Comparing Memory and Executive Function Performance in Coronary Artery Disease Patients Dichotomized into Low and High Cortisol Groups over 1 year of Cardiac Rehabilitation

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A thesis submitted in conformity with the requirements for the degree of Master of Science (MSc.)

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Abstract

Cognitive impairment in coronary artery disease (CAD) patients can predict poorer quality of life, dementia, and increased mortality. This study aimed to determine the association between long-term cortisol elevations and cognitive function in CAD patients. Participants were recruited at the beginning of a 1 year cardiac rehabilitation program and followed forward. Composite Z-scores were computed from tests measuring memory and executive function at baseline and 1 year. Cortisol deposition (3 months) was measured from a 20 mg, 3 cm hair sample. Analyses of covariance showed less improvement in memory function ($F_{1,50}=4.721, p=0.035$) but not executive function ($F_{1,40}=0.318, p=0.575$) in patients dichotomized into a high cortisol group based on a previously established reference range. Prolonged cortisol elevation may be associated with cognitive changes in subjects with CAD.
Acknowledgements

The past 2 years that I have spent at Sunnybrook have been a great learning experience. I have grown both professionally and personally due to the guidance and mentorship I have received under the supervision of Drs. Krista Lanctôt and Nathan Herrmann. I have developed important skills such as critical thinking, teamwork and problem solving, which will be useful in all aspects of my life. This is an important milestone that has been both challenging and rewarding to achieve and it would not have been possible without everyone that has been a part of this experience.

To my supervisor, Dr. Krista Lanctôt: First and foremost, I would like to sincerely thank you for taking a risk on me and giving me a last minute position in your lab. You have always encouraged me to think critically and find “the hole in the literature that can be filled,” which has always led me to think about what is important and how I can make meaningful contributions to my field of research. In addition, you have created an environment that promotes intellectual freedom, which has allowed me to explore new ideas and create opportunities for the future. You have also been really helpful and resourceful and have always provided me with the best tools to conduct my research.

To my advisor, Dr. Herrmann: Thank you for always making the time to give me your input and advice whenever I have needed it. Your discipline and dedication to your profession is truly inspiring and makes you a great example to look up to. Your hard questions have always pushed me to probe further and strive to be well-versed in my research area. They have also taught me to apply my knowledge to the real world where it is really relevant.

To Dr. Oh, Susan Marzolini and the TRI staff: Thank you for taking time out of your busy schedules to help with the patient recruitment.

To Dr. Stan van Uum and Rachel Gow: Thank you for carrying out the hair cortisol analysis.

To Abby Li: You have always been a great friend before a colleague. Thank you for always being there to help with everything and anything.

To Walter Swardfager: Thank you for being a great mentor and really taking me under your wing and teaching me what you know. You have always been a great support and have always motivated me to be the best that I can be. Your breadth of knowledge and skill is impressive and I look forward to hearing more about you in the future.

To the rest of the Neuropsychopharmacology team: Thank you for all your support when I have needed it. Thank you for all the fun times: the birthday lunches and the basketball games. These are memories that I will always remember.

To my family: I have no words to express my gratitude for your undying support and encouragement. I would like to especially thank my mother for always being encouraging and supportive. You have always taught me to believe in myself and work hard for what I want to achieve.
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<th>Full Form</th>
<th>Description</th>
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<tbody>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
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<tr>
<td>MCI</td>
<td>mild cognitive impairment</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>HPC</td>
<td>hippocampus</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
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<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>CVRF</td>
<td>cardiovascular risk factor</td>
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<tr>
<td>HF</td>
<td>heart failure</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<tr>
<td>GC</td>
<td>glucocorticoid</td>
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<tr>
<td>PVN</td>
<td>paraventricular nucleus</td>
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<tr>
<td>CRH</td>
<td>corticotrophin-releasing hormone</td>
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<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
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<tr>
<td>CBG</td>
<td>corticosteroid binding globulin</td>
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<tr>
<td>MR</td>
<td>mineralocorticoid receptor</td>
<td></td>
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<tr>
<td>TRI</td>
<td>Toronto Rehabilitation Institute</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>cardiac rehabilitation</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CVLT-II</td>
<td>California Verbal Learning test, 2nd ed.</td>
<td></td>
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<tr>
<td>SCID</td>
<td>Structured clinical interview for depression</td>
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<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>ANCOVA</td>
<td>analysis of covariance</td>
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<tr>
<td>ACS</td>
<td>acute coronary syndrome</td>
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<tr>
<td>ISHD</td>
<td>ischemic heart disease</td>
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<tr>
<td>PCI</td>
<td>percutaneous coronary intervention</td>
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<tr>
<td>SDFR</td>
<td>short-delay free recall</td>
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<tr>
<td>LDFR</td>
<td>long-delay free recall</td>
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<tr>
<td>bpm</td>
<td>beats per minute</td>
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<tr>
<td>CES-D</td>
<td>Center for Epidemiological Studies depression scale</td>
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<tr>
<td>PSS</td>
<td>Perceived stress scale</td>
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<tr>
<td>SRRS</td>
<td>Social readjustment rating scale</td>
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<tr>
<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor</td>
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1 Introduction

1.1 Statement of Problem

Cognitive performance is particularly critical in aging populations where deficits are predictive of functional decline (Boyle et al. 2004, Jefferson et al. 2006), progression to dementia (Griffith et al. 2006, Marcos et al. 2006, Tabert et al. 2006) and increased mortality (Gale et al. 1996, Tilvis et al. 2004). Patients with coronary artery disease (CAD) are at a higher risk of developing cognitive impairment as various indicators of CAD such as arterial thickening, coronary artery calcification and the presence of atherosclerotic plaques, and related cardiovascular risk factors (CVRFs) such as hypertension, diabetes, and dyslipidemia, have been associated with increased risks of Mild Cognitive Impairment (MCI) and progression to Alzheimer’s Disease (AD) (Agatisa et al. 2005, Breteler et al. 1994, Hofman et al. 1997, Panza et al. 2006, Roberts et al. 2008, Rosano et al. 2005, Tiemeier et al. 2004, Vinkers et al. 2005). For CAD patients, cognitive impairment represents significant detriments to long-term quality of life (Kiessling and Henriksson 2005) as exemplified by their association with poor outcomes including unemployment and mortality (Gale et al. 1996, Kiessling and Henriksson 2005). In addition, in a large observational study, memory performance was a significant mediator of the association between blood pressure elevations and physical disability (Elias et al. 2010).

Cognitive impairment in CAD is represented by deficits in both memory and executive function. Memory is largely mediated by the hippocampus (HPC) (Kohler et al. 1998, Muller et al. 2005, Mungas et al. 2001, Wilson et al. 1996) while executive function is thought to be mediated by the prefrontal cortex (PFC) (Hommet et al. 2010). Therefore, impairments in memory and executive function reflect predominantly hippocampal and cortical function (Bremner et al. 2000, Magarinos et al. 1996, Starkman et al. 1992). Morphological changes and atrophy shown to occur in both the right and left HPC is thought to precede MCI, which precedes
dementia (Henneman et al. 2009). Hippocampal atrophy is also a risk factor for dementia (Dubois et al. 2007).

In summary, the mechanisms underlying changes in brain regions mediating memory and executive function and subsequent cognitive impairment in CAD are unknown. This information can lead to the development of novel disease-modifying treatments that might slow the progression of cognitive decline, prevent and reduce disability and improve the overall quality of life of subjects with CAD.

1.2 Purpose of Study and Objective

A large body of evidence shows an association between higher cortisol concentrations and hippocampal atrophy (De Kloet et al. 1991, Sapolsky et al. 1987, Sapolsky 1996) and subsequent memory impairment (Pruessner et al. 2005, Pruessner et al. 2007, Starkman et al. 1992, Wolf et al. 2002). Since higher cortisol concentrations have been associated with CAD (Alevizaki et al. 2007, Krantz et al. 1996, Mittleman et al. 1995, Mittleman et al. 1993, Rosmond and Bjorntorp 2000) and also associated with cognitive impairment in healthy elderly individuals (Jameison and Dinan 2001, Lee et al. 2007, Lupien et al. 1994, Newcomer et al. 1999), it is possible that cortisol might mediate the relationship between cognitive deficits and CAD. In keeping with this, cognitive impairment in CAD is both persistent and related to changes in brain regions thought to be susceptible to the damaging effects of cortisol. Previous research with cortisol has been hampered by the lack of a validated method to determine long-term cortisol exposure. This study used a relatively new method of measuring cortisol in the hair to obtain long-term measurements of cortisol secretion to represent the effects of chronic exposure of cortisol.
The overall objective of this study was to determine the association between a dysregulated hypothalamic-pituitary-adrenal (HPA) axis and cognitive function in subjects with CAD. Specifically, the primary objective of this study was to determine the differences in memory performance between patients with low and high cortisol. Also, since morphological changes with cortisol have been shown to occur in the PFC (McEwen 2000) largely known to mediate executive function (Li et al. 2006, Lupien et al. 1994), a secondary objective of the study was to determine the differences in executive function in patients with low and high cortisol.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

1.3.1 Primary Hypothesis

| CAD patients divided into the high cortisol group (hair cortisol concentrations ≥ 153.2 ng/g) |
| based on a previously established cortisol reference range will have worse memory performance |
| compared to those in the low cortisol group, represented by lower composite verbal memory |
| change Z-scores over 1 year of cardiac rehabilitation. |

Previously, studies have shown that reductions in hippocampal volume in healthy elderly individuals are associated with worse memory performance (Pruessner et al. 2005, Pruessner et al. 2007, Starkman et al. 1992, Wolf et al. 2002). Recently, reductions in hippocampal volume in CAD patients were also associated with memory deficits (Koscheck, Irle 2005). Various studies have also indicated that CAD patients show altered cortisol secretion, which results in higher cortisol concentrations compared to healthy controls (Alevizaki et al. 2007, Krantz et al. 1996, Mittleman et al. 1995, Mittleman et al. 1993, Rosmond and Bjorntorp 2000). Due to the high density of glucocorticoid receptors (GR) in the hippocampus, cortisol is hypothesized to contribute to hippocampal atrophy and may be responsible for the memory impairment seen in
CAD patients. Therefore, studying the differences in memory performance over 1 year between CAD patients with low cortisol compared to those with high cortisol was a primary goal of this study.

### 1.3.2 Secondary Hypothesis

| CAD patients divided into the high cortisol group (hair cortisol concentrations $\geq 153.2$ ng/g) based on a previously established cortisol reference range will have worse executive function performance compared to those in the low cortisol group, represented by lower composite executive function change $Z$-scores over 1 year of cardiac rehabilitation. |

Studies have also shown structural and volumetric changes in the PFC in CAD patients suggesting that deficits in executive function can also occur (McEwen 2000). Various studies including those carried out by our group have indicated deficits in performance on tests of speed of processing and executive function in CAD patients (Selnes et al. 2007, Selnes et al. 2009, Swardfager et al. 2010). Since the PFC also has a high density of GRs, it is suggested that chronic exposure to cortisol could also result in degeneration in this brain region resulting in deficits in executive function. As a result, a secondary goal of this study was to determine the differences in executive function between CAD patients with low and high cortisol.

### 1.4 Review of Literature

#### 1.4.1 CAD and Cognition

CAD affects approximately 19.8% of Canadians over the age of 65 (Lindsay 1999, Turcotte 2007) and is responsible for 30% of all deaths in Canada (Statistics Canada 2010). CAD can also significantly affect quality of life with 60% of those with CAD reporting a reduction in
physical activity and 30% reporting disability and unemployment due to illness or disability (Heart and Stroke Foundation of Canada 2000).

CAD is characterized by the narrowing of arteries that supply blood to the heart known as atherosclerosis. Atherosclerosis is characterized by the formation of multiple plaques, which consist of fatty material, calcium deposits and scar tissue in the arterial wall (Nissen et al. 2006, Wexler et al. 1996). The formation of multiple plaques within the arteries can result in the stenosis or the narrowing of the arterial wall or complete blockage of the artery. Blockage of coronary arteries can result in insufficient blood flow and the restriction of oxygen supply to the heart and can require either revascularization (coronary artery bypass graft (CABG) surgery) or result in ischemic damage and myocardial infarction (MI). In keeping with this, CAD was defined as a history of MI, coronary angiographic evidence of >50% blockage in >1 major coronary artery or prior vascularization (Frasure-Smith et al. 2006) in this study.

A largely under-recognized symptom of CAD is cognitive impairment. Various studies have shown cognitive impairment to be associated with cardiac arrest (Reich et al. 1983), arteriosclerosis (Molsa et al. 1982), MI (Legault et al. 1992), and angina pectoris (Singh-Manoux et al. 2003) in the elderly. Cognitive impairment may also be a complication of cardiac surgery, especially in elderly patients (Hammon et al. 1997, Newman et al. 1994, Selnes et al. 1999, Tuman et al. 1992). Cognitive impairment after CABG surgery has been shown to persist up to 5 years after surgery with an incidence of 42% (Newman et al. 2001). A 4-fold increase in cognitive impairment has also been shown to occur in patients with heart failure (HF) (Cacciatore et al. 1998, Zuccala et al. 1997, Zuccala et al. 2001), especially in memory and learning (Sauve et al. 2009).

Cardiovascular risk factors such as obesity and hypertension are also important as they contribute to the clinical presentation of CAD (Bernick et al. 2005, Chrousos 1995, Elias et al.
Cumulative effects of CVRFs have been observed prospectively on learning and memory (Elias et al. 2003, Gianaros et al. 2006, Gunstad et al. 2006). Hypertension has been suggested to precede impairment in multiple cognitive domains including memory (Gold et al. 2005, Vicario et al. 2005). Clinical markers of abnormal lipid metabolism, including low high-density lipoprotein (HDL), cholesterol levels (Wolf et al. 2004), hypercholesterolemia (Hajjar et al. 2002, Muldoon et al. 2004), and hypertriglyceridemia (Kilander et al. 1997) have been particularly strongly associated with cognitive impairment (Bernick et al. 2005, Hajjar et al. 2002, Morales et al. 2006, Muldoon et al. 2004, Sparks et al. 2006). Diabetes has also been shown to be associated with cognitive impairment in CAD patients (Yaffe et al. 2004). Thus, **CAD and related CVRFs are associated with poorer cognitive performance.**

It has been suggested that cognitive impairment in CAD is predominantly in the learning and executive function domains (Almeida et al. 2008). Epidemiological studies document associations between CAD and CVRFs with poorer memory performance (Hogue et al. 2006, Pugh et al. 2003, Saxton et al. 2000, Stewart et al. 2001) and an increased rate of memory decline (Vinkers et al. 2005). Numerous studies document poorer memory performance at all stages of CAD progression; prior to acute cardiovascular events (Saxton et al. 2000), pre-operatively post-MI (Hogue et al. 2006), and following CABG surgery (Chernov et al. 2006, McKhann et al. 1997, Selnes et al. 2009). Several studies have also indicated that patients with CAD display deficits in executive function (Haley et al. 2007, Saxton et al. 2000, Vinkers et al. 2005). **These cognitive changes are important as they are associated with the development of dementia in later life, loss of independence and increased mortality (Wentzel et al. 2001).**
1.4.2 The Hypothalamic-Pituitary-Adrenal (HPA) axis

Stress can be defined as the repeated exposure to psychosocial hazards resulting in a bodily state that can be damaging to multiple physiological systems (Lee et al. 2007). In a normal stress response, the HPA axis is activated to trigger the release of glucocorticoids (GC), specifically cortisol to promote an adaptive response. Stress related information is usually processed in the limbic circuitry, which contains interconnected hippocampal, amygdaloid and cortical circuits where a novel stressful situation is closely linked to emotional responses and cognitive processes (Aggleton and Brown 2005, LeDoux 2007). At the presentation of a stressor, signals from the limbic circuitry are sent to neurons in the paraventricular nucleus (PVN) of the hypothalamus that produce and release corticotrophin-releasing hormone (CRH) (Antoni 1986, Whitnall 1993) (Figure I). CRH then acts on the anterior pituitary to cause the release of adrenocorticotropic hormone (ACTH), which then acts on the adrenal cortex to induce the synthesis and release of cortisol (Derijk and de Kloet 2008).

Cortisol also exercises negative feedback on the same HPA axis pathways that triggered the initial stress reaction in order to re-establish stability (Buckingham 2006, Lupien et al. 2007, McEwen 1998) by binding to specific sites in the CNS including the pituitary and the hypothalamus, HPC, amygdala and the PFC (Feldman and Weidenfeld 1995, Herman and Cullinan 1997, Herman et al. 2005). Both the HPC and the PFC (Price et al. 1996, Quirk and Beer 2006, Schneider and Koch 2005) inhibit the HPA axis through a network of interneurons that connect the PVN to the hypothalamus (Herman et al. 2003, Smith and Vale 2006).

During the feedback process, cortisol contributes to neuronal plasticity and remodeling of the nerve cells (McEwen 2007). Therefore, rapid secretion of GCs induced by stressful stimuli is a sign of health and resilience as it promotes behavioural adaptation. **If hormone response is inadequate, excessive or prolonged, it can lead to deleterious effects on cognition and**
emotional balance (de Kloet et al. 2005, Joels et al. 2007, McEwen 2007), which have been known to predict functional decline and mortality

Figure I: The HPA axis. The HPA axis is activated in response to a stress stimulus. Signals from the limbic circuitry are sent to neurons in the PVN of the hypothalamus that produce and release CRH. CRH then acts on the anterior pituitary to cause the release of ACTH, which then acts on the adrenal cortex to release cortisol. Cortisol also exerts negative feedback on the HPA axis at various levels to inhibit its own release.

1.4.3 Cortisol

1.4.3.1 Synthesis

Cortisol is produced by the adrenal gland in the zona fasciculate of the adrenal cortex (Schimmer and George 2007). As shown in Figure II, cortisol is synthesized from cholesterol, which translocates from the cytoplasm to the inner mitochondrial membrane for oxidative cleavage to pregnenolone by the action of desmolase. The translocation of cholesterol is the rate-limiting step in steroidogenesis. Pregnenolone is then converted to 17-hydroxypregnenolone by 17α-hydroxylase. 17-hydroxypregnenolone is then converted to 17-hydroxyprogesterone by the
action of 3β-hydroxysteroid dehydrogenase. 21α-hydroxylase then acts on 17-
hydroxyprogesterone to form 11-deoxycortisol, which is then converted to cortisol by the action
of 11β-hydroxylase (Price et al. 1996).

**Figure II: Synthesis of cortisol.** Cortisol is synthesized from cholesterol, which translocates
from the cytoplasm to the inner mitochondrial membrane for oxidative cleavage to pregnenolone
by desmolase. Pregnenolone is then converted to 17-hydroxypregnenolone by 17α-hydroxylase. 17-
hydroxypregnenolone is then converted to 17-hydroxyprogesterone by the action of 3β-
hydroxysteroid dehydrogenase. 21α-hydroxylase then acts on 17-hydroxyprogesterone to form
11-deoxycortisol, which is then converted to cortisol by the action of 11β-hydroxylase.

1.4.3.2 Metabolism

Cortisol is metabolized by the 11β-hydroxysteroid dehydrogenase system (11β-HSD),
which consists of 2 enzymes including 11β-HSD1 and 11β-HSD2 (Schimmer and George 2007).
As shown in Figure III, 11β-HSD1 uses the cofactor nicotinamide adenine dinucleotide
phosphate to convert inactive cortisone to cortisol while 11β-HSD2 uses nicotinamide adenine dinucleotide to convert cortisol to cortisone (Schimmer and George 2007).

![Figure III: The 11β-hydroxysteroid dehydrogenase system.](image)

**Figure III: The 11β-hydroxysteroid dehydrogenase system.** The inactive cortisone is converted into cortisol by 11β-HSD1 while cortisol is converted to the inactive molecule cortisone by 11β-HSD2. [Figure modified from (Schimmer BP, George SR. Adrenocortical Steroid Hormones. In: Kalant H, et al., eds.. Toronto: Saunders Canada; 2007:647-657)]

### 1.4.3.3 Mechanism of Action of Cortisol

Approximately 80% of cortisol is bound to corticosteroid binding globulin (CBG) in the blood, which binds to cortisol with high affinity and biologically inactivates it. Thus, CBG can be considered the main source from which cortisol is made available (Klieber et al. 2007). Albumin also binds 20% of cortisol with low affinity (Enthoven et al. 2008, Schmidt et al. 2005) leaving approximately 10% of cortisol free and biologically active (Rhen and Cidlowski 2005).

Free cortisol acts on two types of corticosteroid receptors, which include the mineralocorticoid receptors (MR) and GRs in the cytoplasm of the target cells (Figure IV). In the absence of corticosteroids, receptors are complexed with regulatory proteins such as heat-shock proteins and immunophilin causing the receptors to be restricted to the cytoplasm in an inactive state (Schimmer and George 2007). At the presentation of corticosteroids, the receptors
dissociate from the regulatory proteins and bind to the corticosteroids to form a hormone-receptor complex (Schimmer and George 2007). The hormone-receptor complex then translocates into the nucleus where it binds to GC response elements leading to transcriptional changes (De Bosscher et al. 2003, Meijer 2006, Pascual-Le Tallec and Lombes 2005) that affect multiple physiological systems in order to meet the energy demands of the body in a stress response (Sapolsky 1996, Sapolsky 1999, Sapolsky et al. 2000).

Cortisol action occurs in three phases. In the basal state, the HPA axis is unstimulated. With the presentation of a stressor, in the stress reactivity phase, the HPA axis activity is heightened and cortisol concentrations increase from the basal state. In the stress recovery phase, which occurs in the aftermath of the stress response, both the HPA axis activity and cortisol concentrations return to the basal state (McEwen 1998).

Figure IV: Cortisol mechanism of action. Cortisol binds to either the MRs or the GRs in the cytoplasm of the target cells. The hormone-receptor complex then translocates into the nucleus where it binds to specific DNA response elements leading to transcriptional changes that affect various physiological systems. [Figure modified from (Purves WK, Orians GH, Heller HC, Sadava D. Life the Science of Biology: The Cell and Heredity. 5th ed. Gordonsville, VA: W.H. Freeman & Company; 1998)]
1.4.3.4 Secretion

Cortisol secretion shows a diurnal rhythm characterized by a peak in cortisol secretion after awakening, steady decline throughout the day, and a trough in secretion at midnight (Curtis 1972, Edwards et al. 2001, Spath-Schwalbe et al. 1993, Weitzman et al. 1971, Wust et al. 2000). It has been shown that chronic stress can alter this rhythm leading to various deleterious effects (Adam and Gunnar 2001, Lightman 1994). Studies of the cortisol diurnal rhythm have shown elevated levels of cortisol in the nadir phase (Dodt et al. 1994, Van Cauter et al. 2000) resulting in a flattening of the rhythm with aging (Magri et al. 1997). Both hyposcretion of morning cortisol leading to a flattened diurnal rhythm and hypersecretion of morning cortisol leading to increased diurnal variability have also been reported in older individuals (Gerritsen et al. 2009). Mean levels of cortisol have also been shown to increase with age (Gotthardt et al. 1995).

While there is a large variation in stress responses within healthy individuals, a subset of depressed individuals differ in their cortisol response to stress compared to healthy individuals. Depressed individuals show an increase in HPA axis activity leading to increased cortisol concentrations (Branchey et al. 1982, Deuschle et al. 1997, Halbreich et al. 1985, Linkowski et al. 1987, Linkowski et al. 1985, Mortola et al. 1987, Pfohl et al. 1985, Sachar et al. 1973). Depressed individuals also tend to have higher cortisol secretion in the afternoon and in the evening suggesting that a higher number of these individuals would have a flat diurnal rhythm compared to healthy individuals (Dori et al. 1994, Sachar et al. 1973, Sachar et al. 1976, Weiner et al. 1997, Young et al. 1994). The flatter diurnal rhythm could be due to various reasons including the underlying disease or other factors related to depression such as medication. As a result, depression is a possible confounder in any study reporting cognition.
1.4.4 Cortisol Receptors

1.4.4.1 Mineralocorticoid receptors

The MRs belong to the steroid hormone receptor family and in the brain, are exclusively located in the limbic system, with a preferential distribution in the HPC, parahippocampal gyrus, and the entorhinal and insular cortices (Funder 2005). In the periphery, MRs are located in sweat glands, distal colon, kidney, and salivary glands where they largely regulate salt homeostasis as a result of the effects of aldosterone, the main corticosteroid acting on the MRs (Funder 2005). MR activation also leads to the regulation of mineral concentrations such as sodium and potassium in the extracellular fluid, which in turn contributes to the regulation of blood pressure (Schimmer and George 2007).

Both aldosterone and cortisol bind to MRs with similar affinity although the MRs are selectively activated by aldosterone to exert specific effects in certain tissues. This specific activation of MRs by aldosterone is due to the presence of 11β-HSD2, which inactivates cortisol by converting it to cortisone in aldosterone responsive cells. As a result, aldosterone can bind to MRs in these cells without competition from cortisol (Schimmer and George 2007).

MRs are activated at lower basal cortisol concentrations during non-stressed periods, that is during the afternoon, evening and nocturnal phases of the cortisol diurnal rhythm (Reul and de Kloet 1985). The MRs also have a 10 fold higher affinity for cortisol compared to GRs and determine the threshold or sensitivity of the stress system and the onset of the stress response (de Kloet et al. 2005).

1.4.4.2 Glucocorticoid receptors

The GRs, which also belong to the steroid hormone receptor family, are more widely distributed in the brain and are present in subcortical structures including the PVN and other hypothalamic nuclei, amygdale, HPC and parahippocampal gyrus as well as cortical structures,
with a preferential distribution in the PFC (De Kloet et al. 1998, McEwen et al. 1986, Reul et al. 1991, Sarrieau et al. 1988, Spencer et al. 1993). The GRs are also widely distributed in the periphery (Schimmer and George 2007). The GRs are low affinity receptors that are activated in periods of stress indicated by elevated concentrations of cortisol and during the morning phase of the diurnal rhythm (Reul and de Kloet 1985). GRs also mediate the negative feedback mechanism of the HPA axis and are involved in the termination of the stress response (de Kloet et al. 2005, Kim and Diamond 2002).

1.4.5 Physiological and Metabolic Effects of Cortisol

As mentioned before, GRs are found in almost every cell in the body and therefore, exert a wide range of physiological actions affecting every organ in the body as shown in Table 1. In response to stressful stimuli, cortisol effects lead to the breakdown of proteins into amino acids in the muscle and other tissues. These amino acids are then carried to the liver, deaminated and converted to glucose in a process known as gluconeogenesis. Cortisol also leads to an increase in the liver glycogen concentrations, fasting blood glucose levels and urinary nitrogen output. Furthermore, cortisol leads to an increase in the mobilization and oxidation of fat deposits. In terms of the immune system, cortisol hinders the intensity of an inflammatory response by reducing the production of vasoactive substances such as cytokines. Cortisol also exerts effects on the renal system to increase water excretion from the kidneys. In relation to the cardiovascular system, cortisol leads to increased cardiac contractility and an enhancement of vascular tone (Schimmer and George 2007).

Walker et al. 1998), which are known CVRFs (Filipovsky et al. 1996, Peeke and Chrousos 1995, Rosmond et al. 1998). Prolonged exposure to cortisol can result in the stimulation of fat storage enzyme activity that can lead to increased fat deposition and obesity (Bjorntorp et al. 1990). Exposure to elevated cortisol concentrations over time can also increase food intake by the stimulation of ghrelin, a gastric hormone, that can act centrally to increase food intake (Espelund et al. 2005) and leptin, an adipokine secreted by adipocytes, which signals to the central nervous system to control food intake in relation to fat stores in the body (Badman and Flier 2007, Dagogo-Jack et al. 1997, Dubuc and Wilden 1986, Laferrere et al. 2002, Laferrere et al. 2000, Porte et al. 2002). In addition, prolonged exposure to elevated cortisol concentrations can cause changes in peptides that are involved in food intake regulation such as insulin and neuropeptide Y (Kuo et al. 2007). Therefore, chronic exposure to cortisol may contribute to the development of CVRFs, which in turn, may be associated with an increased risk of developing CAD.
Table 1: Metabolic and physiological effects of cortisol in response to a stress stimulus.

<table>
<thead>
<tr>
<th>Metabolic Effects</th>
<th>Increase</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Metabolism</td>
<td>• gluconeogenesis</td>
<td>• glucose uptake in muscle and adipose tissue</td>
</tr>
<tr>
<td></td>
<td>• glycogen breakdown in liver</td>
<td></td>
</tr>
<tr>
<td>Protein Metabolism</td>
<td>• breakdown of protein in muscle and other tissues</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• free amino acids in serum</td>
<td></td>
</tr>
<tr>
<td>Lipid Metabolism</td>
<td>• fat breakdown in adipose tissue</td>
<td></td>
</tr>
</tbody>
</table>

| Physiological Effects    |                                                                         |                                                                          |
|--------------------------|                                                                         |                                                                          |
| Bone Formation           |                                                                          | • total body calcium                                                    |
|                          |                                                                          | • calcium uptake from intestine                                          |
| Renal Function           | • calcium excretion                                                     | • water excretion                                                       |
| Heart                    | • cardiac contractility                                                 | • vascular tone                                                         |
|                          | • sensitivity to catecholamines and Angiotensin II                       | • sensitivity to Angiotensin II                                          |
| Inflammation             |                                                                          | • production of vasoactive substances                                   |
|                          |                                                                          | • fibroblast growth                                                     |
1.4.6 Cortisol and Susceptible Brain Regions

The HPC and the PFC are susceptible to changes in neuronal metabolism, morphology and survival as a result of chronic exposure to GCs leading to cognitive deficits (McEwen 2000). Dysregulation of the HPA axis over a long period of time leads to an increase in the exposure of the HPC to higher concentrations of cortisol (Lupien et al. 1998, Starkman et al. 1992). The HPC plays an essential role in declarative memory function, the conscious recall of memories of facts or events (Squire 1992), and is also involved in the feedback regulation of cortisol (Jacobson Sapolsky 1991, Pruessner et al. 2008). Several studies have shown that exposure to GCs negatively impacts declarative memory mediated by the HPC (Sauro et al. 2003). In addition, neuroimaging studies indicate that reduced activation of the medial temporal lobe after exogenous cortisol administration is associated with worse retrieval performance (de Quervain et al. 2003, Oei et al. 2007). These studies suggest that cortisol affects memory retrieval in part by hindering the activation of the medial temporal lobe.

Furthermore, cortisol also modulates synaptic plasticity and inhibits glucose metabolism by hippocampal neurons resulting in changes in dendritic structures that persist for weeks (de Leon 1997, Sapolsky 1993). Neuroimaging studies have also indicated that prolonged exposure to cortisol can alter the morphology and the functional integrity of hippocampal neurons (Joels and de Kloet 1989, McEwen 2001). Since it is suggested that hippocampal function is associated with hippocampal morphology (Bremner et al. 2000, Magarinos et al. 1996, Starkman et al. 1992), changes in hippocampal structure due to exposure to cortisol over time may be responsible for memory impairment seen in aging individuals.

Experimental data suggest that higher cortisol concentrations also make hippocampal neurons more vulnerable to degeneration (Sapolsky et al. 1990). Hypersecretion of cortisol has been correlated to the amount of HPC atrophy (De Kloet et al. 1991, Sapolsky et al. 1987,
Sapolsky 1996) and subsequent HPC-dependent cognitive impairment (Pruessner et al. 2005, Pruessner et al. 2007, Starkman et al. 1992, Wolf et al. 2002). Increases in nighttime cortisol have been associated with reductions in both HPC and temporal lobe volumes (Magri et al. 2000).

Reduced HPC volumes have subsequently been associated with deficits in various cognitive domains including verbal learning (Stoub et al. 2005, Tischler et al. 2006), delayed verbal recall (Kohler et al. 1998, Muller et al. 2005, Mungas et al. 2001), visual recall (Hickie et al. 2005) and verbal fluency (Mungas et al. 2001). Observational studies in non-demented elderly showed an association between various cortisol metrics such as basal cortisol levels (Lupien et al. 2005) and cortisol slope (Li et al. 2006) and memory impairment (Lupien et al. 2005). Impairments in memory corresponding to a 14% reduction in hippocampal volume was also shown to be associated with increasing cortisol over 5-6 years and currently high cortisol in elderly patients (Lupien et al. 1998). These results suggest that a **reduction in hippocampal volume, which is strongly correlated with the degree of cortisol elevation over time and current basal cortisol levels** (Lupien et al. 2005), might be a mechanism through which cortisol may lead to memory impairment in non-demented aging patients.

The effects of cortisol on cognition are also seen in PFC dependent tasks due to the preferential distribution of GRs in that region of the brain. As mentioned before, the PFC has also been implicated the regulation of the HPA axis. Specifically, the medial PFC has been suggested to play a role in negative feedback inhibition (Sullivan et al. 1999). Changes in the negative feedback inhibition can result in chronic dysregulation of cortisol secretion, which can then subsequently lead to deleterious effects (Diorio et al. 1993, McEwen 1999, Sapolsky 2003).

Also, the PFC has been shown to have a role in declarative memory functions mediated by the hippocampus through the encoding and retrieval of episodic memories (Lee et al. 2000).
Neural correlates of vigilance and attention have also been localized to the right PFC suggesting that this brain region may play a key role in the brain response to stress (Sarter et al. 2001). Various cortisol measures such as cortisol slopes and basal cortisol concentrations have been associated with impairments in executive function over time in elderly cognitively normal subjects (Li et al. 2006, Lupien et al. 1994). These results suggest that the PFC might also be susceptible to cortisol-mediated changes and associated with impairment in executive function.

1.4.7 Cortisol and Aging


The main sites of cortisol feedback include the hypothalamus and the hippocampus (Kaneko and Hiroshige 1978). Loss of neurons in these brain regions insinuates a reduction in GRs, which in turn leads to the impaired regulation of the HPA axis (Ball 1977, Mani et al. 1986). With aging, there is also a significant loss of MRs (Armanini et al. 1992, Ritger 1984, Sapolsky et al. 1983, Sapolsky et al. 1985), which maintain the rhythm of the HPA axis (Deuschle et al. 1997) possibly leading to reduced sensitivity of the HPA axis to negative cortisol.
feedback (Issa et al. 1990, Jacobson and Sapolsky 1991, Reul et al. 1988, Ritger 1984, Sapolsky et al. 1983, Sapolsky et al. 1986). This reduction in MR receptors is suggested to contribute to increased cortisol concentrations with age, which is consistent with studies showing changes in cortisol diurnal rhythm (Deuschle et al. 1997, Van Cauter et al. 1996), reduced stress responsivity (Gotthardt et al. 1995), and heightened cortisol response in challenge studies (Heuser et al. 1994).

Changes in the circulating cortisol concentrations have been associated with impairments in a wide range of cognitive domains including attention, perception, and memory (Jameison and Dinan 2001, Lupien et al. 1994, Newcomer et al. 1999). A dose-response relationship indicates that exposure to higher levels of cortisol in persons aged 50-70 is associated with worse performance in different cognitive domains mediated by the HPC and PFC (Lee et al. 2007). The hippocampus has been shown to lose approximately half of the MR binding sites and some GR binding sites (Sapolsky 1993). Changes in GR regulation (Galeeva et al. 2006, Peiffer et al. 1991) and cortisol feedback inhibition (Boscaro et al. 1998, Wilkinson et al. 1997, Wilkinson et al. 2001) shown to be altered during aging, might also increase the vulnerability to stress-related metabolic, cardiovascular and brain diseases (Derijk and de Kloet 2008).

1.4.8 Cortisol and CAD

Mental and physical stresses indicated by elevated cortisol concentrations have been shown to be associated with CAD (Alevizaki et al. 2007, Krantz et al. 1996, Mittleman et al. 1995, Mittleman et al. 1993, Rosmond and Bjorntorp 2000) as well as CVRFs including hypertension (al'Absi and Arnett 2000, Whitworth et al. 2000), diabetes, cholesterol level (Breteler et al. 1994, Desmond et al. 1993, Gale et al. 1996, Launer et al. 1995, Starr et al. 1993,
van Swieten et al. 1991), and waist-hip ratio (Steptoe et al. 2004). Higher cortisol concentrations in diabetic patients have also been linked to the severity of CAD (Dacou-Voutetakis et al. 1998).

In addition, HPA axis dysregulation might be a risk factor for the development of atherosclerosis. Matthews et al. showed that a flatter diurnal rhythm and cortisol area under the curve was associated with a higher incidence of coronary calcification (Matthews, et al. 2006). Recent studies have associated HPA axis dysregulation with inflammatory mechanisms (Miller et al. 2002) that have been implicated in the development and progression of atherosclerosis (Yudkin et al. 2000). High cortisol levels were also associated with the number of atherosclerotic lesions in the coronary arteries (Troxler et al. 1977).

Due to the high density of GC receptors in regions of the brain mediating memory (HPC) and executive function (PFC), elevated concentrations of cortisol in CAD patients could be associated with cognitive dysfunction in these patients. However, other studies suggest that CVRFs themselves may be more closely associated with the predisposition and extent of cognitive impairment (Siuda et al. 2007). For example, hypertensive patients have significantly different cortisol feedback sensitivity, suggesting that HPA function in systemic hypertension is altered (Wirtz et al. 2007).

Low morning cortisol peak has also been associated with the presence and severity of other CVRFs (Rosmond and Bjorntorp 2000). Individuals with upper-body obesity have a greater cortisol response to a stimulus compared to those with lower body obesity (Marin et al. 1992). In another study, it was shown that impairment in cognitive performance in individuals with higher body fat was associated with increased cortisol reactivity in response to a stressor (Mujica-Parodi et al. 2009). Flattened cortisol diurnal rhythm has also been associated with higher waist circumferences (Abercrombie et al. 2004). These results suggest that dysregulation
of the HPA axis is present in patients with CAD and related CVRFs and may be a contributing factor to the cognitive impairment also present in these patients.

1.4.9 Cortisol, CAD and Cognition

Almeida et al. were the first to show loss of grey matter volume across various regions of the brain including the temporal lobe and the frontal cortex involved in working memory, attentional resource allocation, reaction time and episodic memory (Almeida et al. 2008, Ramnani and Owen 2004) in middle aged CAD patients. This suggests that these regions of the brain are particularly susceptible to atrophy, and cognitive decline in CAD patients.

Yin et al. suggested that atrophy and cognitive impairment may be due to toxic effects of irregular cortisol secretion in CAD patients. This group investigated the relationship between the circadian rhythm of perioperative cortisol secretion and cognitive function in 40 male patients undergoing CABG. Subjects were assessed preoperatively, 7-10 days and 3 months postoperatively. 75% of patients showed a disturbed circadian rhythm and deficits were also seen in short-term memory, visual perception and motion, executive function and response inhibitory capacity (Yin et al. 2007). While this study clearly suggests irregular cortisol secretion may be associated with a decline in executive function, the association between irregular cortisol secretion and HPC mediated memory are unclear due to a limited neurocognitive battery. Patients were also only followed 3 months post-operatively while it has been shown that cognitive decline in CAD patients can persist up to 5 years (Newman et al. 2001).

Beer et al. also showed that temporal lobe atrophy and deep white matter hyperintensities were associated with cognitive decline in HF. Cortisol concentrations in HF patients were also shown to be elevated compared to controls (Beer et al. 2009). While this study also suggests that elevated cortisol is associated with neuronal damage and cognitive decline, it is limited by single time point measurements of cortisol. A measure of long-term cortisol secretion may
have led to more robust correlations between cortisol secretion, brain atrophy and cognitive decline.

Therefore, emerging evidence suggests that irregular cortisol secretion in CAD patients is possibly associated with a reduction in temporal lobe and frontal cortex volumes and a decline in cognitive functions mediated by these brain regions (Figure V).

**Figure V: Possible mechanism of the development of cognitive impairment in CAD.** A dysregulated HPA axis associated with both aging and CAD can result in increased cortisol secretion. Cortisol can act on MRs and GRs present in high density in brain regions such as the HPC and PFC and may cause toxicity and subsequent atrophy. This atrophy may then be associated with deficits in HPC-mediated memory as well as executive function.
1.4.10  Cortisol measurements

In the past, cortisol has been measured in the saliva, blood and urine. Urine samples can yield cortisol concentrations in the past 24 hours while saliva and blood samples can show cortisol changes in the past few hours. Therefore, these measures demonstrate acute stress and subsequent short-term changes in cortisol concentrations (Sauve et al. 2007). In order to get a long-term measurement, multiple samples have to be taken, which raises the issues of accuracy and compliance (Kudielka et al. 2003). In addition, due to the diurnal variation in cortisol secretion, samples need to be taken at specific times of the day (saliva or serum) or require an extensive collection method (24 hour urine collection), making them unsuitable for population analysis. Recently, cortisol has been measured in the hair to yield cortisol secretion over months. Thus, measuring cortisol in the hair yields a long-term measurement of cortisol secretion and might be representative of the effects of chronic stress (Sauve et al. 2007).

1.4.10.1  Hair Cortisol

The hair shaft consists of the outer cuticle, cortex and inner medulla (Harkey 1993). During hair production, the follicle undergoes three stages. The stages include the active growing phase (anagen), the remodeling phase (catagen) and the quiescent phase (telogen) (Lavker et al. 2003). The hair follicle is present 3-4 mm below the skin and is associated with arterial capillaries, sebaceous glands and apocrine sweat glands (Cone 1996, Harkey 1993, Henderson 1993). These glands secrete sebum and sweat into the hair follicle (Cone 1996, Henderson 1993).

Several models of cortisol incorporation into the hair have been proposed. One model suggests that cortisol might move into the growing hair cells at the base of the hair follicle by passive diffusion from the bloodstream and then become tightly bound in the protein structure of the hair matrix most likely through both electrostatic forces and van der Waals forces (Larrson and Tjalve 1979, Uematsu et al. 1992). As a result, cortisol incorporation in the hair is dependent
on blood concentrations. This model is also the basis of segmental analysis used to determine the temporal course of cortisol secretion. Since it is assumed that hair grows at a constant rate, the position of cortisol along the hair shaft can be correlated with the time that cortisol was present in the bloodstream (Henderson 1993).

A more complex model of incorporation suggests that cortisol is possibly transferred to the hair from the body from various pools and during various times in the life cycle of the hair (Chittleborough and Steel 1980). This model suggests that cortisol might be incorporated into the hair from the blood during formation, from the sweat and sebum after hair formation and from the external environment after the hair has emerged from the skin (Henderson 1993). External contamination can occur through the use of cortisol containing creams or ointments that can remain on the hands and become bound to the outer hair shaft for example, when hands are run through the hair or when the head is scratched (Thomson et al. 2010). Sweat and sebum can also coat the outside of the hair shaft as they emerge from the scalp (Cone 1996, Henderson 1993). Cortisol can also be transferred into the hair from the tissues surrounding the hair follicle (Henderson 1993).

Recently, it was demonstrated that human hair follicles also contain a functional HPA-axis, which can synthesize cortisol locally and respond to regulatory feedback similar to the central HPA axis (Ito et al. 2005). Hence, this local cortisol production might also contribute to cortisol concentrations measured in the hair (Ito et al. 2005) although the relative contribution of locally produced cortisol may be minimal (Sauve et al. 2007).

So far, cortisol has been measured in the hair in various populations. Hair cortisol has been indicated as a potential biomarker of chronic stress in neonates (Klein et al. 2004, Yamada et al. 2007). Hair cortisol concentrations are also shown to be higher in patients with Cushing’s syndrome (Thomson et al. 2010), alcohol withdrawal (Stalder et al. 2010), and unemployed
individuals (Dettenborn et al. 2010). Elevated cortisol concentrations were also shown in pregnant women in their third trimester (Kalra et al. 2007) and in patients with chronic pain (Van Uum et al. 2008). In both of these populations, hair cortisol concentrations were also associated with perceived stress. On the other hand, Steudte et al. suggest that in the long term, generalized anxiety disorder may be associated with chronic hypocortisolism as measured by hair cortisol concentrations in contrast to previous heterogeneous findings of hypercortisolism or no change in cortisol concentrations measured in the saliva, plasma and urine in this population. The findings by Steudte et al. may more accurately represent HPA axis activity in generalized anxiety disorder as hair cortisol concentrations reflect cortisol secretion over a prolonged period of time (Steudte et al. 2010).

Hair cortisol concentrations have also been measured in patients with CAD. In a previous study by our group, no significant differences in hair cortisol concentrations were found between depressed and non-depressed CAD patients when controlling for age, marital status and history of depression (Dowlati et al. 2010). Recently, it was also shown that MI patients had significantly higher cortisol concentrations compared to controls, which suggested that chronic stress might be a contributing factor in the development of CAD (Pereg et al. 2011).
2 Methods

2.1 Study Design

This study was a longitudinal observational study assessing the association between cortisol measured in the hair and cognitive functioning in CAD patients that were followed over 1 year of cardiac rehabilitation. Consecutive patients meeting the criteria for CAD were invited to participate in this study. Neurocognitive assessments were carried out in all consenting patients at baseline and 1 year. Hair samples were also taken at baseline for cortisol analysis.

2.2 Participant Selection

Patients were recruited from the Toronto Rehabilitation Institute (TRI) Cardiac Clinic. The cardiac rehabilitation (CR) program is a one-year exercise rehab program consisting of both aerobic and resistance training in a group setting under the supervision of exercise and medical specialists. First, patients attend a group intake session in which, patients get oriented to the program consisting of a series of short lectures on the risk factors associated with CAD and the value of exercise. Patients then attend supervised exercise visits that include an aerobic walk or walk/jog once a week for 36 weeks and once a month for the remaining 3 months of the year. Patients are also expected to exercise 5 out of 7 days of the week in between the supervised visits. Depression and cardiopulmonary fitness assessments are carried out at entry, 6 months and 1-year time points. For the purpose of this study, patients were recruited and assessed at entry to the CR program and reassessed at the end of rehab 1 year later.

The research ethics boards of TRI and Sunnybrook Health Sciences Centre approved this study (Appendix A). All patients provided written, informed consent (Appendix B).
2.3 **Inclusion Criteria**

Patients were enrolled in the study if they met the following criteria:

- spoke and understood English
- evidence of CAD based on at least 1 of the following:
  - previous hospitalization for acute MI
  - coronary angiographic evidence of $\geq 50\%$ blockage in $\geq 1$ major coronary artery
  - prior revascularization procedure
- stable CAD based on no recent (last 4 weeks) hospitalization for cardiac events such as acute MI, unstable angina, congestive heart failure, ventricular arrhythmias or coronary revascularization

2.4 **Exclusion Criteria**

Patients were excluded from the study if they met any of the following:

- acute medical illnesses including:
  - drug overdose, uncontrolled hypothyroidism, Parkinson’s disease, Alzheimer’s disease, Huntington’s chorea, brain tumour, frontotemporal degeneration syndromes, history of epilepsy, birth trauma, subdural hematoma, traumatic brain injury, clinical stroke, progressive supranuclear paralysis, Killip Class III or IV states, multiple sclerosis, and severely disrupted liver/ kidney/ lung function
- illnesses affecting the HPA axis such as Cushing’s syndrome, Addison’s disease, and hypopituitarism
- use of drugs affecting:
  - the HPA axis: glucocorticoids and hormone replacement therapy
o cognition: hypnotics, antipsychotics, anticholinergic medication and recreational drugs

• premorbid psychiatric diagnosis of conditions with established cognitive impairment such as schizophrenia or bipolar disorder
• significant cognitive impairment (MMSE ≤ 24 (Cacciatore et al. 2004, Perry et al. 2000))
• Canadian Cardiovascular Society (CCS) Class 4 angina
• baldness

2.5 Demographics and Medical History

Demographics and clinical characteristics including age, gender, marital status, employment status, level of education, smoking status, personal and family psychiatric history, and detailed medical history including comorbidities independent of CAD were collected from patient interviews. Cardiac medical history, concomitant medications and cardiac health indicators including heart rate, blood pressure, body mass index (BMI), height, body mass, VO₂ peak (measure of cardiopulmonary fitness), lipids, and percentage body fat were collected from patient files at the TRI cardiac program.

2.6 Assessments

The neurocognitive battery based on the National Institute of Neurological Disorders and Stroke and Canadian Stroke Network harmonized standards for the investigation of vascular cognitive impairment, were used to assess memory and executive function of patients. Hippocampal-dependent memory was assessed by the California Verbal Learning Test (CVLT-II), which yields multiple measures of HPC function such as learning, immediate and delayed verbal recall. Trails B and Stroop tests (Lee et al. 2007, Li et al. 2006) were used to measure
executive function. The Structured Clinical Interview for Depression (SCID) from the Diagnostic and Statistical Manual of Mental Disorders version IV was used to diagnose depression.

2.6.1 CVLT-II

The CVLT-II word list includes 16 words that fall into 4 different categories. This list of words is read to the subject and then recalled orally by the subject. This procedure is carried out 5 times constituting the 5 learning trials, which are a measure of learning. A distractor list is then read to the subject and recalled orally by the subject after which, the subject is prompted to recall the original list again. This is defined as the short-delay free recall (SDFR) and is a measure of short-term memory. After 20 minutes, the subject is prompted to recall the original word list again, which is defined as long-delay free recall (LDFR) and is a measure of long-term memory. Z-scores for the performance over the 5 learning trials, SDFR and LDFR are provided in the final output after the test is scored electronically. A higher Z-score reflects better performance on the test. The CVLT-II was chosen to assess memory function in this study since it has been shown to be sensitive to cognitive impairment in multiple domains in CAD patients (Alexander et al. 2003, Dickson et al. 2007, Hachinski et al. 2006).

2.6.2 Stroop Test

This test requires the subject to read out 3 different cards. Card 1 requires subjects to name the colours of rows of dots that are presented in four basic colours including red, blue, green and yellow. Card 2 requires the subject to name the colour (same 4 basic colours as in Card 1) of various words written in rows. Card 3 requires the subject to read aloud the colour names printed in conflicting colours, providing a measure of interference. For cards 2 and 3, subjects are required to name the colours and not read the words. The least amount of time required to read Card 3 corresponds to a higher Z-score and better performance on the test.
2.6.3 **Trails B**

This test requires subjects to use straight lines to connect a series of numbers and letters in order (e.g. 1-A-2-B) as quickly as possible. The least amount of time required to connect the series of numbers and letters correctly corresponds to a higher Z-score and better performance on the test.

2.6.4 **SCID**

The SCID from the Diagnostic and Statistical Manual of Mental Disorders version IV was used to diagnose depression in the study participants at baseline. The SCID consists of 9 different questions focusing on the mood and behaviour of the subject in the last month prior to the interview. Criteria for diagnosing a major depressive episode includes one of either depressed mood or anhedonia for 2 weeks or more in the past month along with changes in appetite and/or weight, insomnia or hypersomnia, agitation or retardation, fatigue or loss of energy, feelings of worthlessness or guilt, diminished ability to think or indecisiveness, or current thoughts of own death. Subjects are classified as being majorly depressed if they meet at least 5 of the 9 criteria with one of the criteria being either depressed mood or anhedonia. Subjects are classified as minor depressed if they meet between 3 and 5 of the 9 criteria with one of the criteria being either depressed mood or anhedonia. Both major and minor depressed patients were included in this study.

2.7 **Cortisol measurement**

Cortisol concentrations were measured in the hair to determine the average cortisol secretion over the past 3 months. Since hair cortisol concentrations are a measure of long-term HPA-axis function, this method of measuring cortisol might be a more accurate representation of chronic stress (Sauve et al. 2007). Hair analysis is largely used to measure exposure to drugs of
abuse and environmental toxins (Villain et al. 2004). Incorporation of hormones in the hair is suggested to occur through the blood circulation during the formation of the hair shaft (Cone 1996). Since a balance exists between blood and hair concentrations, measurement of endogenously produced hormones in the hair can reflect average hormonal levels over months (Sauve et al. 2007). Sauve et al. showed that hair cortisol concentrations were significantly associated with 24-hour urine cortisol concentrations ($r=0.33$, $p=0.041$) but not with serum or salivary cortisol concentrations (Sauve et al. 2007). These results support the relevance of hair cortisol measurements as biomarkers of long-term exposure.

A mentioned before, hair has been indicated as a potential biomarker of chronic stress in many different population including neonates (Klein et al. 2004, Yamada et al. 2007), patients with chronic pain (Van Uum et al. 2008), Cushing’s syndrome (Thomson et al. 2010), alcohol withdrawal (Stalder et al. 2010), generalized anxiety disorder (Steudte et al. 2010), unemployed individuals (Dettenborn et al. 2010) and pregnant women in their third trimester (Kalra et al. 2007). Cortisol concentrations have also recently been measured in patients with CAD (Dowlati et al. 2010, Pereg et al. 2011). These studies suggest that elevated hair cortisol concentrations have been detected in different populations and can be reliably measured in the CAD population.

2.7.1 Hair Collection

Hair samples from patients were collected from the posterior vortex region of the scalp, which is located below the crown of the head at baseline (Figure VI). The posterior vortex region has higher hair density and a larger portion of hair in the same growth phase compared to other regions of the head (Harkey 1993, Mangin and Kintz 1993, Villain et al. 2004). The sampling site was in accordance with the consensus statement from the Society of Hair Testing (Society of Hair Testing 1997).
The hair sample consisted of 20 mg of hair, which is approximately 100-150 strands of hair cut as close to the scalp using clean sharp scissors. The roots were not removed from the head in order to ensure that only cortisol stored in the hair was measured. Based on the estimation that there is 1 cm of growth each month (Saitoh et al. 1969), 3 cm of hair was taken to correspond with a measurement of 3 months of cortisol with the first cm of hair corresponding to the most recent month of growth. The hair in the sample was then placed between clean notepad paper with the scalp end being stuck to the sticky end of the notepad in order to identify it clearly. The hair sample was kept in white, labeled and sealed envelopes to protect it from contamination and set aside at room temperature until the time of analysis.

Figure VI: The cutting of the hair sample from the posterior vortex region of the scalp.
2.7.2 Laboratory Preparation

After hair samples were collected from all the patients included in the study, the samples were sent to the lab of Dr. Stan Van Uum (University of Western Ontario) for analysis. Each sample was carefully separated from the notepad paper in a way that the hair strands stayed together and did not get mixed. Each hair sample was weighed and hair was positioned in custom made hair cutting equipment that is able to measure the length of the hair similar to a ruler. This facilitated the cutting of the hair sample into desired segments of 1, 2 and 3 centimeters using surgical scissors.

Once the hair was cut into target segments, it was placed in glass vials. 1 mL of methanol was added to each glass vial and sealed to prevent vapourization. The vials were then incubated overnight on a shaker at 100 RPM for 16 hours at 50°C. After the incubation period, methanol was removed from each sample and placed in a glass tube. The methanol was then evaporated in a dry bath under a stream of nitrogen gas at 50°C until the sample was very dry. Once the methanol was evaporated, 250 uL of phosphate buffered saline solution at pH 8.0 was added to the sample to increase the concentration four-fold and the sample was vortexed until well mixed.

The cortisol measurements were performed using salivary enzyme-linked immunosorbent assay (ELISA) cortisol kits (Alpco Diagnostics, NH, USA) as shown before (Van Uum et al. 2008). The samples were run on the ELISA assays according to instructions from the manufacturer. Both positive and negative controls were used to ensure accuracy and specificity of the measurements. The negative control, which contained buffer only, was used to determine any non-specific binding, which was subtracted from all other values before interpretation. The intra-assay coefficient of variation, which was 3.8% (n=5) and the inter-assay coefficient of variation, which was 8% (n=6), were obtained using a standard hair sample measured over several weeks. The limit of detection of the ELISA cortisol kit was 1.14ng/ml (Alpco
Diagnostics) (Pereg et al. 2011). Cross-reactivity of other steroids with the ELISA kit antibodies were reported as follows: corticosterone 31%, progesterone <2%, deoxycortisol <2%, dexamethasone <2%, estriol, estrone and testosterone <0.001% and cortisone 1%. Therefore, hair cortisol can be reliably tested without cross-reactivity to cortisone (Sauve et al. 2007).

2.8 Statistical Analysis

Samples above 1500ng/g were excluded due to possible contamination before any analyses were conducted. Based on previous experiments, it was expected that cortisol concentrations would be skewed. Therefore, patients were dichotomized into low and high cortisol groups for a more conservative analytical approach. Patients were divided into either the low or high hair cortisol groups based on a reference range of hair cortisol concentrations (17.7-153.2 ng/g) established in 39 healthy, non-obese (BMI<30 kg/m^2) individuals including 19 males and 20 females by our collaborators (Sauve et al. 2007). Patients with hair cortisol below 153.2 ng/g were classified as having low cortisol while patients with hair cortisol concentrations above or equal to 153.2 ng/g were classified as having high cortisol. None of the patients had cortisol concentrations as low as 17.7 ng/g so the lower limit of the range was not used to stratify the patients.

All data analyses were performed using IBM SPSS Statistics 18. Continuous variables were reported as mean ± standard deviation. Associations between demographic data and clinical characteristics were found using analyses of variance (ANOVA) for continuous data and chi-square tests for categorical data. All analyses were 2-tailed with a p-value of 0.05.
2.8.1 Primary Hypothesis

CAD patients divided into the high cortisol group (hair cortisol concentrations ≥ 153.2 ng/g) based on a previously established cortisol reference range will have worse memory performance compared to those in the low cortisol group, represented by lower composite verbal memory change Z-scores over 1 year of cardiac rehabilitation.

Differences in memory performance over 1 year between the low and high cortisol groups were determined by analysis of covariance (ANCOVA). Memory function was measured by composite Z-scores calculated by summing up Z-scores corresponding to scores of the SDFR and LDFR outcomes of the CVLT-II as done before (Swardfager et al. 2010). Change scores were calculated by subtracting composite baseline Z-scores from follow-up Z-scores.

Covariates were determined a priori based on previous research and clinical knowledge that suggested they were potential confounders of the relationship that was tested. A sample size of 56 patients allowed adjustment for 4 covariates in addition to the cortisol groups. The covariates included in the ANCOVA analysis included gender, exercise, baseline verbal memory and depression. Age and total education were not included as covariates because Z-scores are standardized for age and total years of education. Variables that were significantly different between low and high cortisol groups were also included in the ANCOVA in a post-hoc analysis.
2.8.2 Secondary Hypothesis

CAD patients divided into the high cortisol group (hair cortisol concentrations \( \geq 153.2 \text{ ng/g} \)) based on a previously established cortisol reference range will have worse executive function performance compared to those in the low cortisol group, represented by lower composite executive function change Z-scores over 1 year of cardiac rehabilitation.

Differences in executive function over 1 year between the low and high cortisol groups were also determined by an ANCOVA. Executive function was measured by composite Z-scores calculated by summing up Z-scores of the Trails B and Stroop tests as done before (Swardfager et al. 2010). Change scores were calculated in the same manner as the primary analysis. Covariates included in the primary analysis were also included in the secondary analysis.

2.8.3 Covariates

2.8.3.1 Gender

The activity of the HPA axis under both basal and stimulated conditions has been shown to differ between men and women (Kudielka and Kirschbaum 2003, Viau 2002). Men seem to secrete more cortisol in response to different stressors (Earle et al. 1999, Kirschbaum et al. 1992, Kudielka et al. 1998, Kumsta et al. 2007) while women secrete more cortisol in response to ACTH, suggesting that the female adrenal cortex is more sensitive and that women are possibly more responsive to CRH with respect to ACTH secretion compared to men (Roelfsema et al. 1993). Women have also consistently shown a stronger increase and delayed peak in the salivary cortisol awakening response compared to men (Pruessner et al. 1997).

Gender differences in HPA axis functioning have also been demonstrated in the presence of CVRFs. For example, HPA axis function differs between men and women in the presence of
obesity (Pasquali et al. 2002) suggesting that differences in HPA axis function persist in the presence of CVRFs and may be possibly amplified in CAD.

While is it memory retrieval that is largely affected by cortisol (Buchanan et al. 2006, de Quervain et al. 2003, Roozendaal 2002), effects of cortisol during all stages of memory processing including encoding, consolidation and retrieval are gender-dependent (Andreano and Cahill 2006, Buchanan and Tranel 2008, Wolf et al. 2001).

2.8.3.2 Depression

Depressed patients were not excluded in this study. Depressed subject show various impairments in attention, working memory, new learning and executive function compared to controls. These cognitive deficits might be attributed to elevated cortisol concentrations in these individuals as one study demonstrated 53% increase in area under the curve in depressed subjects and a corresponding 6% decrease in hippocampal volume (O'Brien et al. 2004).

In contrast, while it has been hypothesized that persisting hypercortisolemia in aging depressed patients might cause hippocampal damage, cortisol-mediated neurotoxicity was not found to be the major etiological mechanism in 61 depressed patients over the age of 60 (O'Brien et al. 1996). This suggests that other mechanisms might be leading to brain volume changes in depressed subjects. Since there were not enough depressed patients for a subgroup analysis, depression was included as a covariate in all analyses.

2.8.3.3 Exercise

al. 2005, Weuve et al. 2004, Yaffe et al. 2001, Yaffe et al. 1999) studies have shown that active individuals exhibit better cognitive function compared to inactive individuals. For example, a large 5-year prospective study indicated that physical activity was associated with lower risks of cognitive impairment, AD and dementia in general (Laurin et al. 2001). Animal research suggests that exercise can contribute to neuronal survival (Carro et al. 2001, Stummer et al. 1994), increase brain revascularization (Black et al. 1991, Gomez-Pinilla et al. 1998, Isaacs et al. 1992), stimulate neurogenesis (Olson et al. 2006, Uda et al. 2006, van Praag et al. 1999, van Praag et al. 2005) and enhance learning (van Praag et al. 2005, Young et al. 1999), which all lend to the maintenance of cognitive function during aging (Escorihuela et al. 1995).

Physical activity can also be neuroprotective against ischemic neuronal damage in the hippocampus (Stummer et al. 1994) as well as the neostriatum (Dobrossy and Dunnett 2003, Smith and Zigmond 2003), suggesting that it would have beneficial effects on both memory and executive function performance. A 3-month exercise intervention in middle-aged adults was shown to increase cerebral blood volume in the dentate gyrus of the HPC, which was in turn, associated with memory improvement (Pereira et al. 2007). Various studies have also indicated an improvement in executive function as a result of physical activity (Barnes et al. 2003, Kamijo et al. 2004, Kramer et al. 1999, Smith et al. 2010, van Boxtel et al. 1997). Neuroimaging studies indicate that exercise can preserve brain volumes and retain the integrity of neural pathways, especially those associated with the prefrontal cortex (Marks et al. 2007).

In addition, exercise can directly affect cortisol secretion (Russo-Neustadt et al. 2000). As mentioned before, chronic stress can result in morphological changes in hippocampal neurons, which in turn has a direct impact on memory performance (Bremner et al. 2000, Joels and de Kloet 1989, Magarinos et al. 1996, McEwen 2000, McEwen 2001, Starkman et al. 1992). Exercise is commonly believed to be a behavioural intervention to relieve stress (Byrne and
Byrne 1993). Physical activity can influence both the blood concentrations of cortisol (Lehmann et al. 1993, Luger et al. 1987) and the expression of corticosteroid receptors to counteract the deleterious effects of stress on the limbic system (Kraemer and Ratamess 2005).

Hence, the VO$_2$ peak was used as a measure of fitness of individuals in both the low and high cortisol groups. VO$_2$ peak or the maximal oxygen uptake is commonly used to determine the workload of physical exercise and is used to indicate the functional capacity of respiratory, circulatory and metabolic systems (Kashiwara et al. 2009, Wasserman and Whipp 1975).

2.8.3.4 Baseline composite Z-scores

Baseline composite Z-scores of both memory and executive function were included as a covariate in the ANCOVA model to account for cognitive function at baseline. Previous studies have shown that early cognitive impairment may be an indicator of long-term impairment (Newman et al. 2001).

2.9 Sample Size Calculation

Since this was the first study to determine differences in cognitive function between low and high hair cortisol groups, sample size was calculated based on an inferred effect size. For an ANCOVA with 5 pre-determined predictors, in order to detect a large effect size ($f=0.45$) with an $\alpha$ of 0.05, 26 subjects per group and a total of 52 patients were required for the power of 0.86 ($1-\beta$) for the study.
3 Results

3.1 Patient Recruitment

For this study, 360 patients were screened by TRI staff for evidence of CAD at entry in the CR program. Of the patients screened, 43 were excluded based on referral to rehab for other diagnoses such as diabetes or for referral to CR due to the presence of cardiovascular risk factors only. The remaining 317 patients with evidence of CAD were then approached to get consent to be contacted for the study. 89 of the approached patients declined to be contacted by study personnel while 228 patients agreed to be contacted about the study. When contacted, patients were asked to provide consent for participation in the study. 78 patients declined to participate in the study while 150 agreed to participate in the study. From the patients that agreed to participate, 39 patients were excluded based on exclusion criteria. In addition, 8 patients had insufficient hair samples for cortisol analysis and 4 samples were contaminated and were therefore, excluded leaving 99 patients in the study. 43 of these patients were then lost to follow-up due to a number of reasons including death, withdrawal of consent at follow-up or inability of research personnel to contact them. 3 of these patients were also new follow-ups or patients recruited at the end of CR for a previous study and therefore, had no baseline data. This left 56 longitudinal patients that were included in the final analysis and divided into low (n=26) and high cortisol (n=30) groups. The patient inclusion process for the study is shown in Figure VII.
Figure VII: Patient inclusion process for the study.
3.2 Demographics and Clinical Characteristics

Demographics and clinical characteristics of 99 patients enrolled in the CR program at baseline compared to 56 of those patients that completed the program and included in the final analysis are shown in Table 2.

Table 2: Demographic and clinical characteristics of patients enrolled in the study. 99 patients were enrolled in the CR program at baseline compared to 56 of those patients that completed the 1-year CR program.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Sample (n=99)</th>
<th>Follow-up sample (n=56)</th>
<th>Statistic (F or X^2)</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sociodemographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>64±11</td>
<td>66±11</td>
<td>1.031</td>
<td>0.312</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>79</td>
<td>86</td>
<td>1.128</td>
<td>0.288</td>
</tr>
<tr>
<td>Marital status (% married)</td>
<td>73</td>
<td>80</td>
<td>1.125</td>
<td>0.289</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>88</td>
<td>89</td>
<td>0.069</td>
<td>0.793</td>
</tr>
<tr>
<td>Total education (yrs)</td>
<td>17±3</td>
<td>17±3</td>
<td>0.019</td>
<td>0.891</td>
</tr>
<tr>
<td><strong>Smoking History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>4.0</td>
<td>3.6</td>
<td>0.240</td>
<td>0.887</td>
</tr>
<tr>
<td>Cigarette consumption (# per day)</td>
<td>10±12</td>
<td>9±10</td>
<td>0.031</td>
<td>0.861</td>
</tr>
<tr>
<td>Years smoked</td>
<td>13±16</td>
<td>14±16</td>
<td>0.297</td>
<td>0.587</td>
</tr>
<tr>
<td><strong>CAD Severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative stenosis (%)</td>
<td>137.6±73.5</td>
<td>138.5±69.0</td>
<td>0.005</td>
<td>0.945</td>
</tr>
<tr>
<td>Number of vessels stenosed</td>
<td>2±1</td>
<td>2±1</td>
<td>&lt;0.00005</td>
<td>0.990</td>
</tr>
<tr>
<td>Time since ACS (wks)</td>
<td>21±48</td>
<td>25±63</td>
<td>0.231</td>
<td>0.631</td>
</tr>
<tr>
<td><strong>Medical Comorbidities (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>55</td>
<td>59</td>
<td>0.279</td>
<td>0.597</td>
</tr>
<tr>
<td></td>
<td>Baseline Sample (n=99)</td>
<td>Follow-up sample (n=56)</td>
<td>Statistic (F or $X^2$)</td>
<td>p-value (significance at $p \leq 0.05$)*</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------</td>
<td>-------------------------</td>
<td>------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Medical Comorbidities (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>94</td>
<td>100</td>
<td>3.531</td>
<td>0.060</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17</td>
<td>13</td>
<td>0.597</td>
<td>0.440</td>
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<tr>
<td>Depression</td>
<td>29</td>
<td>29</td>
<td>0.009</td>
<td>0.924</td>
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<td>Cardiac History (%)</td>
<td></td>
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<td></td>
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<tr>
<td>ISHD</td>
<td>16</td>
<td>11</td>
<td>0.871</td>
<td>0.351</td>
</tr>
<tr>
<td>PCI</td>
<td>51</td>
<td>50</td>
<td>0.004</td>
<td>0.952</td>
</tr>
<tr>
<td>CABG</td>
<td>29</td>
<td>38</td>
<td>1.102</td>
<td>0.294</td>
</tr>
<tr>
<td>MI</td>
<td>44</td>
<td>48</td>
<td>0.205</td>
<td>0.651</td>
</tr>
<tr>
<td>Angina</td>
<td>25</td>
<td>23</td>
<td>0.080</td>
<td>0.777</td>
</tr>
<tr>
<td>Body Composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8±4.6</td>
<td>27.3±4.2</td>
<td>0.554</td>
<td>0.458</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>28.2±8.2</td>
<td>27.5±7.8</td>
<td>0.213</td>
<td>0.645</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.2±15.1</td>
<td>81.2±13.7</td>
<td>&lt;0.00005</td>
<td>0.991</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>97.8±12.3</td>
<td>97.6±9.4</td>
<td>0.010</td>
<td>0.919</td>
</tr>
<tr>
<td>Resting Physiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>67.1±12.0</td>
<td>67.6±12.2</td>
<td>0.058</td>
<td>0.810</td>
</tr>
<tr>
<td>Resting systolic blood pressure (mm Hg)</td>
<td>127.3±16.4</td>
<td>127.2±15.4</td>
<td>0.001</td>
<td>0.974</td>
</tr>
<tr>
<td>Resting diastolic blood pressure (mm Hg)</td>
<td>72.9±9.4</td>
<td>72.6±8.7</td>
<td>0.039</td>
<td>0.844</td>
</tr>
<tr>
<td>Cardiopulmonary Fitness Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum heart rate (bpm)</td>
<td>119.2±22.4</td>
<td>119.7±20.0</td>
<td>0.021</td>
<td>0.886</td>
</tr>
<tr>
<td>Maximum systolic blood pressure (mm Hg)</td>
<td>175.1±26.3</td>
<td>176.2±25.2</td>
<td>0.063</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>Baseline Sample (n=99)</td>
<td>Follow-up sample (n=56)</td>
<td>Statistic (F or X²)</td>
<td>p-value (significance at p≤0.05)*</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Cardiopulmonary Fitness Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum diastolic blood pressure (mm Hg)</td>
<td>78.0±10.8</td>
<td>78.2±10.2</td>
<td>0.014</td>
<td>0.906</td>
</tr>
<tr>
<td>VO₂ peak (mL/kg/min)</td>
<td>19.5±5.3</td>
<td>19.8±4.6</td>
<td>0.153</td>
<td>0.697</td>
</tr>
<tr>
<td><strong>Concomitant Medications (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>75</td>
<td>75</td>
<td>0.001</td>
<td>0.972</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>20</td>
<td>18</td>
<td>0.126</td>
<td>0.723</td>
</tr>
<tr>
<td>Diuretics</td>
<td>18</td>
<td>21</td>
<td>0.242</td>
<td>0.623</td>
</tr>
<tr>
<td>Anti-hypertensives</td>
<td>61</td>
<td>57</td>
<td>0.178</td>
<td>0.673</td>
</tr>
<tr>
<td>Anti-diabetics</td>
<td>14</td>
<td>11</td>
<td>0.374</td>
<td>0.541</td>
</tr>
<tr>
<td>Statins</td>
<td>94</td>
<td>100</td>
<td>3.531</td>
<td>0.060</td>
</tr>
<tr>
<td><strong>Psychotropic Medications (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>9</td>
<td>7</td>
<td>0.177</td>
<td>0.674</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>9</td>
<td>7</td>
<td>0.177</td>
<td>0.674</td>
</tr>
<tr>
<td><strong>Scales</strong></td>
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</tr>
<tr>
<td>CES-D</td>
<td>12±11</td>
<td>12±12</td>
<td>0.109</td>
<td>0.742</td>
</tr>
<tr>
<td>PSS</td>
<td>14±8</td>
<td>14±8</td>
<td>0.353</td>
<td>0.553</td>
</tr>
<tr>
<td>SRRS</td>
<td>225±125</td>
<td>200±120</td>
<td>1.442</td>
<td>0.235</td>
</tr>
</tbody>
</table>

Abbreviations: ACS=acute coronary syndrome, ISHD=ischemic heart disease, PCI=percutaneous coronary intervention, CABG=coronary artery bypass graft, MI=myocardial infarction, bpm=beats per minute, CES-D=Centre for Epidemiological Studies depression scale, PSS=Perceived stress scale, SRRS=Social readjustment rating scale

There were no significant differences in terms of demographics and clinical characteristics between subjects that were initially enrolled in the study (baseline sample) and the subjects that finished the CR program (follow-up sample).
3.3 Cortisol Analysis

Hair cortisol samples from the 99 patients at the beginning of CR program, 56 of which finished the program were sent for analysis. In the baseline sample, 13 were black, 44 were grey, 24 were brown, 15 were white and 3 were blonde. In the follow-up subsample, 7 were black, 27 were grey, 14 were brown and 8 were white. As shown in Table 3, there were no differences in cortisol concentrations between the two groups. There were also no differences in cortisol concentrations between samples of different hair colour between the baseline and follow-up groups and between different hair coloured samples within each group. Performance on cognitive tests measuring memory (CVLT-II) and executive function (Trails B and Stroop) as well as composite Z-scores were also not different between the baseline and follow-up samples as shown in Table 4.

Table 3: Cortisol concentrations in the baseline and follow-up groups.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Sample (n=99)</th>
<th>Follow-up sample (n=56)</th>
<th>Statistic (F or X²)</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/g)</td>
<td>198.6±153.6</td>
<td>233.2±173.0</td>
<td>1.654</td>
<td>0.200</td>
</tr>
</tbody>
</table>
Table 4: Mean cognitive test scores in the baseline and follow-up sample at entry into the CR program.

<table>
<thead>
<tr>
<th>Baseline Cognitive test Z-scores</th>
<th>Baseline Sample (n=99)</th>
<th>Follow-up sample (n=56)</th>
<th>Statistic (F or X²)</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT-II</td>
<td>SDFR 0.01±1.02</td>
<td>0.05±1.08</td>
<td>0.062</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>LDFR -0.07±1.06</td>
<td>-0.07±1.18</td>
<td>0.001</td>
<td>0.975</td>
</tr>
<tr>
<td>Stroop</td>
<td>0.31±1.00</td>
<td>0.36±0.98</td>
<td>0.066</td>
<td>0.798</td>
</tr>
<tr>
<td>Trails B</td>
<td>-0.14±0.84</td>
<td>0.00±0.83</td>
<td>1.060</td>
<td>0.305</td>
</tr>
<tr>
<td>Composite memory</td>
<td>-0.06±1.97</td>
<td>-0.02±2.16</td>
<td>0.012</td>
<td>0.912</td>
</tr>
<tr>
<td>Composite executive function</td>
<td>0.17±1.59</td>
<td>0.36±1.54</td>
<td>0.494</td>
<td>0.483</td>
</tr>
</tbody>
</table>

Abbreviations: CVLT-II=California Verbal Learning test, 2nd edition, SDFR=short delay free recall, LDFR=long delay free recall

3.4 Follow-up Sample

3.4.1 Cognitive Performance

As shown in Figure VIII, Z-scores corresponding to performance on the CVLT-II used to measure memory and the Trails B test used to measure executive function at baseline reflected a continuous range in cognitive performance in 56 CAD patients that completed the 1 year CR program. Similar to other studies (Bauer and Pozehl 2010, Vogels et al. 2007), a cutoff of Z-scores less than or equal to -1.34 on the CVLT-II and the Trails B test was used to define impairment in memory and executive function. Although 14.3% of the CAD patients showed memory impairment and 5.4% showed executive dysfunction, the mean value of Z-scores on both the CVLT-II (-0.071±1.18) and the Trails B test (0.00±0.83) were within established norms.

Table 5 shows scores of cognitive tests assessing memory (CVLT-II) and executive function (Stroop and Trails B) performance at entry into the CR program and follow-up a year
later at the end of the rehab program. Study participants improved significantly in performance on all tests over 1 year, especially the CVLT-II. Composite memory and executive function scores also significantly improved over 1 year as shown in Table 6.

a)

Figure VIII: Cognitive impairment in CAD patients in the follow-up sample. Impairment was defined as Z-scores \( \leq -1.34 \) (left of the red dotted line); a) 14.3% of CAD patients had verbal memory impairment measured by the CVLT-II while b) 5.4% were impaired in executive function measured by the Trails B test. However, mean Z-scores corresponding to performance on the CVLT-II (-0.071±1.18) and the Trails B test (0.00±0.83) were within established norms.
Table 5: Cognitive test scores at entry and at the end of CR in the follow-up sample.

<table>
<thead>
<tr>
<th>Cognitive Test</th>
<th>Baseline Z-scores</th>
<th>Follow-up Z-scores</th>
<th>t</th>
<th>df</th>
<th>p-value (significance at $p \leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT-II (Z-scores)</td>
<td>SDFR</td>
<td>0.05±1.08</td>
<td>0.61±1.32</td>
<td>-4.149</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>LDFR</td>
<td>-0.07±1.18</td>
<td>0.69±1.09</td>
<td>-6.788</td>
<td>55</td>
</tr>
<tr>
<td>Stroop Z-scores</td>
<td>0.36±0.98</td>
<td>0.61±1.01</td>
<td>-2.643</td>
<td>54</td>
<td>0.011*</td>
</tr>
<tr>
<td>Trails B Z-scores</td>
<td>0.00±0.83</td>
<td>0.21±0.78</td>
<td>-2.586</td>
<td>54</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

Abbreviations: CVLT-II=California Verbal Learning test, 2nd edition, SDFR=short delay free recall, LDFR=long delay free recall

Table 6: Composite memory and executive function Z-scores at entry and at the end of CR in the follow-up sample.

<table>
<thead>
<tr>
<th>Composite Z-scores</th>
<th>Baseline</th>
<th>Follow-up</th>
<th>t</th>
<th>df</th>
<th>p-value (significance at $p \leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>-0.02±2.16</td>
<td>1.29±2.33</td>
<td>-5.864</td>
<td>55</td>
<td>&lt;0.00005*</td>
</tr>
<tr>
<td>Executive function</td>
<td>0.36±1.54</td>
<td>0.82±1.53</td>
<td>-3.444</td>
<td>54</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

3.4.2 Cortisol and Cognitive Performance

Before testing the hypotheses of the study and dividing CAD patients into low and high cortisol groups for the proposed analyses, the follow-up sample was first analyzed as one group to determine the associations between cortisol concentrations as a continuous variable and cognition, specifically, memory and executive function performance over 1 year.

Memory function in patients over 1 year was assessed by change in composite memory Z-scores. Change in executive function over 1 year was assessed by change in composite executive function Z-scores. In order to assess whether cortisol concentrations at entry predicted memory performance over 1 year in the follow-up sample, backward linear regression was
conducted. Gender, VO₂ peak, depression and baseline composite memory Z-scores were included as covariates in the model. A summary of the significant model is shown in Table 7. As shown in Table 8, baseline composite memory Z-scores were the only significant predictor of change in memory of CAD patients in the follow-up sample over 1 year (β=-0.279, p=0.037). Table 9 shows all the excluded predictors in the backward linear regression analysis, including cortisol concentrations (β=-0.002, p=0.991).

**Table 7: Summary of the backward linear regression model looking at the association between cortisol concentrations and memory performance over 1 year in the follow-up sample.** Covariates entered in the model included gender, VO₂ peak, depression and baseline composite memory Z-scores.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted R²</th>
<th>F</th>
<th>df</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.061</td>
<td>4.557</td>
<td>55</td>
<td>0.037*</td>
</tr>
</tbody>
</table>

**Table 8: The final model in the backward linear regression analysis looking at the association between cortisol concentrations and executive function over 1 year in the follow-up sample.**

<table>
<thead>
<tr>
<th>Model</th>
<th>β</th>
<th>t</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>6.033</td>
<td>&lt;0.00005</td>
</tr>
<tr>
<td>Baseline composite memory Z-scores</td>
<td>-0.279</td>
<td>-2.135</td>
<td>0.037*</td>
</tr>
</tbody>
</table>
Table 9: Excluded variables in the backward linear regression analysis looking at the association between cortisol concentrations and memory performance over 1 year in the follow-up sample.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$</th>
<th>$t$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.078</td>
<td>0.590</td>
<td>0.558</td>
</tr>
<tr>
<td>VO$_2$ peak</td>
<td>0.231</td>
<td>1.788</td>
<td>0.080</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.040</td>
<td>-0.304</td>
<td>0.762</td>
</tr>
<tr>
<td>Cortisol</td>
<td>-0.002</td>
<td>-0.012</td>
<td>0.991</td>
</tr>
</tbody>
</table>

Backward linear regression analysis was also conducted to determine whether cortisol concentrations at entry predict executive function performance over 1 year. Gender, VO$_2$ peak, depression and baseline composite executive function Z-scores were also included as covariates in the model. A summary of the significant model is shown in Table 10. As shown in Table 11, baseline composite executive function Z-scores were the only significant predictor of change in executive function of CAD patients in the follow-up sample over 1 year ($\beta$=-0.336, p=0.012).

Table 12 shows all the excluded predictors in the backward linear regression analysis, including cortisol concentrations ($\beta$=0.084, p=0.519).

Table 10: Summary of the backward linear regression model looking at the association between cortisol concentrations and executive function over 1 year in the follow-up sample. Covariates entered into the model included gender, VO$_2$ peak, depression and baseline composite executive function Z-scores.

<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>F</th>
<th>df</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall model</td>
<td>0.096</td>
<td>6.757</td>
<td>54</td>
<td>0.012*</td>
</tr>
</tbody>
</table>
Table 11: The final model in the backward linear regression analysis looking at the association between cortisol concentrations and executive function over 1 year in the follow-up sample.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$</th>
<th>t</th>
<th>p-value (significance at $p \leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-</td>
<td>4.120</td>
<td>&lt;0.00005</td>
</tr>
<tr>
<td>Baseline composite executive function Z-scores</td>
<td>-0.336</td>
<td>-2.599</td>
<td>0.012*</td>
</tr>
</tbody>
</table>

Table 12: Excluded variables in the backward linear regression analysis looking at the association between cortisol concentrations and executive function over 1 year in the follow-up sample.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\beta$</th>
<th>t</th>
<th>p-value (significance at $p \leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>0.097</td>
<td>0.746</td>
<td>0.459</td>
</tr>
<tr>
<td>VO$_2$ peak</td>
<td>0.073</td>
<td>0.558</td>
<td>0.579</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.125</td>
<td>-0.937</td>
<td>0.353</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.084</td>
<td>0.649</td>
<td>0.519</td>
</tr>
</tbody>
</table>

3.5 Analyses to Test Hypotheses

3.5.1 Demographics and Clinical Characteristics

Demographics and clinical characteristics of 56 CAD patients that were followed over 1 year of CR divided into low (n=26) and high (n=30) cortisol groups based on hair cortisol reference ranges as mentioned before, are shown in Table 13.
Table 13: Demographics and clinical characteristics of the follow-up sample (n=56) divided into low and high cortisol groups.

<table>
<thead>
<tr>
<th></th>
<th>Low Cortisol (n=26)</th>
<th>High Cortisol (n=30)</th>
<th>Statistic (F or X²)</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sociodemographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>67±12</td>
<td>65±11</td>
<td>0.624</td>
<td>0.433</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>92.3</td>
<td>80</td>
<td>1.723</td>
<td>0.189</td>
</tr>
<tr>
<td>Marital status (% married)</td>
<td>88.5</td>
<td>73.3</td>
<td>1.619</td>
<td>0.203</td>
</tr>
<tr>
<td>Ethnicity (% Caucasian)</td>
<td>84.6</td>
<td>93.3</td>
<td>1.016</td>
<td>0.313</td>
</tr>
<tr>
<td>Total education (yrs)</td>
<td>16.5±3</td>
<td>17±3.5</td>
<td>0.585</td>
<td>0.448</td>
</tr>
<tr>
<td><strong>Smoking History</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>3.8</td>
<td>3.3</td>
<td>0.249</td>
<td>0.883</td>
</tr>
<tr>
<td>Cigarette consumption (# per day)</td>
<td>9±10</td>
<td>10±10</td>
<td>0.109</td>
<td>0.742</td>
</tr>
<tr>
<td>Years smoked</td>
<td>13±16</td>
<td>16±16</td>
<td>0.434</td>
<td>0.513</td>
</tr>
<tr>
<td><strong>CAD Severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative stenosis (%)</td>
<td>123.4±61.8</td>
<td>153.0±73.6</td>
<td>2.509</td>
<td>0.119</td>
</tr>
<tr>
<td>Number of vessels stenosed</td>
<td>2±1</td>
<td>2±1</td>
<td>0.911</td>
<td>0.344</td>
</tr>
<tr>
<td>Time since ACS (wks)</td>
<td>22±42</td>
<td>30±81</td>
<td>0.206</td>
<td>0.652</td>
</tr>
<tr>
<td><strong>Medical Comorbidities (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>53.8</td>
<td>63.3</td>
<td>0.518</td>
<td>0.472</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7.7</td>
<td>16.7</td>
<td>1.026</td>
<td>0.311</td>
</tr>
<tr>
<td>Depression</td>
<td>26.9</td>
<td>30</td>
<td>0.065</td>
<td>0.799</td>
</tr>
<tr>
<td><strong>Cardiac History (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISHD</td>
<td>11.5</td>
<td>10</td>
<td>0.034</td>
<td>0.853</td>
</tr>
<tr>
<td>PCI</td>
<td>57.7</td>
<td>43.3</td>
<td>1.149</td>
<td>0.284</td>
</tr>
<tr>
<td>CABG</td>
<td>23.1</td>
<td>50</td>
<td>4.308</td>
<td>0.038*</td>
</tr>
<tr>
<td></td>
<td>Low Cortisol (n=26)</td>
<td>High Cortisol (n=30)</td>
<td>Statistic (F or X²)</td>
<td>p-value (significance at p≤0.05)*</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Cardiac History (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>57.7</td>
<td>40</td>
<td>1.746</td>
<td>0.186</td>
</tr>
<tr>
<td>Angina</td>
<td>23.1</td>
<td>20.7</td>
<td>0.046</td>
<td>0.831</td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±5.0</td>
<td>27.4±3.5</td>
<td>0.104</td>
<td>0.748</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>26.1±8.4</td>
<td>28.8±7.2</td>
<td>1.686</td>
<td>0.200</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.8±15.4</td>
<td>82.4±12.3</td>
<td>0.470</td>
<td>0.496</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>96.2±10.1</td>
<td>98.8±8.8</td>
<td>1.133</td>
<td>0.292</td>
</tr>
<tr>
<td><strong>Resting Physiology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>66.7±12.4</td>
<td>68.4±12.1</td>
<td>0.270</td>
<td>0.606</td>
</tr>
<tr>
<td>Resting systolic blood pressure (mm Hg)</td>
<td>124.4±17.6</td>
<td>129.6±12.9</td>
<td>1.598</td>
<td>0.212</td>
</tr>
<tr>
<td>Resting diastolic blood pressure (mm Hg)</td>
<td>71.4±7.4</td>
<td>73.7±9.7</td>
<td>1.013</td>
<td>0.319</td>
</tr>
<tr>
<td><strong>Cardiopulmonary Fitness Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum heart rate (bpm)</td>
<td>118.0±18.1</td>
<td>121.2±21.7</td>
<td>0.360</td>
<td>0.551</td>
</tr>
<tr>
<td>Maximum systolic blood pressure (mm Hg)</td>
<td>175.7±23.5</td>
<td>176.6±27.0</td>
<td>0.016</td>
<td>0.899</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure (mm Hg)</td>
<td>76.2±9.0</td>
<td>79.9±11.1</td>
<td>1.863</td>
<td>0.178</td>
</tr>
<tr>
<td>VO₂ peak (mL/kg/min)</td>
<td>19.7±4.5</td>
<td>19.6±4.6</td>
<td>0.001</td>
<td>0.975</td>
</tr>
<tr>
<td><strong>Concomitant Medications (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>80.1</td>
<td>70</td>
<td>0.862</td>
<td>0.353</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>26.9</td>
<td>10</td>
<td>2.719</td>
<td>0.099</td>
</tr>
<tr>
<td>Diuretics</td>
<td>23.1</td>
<td>20</td>
<td>0.078</td>
<td>0.780</td>
</tr>
<tr>
<td>Anti-hypertensives</td>
<td>50</td>
<td>63.3</td>
<td>1.011</td>
<td>0.315</td>
</tr>
<tr>
<td>Low Cortisol (n=26)</td>
<td>High Cortisol (n=30)</td>
<td>Statistic (F or X^2)</td>
<td>p-value (significance at p≤0.05)*</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------</td>
<td>-----------------------</td>
<td>----------------------------------</td>
<td></td>
</tr>
<tr>
<td>Concomitant Medications (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-diabetics</td>
<td>3.8</td>
<td>16.7</td>
<td>2.393</td>
<td>0.122</td>
</tr>
<tr>
<td>Statins</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Psychotropic Medications (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidepressants</td>
<td>3.8</td>
<td>10</td>
<td>0.795</td>
<td>0.373</td>
</tr>
<tr>
<td>Anxiolytics</td>
<td>11.5</td>
<td>3.3</td>
<td>1.414</td>
<td>0.234</td>
</tr>
<tr>
<td>Scales</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CES-D</td>
<td>13±13</td>
<td>11±11</td>
<td>0.303</td>
<td>0.584</td>
</tr>
<tr>
<td>PSS</td>
<td>14±8</td>
<td>13±9</td>
<td>0.290</td>
<td>0.592</td>
</tr>
<tr>
<td>SRRS (at follow-up)^1</td>
<td>192±165</td>
<td>180±111</td>
<td>0.093</td>
<td>0.762</td>
</tr>
<tr>
<td>Composite Cognitive Z-scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline memory</td>
<td>-0.2±2.0</td>
<td>0.2±2.3</td>
<td>0.468</td>
<td>0.497</td>
</tr>
<tr>
<td>Follow-up memory</td>
<td>1.6±2.0</td>
<td>1.1±2.6</td>
<td>1.083</td>
<td>0.303</td>
</tr>
<tr>
<td>Baseline executive function</td>
<td>0.4±1.2</td>
<td>0.3±1.8</td>
<td>0.011</td>
<td>0.918</td>
</tr>
<tr>
<td>Follow-up executive function</td>
<td>0.9±1.7</td>
<td>0.8±1.4</td>
<td>0.112</td>
<td>0.739</td>
</tr>
</tbody>
</table>

Abbreviations: ACS=acute coronary syndrome, ISHD=ischemic heart disease, PCI=percutaneous coronary intervention, CABG=coronary artery bypass graft, MI=myocardial infarction, bpm=beats per minute, CES-D=Centre for Epidemiological Studies depression scale, PSS=Perceived stress scale, SRRS=Social readjustment rating scale

A higher proportion of patients in the high cortisol group had CABG (X^2=4.308, p=0.038) compared to the patients in the low cortisol group. There were no other differences between the low and high cortisol groups in terms of demographics and clinical characteristics.

^1 SRRS follow-up scores represent exposure to stressful life events over the 1 year study period.
3.5.2 Cortisol Analysis

Hair samples collected from 56 patients that finished the CR program were sent for analysis. Of these samples, 26 samples were classified as low cortisol hair samples while 30 samples were classified as high cortisol hair samples. Cortisol assay results are reported in Table 14 and represented in Figure IX. Cortisol concentrations were significantly different between the low and high cortisol groups.

Table 14: Hair cortisol concentrations in the low and high cortisol groups.

<table>
<thead>
<tr>
<th></th>
<th>Low Cortisol</th>
<th>High Cortisol</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/g)</td>
<td>113.5 ±17.8</td>
<td>339.6±182.9</td>
<td>&lt;0.00005*</td>
</tr>
</tbody>
</table>

Figure IX: Cortisol concentrations in the low and high cortisol groups.
3.5.3 Cognitive function

ANOVA analyses were conducted in order to compare changes in memory and executive function between low and high cortisol groups. The ANOVAs showing differences in change in memory and executive function over 1 year between high and low cortisol groups are reported in Table 15 and 16 respectively and represented in Figures X and XI. While both groups improved in memory and executive function over 1 year, there was significantly less improvement in memory in the high cortisol group compared to the low cortisol group ($F_{1,54}=4.144$, $p=0.047$). There were no differences in change in executive function over 1 year between the 2 groups ($F_{1,54}=0.122$, $p=0.728$).
Table 15: ANOVA analysis showing significant differences in composite memory change Z-scores over 1 year between low and high cortisol groups.

<table>
<thead>
<tr>
<th>Change Scores</th>
<th>Low Cortisol</th>
<th>High Cortisol</th>
<th>F</th>
<th>df</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite memory Z-scores</td>
<td>1.8±1.5</td>
<td>0.9±1.7</td>
<td>4.144</td>
<td>54</td>
<td>0.047*</td>
</tr>
</tbody>
</table>

Figure X: Change in memory function over 1 year in the low and high cortisol groups. There is less improvement in memory function over 1 year in the high cortisol group (green line) compared to the low cortisol group (blue line).
Table 16: ANOVA analysis showing no differences in composite executive function change Z-scores over 1 year between low and high cortisol groups.

<table>
<thead>
<tr>
<th>Change Scores</th>
<th>Low Cortisol</th>
<th>High Cortisol</th>
<th>F</th>
<th>df</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite executive function Z-scores</td>
<td>0.5±1.0</td>
<td>0.4±1.0</td>
<td>0.122</td>
<td>54</td>
<td>0.728</td>
</tr>
</tbody>
</table>

Figure XI: Change in executive function over 1 year in the low and high cortisol groups. Both groups improved in executive function over 1 year but there were no differences in improvement between the 2 groups.
3.5.4 Primary Hypothesis

CAD patients divided into the high cortisol group (hair cortisol concentrations ≥ 153.2 ng/g) based on a previously established cortisol reference range will have worse memory performance compared to those in the low cortisol group, represented by lower composite verbal memory change Z-scores over 1 year of cardiac rehabilitation.

In order to compare changes in memory function over 1 year between patients in low and high cortisol groups, an ANCOVA was conducted with composite memory Z-scores as the dependent variable and high or low cortisol group as the factor. Gender, VO₂ peak, depression and baseline memory composite Z-scores were also added in the analysis as covariates. As shown in Table 17, patients with high cortisol differed significantly in memory performance over 1 year from those with low cortisol (F₁,₅₀ = 4.721, p = 0.035).
Table 17: Coefficients of an ANCOVA analysis detecting differences in composite memory change Z-scores over 1 year between low and high cortisol groups. Covariates entered into the model included gender, VO₂ peak, depression and baseline composite memory Z-scores².

<table>
<thead>
<tr>
<th>Source</th>
<th>df error</th>
<th>F</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>50</td>
<td>2.931</td>
<td>0.021</td>
</tr>
<tr>
<td>Intercept</td>
<td>50</td>
<td>0.594</td>
<td>0.445</td>
</tr>
<tr>
<td>Gender</td>
<td>50</td>
<td>2.063</td>
<td>0.157</td>
</tr>
<tr>
<td>VO₂ peak</td>
<td>50</td>
<td>4.794</td>
<td>0.033*</td>
</tr>
<tr>
<td>Depression</td>
<td>50</td>
<td>0.459</td>
<td>0.501</td>
</tr>
<tr>
<td>Baseline Composite Memory Z-Scores</td>
<td>50</td>
<td>5.906</td>
<td>0.019*</td>
</tr>
<tr>
<td>High/Low Cortisol</td>
<td>50</td>
<td>4.721</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

Covariates including VO₂ peak and baseline composite memory Z-scores were also significantly associated with change in memory over 1 year. A higher VO₂ peak was significantly associated with improvement in composite memory Z-scores over 1 year in the high cortisol group (r=0.398, p=0.029) as shown in Figure XII.

² Using 1 year change CES-D (a 20-item questionnaire asking about perceived mood and function within the past 7 days scored on a scale of 0-60) scores to represent the change in depressive symptoms over 1 year improved the association between 1 year composite memory change Z-scores and baseline cortisol concentrations (F=5.462, p=0.024) suggesting that CES-D scores should be used in future analyses instead of a dichotomous classification of depression in this population.
Figure XII: Association between VO$_2$ peak and composite memory change Z-scores in the low and high cortisol groups. Higher VO$_2$ peak was associated with significant improvement in memory over 1 year in the high cortisol group ($r=0.398$, $p=0.029$; green line) compared to the low cortisol group ($r=-0.070$, $p=0.734$; blue line).
### 3.5.5 Secondary Hypothesis

CAD patients divided into the high cortisol group (hair cortisol concentrations $\geq 153.2$ ng/g) based on a previously established cortisol reference range will have worse executive function performance compared to those in the low cortisol group, represented by lower composite executive function change Z-scores over 1 year of cardiac rehabilitation.

In order to compare change in executive function over 1 year between patients in low and high cortisol groups, an ANCOVA was conducted with composite executive function Z-scores as the dependent variable and high or low cortisol group as the factor. One patient was excluded from the analysis due to an inability to complete the Trails B and Stroop tests as a result of visual impairment. Gender, VO$_2$ peak, depression and baseline executive function composite Z-scores were also added in the analysis as covariates. As shown in Table 18, there are no differences between the low and high cortisol groups in terms of executive function ($F_{1,40} = 0.318$, $p=0.575$).
Table 18: Coefficients of an ANCOVA analysis detecting differences in composite executive function change Z-scores over 1 year between low and high cortisol groups. Covariates entered into the model included gender, VO\textsubscript{2} peak, depression and baseline composite executive function Z-scores\textsuperscript{3,4}.

<table>
<thead>
<tr>
<th>Source</th>
<th>df error</th>
<th>F</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>49</td>
<td>1.822</td>
<td>0.126</td>
</tr>
<tr>
<td>Intercept</td>
<td>49</td>
<td>0.014</td>
<td>0.905</td>
</tr>
<tr>
<td>Gender</td>
<td>49</td>
<td>1.029</td>
<td>0.301</td>
</tr>
<tr>
<td>VO\textsubscript{2} peak</td>
<td>49</td>
<td>0.761</td>
<td>0.387</td>
</tr>
<tr>
<td>Depression</td>
<td>49</td>
<td>1.164</td>
<td>0.286</td>
</tr>
<tr>
<td>Baseline Composite Executive function Z-Scores</td>
<td>49</td>
<td>5.643</td>
<td>0.021*</td>
</tr>
<tr>
<td>High/Low Cortisol</td>
<td>49</td>
<td>0.318</td>
<td>0.575</td>
</tr>
</tbody>
</table>

3.6 Post-hoc analyses

Since CABG was significantly different between the low and high cortisol groups (X\textsuperscript{2}=4.308, p=0.038; Table 13), it was also added to the ANCOVA along with the other covariates in a post-hoc analysis to determine whether it would have any influence on the

\textsuperscript{3} Using 1 year change CES-D (a 20-item questionnaire asking about perceived mood and function within the past 7 days scored on a scale of 0-60) scores to represent the change in depressive symptoms over 1 year improved the association between 1 year composite executive function change Z-scores and baseline cortisol concentrations (F=0.216, p=0.644) suggesting that CES-D scores should be used in future analyses instead of a dichotomous classification of depression in this population.

\textsuperscript{4} In order to assess whether speed of processing had any impact on the association between higher cortisol concentrations and executive function performance over 1 year, Digit Symbol Coding test (a direct measure of processing speed and activation) 1 year change Z-scores were included in the ANCOVA model in addition to the high/low cortisol groups and the covariates including gender, VO\textsubscript{2} peak, and depression. There were no differences in composite executive function change Z-scores over 1 year between the low and high cortisol groups when controlling for speed of processing (F\textsubscript{1,39}=0.072, p=0.789).
differences in memory and executive function performance over 1 year in patients in the low and high cortisol groups. As shown in Table 19, there was a trend for a difference in memory function between the low and high cortisol groups after CABG is added as an additional covariate in the analysis ($F_{1,49}=3.444$, $p=0.070$).

Table 19: Coefficients of a post-hoc analysis detecting differences in composite memory change $Z$-scores over 1 year between low and high cortisol groups with CABG as an additional covariate.

<table>
<thead>
<tr>
<th>Source</th>
<th>df error</th>
<th>F</th>
<th>p-value (significance at $p \leq 0.05$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>49</td>
<td>2.452</td>
<td>0.037</td>
</tr>
<tr>
<td>Intercept</td>
<td>49</td>
<td>0.341</td>
<td>0.562</td>
</tr>
<tr>
<td>Gender</td>
<td>49</td>
<td>1.508</td>
<td>0.225</td>
</tr>
<tr>
<td>VO\textsubscript{2} peak</td>
<td>49</td>
<td>4.523</td>
<td>0.038*</td>
</tr>
<tr>
<td>Depression</td>
<td>49</td>
<td>0.554</td>
<td>0.460</td>
</tr>
<tr>
<td>Baseline Composite</td>
<td>49</td>
<td>5.795</td>
<td>0.020*</td>
</tr>
<tr>
<td>Memory $Z$-Scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>49</td>
<td>0.272</td>
<td>0.604</td>
</tr>
<tr>
<td>High/Low Cortisol</td>
<td>49</td>
<td>3.444</td>
<td>0.070</td>
</tr>
</tbody>
</table>

CABG was also added to the secondary analysis along with the other covariates in a post-hoc analysis. As shown in Table 20, there were no differences in executive function over 1 year between the low and high cortisol groups with CABG as an additional covariate ($F_{1,48}=0.231$, $p=0.633$).
Table 20: Coefficients of a post-hoc analysis detecting differences in composite executive function change Z-scores over 1 year between low and high cortisol groups with CABG as an additional covariate.

<table>
<thead>
<tr>
<th>Source</th>
<th>df error</th>
<th>F</th>
<th>p-value (significance at p≤0.05)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>48</td>
<td>1.492</td>
<td>0.201</td>
</tr>
<tr>
<td>Intercept</td>
<td>48</td>
<td>0.024</td>
<td>0.876</td>
</tr>
<tr>
<td>Gender</td>
<td>48</td>
<td>0.906</td>
<td>0.346</td>
</tr>
<tr>
<td>VO$_2$ peak</td>
<td>48</td>
<td>0.719</td>
<td>0.401</td>
</tr>
<tr>
<td>Depression</td>
<td>48</td>
<td>1.161</td>
<td>0.287</td>
</tr>
<tr>
<td>Baseline Composite Executive function Z-Scores</td>
<td>48</td>
<td>5.493</td>
<td>0.023*</td>
</tr>
<tr>
<td>CABG</td>
<td>48</td>
<td>0.021</td>
<td>0.885</td>
</tr>
<tr>
<td>High/Low Cortisol</td>
<td>48</td>
<td>0.231</td>
<td>0.633</td>
</tr>
</tbody>
</table>
4 Discussion and Conclusion

The present study is the first study to look at the association between long-term secretion of cortisol and cognition in CAD patients. Using a novel method of measuring the long-term activity of the HPA axis by measuring cortisol in the hair, this study found differences in composite memory change Z-scores between patients in the low and high cortisol groups when controlling for gender, VO₂ peak, depression and baseline composite memory scores. Patients in the high cortisol group showed less improvement in memory performance over 1 year compared to those with low cortisol. No differences between the 2 groups were found in terms of executive function. This study is important as it was looking at the relationship between persistently elevated levels of cortisol and cognitive function in the CAD population, which if found, can lead to the development of new therapeutic approaches to alleviate cognitive symptoms in CAD.

4.1 Cortisol and Memory

As mentioned before, various studies have shown that reductions in hippocampal volume in CAD patients are associated with memory impairment (De Kloet et al. 1991, Hickie et al. 2005, Kohler et al. 1998, Muller et al. 2005, Mungas et al. 2001, Pruessner et al. 2005, Pruessner et al. 2007, Sapolsky et al. 1987, Sapolsky 1996, Starkman et al. 1992, Stoub et al. 2005, Tischler et al. 2006, Wolf et al. 2002). Various studies have also indicated that CAD patients show altered cortisol secretion, which results in higher cortisol concentrations compared to healthy controls (Alevizaki et al. 2007, Krantz et al. 1996, Matthews et al. 2006, Mittleman et al. 1995, Mittleman et al. 1993, Rosmond and Bjorntorp 2000, Troxler et al. 1977). Due to the high density of GRs in the hippocampus, cortisol is hypothesized to contribute to hippocampal atrophy and may be responsible for the memory impairment seen in CAD patients (Figure V).
While it was hypothesized that worse memory performance would be found in patients with high cortisol compared to those with low cortisol, all patients improved over 1 year on the CVLT-II used to assess memory performance. A similar pattern of improvement was also seen with composite memory change Z-scores over 1 year. In addition, when controlling for gender, VO2 peak, depression and baseline composite scores, cortisol was not a significant predictor of changes in composite memory change Z-scores over 1 year in all patients.

However, when patients were divided into low and high cortisol groups, it was found that patients in the high cortisol group showed less improvement in memory performance over 1 year compared to patients with low cortisol when controlling for gender, VO2 peak, depression and baseline composite memory scores. Both VO2 peak and baseline composite memory scores were also significant in the model indicating some contribution to memory performance over 1 year.

Upon further exploration, it was found that higher VO2 peak at baseline was significantly associated with more improvement in composite memory change Z-scores over 1 year in the high cortisol group, which is consistent with previous findings (Barnes et al. 2003). Exercise has consistently been shown to be an important indicator of improved cognitive function (Berkman et al. 1993, Blomquist and Danner 1987, Brown et al. 2010, Chodzko-Zajko 1991, Chodzko-Zajko and Moore 1994, Elsayed et al. 1980, Etnier et al. 1999, Gordon et al. 2008, Hill et al. 1993, Hillman et al. 2006, Larrieu et al. 2002, McAuley et al. 2004, Podewils et al. 2005, Rogers et al. 1990, Singh-Manoux et al. 2005, Weuve et al. 2004, Yaffe et al. 2001, Yaffe et al. 2009). Physical activity has been associated with lower risks of cognitive impairment and development of dementia (Laurin et al. 2001), more specifically AD (Friedland et al. 2001). These findings support the suggestion that the exercise intervention in these patients might be associated with improved memory performance and might protect the brain against insults from elevated cortisol concentrations.
As mentioned before, prolonged exposure to stress hormones leads to a reduction in neuronal health and survival, especially in the hippocampus (De Kloet et al. 1991, Magri et al. 2000, Sapolsky et al. 1987, Sapolsky 1996, Sapolsky et al. 1990). In response to chronic stress, neurons undergo morphological changes, which have a deleterious impact on brain plasticity (Joels and de Kloet 1989, McEwen 2000, McEwen 2001). Chronic stress also decreases adult hippocampal neurogenesis and animal studies have also shown that cortisol can decrease the availability of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in the HPC (Schaaf et al. 2000). Regular aerobic exercise has been shown to down-regulate the HPA axis (Russo-Neustadt et al. 2000) and various studies have shown lower plasma cortisol and ACTH responses to CRH in trained individuals (Lehmann et al., Luger et al.). Exercise also promotes neurogenesis (Adlard et al. 2005, Uda et al. 2006, van Praag et al. 1999, van Praag et al. 1999, van Praag et al. 2005), angiogenesis (Black et al. 1991, Gomez-Pinilla et al. 1998, Isaacs et al. 1992), and synaptic plasticity, particularly in the HPC (Bugg and Head 2009). As a result, exercise may protect against the deleterious effects of cortisol by reducing the activity of the HPA axis, and increasing neuronal survival and plasticity.

In addition, exercise may also directly increase neurotrophic factors. Animal studies suggest that exercise leads to an increase in neurotrophic factors such as BDNF (Berchtold et al. 2001, Neper et al. 1995, Neper et al. 1996, Vaynman et al. 2004) and insulin-like growth factor (IGF-1) (Ang and Gomez-Pinilla 2007, Cotman and Berchtold 2002). Exercise has been demonstrated to be a key determinant of BDNF levels in the hippocampus (Russo-Neustadt et al. 2000) and known to increase the hippocampal expression of BDNF in animal models (Adlard et al. 2005). BDNF has been shown to promote growth and survival of neurons in various brain regions, especially neurons involved in hippocampal memory mechanisms (Ghosh et al. 1994,
Kang et al. 1997) due to the fact that BDNF is present in highest concentrations within the hippocampus (Hofer et al. 1990).

IGF-1 is also a survival factor for brain cells and participates in neuronal growth and differentiation in the brain (van Praag et al. 1999, van Praag et al. 2005). Exercise increases the uptake of circulating IGF-1 in the brain (Anderson et al. 2002), which leads to an increase in BDNF gene expression (Ding et al. 2006). Since IGF-1 induces BDNF expression (Fabel et al. 2003), an increase in IGF-1 levels is also associated with increased proliferation of neurons in the hippocampus and is therefore, important for exercise-stimulated neuroprotection and neuronal plasticity (Trejo et al. 2002, Trejo et al. 2001).

Furthermore, recent evidence suggests that exercise might positively affect the structural integrity of the brain. Various cross-sectional studies have found that exercise, particularly, aerobic fitness training protects against the effects of age and significantly increases brain volumes in the prefrontal, parietal and temporal cortices and anterior white matter tracts (Colcombe et al. 2003, Colcombe et al. 2006).

Various longitudinal studies and randomized controlled trials also provide evidence of an association between physical activity, cardiovascular fitness and cognitive function (Abbott et al. 2004, Bakken et al. 2001, Laurin et al. 2001, Richards et al. 2003, Sturman et al. 2005). Positive associations between cardiopulmonary fitness and medial temporal regions in healthy older adults (Gordon et al. 2008) and in individuals with AD (Honea et al. 2009) provide early evidence of the benefits of exercise in aging populations. As a result, the exercise intervention in the study may have contributed to the improvement in cognitive function over 1 year by activating mechanisms that increased neuronal survival and neurogenesis in vulnerable regions of the brain, which worked to counter the damaging effects of cortisol.
However, less improvement in memory function seen in the high cortisol group compared to the low cortisol group indicates that exercise may not have been able to fully protect against the effects of cortisol. It is possible that the brain and cognitive reserve of the individuals in the high cortisol group may have been compromised due to the prolonged exposure to elevated concentrations of cortisol. Brain reserve can be defined as the neuroprotective brain capacity, which can be induced by chronic mental and physical activity (Katzman 1993, Mortimer 1997, Satz et al. 1993, Schmand et al. 1997, Stern et al. 1995, Valenzuela 2008, Valenzuela and Sachdev 2006, Valenzuela and Sachdev 2006) while cognitive reserve can be defined as the increase in cognitive function and enhanced complex mental activity (Andel et al. 2006, Le Carret et al. 2005, Mortimer et al. 2005, Richards and Deary 2005, Scarmeas and Stern 2003, Scarmeas and Stern 2004, Scarmeas et al. 2003, Staff et al. 2004, Stern 2002, Stern et al. 2005, Whalley et al. 2004).

Both brain and cognitive reserve have been shown to be protective against dementia, especially AD and other brain disorders (Caamano-Isorna et al. 2006, Valenzuela 2008, Valenzuela and Sachdev 2006, Valenzuela and Sachdev 2006). Exercise has been shown to contribute to the development of brain and cognitive reserve through the promotion of neurogenesis (Kempermann 2008, Kempermann et al. 1997, Steiner et al. 2008, Suh et al. 2009, van Praag et al. 1999, van Praag et al. 2005) and synaptic plasticity (Duffy et al. 2001, Foster et al. 1997, Foster and Dumas 2001) as well as increasing the expression neurotrophic factors, especially BDNF (Ickes et al. 2000, Torasdotter et al. 1998, Zhu et al. 2006) as mentioned above. Therefore, it is possible that cognitive function and the ability to improve over 1 year in the CAD patients in this study may have been dependent on the balance between opposing mechanisms activated by cortisol and exercise.
4.2 Cortisol and Executive Function

In this study, no differences between the low and high cortisol groups in terms of executive function were found. As mentioned before, aging is associated with reductions in white and grey matter in the prefrontal cortex (Raz and Rodrigue 2006), which subsequently leads to deficits in executive functions such as task coordination, planning, goal maintenance, working memory, and task switching (Daniels et al. 2006).

However, executive functions appear to be the most amenable to the positive effects of exercise (Erickson and Kramer 2009, Kramer and Erickson 2007). A meta-analysis also suggested that executive function was the most improved cognitive domain after an aerobic exercise intervention (Colcombe and Kramer 2003). In a study by Kramer et al. (Kramer et al. 1999), participants were randomly assigned to either an aerobic exercise intervention or control (stretching and toning). Cognitive testing was conducted before and after 6 months in the exercise program. Over the 6-month period, participants in the aerobic training group showed enhanced executive function compared to the control group. From this study, it can be concluded that 6 months of moderate aerobic exercise can reliably reverse age-related cognitive decline and that the benefits of aerobic exercise were disproportionately greater for executive tasks suggesting some specificity of the effects of exercise on certain brain regions. As a result, since the prefrontal cortex is more sensitive to the effects of exercise, the CR regimen in the CAD patients in both the low and high cortisol groups might have been discriminately protective against the damaging effects of prolonged exposure to cortisol in the PFC leading to no detectable change between the 2 groups in terms of executive function.
4.3 Post-hoc analyses

Since the incidence of CABG was significantly different between the low and high cortisol groups, CABG was added as an additional covariate in the ANCOVA models assessing differences in memory performance and executive functions over 1 year between patients in the low and high cortisol groups in post-hoc analyses. CABG was not a significant predictor of either the differences in memory or executive function between the low and high cortisol groups. The presence of CABG in the ANCOVA model caused the difference in composite memory Z-scores between the 2 groups to trend towards significance while the presence of CABG in the model had no effect on the differences between the 2 groups in terms of executive function.

The association between CABG and cognitive deficits are somewhat unclear to date due to differences in populations studied, the neurocognitive tests used, the timing of post-operative methods, surgical techniques and most importantly, the definition of cognitive impairment (Boodhwani et al. 2006, Bruggemans et al. 1995). There is evidence suggesting that CABG may impair cognitive function including deficits in learning and memory, reduced ability to pay attention and concentrate, and psychomotor speed (Hammeke and Hastings 1988, Newman et al. 2001, Shaw et al. 1987). Studies have found that the incidence of decline is highest at discharge when it is approximately 50 to 80%, reduced to 20-50% at 6 weeks and 10-30% at 6 months (Blumenthal et al. 1995, Newman et al. 1995, Selnes et al. 1999). In addition, 42% of patients that show cognitive impairment at discharge show long-term cognitive impairment up to 5 years after CABG (Newman et al. 2001). These results suggest that early cognitive changes after CABG are clinically important and indicators of long-term cognitive impairment although the reasons for this late decline remain unclear.
Various studies suggest that surgery-related factors might be responsible for post-operative cognitive dysfunction in CABG patients (Grigore et al. 2002, Hammon et al. 1997, Moody et al. 1995, Taylor 1998). However, some studies show that there are no differences in cognitive function between patients that have undergone CABG and patients with non-surgical CAD (McKhann et al. 2005) or healthy controls (Selnes et al. 2003) suggesting that the majority of the cognitive deficits found in these patients may be due to the underlying pathology rather than a result of the surgical procedure (Boodhwani et al. 2006). It is possible that the stress of the surgical procedure in these patients may unmask underlying pathologies that may put the patients at risk of long-term cognitive decline (Selnes et al. 2007).

As mentioned before, Yin et al. (Yin et al. 2007) suggested that atrophy and neuropsychological dysfunction may be due to toxic effects of irregular cortisol secretion in CAD patients. In that study, 75% of patients undergoing CABG showed a disturbed circadian rhythm and deficits were also seen in short-term memory, visual perception and motion, executive functions and response inhibitory capacity. Roth-Isigkeit et al. (Roth-Isigkeit and Schmucker 1997) also indicated irregularities in HPA-axis function postoperatively. Although ACTH levels were shown to return to preoperative basal levels, cortisol levels remained elevated up to 2 days after CABG surgery suggesting that the negative feedback mechanism responsible for inhibiting ACTH secretion and responsiveness to ACTH is altered during the early post-operative period. These results further support the suggestion that cognitive deficits seen in CABG patients could be due to underlying pathology, especially irregularities in HPA axis function rather than the surgical procedure.

Furthermore, the ANCOVA models used to detect differences in memory and executive function performance included more than the allowed covariates. Since there were 56 patients included in the study, according to the 10 to 1 rule, only 5 predictors could be included in the
model. The trend towards significant differences in memory function between the low and high cortisol groups suggests that with a larger number of patients, the original differences in memory performance over 1 year between the low and high cortisol groups might be evident. Therefore, even though it has been suggested that CABG is a predictor of cognitive deficits, this was not the case in the present study.

4.4 Limitations

One of the limitations of this study is sample size. While enough patients were recruited to have adequate power for the study, a larger number of patients would have allowed for the inclusion of more covariates. As mentioned before, various CVRFs such as hypertension and dyslipidemia are associated with both cognitive function and cortisol concentrations (Bernick et al. 2005, Elias et al. 2003, Gianaros et al. 2006, Gold et al. 2005, Gunstad et al. 2006, Hajjar et al. 2002, Kilander et al. 1997, Morales et al. 2006, Muldoon et al. 2004, Sparks et al. 2006, Vicario et al. 2005, Wolf et al. 2004). In addition, there is evidence that CABG is also associated with cognitive dysfunction (Hammeke and Hastings 1988, Newman et al. 2001, Shaw et al. 1987). Including these potentially important factors as covariates in the ANCOVA models would have further clarified the relationship between elevated cortisol concentrations and changes in memory and executive function in this study.

In addition, in this study, we did not know the details of the CABG surgeries. Some studies suggest that patients that have undergone off-pump CABG surgery have better cognitive outcomes compared to those that have undergone on-pump CABG surgery up to 6 months after surgery (Stroobant et al. 2002, Van Dijk et al. 2002, Zamvar et al. 2002) while other studies have not found a difference in cognitive function between the 2 groups after these 6 months (Jensen et al. 2008, McKhann et al. 2005, Takagi et al. 2007). It has been suggested that these contradictory
findings with respect to cognitive changes after on and off-pump CABG surgery could be due to the large variation in the CABG procedure within and between institutions (Bruce et al. 2008). Thus, details of the CABG surgeries of patients included in this study would have led to more clear conclusions about the contribution of CABG to cognitive outcomes.

Moreover, depressed patients were not excluded in this study due to a small sample size. Hyperactivity of the HPA axis is established in depressed subjects, who also show various impairments in memory and executive function (Alexopoulos et al. 1997, Elderkin-Thompson et al. 2003, Krishnan et al. 1997, Lockwood et al. 2002, Nebes et al. 2001). These cognitive deficits might be attributed to elevated cortisol concentrations in these individuals as one study demonstrated 53% increase in area under the curve in depressed subjects and a corresponding 6% decrease in hippocampal volume (O'Brien et al. 2004). Since depressed patients show elevated cortisol concentrations, they were largely included in the high cortisol group, which might have affected the final results although there were no differences in the incidence of depression between the 2 groups. Also, some of the depressed patients were taking anxiolytics and antidepressants, which are known to reduce HPA-axis activity (Holsboer and Barden 1996). This may have also affected the cognitive outcomes being measured in the study.

4.5 Recommendations for Future Studies

This study found that elevated levels of cortisol in CAD patients may be responsible for the cognitive impairment seen in these patients. These results suggest that dysregulation of the HPA-axis and the resulting elevations in cortisol concentrations are associated with cognitive impairment in CAD patients and indicate that additional research in this area will further elucidate the mechanisms underlying cognitive symptoms in CAD.

Since reductions in brain volumes of susceptible brain regions such as the HPC and the
PFC are associated with cognitive impairment (Lupien et al. 2005, McEwen 2000), an interesting next step of this study would be to measure changes in hippocampal and frontal cortical volumes over time through magnetic resonance imaging and determine whether higher long-term cortisol concentrations are associated with brain volume changes and whether these changes are subsequently associated with cognitive function.

In addition, inter-individual differences in basal cortisol concentrations are partly due to the environment and partly due to genetics (Burleson et al. 2003, Huizenga et al. 1998, Petrides et al. 1994, Stone et al. 2001). MR gene variants have been shown to change cortisol signaling in-vitro (Arai et al. 2003) while GR gene variants change HPA axis reactivity after a dexamethasone (synthetic GC) suppression test (used to test HPA axis response to negative feedback) or a psychosocial stressor (Huizenga et al. 1998, van Rossum et al. 2002, van Rossum et al. 2003), suggesting that genetic variants of both the MR and the GR can possibly determine individual stress-responsivity and coping style, affecting vulnerability to disease (Derijk de Kloet 2008). Therefore, tissue hypersensitivity determined by MR and GR gene polymorphisms might be increasing the susceptibility of brain regions to damage in CAD patients possibly leading to neurocognitive dysfunction. As a result, in the future, subjects can be genotyped for MR and GR gene variants that have been correlated with CAD and contributing CVRFs to assess the contribution of genetics to increased sensitivity to cortisol and neuropsychological function.

Sauve et al. showed that hair cortisol concentrations were associated with 24-hour urine cortisol suggesting the relevance of hair cortisol as a biomarker of chronic stress (Sauve et al. 2007). To build on these findings, hair cortisol measurements can also be compared to alternative metrics of long-term cortisol such as area under the curve obtained from multiple salivary or blood cortisol measurements (Lupien et al. 1996) with respect to cognitive outcomes and
changes in brain volumes in order to determine the predictive value of these cortisol measures in future studies.

4.6 Implications for future Research

Since cognitive impairment in CAD patients has been associated with significant decline in long-term quality of life (Kiessling and Henriksson 2005), increased unemployment and mortality (Gale et al. 1996, Kiessling and Henriksson 2005), it is important to find new therapeutic interventions that might slow the progression of cognitive decline, prevent and reduce disability and improve the overall quality of life of subjects with CAD. Cortisol antagonism would be expected to reduce neuronal cell death in vulnerable regions of the brain preserving cognitive function in at risk populations such as the CAD population. However, cortisol plays a fundamental role in the stress response and has important effects on cellular growth and differentiation as well as immune modulation. Antagonizing cortisol may risk adrenal insufficiency at times of stress and therefore is not a feasible option (Hughes et al. 2008).

On the other hand, cardiac rehabilitation involving an exercise regimen as secondary prevention can be beneficial in alleviating cognitive symptoms as shown in this study and prevent progression to dementia and AD. Various prospective observational studies found a reduced risk of AD and other forms of dementia for individuals that were physically active (Abbott et al. 2004, Barnes et al. 2003, Laurin et al. 2001, Podewils et al. 2005, Scarmeas et al. 2003). For example, in a study by Larson et al. (Larson et al. 2006), individuals over 65 that exercised more than 3 times a week were 34% less likely to be diagnosed with dementia compared to those who exercised less than 3 times a week over a mean period of 6 years.

In addition, pharmacological agents that can act as pharmacomimetics of exercise are also promising options to alleviate cognitive symptoms in CAD patients. Exercise has been
shown to increase the levels of CBG, which binds to cortisol and inhibits its biological actions (Droste et al. 2003). Therefore, agents that can increase CBG levels would potentially reduce the damaging effects of cortisol on vulnerable brain regions that are also associated with cognitive impairment (Stranahan et al. 2009). Non-steroidal GR ligands including quinol-4-ones (Regan et al. 2006) and aryl pyrazoles (Clackers et al. 2007) have also been recently developed and may possibly mimic the effects of exercise.

Furthermore, other pathways involved in neuronal plasticity and neurogenesis can also be targeted. Drugs activating the downstream pathways of BDNF can mimic the effects of BDNF (Boado et al. 2007, Fletcher and Hughes 2006, Fletcher and Hughes 2009) and increase neuronal plasticity and neurogenesis in the brain. Also, IGF-1 protein has been administered previously for multiple disorders and is able to cross the blood-brain barrier (Clark and Robinson 1996). Hence, administering IGF-1 in at-risk patients may upregulate BDNF expression and promote neurogenesis and neuronal plasticity as mentioned before.

4.7 Conclusion

This study found that patients with elevated cortisol concentrations showed less improvement in memory performance over 1 year. Prolonged exposure to elevated cortisol concentration may compromise regions of the brain that mediate memory and executive function resulting in cognitive changes. It has been suggested that vascular pathology may be one of the underlying causes of the changes occurring in AD and vascular dementia (Singh-Manoux et al. 2003). Cognitive decline also occurs with aging making aging CAD patients at a higher risk of cognitive impairment and subsequent dementia. Since cognitive impairment is a predictor of functional decline (Boyle et al. 2004, Jefferson et al. 2006), and mortality (Gale et al. 1996, Kiessling and Henriksson 2005), the identification of possible mechanisms through which
impairment occurs may be of profound clinical significance. The findings of this study suggest that further research in this area is warranted and that the development of pharmacological agents that can alleviate cognitive symptoms in CAD patients might be beneficial.


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List of Publications and Abstracts

Peer Reviewed Publications


Submitted Publications


Published Abstracts

Appendices

Appendix A - REB Approval
August 4, 2010

Dr Paul Oh
Toronto Rehabilitation Institute
TRI - Rumsey Centre (Cardio)
347 Rumsey Road
Toronto, ON
M4G 1R7

Dear Dr. Oh:

**RE: TRI REB # 07-080**

The Role of Cytokines and Kynurenine in the Mood and Cognitive Symptoms of Coronary Artery Disease

The above-named study has received continued approval from the Toronto Rehab Research Ethics Board until the expiry date noted below. If the study is expected to continue beyond the expiry date, you are responsible for ensuring the study receives re-approval. The REB must also be notified of the completion or termination of this study and a final report provided.

If, during the course of the research, there are any serious adverse events, changes in the approved protocol or consent form or any new information that must be considered with respect to the study, these should be brought to the immediate attention of the Board.

Sincerely,

[Signature]

[ ] Paul Oh MD, MSc, FRCPC
Chair, Research Ethics Board
Toronto Rehabilitation Institute

[ ] Ann Heesters BEd, BA, MA, PhD(ABD)
Vice Chair, Research Ethics Board
Toronto Rehabilitation Institute

July 25, 2008
Date of Initial REB Approval

July 25, 2011
Expiry Date of REB Approval

TRI REB conforms with the **Tri-Council Policy Statement Ethical Conduct for Research Involving Humans** and **Ontario Privacy Legislation PHIPA**
Appendix B - Study Consent Form
The Role of Cytokines and Kynurenine in the Mood and Cognitive Symptoms of Coronary Artery Disease

Subject Information and Consent

Information for Subject:

You are being invited to participate in a research study conducted at the Toronto Rehabilitation Institute and Sunnybrook Health Sciences Centre under the supervision of the above investigators. Participation is completely voluntary. A description of this study follows.

1) Description and purpose of the trial:

This study is being conducted to determine if there is a relationship between cognition (in particular, memory) or mood and levels of certain chemicals in the blood that may be related to cell health (i.e. cytokines and kynurenine). We are also interested in a structure in the brain that is important for memory function, called the hippocampus, which may be affected by these chemicals. It is currently unknown whether these blood or brain markers are related to memory or mood in subjects with heart disease.
2) Study Details:

Subjects entering rehab undergo fitness assessments using the exercise test and they are given an exercise prescription. The rehab program also provides resources to assist with diet, mood and other aspects of lifestyle modification. The rehab team also collects information such as demographic data (age, gender and diagnoses) and what medications you are using and administers a green questionnaire pertaining to mood symptoms that you might be experiencing. If you choose to participate in this study, we will notify your TRI physician and your TRI-Cardiac rehab team of your involvement.

This study will not interfere with any of the usual care received in rehab or from your family physician. If you agree to participate in this study, we would ask to review information that you have provided to the rehab team including demographic data (age, gender and diagnoses), what medications you are using, the results of your exercise test, and to review the green questionnaire pertaining to mood symptoms that you completed. If you agree to participate, you will be asked to undergo an assessment with a trained researcher that will take about 1 hour. This will include assessments of memory and thinking speed, and a screening interview for depression or substance abuse. With your permission, we would notify your Toronto Rehabilitation team if the results of this interview suggest you might benefit from the resources that are already in place to assist subjects showing signs of depression. These resources include the opportunity to make appointments with a psychologist on staff at the Toronto Rehab.

If you choose to participate in this research study, it will be necessary to collect some samples for analysis. We will collect a small (20 mg) hair sample from the top of your head close to the scalp for measuring your levels of cortisol, a common stress hormone. A swab of your inner cheek will be collected for a genetic test related to cholesterol. Finally, about 34 ml (2 ⅓ tablespoons) of blood will be collected following an overnight fast, which will be analyzed only for markers of inflammation. All samples will be identified by a unique number only (not you name). All samples will be analyzed for only these markers and then destroyed once the assay is complete.

As part of your participation in this research study, you will have two Magnetic Resonance Imaging (MRI) brain scans at Sunnybrook, one around the time you begin the rehabilitation program and one near its completion. If you decide not to complete rehabilitation, with your permission, we would still contact you to arrange a follow-up scan. MRI scanning is a method of making pictures of the brain using magnetic waves. This gives us information about the structure of the brain, including the hippocampus. The scan will take less than one hour. The scan will involve lying still with a simple device placed around your head/neck. The MRI machine looks like a long narrow tube. Even though the tube is open, some people feel confined in small places. If this bothers you, please notify us. You may end the scan at any time by telling the MRI staff, who will be able to see you, hear you, and communicate with you at all times. When MRI pictures are taken, it is normal for the MRI machine to make noises (banging and clicking). You will be asked to wear earplugs or headphones for your comfort during the scan.

The scans performed in this study are not optimized to find clinical abnormalities. However, on occasion, a member of the research team may notice a finding on a scan that seems abnormal, in which case we will consult a neurologist as to whether the abnormality merits further investigation.
If this is done, a member of the research team will contact you. The decision as to whether to proceed with further clinical examination or treatment will be yours; with your permission, we would forward your results to your family physician for further follow-up.

We would also like to repeat the cognitive and mood assessments around the time of your 6-month exercise test and again when you complete rehabilitation. These two follow-up assessments will be similar to the initial assessment, and they will also take approximately 60 minutes. Around the time of the final assessment, we would like to repeat the research MRI scan. This will take an additional hour. Further blood and hair samples will be taken at these two followup assessments for analysis of the same stress-related and blood chemicals. If you decide not to continue with the rehabilitation program, then with your permission, we would contact you around these times to arrange visits to Sunnybrook Health Sciences Centre to complete the study. If you consent, we would like you to continue with this study even if you decide not to complete rehab. However, if you decide that you no longer wish to participate in this study, you can withdraw at any time and your care at the rehab program will not be affected and we will not contact you to arrange further visits to Sunnybrook.

3) Benefits:

You will not benefit directly from participation in this study, but knowledge gained from this study may be helpful to subjects in the future in the management of depressive symptoms or cognitive changes resulting from heart disease. The testing in this study, including MRI scanning, is of no diagnostic value so individual results will not be made available. However, the group results will be published, and if you wish, we will be happy to forward to you a copy of any publication(s) that may arise from this work.

4) Risks:

**Blood draw:** As with any blood test, you may experience slight discomfort or bruising.

**MRI:** The effects of magnetic fields in an MRI scanner have been extensively studied. You may be bothered by feelings of confinement (claustrophobia), and by clicking and banging noises made by the magnet during the procedure. Because MRI uses strong magnetic fields, you may not participate in MRI scans if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. An interview will be conducted prior to your MRI to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye. There is a small chance that we may observe something abnormal on your MRI. If this is the case, we will inform you, which may cause you anxiety, and suggest the need for further tests.

**Cognitive testing:** You may experience very mild temporary mental stress as a result of memory or timed tasks (each task takes less than 2 min).

**Mood interview:** The mood interview takes less than 10 min. Some questions may prompt you to consider unpleasant thoughts.
Hair sample collection: Collection of the hair sample will take less than 30 seconds. This small amount of hair will not be disfiguring.

Cheek swab: The cheek swab involves a rubbing of a dacron swab against the inside of your cheek 10 times. No risk is anticipated.

5) Costs:

You will be reimbursed $23.00 for parking expenses each time you visit Sunnybrook to complete an MRI scan.

6) Participation/Termination:

Your participation in this study is voluntary. Thus, if you do not wish to take part in this study or wish to withdraw at any time after commencing the study, you may do so, and your care will not be affected in any way.

7) Confidentiality:

Your identity in this study will be treated as confidential. Certain Toronto Rehabilitation Institute and Sunnybrook research staff involved in this study may need to review your medical chart. We will have access to your medical chart for information on: blood pressure, heart rate, medications, mood symptoms and cardiopulmonary assessments for 1 year. Data will be kept in password protected computer files and locked filing cabinets in a secure area until 6 months after study completion. Data will only be accessed by the study investigators and by the research staff under their direct supervision. On all data collected for this study, you will be identified only by a unique assigned number. In the future, only the study investigators and certain members of the Research Ethics Board will have access to your study information. The only legal limit to confidentiality during this study will be if you disclose intention to harm yourself or others.

8) Contacts:

If you have any questions about this study or for more information you may contact the Study Co-ordinator, Walter Swardfager (416-480-6100 x3185), Dr. Krista Lanctôt (416-480-6100 x2241) or Dr. Paul Oh (416-597-3422 x5263).

Should you have any questions about your rights as a research subject, you may contact the Chair of the Toronto Rehabilitation Institute Research Ethics Board at (416) 597-3422x3730 or the Sunnybrook Health Sciences Centre Research Ethics Board at (416) 480-4276.
The Role of Cytokines and Kynurenine in the Mood and Cognitive Symptoms of Coronary Artery Disease

Consent to Participate in this Study:

I, (subject’s name) __________________________________ have read the above information and fully understand the nature and the purpose of the study in which I have been asked to take part. The explanation I have been given has mentioned both the possible risks and benefits of the study. I understand that I will be free to withdraw from the study at any time without affecting my subsequent treatment by my doctor in any way. I voluntarily consent to participate in this study.

_________________________________
Name of Subject (typed or printed)

_________________________________
Signature of Subject                     Date

_________________________________
Name of Person obtaining Consent

_________________________________
Signature of Person obtaining Consent   Date