questions related to somatic symptoms. The study by Brunner et al suggests it would be valuable to explore other processes in the determination of cardiovascular disease. There are two very different types of stroke - ischaemic and haemorrhagic - and this may partly explain the study findings by Brunner et al. While ischaemic stroke may share a common pathophysiology with ischaemic heart disease, haemorrhagic stroke may have different determinants.

Strengths and limitations

- **Power**. This dissertation is based on secondary analysis. I have not carried out any sample size calculation before the design of any of the presented studies because their data had already been collected before I became involved in them. The original sample size calculation for none of the studies was based on my original research hypothesis. Study one is probably underpowered to address my research hypothesis; however, although the P values for the statistical tests were non-significant, the results showed a positive trend in the interaction, meaning that the lower the socioeconomic status of the individuals, the higher the measure of effect (odds ratio) for the association between mental stress and heart disease. Studies two and three had sufficient power to test my research hypothesis as they have showed the expected statistically significant trends.
- **Information bias.** Possible measurement errors in the key variables can distort the results if they are differential (informative), i.e. if they happen to a greater or lesser extent in certain categories of study participants. For example, if people with high levels of mental stress who then develop heart disease are wrongly diagnosed as not having the disease, the effect is underestimated (lower relative risk than the true one); whereas if people with low stress who then develop heart disease are wrongly diagnosed as not having the disease, the effect is overestimated (higher relative risk than the true one). Additionally, if people with high levels of stress who then do not develop heart disease are wrongly diagnosed as having the disease, the effect is overestimated, whereas if people with low levels of stress who then

do not develop heart disease are wrongly diagnosed as having the disease, the effect is underestimated. My results would be due to those kinds of distortions if errors tending to overestimate the results happened more often in low socio-economic status groups and/or if errors tending to underestimate the results happened more often in high socio-economic status groups. This is unlikely to have happened because the outcome ascertainment for all studies was blind, i.e. the researchers or clinicians who recorded the outcome did not know the levels of mental stress and the socioeconomic position of the study participants. Instead, non-differential (non-informative) measurement errors are those that happen randomly or for reasons that are not linked with the research hypothesis. In this case they do not distort the results, but they do create some "noise" in the data, i.e. they decrease the power of the analysis and therefore they increase the P values. As a consequence, when a P value is small and non-differential information bias is present the results acquire more importance. As I have explained in the chapters pertaining to each study, it is very likely that if there were measurement errors in any of the presented studies they would be of the non-differential type. I have measured the exposure variable only at baseline for studies two and three, and therefore I could not account for changes of those measures during the follow-up period, which would introduce non-differential misclassification. However, my third study had a relatively short follow-up time, and hence had a reduced probability of such bias. Moreover my first study was cross-sectional and was carried out in laboratory-based experimental conditions, and therefore the measurement of the exposure variable was optimal in that case. The outcome variable and

the effect modifier could also be affected by measurement error; however the three studies had three different methods of ascertainment for both measures, and the results were consistent between them.

- **Selection bias and generalisability.** Selection bias can distort the results in two main ways: if potential participants with certain patterns of association are more/less likely to be recruited and selected for a study, and if they are more/less likely to drop out from the study once they are in. It is improbable that these potential distortions account for my results because participants' recruitment for all studies was blind towards my research hypothesis and because it is very unlikely that people with higher vulnerability to mental stress were more prone to be selected or less prone to drop out, rendering them artificially more frequent in my datasets than they are in the general population. Moreover the sample of my second study was representative of the national population, thus rendering selection bias less likely and increasing the generalisibility of my results.
- **Heterogeneity of measures and settings.** My research hypothesis was confirmed in three separate studies that had different key features from each other such as the study design, the index for mental stress, socio-economic status, and heart disease used, the follow-up time resolution and duration, the reference population, the calendar time (time of data collection), and the severity of the events (fatal and non-fatal). Therefore my research results appear to have considerable validity and generalisibility.
- **Confounding.** Confounding is a common issue in studies assessing the association between two variables. The results can be confounded if a third factor is associated with the exposure variable and is a risk factor for the

outcome variable, therefore explaining the results by being the real determinant of the outcome and disproving a causal relationship between the exposure and the outcome. For example, chronic inflammation may cause both heart disease and mental disease, thus explaining the association between those two. Smoking is a risk factor for heart disease and can be another confounder, if people who are more stressed tend to smoke more. My analyses have been adjusted for several determinants of heart disease such as the ones that I have mentioned, although for my third study the covariates may have not been robust enough. Further confounding may have come from unmeasured variables. For example, there could be genetic polymorphisms that affect vulnerability to stress, and these polymorphisms could be over-represented in the lower SES groups, thus explaining not only the association between mental stress and heart disease but also the interaction between mental stress and socio-economic status. Childhood infections may also give a similar explanation, if some of them are able to cause augmented vulnerability to mental stress (through immune function dysregulation for example) and if they cluster in lower socioeconomic sectors of the population.[249]

- **Interaction**. It is theoretically possible that a fourth variable can act as a further effect modifier, resulting in a four-dimensional interaction. For example, the augmented effect of mental stress for heart disease seen in people from low socioeconomic backgrounds can be further increased if these people are females, or older, or have some other characteristics. The possibility of gender interaction has been evaluated by restricting one analysis to female participants, and the results were consistent. We did not

carry out any other four-dimension analysis because it has been shown that post hoc subgroup analysis can lead to false positive results simply due to chance, and therefore there has to be a strong biological plausibility, along with an increased sample size, for these sub-analyses to be valid.

- **Reverse causality.** In studies for the association between two variables the issue of reverse causality is never ruled out completely. Unless the exposure variable is experimentally and randomly assigned (randomised controlled trials), there is always a chance that variable A is the effect of variable B, and not its cause. My three studies were all observational and therefore I cannot rule out the problem of reverse causality. It is possible that people at early stages of heart disease felt more stressed, as a consequence of metabolic unbalances due to coronary disease for example. Reverse causality is a major limitation of my research and it may also explain the interaction effect that I have found. In study one for example people with subclinical or initial ischaemic heart disease could have had alterations in their cortisol reactivity, and people in higher socioeconomic conditions may have engaged less in the performance of the stress tasks. In study two and three, people at initial stages of ischaemic heart disease, or people with conditions that are prodromes of ischaemic heart disease, may have had higher chances of answering questions related to anxiety or depression positively, and, most importantly, people from different socioeconomic sectors may have answered the subjective measurements (questionnaires) with differential accuracy. To tackle this issue, in my two cohort studies I carried out sensitivity analyses by excluding study participants who developed the outcome soon after their recruitment, and who might have been thought to

be already affected by subclinical heart disease. In study two I excluded the 51/1,007 (5.1%) participants who experienced the outcome within one year from recruitment. In study three I excluded the 65/569 (11.4%) participants who experienced the outcome within six months from recruitment. The results from these sensitivity analyses were similar to those from the main analyses. Also, by checking the proportional hazards - as explained in the Methods section of the two studies (p.122-123, 144) - we observed that there was no evidence of changes in the incidence rates during the follow-up times. If the samples included considerable numbers of people who were already affected by heart disease at the beginning, the incidence rates would have appeared higher in the first period of time, then lower, and then higher again, whereas the rates appeared always stable in time.

- **Types of outcome.** For this dissertation I have used three different CVD outcomes. For study 1 I have used HS-CTnT measured in a laboratory-based experimental setting, for study 2 I have measured mortality for IHD using death records from the Office for National Statistics, and for study 3 I have measured hospitalisation rates for IHD using Hospital Episode Statistics records. The results from those different sources of outcome data were consistent and this gives my findings particular value. While for studies 2 and 3 the outcome variable was a binary variable measuring the incidence of a real event, study 1 had a continuous outcome variable that is routinely used to mark cardiac damage in the context of emergency diagnosis of AMI. The finding of troponin abnormalities is the condition sine qua non for a conclusive diagnosis of AMI: an AMI is confirmed if an elevation or a decline in troponin concentration above the 99th percentile upper reference limit is

detected in the peripheral blood plasma of an individual, and if at least one of the following conditions is also present: symptoms of ischaemia; electrocardiographic abnormalities; imaging evidence of new loss of viable myocardium or new regional wall motion abnormality; identification of an intracoronary thrombus by angiography or autopsy.[250] However causes of troponin elevation not related to AMI have become common findings with the advent of high-sensitivity assays, not only in patients with chest pain but also in people with no symptoms. People who do strenuous physical exercise such as a marathon for example can have troponin elevations. All the conditions leading to troponin release in the absence of AMI are largely unknown, and wrong diagnosis (false positive) may occur if one of such conditions couples with another spurious condition (e.g. physical exercise + chest pain due to heartburn).[22] Although experimental data strongly suggest that troponin leaks out of the cell only after membrane disruption following myocardial cell death,[251] the detection of brief rise and subsequent fall of troponin concentration during marathon running[252] and rise after inducible myocardial ischaemia[253] has cast some doubts on the hypothesis that troponin is released only upon irreversible damage. Although the hypothesis that there may be a leakage of troponin through myocyte membranes without necrosis is not implausible, there is no scientific evidence supporting this theory yet and troponin abnormalities have to be considered as a sign of cardiac damage.[254] Moreover, in healthy people not fulfilling any diagnostic criterion for AMI, greater HS-CTnT is associated with a greater incidence of structural and functional heart disease, cardiovascular mortality, and all-cause mortality.[176,177] Therefore, although the outcome for study 1 does not

mark an incident event, it is a prodrome of IHD that is relevant to my research hypothesis and hence the findings from study 1 support the findings from the other studies.

Comparison with other studies

Other studies have had a similar research hypothesis to mine. Out of the eleven observational studies found through my literature review, five found an interaction and confirm our results, four found no interaction, one study on a male population found a negative interaction, with high-SES participants being more vulnerable to stress, and one study found positive interaction in men and negative in women. In the following paragraphs I will go through the papers that do not confirm my results and will evaluate the differences to my studies.

Kuper and Marmot (2003) found no interaction between job strain and employment grade on around 10,000 participants from the Whitehall II study.[155] Their result may be due to the fact that their study was underpowered to detect an interaction, since some of my results showed borderline significance (P around 0.05) in spite of having more than six times (for study two) and more than eight times (for study three) the sample size of Kuper&Marmot's study.

Janszky et al. (2010) had a sample size that was comparable to mine (about 50,000 men).[157] However, they measured mental stress (adolescent depression or anxiety) and SES (self-reported occupation of the head of the household during participants' childhood) only at baseline for a follow-up of 37 years of duration on average. Those measures may have been less relevant than more contemporary measures of SES,

introducing information bias and therefore a decrease in power. Moreover, the authors do not report the results from any statistical test for interaction.

Toivanen (2011) had a smaller sample size (about 2,600 persons),[159] as well as possible information bias leading to further power reduction, since they have considered self-reported health as an outcome. Although self-reported health is a validated end-point in epidemiological studies, its measurement has less precision and accuracy than hard clinical outcomes such as acute myocardial infarction or other IHD events.

Tsutsumi et al. (2011) found a positive interaction in men (the lower the SES, the higher the vulnerability) and a negative in women (the lower the SES, the lower the vulnerability).[160] This study had also a relatively small sample size (about 6,400 people) and the authors did not carry out any formal test for interaction; their conclusions are drawn solely from observing stratified results. A further limitation may have arisen from the choice of the variables and from the adjustments. The authors considered occupational stress as the exposure variable and occupational class as the effect modifier, and their analysis was adjusted for level of education. All these three variables may be correlated with each other (e.g. education is also an index of SES as occupational class is), hence introducing potential bias and overadjustment.

A negative interaction was found in men by Suadicani et al. in 2011.[161] Their sample size was relatively small (about 4,900) and the follow-up was long (about 30 years), thus introducing information bias and power reduction. Moreover their exposure

variable (perceived psychological work pressure) was measured using a non-validated questionnaire.

Mittag et al. (2012) had an adequate sample size (about 64,000).[162] The main limitation of this study is that it was cross-sectional, and the exposure variable, the effect modifier, and the outcome were self-reported using non-validated questionnaires (e.g. the existence of IHD was assumed if any of these items were answered positively: “has a doctor ever told you that you had angina pectoris or coronary artery disease? Has a doctor ever told you that you had a myocardial infarction or heart attack?”).

Therefore my research has several advantages over the previous studies. For studies two and three my sample size was considerably larger than most of the previous research in this specific research area. Study two was representative of the English national population and is therefore stronger than the previous literature in terms of generalizability. In all my studies, all three main variables were uncorrelated with each other, to the contrary of most previous studies that had a focus on job stress. Studies two and three had different follow-up times; a short follow-up time has the disadvantage of being more prone to reverse causality, whereas a long follow-up time has the disadvantage of being more prone to information bias (change in the exposure variable), so by having a study with a long follow-up time and a study with a short follow-up time my results acquire more validity. The quality of the measurements in my studies is considerably higher than most of the studies that attempted to address my research hypothesis.

This dissertation advances the scientific knowledge not only in the area of psychosocial research, but also in the study of the association between socioeconomic status and health, and has helped to establish a link between the two areas. We have seen how mental stress may influence human health though chronic inflammation and neuroendocrine-immune interactions; but also socioeconomic status is traditionally seen as a major determinant of health and well-being, and psychoneuroimmunological processes may contribute to the links between socioeconomic status and health outcomes. There is extensive population evidence that socioeconomic status is inversely associated with chronic inflammation indexed by markers such as C-reactive protein, interleukin-6, and fibrinogen. Both childhood and adult socioeconomic status contribute to this pattern. Evidence for socioeconomic status differences in acquired immunity is more sparse, but it also implicates lower SES in the dysregulation of immune responses. However, while there is an inverse correlation between SES and seropositivity for common infections acquired early in the life course that may in turn contribute to low-grade inflammation in adult life, there is limited experimental data from humans showing that lower SES is associated with heightened inflammation following acute stress. It has been argued that SES differences in immunity and inflammation are also mediated in part by variations in health-related behaviours such as smoking and energy balance, and by direct stress-related processes, and that longitudinal research linking SES, psychosocial adversity, immune function, is needed.[255] Therefore my research covers an area of investigation that has been previously indicated as important and substantially unexplored, and creates important connections between areas of knowledge.

Possible explanation of the findings: putting results in context.

Health disparities associated with SES are in part due to differences in the distribution of basic resources such as health care, nutrition, and sanitary environments.[152] This focus may be particularly relevant to explaining poor health in groups characterised by poverty, but the effect of SES on health is not only at the poverty line. Rather, health discrepancies have a monotonic relationship with SES, so that even relatively affluent groups exhibit worse health than their higher SES counterparts. Thus, numerous interconnected factors appear to contribute to SES disparities in health and we have seen how one prominent explanation is that cognitive and emotional factors and disorders play a role in understanding how low SES results in augmented risk of morbidity and mortality. But although SES is consistently related to cognitive and emotional symptoms, which predict worse health outcomes, these three components cannot be considered to be on a unidimensional causal cascade to determine a mechanism of mediation. Rather, we speculate that low levels of tangible, interpersonal, and intrapersonal resources, i.e. a deficient reserve capacity associated with low SES, may exacerbate the effect of SES-associated stress on negative emotions and attitudes, recognising that low SES can both deplete resources and impede the development and restoration of the resource bank.[152] There is indeed evidence that SES is associated with low reserve capacity. For example, individuals with low SES have access to fewer financial and material goods, which might otherwise offset tangible stressors such as job loss, illness, or disability.[256] Low-SES environments also contain deficient community resources, such as safe neighbourhoods, parks, transportation, and child care.[257–259] If present, these resources might reduce chronic or daily stress. SES is also associated

with diverse aspects of social functioning, including contact with others, network size, reciprocity in relationships, satisfaction with support, the tendency to seek social support, work support, and generalized support perceptions. [260–272] Furthermore, neighbourhood as well as individual SES appears to influence social experiences.[273,274] Social stressors typical of low-SES environments (e.g., crowding, violence, high crime rates) may interfere with the development of supportive contacts by discouraging interpersonal trust[265,275,276] In addition, individuals with low SES may be vulnerable to factors that could degrade social support and increase social stress, such as marital instability,[277] domestic violence,[278–281] substance abuse,[282] and single parenting.[283] Furthermore, the association between low educational attainment and cognitive decline linked to aging is not simply due to the fact that less educated people have lower mental abilities throughout their lives but that education encourages the development of a set of reserves that allows more efficient processing or develops compensatory processes to protect against age-related decrements in functioning.[284,285]

My results are therefore consistent with the reserve capacity model proposed by Matthews and Gallo, which I have detailed in Chapter 1.7. The theory that they have described from a sociological and a psychological perspective seems to have implications in the real world and impact on robust clinical outcomes such as heart disease measured using an epidemiological approach. That model focuses on interpersonal and intrapersonal resources as modifiers of the effect of environmental demands and stresses on emotional and physiological responses. Low-SES individuals may maintain a smaller bank of resources to deal with stressful events when compared to their higher-SES counterparts.

The complex pathophysiology of heart disease and the dynamics through which mental health affects cardiovascular health cannot explain my findings alone. This is sustained by several arguments: (a) in my first study I have adjusted for coronary calcification and for factors that link mental stress with heart disease (cytokines, inflammatory factors, etc.) but the interaction trend remained; (b) in study two, I carried out an analysis on all-cause mortality along with IHD-mortality (thus including diseases with different pathophysiology compared to heart disease), and the interaction was similarly present; (3) in my third study, I adjusted for inflammatory conditions that are linked with both mental stress and heart disease (osteoarthritis, osteoporosis) and the interaction remained evident. Although I could not directly measure the reserve capacities of my study participants, i.e. their interpersonal and intrapersonal resources, the reserve capacity model is the most plausible explanation for my study findings, but needs fuller investigation, also in connection with the clinical determinants of heart disease. Other researchers have in fact tested the reserve capacity model, with mixed results. For example in a study on socioeconomic status, cognitive-emotional factors, and health status following myocardial infarction, partial support for the reserve capacity model emerged: for physical health status, results supported direct rather than indirect effects of socioeconomic status.[286] However, that study had limited sample size (about 2,000) and the data were analysed using mediation models rather than interaction tests.

A challenge of any model of SES and mental stress is to consider the potential for bidirectional relationships: emotions and cognitions can influence the availability of resources, which can alter interpretations of stressful circumstances; there can be a connection from health outcomes, or intermediate paths, back to emotions and

cognitions; health endpoints such as IHD can affect the intermediate psychosocial factors included in the model, as well as socioeconomic standing.[287] However, IHD and premature mortality represent distal health outcomes that typically occur late in the natural history of disease, well after the usual establishment of socioeconomic position.

Another potential limitation of the reserve capacity model is the fact that the supposed interaction operates for all forms of psychosocial adversity in an unspecific way. On page 77 I showed a diagram for the reserve capacity model (Figure 1.8). In that representation of the dynamics linking socioeconomic status, psychological conditions, and physical health, the reserve capacity interferes (line D) in the association between objective experiences and perceived emotions (line B). In people with abundant resources, the actual harms are more efficiently buffered and therefore they raise less negative emotions. However the reserve capacity model does not disentangle the different types of stimuli and responses. As I have described in the Introduction of this thesis (p.68), some of the affective and behavioural conditions that are linked with ischaemic heart disease appear to be different or sometimes opposite from each other. Anxiety and depression are an example of that. Anxiety is an unpleasant state that is often accompanied by nervous behaviour and a feeling of worry that usually lead to overreaction to normal situations (high arousal), whereas depression is a persistent sadness or low mood with associated loss of interest in activities and often failure to react to situations (low arousal). In spite of those differences, both conditions are positively associated with higher chances of IHD. It has been argued that affective dispositions can overlap and that a general

disposition toward negative affectivity may be more important for disease risk than any specific negative affect.[107]

The reserve capacity model is therefore compatible with the concept of general disposition toward negative affectivity. My results also support this idea, since I have tested different exposure variables and got similar results for all of them: cortisol response to stress (Study one) can be seen as an index of anxious temperament because it marks physical overreaction to mild stressors; GHQ-12 (Study two), contains items of both anxiety and depression (see Appendix two for the list of items); the hopelessness/helplessness index (Study three) includes two items linked to depression only. The measure in study 3 may be considered as a subtype of the mental stress measured in study 2 and it may therefore be possible that the results from study 2 are driven by the same items of study 3. However the effect estimates from the two studies were similar and this goes against that hypothesis. If the relevant items were those related to depression only, I would have found higher estimates in study 3 compared to study 2 because these latter estimates would have been diluted but the additional presence of less relevant items; if, conversely, the items related to anxiety were the more relevant I would have found higher estimates in study 2; if anxiety and depression interacted with each other I would have found higher estimates in study 2. So the most likely explanation of my results is that general disposition toward negative affectivity may be more important for disease risk than any specific negative affect.[107] However, my analyses had just enough power to detect the interaction between mental stress and SES and it therefore likely that the power to make these finer comparisons between the different dimensions of mental stress is not enough in my samples and that the similarity in the estimates

found between studies 2 and 3 may be a chance finding. Moreover, it would have been interesting to conduct separate analyses for the different items in the GHQ-12 score (anxiety items versus depression items) and compare their results with each other and with the other studies. Unfortunately we did not have access to the scores of each individual item.

Cortisol reactivity has been studied in conjunction with very different outcomes, for example tobacco and alcohol dependence,[288–290] exercise addiction,[291] depression,[292] bulimia,[293] and motivation.[294] Although it is difficult to see the commonality among some of these apparently diverse outcomes, it has recently been argued that they all are different manifestations of the same underlying central corollary of a deficient peripheral stress response and that all these outcomes, to different degrees, reflect motivational dysregulation, that is, a dysfunction of the neural systems that support motivated behavior.[294] The results from those studies are apparently opposite from mine: they imply that low or blunted cardiovascular and/or cortisol reactions to acute psychological stress are associated with higher occurrence of those conditions. However, from the perspective of the allostasis theory those results are compatible with mine. The notion of allostasis has drawn attention to the complex system of neuroendocrine responses to environmental challenges that is characteristic of living organisms. It has been conceptualised as the process through which organisms actively adjust to both predictable and unpredictable external events through the anabolism and catabolism of mediators, i.e. the way in which they maintain stability through change.[295] From this perspective, serious pathophysiology may occur when chronic overload resulting from sustained stress stimulates prolonged allostatic actions that in the long term lose their effectiveness and ability to respond.[296] The

allostatic model suggests that sustained load is characterised by changes in the morphology of responses that are manifest in chronically-heightened basal levels, inadequate biological responses (blunted stress reactivity), and impaired post-stress recovery.[297] Study one was carried out on healthy older individuals who have perhaps not yet encountered prolonged allostatic actions that decreased their reactivity, whereas the studies that found blunted responses in at-risk people were carried out on samples drawn from the general population (including diseased people) or deliberately on people with manifest diseases.

Future work

Mental stress is becoming increasingly recognised as a risk factor and trigger for heart disease and some experts in the field have argued that the effect that mental stress has on the likelihood of developing heart disease may have a similar magnitude compared to that of more conventional risk factors such as high blood cholesterol and high blood pressure.[54,55,167] However, unlike for these other risk factors, guidelines for screening strategies or preventive cures for mental stress have never been developed, in spite of this risk factor being very frequent in the general population and therefore having a large impact on public health. In contemporary western life-style conditions, mental stress is difficult to avoid or attenuate, unlike other risk factors for heart disease such as high blood cholesterol, diabetes, etc. for which effective treatments are available. Risk assessment coupled with tailored protective intervention is therefore a possible preventive strategy for those numerous heart attacks and strokes triggered by mental stress and, as a consequence, mass screening for vulnerability to mental stress would be extremely beneficial, may have considerable public health effects, and save many lives.

I have demonstrated that the effect that mental stress exerts on heart disease is not the same for everybody, and in some individuals it is greatly amplified. In particular I found that low-income individuals are much more vulnerable to mental stress. While, for example, the average effect of mental stress for heart disease is mild in the general population, the estimates diverge after stratification by socioeconomic status, and I found the effect to be almost null in high-income sectors and strong in low-income.[228,298] It would be valuable to study these interactions more deeply, as well as other possible interactions, because these results still do not justify the implementation of targeted interventions. This is due to two reasons: (a) the target population (low-income sectors) would still be too wide and too difficult to define for public health interventions; (b) the relative incidence rates are still too small and the NNT (number needed to treat) to obtain public health benefits would consequently be too large, and therefore screening would not be cost-effective.

It would be desirable to further restrict the target population to very vulnerable individuals (higher effects) by answering questions such as: what socioeconomic aspects are more relevant? What types of mental stress are more deleterious? Are there any environmental, clinical, or genetic factors that predispose these negative effects or are independent effect modifiers? Therefore an important long-term research aim is to identify the demographic, clinical, environmental, and genetic variables that increase susceptibility to mental stress and may serve to identify people who are particularly vulnerable to stressful events, so that mass screening programs and consequent tailored interventions would become feasible, effective, and cost-effective.

It is plausible that the resources that modify the effect of mental stress develop during one's childhood or may even be genetically determined. Therefore the ideal resolution to address these issues would be the development of a new birth cohort. A birth cohort would also have the additional advantage that it would be possible to assess outcomes other than heart disease, or outcomes that are precursor of heart disease such as health behaviour or immune dysregulation for example, thus explaining these interactions not only for the sake of public health policies but also in terms of basic research in human pathophysiology. However the research that I have outlined in this thesis does not identify new potential effect modifiers or outcomes or precursors of outcomes and it would be appropriate to explore other existing datasets before new cohorts are developed. The results from my present work and from these eventual new studies may then serve as pilot studies for the development of a birth cohort to test these specific issues. For these other analyses, one possibility would be to extract data from UK-Biobank, MINAP, and possibly other databases, and analyse them prospectively (cohort studies). This means that those databases would constitute the baseline of cohorts, and incident outcomes would be recorded during a follow-up time using records from the Hospital Episode Statistics and the Office for National Statistics, as I have done for the present thesis.

The UK-Biobank questionnaire can be categorised into the following broad topic areas of interest: sociodemographics and occupation; lifestyle exposures (including smoking, alcohol, physical activity and diet); early life exposures; psychological state; cognitive function; family history of illness; and medical history and general health. Therefore UK-Biobank routinely measure variables related to psychological and cognitive state. With respect to psychological state, the approach in the UK-Biobank

questionnaire has been to assess psychological traits and moods based on standardized questionnaires, and to record serious life events and medical presentations for psychological symptoms.[299]

MINAP (Myocardial Ischaemia National Audit Project) is a national clinical audit of the management of heart attack. It supplies participating hospitals with a record of their management and compares this with nationally and internationally agreed standards. MINAP provides comparative data to help clinicians and managers to monitor and improve the quality and outcomes of their local services. Although the main focus of my proposed research is on healthy people for the primary prevention of cardiovascular acute events due to mental stress and not on patients, it is biologically plausible that patients' recovery from those events, their prognosis, and their reoccurrence rates also depend on mental stress and on its interactions with other clinical, environmental, and genetic factors. MINAP has a dedicated section for heart conditions that do not require coronary intervention such as the increasingly recognised syndrome of Takotsubo cardiomyopathy.[300] This condition, presenting as a heart attack – often following an extremely stressful event – appears not to be related to coronary artery occlusion at all. Rather the heart damage, from which a full recovery is possible, is driven by the effects of naturally occurring though excessive catecholamine (e.g. adrenaline) stimulation due to mental stress.[189,301] MINAP now provides additional detailed information about such cases[300] and therefore MINAP will be an excellent source of data for a new research project.

Conclusion

In this thesis I have shown the potential importance of the interaction between SES and stress for understanding human disease and cardiovascular disease in particular. The epidemiological approach to investigating these issues has proven to be valid compared to other approaches such as clinical and animal research, especially with regards to statistical power in the ascertainment of hard endpoints. I hope that this research programme will stimulate new work focused on understanding the processes better at a mechanistic level, on devising instruments that will identify vulnerable individuals with greater precision, and on developing interventions aimed at reducing these negative influences on health and survival.

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Appendix 1 - Sensitivity analyses for Study 1.

This appendix presents the results from sensitivity analyses carried out for Study 1. The multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks and plasma detectable high-sensitivity cardiac troponin T were conducted using different settings. For each table, the changed setting is underlined.

Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks (<u>continuous exposure variable calculated as the difference</u> between post-tasks and pre-task values) and plasma detectable high-sensitivity cardiac troponin T (binary outcome).

Model for detectable HS-cTnT (outcome)	**OR for cortisol response (exposure)**	**P**	**(95% CI)**	
Crude association	1.10	0.004	(1.03	1.17)
Adjusted*	3.68	0.001	(1.75	7.76)

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin, CRP, IL-6 and coronary calcification score, treated as a binary variable with cut-off at 0. This level of adjustment is parallel to Model 6a in Table 5.3

Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks (continuous exposure variable calculated as the ratio between post-tasks and pre-task values) and plasma detectable high-sensitivity cardiac troponin T (binary outcome).

Model for detectable HS-cTnT (outcome)	OR for cortisol response (exposure)	P	(95% CI)	
Crude association	1.38	0.005	(1.10	1.73)
Adjusted*	1.13	0.024	(1.03	1.23)

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin, CRP, IL-6 and coronary calcification score, treated as a binary variable with cut-off at 0. This level of adjustment is parallel to Model 6a in Table 5.3

Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks (binary exposure) and plasma detectable high-sensitivity cardiac troponin T (binary outcome).

Model for detectable HS-cTnT (outcome)	OR for cortisol response (exposure)	P	(95% CI)	
Adjusted*	1.13	0.024	(1.03	1.23)

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin, CRP, IL-6, coronary calcification score treated as a binary variable with cut-off at 0, physical activity, BMI, diastolic blood pressure, triglycerides, LDL, and alcohol consumption.

Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks (binary exposure) and plasma detectable high-sensitivity cardiac troponin T (binary outcome), restricted to male participants.

Model for detectable HS-cTnT (outcome)	OR for cortisol response (exposure)	P	(95% CI)	
Crude association	1.15	0.008	(1.12	8.75)
Adjusted*	1.98	0.007	(1.24	9.00)

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin, CRP, IL-6 and coronary calcification score, treated as a binary variable with cut-off at 0. This level of adjustment is parallel to Model 6a in Table 5.3

Multiple logistic regression models for the association between salivary cortisol response to standard laboratory mental stress tasks (binary exposure) and plasma detectable high-sensitivity cardiac troponin T (binary outcome), restricted to female participants.

Model for detectable HS-cTnT (outcome)	OR for cortisol response (exposure)	P	(95% CI)	
Crude association	3.56	<0.001	(1.96	9.62)
Adjusted*	5.80	0.001	(2.14	21.70)

*Adjusted for baseline salivary cortisol (pre-task), time of the session (am or pm), age, gender, smoking, systolic blood pressure, total cholesterol/HDL ratio, and glycated haemoglobin, CRP, IL-6 and coronary calcification score, treated as a binary variable with cut-off at 0. This level of adjustment is parallel to Model 6a in Table 5.3

Appendix 2 - The 12-item General Health Questionnaire.

Twelve-item General Health Questionnaire

We want to know how your health has been in general over the last few weeks.

Please read the questions below and each of the four possible answers.

Circle the response that best applies to you.

Thank you for answering all the questions.

Have you recently:

1	-	been able to concentrate on what you're doing?	Better than usual	Same as usual	Less than usual	Much less than usual
2	-	lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
3	-	felt that you are playing a useful part in things?	More so than usual	Same as usual	Less so than usual	Much less than usual
4	-	felt capable of making decisions about things?	More so than usual	Same as usual	Less so than usual	Much less than usual
5	-	felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
6	-	felt you couldn't overcome your difficulties?	Not at all	No more than usual	Rather more than usual	Much more than usual
7	-	been able to enjoy your normal day to day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual
8	-	been able to face up to your problems?	More so than usual	Same as usual	Less so than usual	Much less than usual
9	-	been feeling unhappy or depressed?	Not at all	No more than usual	Rather more than usual	Much more than usual
10	-	been losing confidence in yourself?	Not at all	No more than usual	Rather more than usual	Much more than usual
11	-	been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
12	-	been feeling reasonably happy, all things considered?	More so than usual	Same as usual	Less so than usual	Much less than usual

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Appendix 3 - Sensitivity analyses for Study 2.

This appendix presents the results from sensitivity analyses carried out for Study 2. They are multiple Cox regression models for the association of mental stress (GHQ-12), socioeconomic status (profession), and an interaction parameter with either IHD mortality or all-cause mortality.

Modified Table 6.7 (two more covariates were included). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.28	(1.12	1.47)	<0.001
Unit increase in SES going from high to low (3 categories)	1.20	(1.07	1.34)	0.001
Interaction SESxGHQ-12 (3x3)	1.07	(1.01	1.11)	0.047
Gender male	1.58	(1.47	1.70)	<0.001
One-year increase in age	1.11	(1.10	1.11)	<0.001
Current smoking	1.43	(1.32	1.54)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.61	(2.08	3.27)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.00	(0.92	1.09)	0.963
Diabetes	1.48	(1.28	1.71)	<0.001
Physical activity	0.94	(0.92	0.96)	<0.001
Hypertension	1.15	(1.06	1.24)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.8 (two more covariates were included). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.19	(1.02	1.89)	0.039
Unit increase in SES going from high to low (3 categories)	1.21	(1.05	2.15)	0.024
Interaction SESxGHQ-12 (3x3)	1.05	(1.00	1.14)	0.052
Gender male	2.36	(1.91	2.93)	<0.001
One-year increase in age	1.11	(1.10	1.12)	<0.001
Current smoking	1.29	(1.04	1.60)	0.023
Unit increase in BMI	1.03	(1.00	1.05)	0.021
Diabetes	2.69	(1.95	3.73)	<0.001
Physical activity	0.93	(0.87	0.99)	0.020
Hypertension	1.12	(0.90	1.39)	0.325

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.7 (the analysis was restricted to females). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.23	(1.06	1.43)	0.005
Unit increase in SES going from high to low (3 categories)	1.19	(1.06	1.34)	0.003
Interaction SESxGHQ-12 (3x3)	1.02	(1.00	1.04)	0.049
One-year increase in age	1.11	(1.11	1.12)	<0.001
Current smoking	1.50	(1.40	1.61)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.63	(2.19	3.17)	<0.001
BMI 30+ versus BMI 18.5-29.9	0.99	(0.91	1.07)	0.763
Diabetes	1.75	(1.51	2.05)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.8 (the analysis was restricted to females). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	95%CI		P
Unit increase in GHQ-12 (3 categories)	1.27	(1.20	1.35)	0.031
Unit increase in SES going from high to low (3 categories)	1.21	(1.15	1.30)	0.030
Interaction SESxGHQ-12 (3x3)	1.01	(0.99	1.03)	0.077
One-year increase in age	1.13	(1.12	1.14)	0.000
Current smoking	1.58	(1.27	1.97)	0.000
Unit increase in BMI	1.01	(0.99	1.03)	0.428
Diabetes	3.82	(2.70	5.40)	0.000

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.7 (the analysis was restricted to males). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.35	(1.18	1.54)	<0.001
Unit increase in SES going from high to low (3 categories)	1.28	(1.15	1.43)	<0.001
Interaction SESxGHQ-12 (3x3)	1.03	(1.01	1.05)	0.038
One-year increase in age	1.11	(1.11	1.11)	<0.001
Current smoking	1.49	(1.37	1.62)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.66	(1.95	3.62)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.08	(0.99	1.17)	0.090
Diabetes	1.47	(1.29	1.67)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.8 (the analysis was restricted to males). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	95%CI		P
Unit increase in GHQ-12 (3 categories)	1.10	(1.05	1.15)	0.006
Unit increase in SES going from high to low (3 categories)	1.07	(1.02	1.14)	0.006
Interaction SESxGHQ-12 (3x3)	1.08	(1.02	1.14)	0.037
One-year increase in age	1.11	(1.10	1.12)	0.000
Current smoking	1.48	(1.19	1.84)	0.000
Unit increase in BMI	1.04	(1.02	1.06)	0.000
Diabetes	1.92	(1.41	2.62)	0.000

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.7 (the analysis was restricted to the participants who have not experience the outcome within one year from recruitment). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality			
	HR	(95%CI)		P
Unit increase in GHQ-12 (3 categories)	1.28	(1.16	1.42)	<0.001
Unit increase in SES going from high to low (3 categories)	1.26	(1.16	1.36)	<0.001
Interaction SESxGHQ-12 (3x3)	1.04	(1.02	1.06)	0.010
Gender male	1.50	(1.42	1.58)	<0.001
One-year increase in age	1.11	(1.11	1.11)	<0.001
Current smoking	1.47	(1.39	1.55)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.52	(2.12	2.98)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.05	(0.98	1.11)	0.151
Diabetes	1.60	(1.44	1.77)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.8 (the analysis was restricted to the participants who have not experience the outcome within one year from recruitment). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality		
	HR	95%CI	P
Unit increase in GHQ-12 (3 categories)	1.22	(1.12 1.32)	0.017
Unit increase in SES going from high to low (3 categories)	1.17	(1.08 1.13)	0.016
Interaction SESxGHQ-12 (3x3)	1.02	(1.00 1.04)	0.048
Gender male	2.13	(1.83 2.48)	<0.001
One-year increase in age	1.12	(1.11 1.12)	<0.001
Current smoking	1.50	(1.28 1.75)	<0.001
Unit increase in BMI	1.03	(1.01 1.05)	<0.001
Diabetes	2.52	(1.99 3.19)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Modified Table 6.7 (the analysis was rerun after recoding GHQ-12 missing value to valid values scoring 4). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for all-cause mortality.

Factor at the beginning of the follow-up	Mutually-adjusted all-cause mortality		
	HR	(95%CI)	P
Unit increase in GHQ-12 (3 categories)	1.39	(1.27 1.51)	<0.001
Unit increase in SES going from high to low (3 categories)	1.20	(1.15 1.25)	<0.001
Interaction SESxGHQ-12 (3x3)	1.07	(1.02 1.11)	0.019
Gender male	1.46	(1.39 1.53)	<0.001
One-year increase in age	1.12	(1.12 1.12)	<0.001
Current smoking	1.41	(1.34 1.48)	<0.001
BMI <18.5 versus BMI 18.5-29.9	2.38	(2.03 2.79)	<0.001
BMI 30+ versus BMI 18.5-29.9	1.09	(1.03 1.16)	0.006
Diabetes	1.65	(1.51 1.80)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

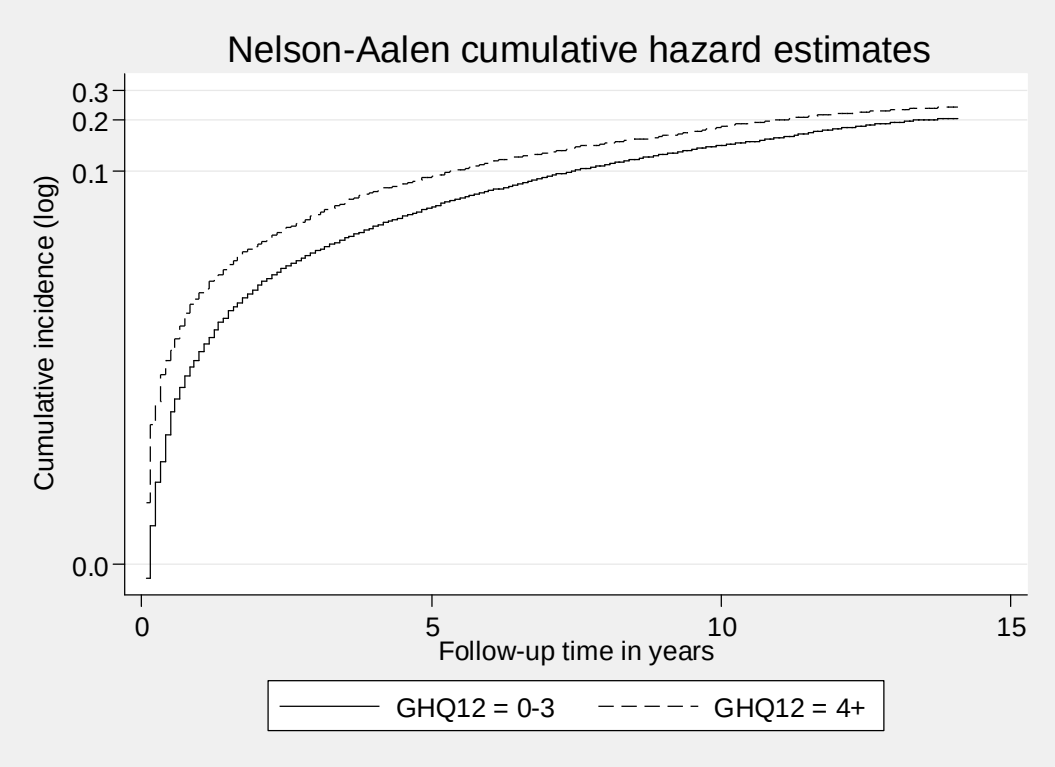
Modified Table 6.8 (the analysis was rerun after recoding GHQ-12 missing value to valid values scoring 4). Multivariate Cox regression model showing hazard ratios, 95% confidence intervals, and P values for IHD mortality.

Factor at the beginning of the follow-up	Mutually-adjusted IHD mortality			
	HR	95%CI		P
Unit increase in GHQ-12 (3 categories)	1.19	(1.16	1.30)	<0.001
Unit increase in SES going from high to low (3 categories)	1.16	(1.14	1.18)	<0.001
Interaction SESxGHQ-12 (3x3)	1.04	(1.02	1.06)	0.034
Gender male	2.13	(1.84	2.47)	<0.001
One-year increase in age	1.10	(1.09	1.11)	<0.001
Current smoking	1.50	(1.28	1.75)	<0.001
Unit increase in BMI	1.03	(1.02	1.05)	0.003
Diabetes	2.53	(2.01	3.18)	<0.001

The interaction between SES and GHQ-12 was calculated manually.

Appendix 4 - Example of Nelson-Aalen plot

This figure shows an example of a Nelson-Aalen plot, created to give a visual impression of the proportionality of a hazard function during the follow-up time, i.e. to see if the effect of a variable on an outcome is stable throughout the follow-up time. A bias is introduced if the effect of a risk factor on an outcome changes after a certain time. In this example, mental stress (GHQ-12) is the risk factor and all-cause mortality is the outcome. The X-axis shows the follow-up time in years and the Y-axis the cumulative hazard on a logarithmic scale. The cumulative hazard ratio is represented by the distance between the two lines. The dotted line (high mental stress) is always above (higher hazard) than the solid line (low mental stress), and the two lines run roughly in parallel, thus the proportionality assumption is satisfied.



Appendix 5 - Sensitivity analysis for Study 3.

This appendix presents the results from a sensitivity analysis carried out for Study 3. The multivariate Cox regression model for the association between hopelessness/helplessness index (used as a marker of mental stress), the index of multiple deprivation (used as a marker of socioeconomic status), and a multiplication of those two factors (interaction) for the determination of hospitalisation for acute ischemic heart disease event was restricted to participants who experienced the outcome at least after six month from recruitment.

Modified Table 7.5 (the analysis was restricted to participants who experienced the outcome at least after six months from recruitment). Multivariate Cox regression model showing adjusted hazard ratios, 95% confidence intervals, and P values for IHD event incidence.

Variable at the beginning of the follow-up	Mutually-adjusted HR for IHD event	95%CI		P
HH Index (one point increase)	1.12	(1.01	1.24)	0.030
IMD Index (one decile increase)	1.07	(1.01	1.14)	0.029
Continuous interaction between IMD and HH	1.03	(1.01	1.04)	0.039
Age (one year increase)	1.05	(1.04	1.07)	<0.001
Current Smoker	1.28	(1.07	1.53)	0.007
High Blood Pressure	1.45	(1.19	1.76)	<0.001
Osteoarthritis	1.39	(1.13	1.72)	0.002
Osteoporosis	1.36	(1.00	1.85)	0.052
BMI (one unit increase)	1.03	(1.01	1.05)	0.002
High blood cholesterol	1.31	(1.07	1.61)	0.009

The interaction between HH and IMD was calculated manually.

Appendix 6 – Study 3 - Histogram of the Hopelessness/Helplessness Index.

Histogram of the Hopelessness/Helplessness Index for 80,197 participants drawn from the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) study, recruited from July 2005 to December 2012.