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Basal and Stress-Reactive Cortisol Levels in Patients with Inflammatory Bowel Diseases

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TABLE OF CONTENTS

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CONTRIBUTION OF AUTHORS TO THE MANUSCRIPT

"Choosing your friends: Blunted cortisol reactivity to psychological stress in patients with Inflammatory Bowel Disease and their friends when compared to non-friends"

Benjamin W.B. Lai assisted in writing the study protocol, managed the bulk of literature searches and analyses, coordinated the study and tested the majority of the participants, undertook the statistical analyses and wrote the first draft of the manuscript.

Mehereen Wadiwalla and *Kamala Pilgrim* assisted in the coordination of and the actual testing of study participants.

Sonia J. Lupien designed the study and wrote the original study protocol. Dr. Lupien contributed significantly to the management of literature searches and analyses, undertook and approved the final statistical analyses. Dr. Lupien was the principal coordinator of the TSST protocol at the Douglas Hospital Research Centre and also approved the final draft of the manuscript.

Gary E. Wild designed the study and assisted in writing the study protocol. Dr. Wild provided all of the IBD patients of the study and performed medical evaluations on all participants to ensure eligibility of participation. Dr. Wild contributed to the management of literature searches and approved the final draft of the manuscript.

ABSTRACT

Inflammatory Bowel Diseases (IBD) are chronic remitting-relapsing diseases of the gastrointestinal tract. Psychological stress has long been suspected to play a role in the pathogenesis and symptom exacerbation of IBD. The goal of this study was to examine whether or not there exist any differences in the stress reactivity in patients with IBD when compared to healthy controls. Salivary cortisol was employed as a biomarker to assess the activity of the hypothalamic-pituitary-adrenal (HPA) axis – normally activated upon the perception of a psychological stressor – in IBD patients and healthy controls. Ten quiescent IBD patients were recruited and each was asked to identify a healthy age- and gender-matched friend to participate in the study to act as a control. In addition, 10 healthy age- and gender-matched subjects who were not friends of the IBD patients were recruited as a second control group. All subjects completed 4 psychological questionnaires: the NEO Personality Five Factor Inventory; the Rosenberg Self-Esteem Scale; the Perceived Stress Scale; and the Coping Inventory for Stress Situations. Subjects were asked to sample their saliva at home in order to discern their basal salivary cortisol secretion pattern. As well, subjects underwent a psychosocial stress protocol, the Trier Social Stress Test (TSST), during which saliva samples were obtained to determine their stress-reactive cortisol levels. Results from this study indicate that IBD patients and their friends are significantly more agreeable in personality and showed a blunted HPA response to the TSST, when compared to healthy non-friends. These results point to a distinct personality profile and a possible social support phenomenon among IBD patients and their friends, which may modulate HPA reactivity to psychological stress and contribute to the differential cortisol response observed.

RÉSUMÉ

Les maladies inflammatoires de l'intestin (MII) sont des conditions chroniques du tube digestif qui provoquent de l'inflammation intermittente. Le stress psychologique est impliqué dans la pathogénie et l'aggravation des symptômes des MII. Le but de cette étude était d'examiner s'il existe des différences dans la façon dont les patients avec MII réagissent au stress psychologique comparativement à des individus en santé. Les niveaux de cortisol salivaire de patients avec MII et d'individus en santé ont été mésurés afin d'examiner l'activité de l'axe hypothalamo-hypophysio-surrénalien (HHS) – normalement activé par la perception d'un stress psychologique. Dix patients avec MII en rémission ont été recrutés; chaque patient ont identifié un(e) ami(e) en bonne santé, de même sexe et âge, pour participer à titre de sujet-contrôle. De plus, 10 sujets sains additionnels, appariés quant à l'âge et au sexe, et n'étant pas ami(e)s des patients avec MII, ont été recrutés dans un deuxième groupe contrôle. Tous les participants ont rempli 4 questionnaires psychologiques: l'Inventaire des 5 dimensions de personnalité NEO; l'Échelle de l'estime de soi de Rosenberg; l'Échelle du stress perçu; et l'Inventaire de stratégie d'adaptation pour les situations stressantes. Les participants ont pris des échantillons de salive à la maison pour déterminer le profil de sécrétion de base de cortisol. Tous les participants ont été soumis à un protocole de stress psychosocial, le Trier Social Stress Test (TSST), au cours duquel des échantillons de salive ont été pris afin de déterminer les niveaux réactifs de cortisol. Les résultats de cette étude indiquent que les patients avec MII et leurs amis ont une personnalité significativement plus agréable que les sujets non-amis du groupe contrôle. Les patients et leurs amis ont démontré également une hyporéactivité cortisolémique au stress psychosocial par rapport aux sujets non-amis du groupe contrôle. Cette étude propose un profil de personnalité unique chez les patients avec MII et leurs amis en santé, ainsi qu'un phénomène de soutien social parmi eux. Ces effets pourraient moduler la réactivité de l'axe HHS aux stresseurs psychosociaux et expliquer les différences de réponse cortisolémique chez les participants de cette étude.

LIST OF ABBREVIATIONS

ACTH:	Adrenocorticotropic hormone
CD:	Crohn's Disease
CDAI:	Crohn's Disease Activity Index
CISS:	Coping Inventory for Stressful Situations
CRF:	Corticotrophin releasing factor
dBP & sBP:	Diastolic blood pressure & systolic blood pressure
DHRC:	Douglas Hospital Research Centre
EIA:	Enzyme immunoassay
GC:	Glucocorticoid
GI:	Gastrointestinal
GR:	Glucocorticoid receptor
gr:	Type 2 glucocorticoid receptor
GRE:	Glucocorticoid response element
HF:	Healthy friend control subjects
HPA:	Hypothalamic-Pituitary-Adrenal axis
HR:	Heart rate
IBD:	Inflammatory Bowel Diseases
IBS:	Irritable Bowel Syndrome
IL:	Interleukin
MR:	Type 1 glucocorticoid receptor (mineralocorticoid receptor)
MUHC:	McGill University Health Centre
NC:	Non-friend control subjects
NEO:	NEO Personality Five Factor Inventory
PSS:	Ten-Item Perceived Stress Scale
RIA:	Radioimmunoassay
RSE:	Rosenberg Self-Esteem Scale
sAA:	Salivary alpha-amylase
SNS:	Sympathetic nervous system
TSST:	Trier Social Stress Test
UC:	Ulcerative colitis

1. INTRODUCTION

Inflammatory Bowel Diseases (IBD), encompassing Crohn's Disease (CD) and Ulcerative Colitis (UC), are chronic remitting-relapsing inflammatory diseases of the gastrointestinal (GI) tract. It is generally characterized by increased GI permeability, secretion, and mucus release, as well as a hyperactive immune response to numerous environmental factors in genetically susceptible hosts.[1] With approximately 170,000 affected individuals, Canada has one of the highest incidence rates of IBD in the world.[2]

The severity of IBD can be assessed using various activity indices, such as the Crohn's Disease Activity Index (CDAI) and the Rachmilewitz Index for Ulcerative Colitis,[3, 4] which take into account the frequency and severity of physical symptoms experienced by patients, as well as endoscopic, histological and hematological findings. When in clinical remission (i.e. during quiescent phases of the disease), IBD patients generally do not experience any physical gastrointestinal symptoms, although evidence of persistent low-grade gastrointestinal inflammation have been reported.[5, 6] Common symptoms during the active phases of IBD include diarrhea, weight loss, abdominal pain, fatigue, and GI bleeding. Many of these active symptoms are similar to those seen in patients with Irritable Bowel Syndrome (IBS), which refers to a group of non-communicable, persistent or recurring physical symptoms, including gut motility changes and visceral hypersensitivity, with no apparent associations with any anatomical, histological or biochemical abnormalities.[7] Despite some shared symptomologies, IBD and IBS are distinct disorders, with the former involving gastrointestinal inflammation and ulcerations, and the latter usually not associated with any pathophysiological manifestations.

Although the exact etiologies of IBD are still unknown, a host of genetic and environmental contributing factors have been identified – illustrating the complex and multi-factorial nature of the disease. Despite the enormous progress in IBD-related genetics research, which led to the identification of such genes as CARD15/NOD2 in the pericentromeric region of chromosome 16, little is known

about the ways in which environmental factors contribute to the pathogenesis of IBD.[8, 9]

Psychological stress, as defined as any perceived threat to the homeostasis of an organism,[10] has long been suspected by clinicians and patients to exacerbate IBD symptomologies.[11, 12] Indeed, the chronic nature of the disease and the subsequent physical, psychological and social constraints imposed on those who suffer from IBD mean that patients are often faced with the classic elements that characterize a stressful situation, namely novelty, unpredictability, threat to the ego, loss of control, and social evaluative threat.[13, 14] Several recent human studies lend support to the contention that psychological stress can lead to relapses in IBD.[15-17] Levenstein et al. (2000), for example, found that long-term, but not short-term, perceived stress significantly augments the risk of symptom exacerbation in UC patients up to several years following the initial assessment of perceived stress.[18] Conclusions from these studies are concordant with those employing experimentally induced forms of IBD in murine models, which have demonstrated that environmental stressors contribute to the reactivation of gut inflammation.[19-22] For example, in a study by Collins et al. (1996) in which acute colitis was previously induced in rats, the subsequent administration of a repeated social stressor after recovery from the colitis resulted in alterations of enteric nerve function and increased the levels of certain inflammatory parameters, when compared to rats that did not undergo induced colitis.[23] Such studies provide compelling evidence that repeated stressors lead to IBD symptom exacerbation, and highlight the role of stress-related mediators, such as stress hormones, in modulating immune and GI functions in IBD. Due to this psycho-physiological interplay, perhaps it would be most useful to employ a biopsychosocial approach to further examine the role of psychological stress in IBD.[24]

1.1 The Neuroendocrine Response to Psychological Stress

The common stress response involves the rapid activation of the sympathetic branch of the autonomic nervous system, which leads to the systemic release of catecholamines (epinephrine and norepinephrine) from chromaffin cells

of the adrenal medulla. While norepinephrine is primarily produced as a neurotransmitter by sympathetic ganglia and neurons, the main endocrine product of the adrenal medulla – due to the presence of N-methyltransferase – is epinephrine. Norepinephrine is normally produced in much smaller quantities in the adrenal medulla and only spills over into the general circulation after intense activation of the sympathetic nervous system. Catecholamines released into the systemic circulation binds adrenergic receptors in target tissues to bring about enhanced arousal, metabolic changes, as well as cardiovascular changes.[25, 26]

The hypothalamic-pituitary-adrenal (HPA) axis also becomes activated shortly after the activation of the sympathetic nervous system in response to stress, thus culminating in the release of corticotropin releasing factor (CRF) from the parvocellular portion of the hypothalamic paraventricular nucleus. CRF stimulates the release of adrenocorticotropic hormone (ACTH) from the adenohypophysis. In turn, ACTH triggers the systemic release of glucocorticoids (GCs), which are cholesterol-derived steroid hormones, from the zona fasciculata of the adrenal cortex. The majority of GCs released into the systemic circulation are quickly bound to carrier proteins; only 5% remain unbound in the blood. Most of the circulating GCs are bound to corticosteroid-binding globulin (CBG; also known as transcortin), which is a specific glucocorticoid-binding α_2-globulin normally expressed at a concentration of 3-4mg/dL. In addition, some circulating GCs bind albumin instead of transcortin, albeit less tightly.[26]

1.2 The Binding of Glucocorticoids to Their Receptors

The lipophilic properties of GCs allow them to passively diffuse into target cells to bind intracellular glucocorticoid receptors (GRs) in the cytosol, after which the hormone-receptor complex translocates into the nucleus to effect transcriptional changes through interactions with glucocorticoid response elements (GREs). Only free-flowing circulating GCs can enter cells, thus GCs bound to serum carrier proteins must first dissociate from these proteins before diffusing across the plasma membrane of target cells. In fact, GCs can freely diffuse into all cells although they are only sequestered within cells that express

GRs, which generally have at least a 10-fold greater affinity for GCs than serum carrier proteins.[27] GCs bind two types of intracellular GRs: Type I (mineralocorticoid) receptors (MR) and Type II (glucocorticoid) receptors (gr), with the former having a higher affinity for GCs than the latter.[26]

1.3 Metabolic Functions of Glucocorticoids

The critical role that GCs play in glucose metabolism under the fasted state is well established. The metabolic actions of GCs serve to increase and subsequently maintain normal concentrations of glucose in the blood. These include the stimulation of gluconeogenesis, especially in the liver, whereby glucose is synthesized from non-hexose substrates, such as amino acids and lipids. GCs also promote the mobilization of amino acids from extrahepatic protein sources while reducing extrahepatic protein synthesis, thus providing additional substrates for gluconeogenesis. In addition to facilitating glucose synthesis, GCs inhibit glucose uptake and utilization by muscles and adipose tissues. GCs stimulate lipolysis; the resulting fatty acids are used for energy production in extrahepatic tissues, whereas the glycerol released are utilized in gluconeogenesis. Orexigenic effects of GCs resulting from the stimulation of hypothalamic neuropeptide Y have also been documented.[28]

1.4 Glucocorticoid's Role in the Regulation of HPA Activity

In addition to their metabolic functions, circulating GCs regulate HPA activity through negative feedback mechanisms in the central nervous system during stress-reactive conditions to inhibit further CRF and ACTH release at the level of the hypothalamus and the adenohypophysis, respectively,[26] thus restoring physiological status quo in the HPA axis once stressors are no longer present. Under basal (no-stress) conditions, endogenous GC secretion follows a circadian pattern, whereby the highest level of secretion occurs around the time of awakening (acrophase) and gradually decreases throughout the day until the lowest level of secretion is reached at night time (nadir).[29] The morning awakening GC peak has been shown to be a reliable measure of adrenal cortical

function and basal HPA activity.[30, 31] Pharmacological studies employing selective antagonists of Type I and Type II GRs demonstrated that Type I receptors are largely responsible for maintaining the low basal GC levels during the circadian trough, while Type II receptors, in conjunction with Type I receptors, play a significant role in regulating GC levels during the circadian peak and in times of acute stress.[32-35]

1.5 The Immune System

There are two major types of immune responses. The *innate immune response* – associated with such cells as polymorphonuclear neutrophils and monocytes – launches the same response regardless of the nature and identity of the pathogen, and does not form specific immunologic memory. Conversely, the *adaptive immune response*, associated with lymphocytes and antigen-presenting cells, launches immune responses with specific immune players and mediators depending on the nature of the infection. Moreover, immunologic memory is formed. The adaptive immune response can be classified into the CD4+ T_{H2} lymphocyte-associated *humoral immunity*, leading to the clonal selection and expansion of specific B-lymphocytes as well as the formation of antibodies, and the CD4+ T_{H1} lymphocyte-associated *cell-mediated immunity*, which leads to the activation of specific CD8+ cytotoxic T-lymphocytes. The cell-mediated response generally does not result in antibody production, but it is associated with the production of proinflammatory cytokines, such as TNFα and INFγ; the ultimate targets of this type of response are host cells that have either become cancerous or been infected by intracellular pathogens, such as the majority of viruses. The humoral response, on the other hand, directly targets pathogens that reside outside of host cells, such as the majority of bacteria.[36]

1.6 The Role of Glucocorticoids in the Immune System

Cortisol, the main effector GC product of the HPA axis in humans, can be used as a biomarker of HPA axis functioning both under basal and experimentally induced stressful (reactive) conditions, thus allowing for the assessment of stress

reactivity in an individual. Such an assessment could be performed in IBD patients to yield vast insight into the activity of the HPA axis in response to environmental stressors within the context of a chronic inflammatory disease. Indeed, this is a crucial aspect worthy of consideration, since proinflammatory cytokines are able to enhance the bioavailability of cortisol by inhibiting the synthesis of CBG, as well as to upregulate the activity of the HPA axis itself.[37] This leads to an increased release of adrenal GCs, which in turn, have anti-inflammatory properties.[38, 39] In fact, GCs have been found to downregulate inflammatory cytokine expression through modulating the NF-κB system and AP-1 activity, which are proinflammatory cytokine transcription factors. As well, GCs inhibit cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS), thereby reducing the level of secondary inflammatory signals, while they decrease histamine release by mast cells.[40-44] High concentrations of GCs have also been found to promote a shift towards a T_{H2}-type immune response (not associated with proinflammatory cytokine production) and upregulate anti-inflammatory mediator synthesis.[40, 45, 46] In sum, through the actions of these important mediators, an immune-endocrine feedback loop is established.

In light of the anti-inflammatory properties of GCs, one would expect HPA activation resulting from psychological stress to attenuate inflammatory processes. Many research findings to date, however, seem to suggest the contrary. Kiecolt-Glaser et al. (2003), for example, found that older adults who provide long-term care for a spouse with dementia exhibit a much greater annual increase in IL-6 levels (a proinflammatory cytokine) when compared to non-caregivers.[47] Further, Maes et al. (1998) found evidence of a T_{H1} (proinflammatory) shift in medical students exposed to academic stress and stress-induced anxiety, as reflected in lipopolysaccharide-induced cytokine production in whole blood taken one day before an academic examination.[48] Conceivably, other variables and mechanisms are at play and/or there may exist a disconnect between the psychoneuroendocrine-immune cross-talk. The gut, an organ with extensive neural innervations that expresses receptors for a plethora of

neuroendocrine and immune mediators, provides a perfect milieu for the pathophysiological presentations of such a systems disconnect.[49]

The interplay between the neuroendocrine and the immune systems is widely documented, as both systems form intimate effector junctions and share a range of important ligands and receptors.[50, 51] As an illustration of this interplay, recent studies have found that glucocorticoids play a role in regulating toll-like receptor (TLR) expression, suggesting a role for glucocorticoids in the innate immune response.[52] Moreover, findings that adrenal cortical cells express cytokines, cytokine receptors, major histocompatibility complex (MHC) class II, as well as TLRs, further confirm the existence of a significant immune-adrenal cross-talk network.[53, 54] The deleterious effects of psychological stress on the immune system provide yet another clear example of the neuroendocrine-immune relationship. In addition to the promotion of inflammation, chronic stress and persistent HPA axis activation are associated with the downregulation of natural killer cell and cytotoxic T-lymphocyte activities – both of which play a vital role in immune surveillance and tumour detection.[55] In consequence to these immunosuppressive effects, stress has been associated with increased risk of cancer and autoimmune disease development, as well as viral infections.[55-57]

1.7 Psychological Stress and Gastrointestinal (GI) Physiology

In terms of GI function, psychological stress increases intestinal permeability via the promotion of transcellular and paracellular transport across the intestinal epithelium, thus encouraging the infiltration of luminal antigens into the intestinal mucosa to activate previously sensitized T-lymphocytes.[58-61] The predictive value of increased intestinal permeability in IBD symptom relapses was demonstrated in CD patients and its potential as a surrogate marker of intestinal inflammation has been proposed.[6] In addition, decreased water absorption, reversal of net Na^+ and Cl^- fluxes in the jejunum, increased colonic mucin release and goblet cell depletion – all of which could precipitate to GI damage and inflammation – have been associated with psychological stress.[62, 63] Recently, Reber et al. (2006) exposed subordinate mice to chronic social stress, and

observed clear physiological and histological evidence of adrenal insufficiency and colonic inflammation in these animals, thus further confirming the deleterious effects of psychological stress on gastrointestinal functioning.[64]

Psychological stress has also been implicated in the inhibition of upper GI motility, while it potentiates lower GI motor functioning.[65] Moreover, changes to the intestinal flora and increased bacterial adherence in response to psychological stress have been demonstrated in animals.[66, 67] Taken together, these studies provide strong evidence for the existence of a reciprocal neuroendocrine-immune and brain-gut relationship, whereby psychological stress elicits a neuroendocrine response, which subsequently modulates an array of immune and gastrointestinal parameters. In addition to these top-down processes, the resulting physical symptoms can heighten the psychological stress experienced by IBD patients, thereby creating a bottom-up effect, which could further exacerbate the inflammatory status of the GI tract. Indeed, these findings emphasize the importance of employing a biopsychosocial approach to more clearly define the role of psychological stress in the treatment and management of such chronic inflammatory diseases as IBD.

1.8 Psychological Stress in IBD

Given the chronic, unpredictable and uncontrollable nature of symptom relapses, IBD patients are likely to be under continual stress. This premise is bolstered by reports of patients grieving after receiving their diagnosis,[68] as well as studies indicating a lower quality of life in IBD patients compared to healthy control subjects, especially those with severe symptoms and low levels of social support.[69-71] Whether or not this chronic state of psychological stress is reflected through higher HPA axis activity in IBD patients under everyday (basal) conditions has yet to be examined. Furthermore, it remains to be determined whether the HPA axis of IBD patients would react differently from healthy individuals upon encountering acute psychosocial stressors. As reviewed by Fries et al. (2005), studies examining chronic stress found that many patients with stress-related disorders, such as chronic fatigue syndrome and fibromyalgia,

exhibit a hypofunctional HPA axis.[72] Such a shift in HPA activity may have been elicited by chronic or excessive stimulation of the HPA axis earlier in life due to severe psychological stress, which results in receptor downregulation at the level of the HPA axis, decreased biosynthesis or depletion of HPA hormones, and/or enhanced sensitivity of the HPA axis to glucocorticoid negative feedback. As Fries et al. suggested, these changes may be part of an allostatic mechanism employed by the body to help counteract and prevent the deleterious physiological effects of a constantly hyperactive HPA axis.

With regards to IBD, evidence of reduced levels of hypothalamic CRF mRNA expression, as well as the uncoupling of the sympathetic nervous system and the HPA axis have been found.[73, 74] Since exogenous sources of GCs are often prescribed to IBD patients as a mainstay treatment to induce remission, this is likely to further contribute to changes in HPA activity in this patient population. For example, Desramé et al. (2002) found that IBD patients on long-term corticosteroid therapy demonstrated suppressed activity of the HPA axis.[75] Such recent experimental findings have prompted some to hypothesize that IBD patients are likely to exhibit a blunted HPA response to further inflammation and acute psychological stressors (i.e. having lower *stress-reactive* cortisol levels).[38] This is likely to be mediated by the affinity modification and downregulation of CRF and/or ACTH receptors caused by chronically elevated levels of proinflammatory cytokines in IBD, as many of these proinflammatory cytokines act as potent CRF and ACTH secretagogues.[76, 77] It is also possible that chronic elevation of proinflammatory cytokines could result in the downregulation of cytokine receptors in the brain, which in turn, could further reduce the sensitivity of the HPA axis to the upregulatory effects of proinflammatory cytokines. Moreover, studies have reported that long-term exposure to proinflammatory cytokines could lead to the malfunctioning of central noradrenergic and serotonergic neurotransmission systems.[78] Due to the important role that these neurotransmission systems play on the HPA axis, any damage to these neurotransmission systems could directly affect the activity and responsiveness of the HPA axis.[79, 80]

Studies by Stark et al. (2001 and 2002) revealed GC resistance in splenic macrophages when subordinate mice were exposed to chronic social disruption through the introduction of a dominant mouse into their cages.[81, 82] As a result, splenic macrophages of these subordinate mice were less sensitive to the anti-proliferative effects of corticosterone (the principal GC in rodents), and secreted more IL-6 in comparison to cells of non-stressed mice. Human studies on chronically-stressed individuals produced similar results – indicating diminished GC sensitivity in peripheral lymphocytes of individuals under chronic psychological stress, such as parents of cancer patients and elderly caregivers of dementia patients.[83, 84]

GC receptor (GR) downregulation and affinity modification in peripheral immune cells have been proposed as possible mechanisms of GC resistance due to chronic HPA activation caused by long-term stress exposure.[85] Specifically, Type II GRs are involved, since this is the primary GR subtype found in lymphoid tissues.[86] Given the important role that elevated cytosolic free Ca^{2+} concentration plays in the activation of murine lymphocytes in response to acute stress, it is possible that GR downregulation in peripheral immune cells could, at least in part, be Ca^{2+}-mediated.[87] Furthermore, Wild et al. (2003) demonstrated increased intestinal permeability, elevated mucosal TNFα levels and NF-κB expression, as well as reduced IκB expression in a subset of quiescent CD patients – all of which are indicative of GI inflammation despite the asymptomatic state of these patients.[6] This study illustrates that HPA activity under basal condition in quiescent IBD patients may not only be enhanced by chronic stress perception, but also by the possibility of persisting inflammation and elevated proinflammatory cytokine production even when these patients do not have any physical symptoms. This further contributes to GC resistance in peripheral immune cells via the upregulatory effects of proinflammatory cytokines on the HPA axis to result in greater GC production.

Taken together, one would expect increased proinflammatory cytokine production in peripheral immune cells of chronically stressed individuals, including IBD patients, despite already high levels of circulating GCs. These

cytokines should further upregulate HPA activity – leading to even higher circulating GC levels. Hence, one would expect a hypercortisolemic state in these individuals under *basal* conditions, especially those with a chronic inflammatory disease, such as IBD, who are prescribed GCs as a mainstay treatment for their condition. Exogenous sources of GCs, such as those prescribed to IBD patients, can decrease GC sensitivity even further. Such findings have already been made in asthmatic patients.[88]

Interestingly, Decorti et al. (2006) observed a higher frequency of a particular mutation in the second intron of the Bc/l gene in CD patients.[89] This particular Bc/l polymorphism has been reported to be associated with increased central and peripheral GC sensitivity and higher cortisol suppression to dexamethasone (a synthetic GC).[90] All this suggests that CD patients may actually be more sensitive to the effects of GCs, which seems to be in contrast to the research evidence reviewed above indicating GC resistance. It is important to note, however, that Decorti et al.'s study employed a small sample of participants and the IBD patients in the study were extremely young, with a mean age of 13.8 years (ranging from 1 to 45 years). Since the typical onset of IBD is in the second or third decade of life, at least some of the subjects in Decorti et al.'s study appear to be those suffering from early-onset IBD, which has been shown to present a distinct phenotype.[91] Hence, a higher incidence of Bc/l polymorphisms and the subsequent increased GC sensitivity might merely be a unique feature among those with early onset IBD. Surely, studies recruiting larger and more representative samples are required to confirm Decorti et al.'s findings.

Another plausible hypothesis concerning basal cortisol levels in IBD arose from findings in a study by Straub et al. (1998), which did not find any significant relation between serum cortisol levels and serum IL-6 levels or disease activity in UC patients.[92] These results seem to run counter to many studies to date, which have indicated a relationship between cortisol and proinflammatory cytokines. However, it must be emphasized that some of the patients in this study were receiving GCs for the treatment of active symptoms at the time of the study. Irrespective, a later study found that the mean serum cortisol concentration in

both active and inactive IBD patients were significantly lower than healthy control subjects – an intriguing finding, especially since serum TNF and IL-6 levels of active IBD patients in this study were elevated, which normally should increase cortisol secretion.[74] All this suggests a decline in the sensitivity of the HPA axis in response to increased levels of proinflammatory cytokines, thus leading to a hypocortisolemic state in IBD patients under *basal* conditions. Therefore, in contrast to the first hypothesis, which suggests an immune-endocrine disconnect at the level of the peripheral immune cells resulting in a hypercortisolemic state under basal conditions, this hypothesis posits a disconnect at the level of the HPA axis – leading to a hypocortisolemic state under basal conditions.

Clearly, much remains to be resolved. Moreover, despite suggestion of a blunted HPA (reactive) response in IBD patients when challenged by an acute stressor,[38] no studies have yet directly explored the reactivity of the HPA axis in the IBD patient population to psychological stress.

1.9 Personality and Stress Reactivity

Personality can be very broadly defined as the specific characteristics that an individual possesses, which accounts for the consistent patterns of feeling, thinking and behaving.[93] Early notable research in personality dates back to the nineteenth century when Sigmund Freud first formulated his psychoanalytic theory on personality. Since that time, much progress has been made and there is now convincing and unequivocal evidence of a link between personality characteristics and stress reactivity. Neuroticism, for example, which can be described as a tendency to become anxious, emotionally unstable, self-conscious and easily upset,[94] has been found to be implicated in an array of stress-related disorders, such as depression and pain syndromes.[95, 96] As well, neuroticism has been reported to be related to increased stress reactivity.[97, 98] This may be due to the fact that neuroticism is often associated with increased likelihood of experiencing stressful situations and interpreting an event as stressful.[99-101] With regards to IBD patients, several studies highlighted a greater prevalence of

neuroticism in this population.[12, 102] In turn, these patients may be more likely to interpret situations with more negative emotions, thus they experience greater levels of distress, which could increase their risk of symptom exacerbation and lower their health-related quality of life.[103]

Self-esteem, which can be characterized as the value that one places on oneself, is also a crucial predictor of stress reactivity, as those with low self-esteem were shown to have higher levels of cortisol when confronted by a stressor.[104, 105] Gruenewald et al. (2004) observed greater increases in salivary cortisol among healthy college students who experienced greater decreases in self-esteem and greater increases in shame when under social-self threat conditions in the laboratory psychosocial stress paradigm, the Trier Social Stress Test (TSST).[106] A recent study also revealed that self-esteem is an important predictor of health-related quality of life in adolescents with IBD.[107]

In addition, IBD patients – at least those that seek medical treatment for their symptoms and condition – have been found to be more introverted, exhibit greater obsessional symptoms, and are more dependent on others.[12, 108, 109] In fact, it has been suggested that patients with IBD often define their own identity in terms of approval given by key figures in their lives from whom they rely heavily on for advice, direction and protection from the demands of others.[109]

In a study by Simren et al. (2002), it was shown that quiescent IBD patients who experience Irritable Bowel Syndrome (IBS)-like symptoms have higher levels of anxiety and depression when compared to those without IBS-like symptoms.[70] What is more, anxiety was found to independently predict the presence of IBS-like symptoms in IBD. Owing to recent findings of low-grade GI inflammation in some IBS patients and subsequent contentions that IBS may be a mild form of IBD,[110-114] it seems plausible that IBD patients who experience IBS-like symptoms could share similar personality and psychological characteristics with IBS patients, such as high levels of distress and neuroticism.[115, 116] In fact, it has been found that both IBS and IBD patients score higher in an array of psychological variables, such as depression and social introversion, when compared to healthy non-patient controls, and that IBS patients score higher in the

same variables than IBD patients.[117] Mittermaier et al. (2004) also observed that in a population of patients with clinically inactive IBD, there were significant correlations between depression and anxiety scores with frequency of relapses.[17] Taken together, this suggests that GI symptoms observed in IBS patients and IBD patients exhibiting IBS-symptoms may be mediated by specific psychological and personality characteristics.

Support for the influence of personality variables on immune physiology has also been growing. For example, individuals scoring low on extraversion, high on optimism, and having high levels of hostility were shown to have greater levels of natural killer cell cytotoxicity.[118-120] Additionally, optimism was found to be associated with higher number of T-helper lymphocytes,[119] while negative affect has been demonstrated to attenuate natural killer cell activity,[121, 122] and result in lower levels of secretory immunoglobulin A antibodies detected in the saliva after the ingestion of foreign proteins.[123] As reviewed by Kiecolt-Glaser et al. (2002), negative emotions can directly influence the level of proinflammatory cytokine expression, as well as prolonging infections and delaying wound healing.[124] In short, aspects of personality give rise to wide individual variations in physiological and psychological reactivity to stressors, which in turn, differentially predispose individuals to pathophysiological states and disease processes.

Certainly, from a psychological standpoint, personality characteristics can influence the predominant coping style that one employs when exposed to a stressful situation, thus determining the level of perceived stress that one experiences. In turn, these factors can collectively affect physiological and disease outcomes. For example, it is well established that individuals high on neuroticism tend to employ lower levels of problem solving strategies in coping, but often cope with difficult situations via escape avoidance, self-blame, and emotional expression.[125-127] Those high on extraversion, however, are more likely to engage in more constructive coping strategies, such as active problem solving and cognitive reframing.[126, 127] It was also found that highly conscientious individuals were more likely to utilize problem solving coping strategies with

non-interpersonal stressors, when compared to those who scored lower in conscientiousness.[128]

The use of different coping strategies can, in turn, either enhance or diminish perceived stress levels, which could affect quality of life and the course of a disease. For example, confrontational coping, in which an individual objectively looks at a problem and attempts to tackle it from all sides, has been found to be associated with positive long-term health outcomes in patients who suffered from a myocardial infarction, slower disease progression in HIV+ men, as well as a better quality of life in patients hospitalized for chronic dermatologic diseases.[129-131] A five-year longitudinal study by Denollet et al. (2006) on patients with coronary heart disease revealed that those with a type D personality, defined as having a tendency to experience negative emotions and to inhibit these emotions during social interactions, had a significantly greater risk of experiencing a major adverse cardiac event than those with non-type D personalities, after adjusting for gender, age, and biomedical risk factors.[132] In terms of IBD, Smith et al. (2002) recently found that CD patients had a higher tendency to utilize maladaptive coping mechanisms, which include emotion-focused and avoidance coping, when compared to psoriasis patients (disease control group) and healthy controls.[133]

The aforementioned research findings bring to light the significance of personality traits in predicting coping styles, stress reactivity, immune functions, disease activity, quality of life and long-term health outcomes. Indeed, the fact that personality can influence such a diverse range of physiological and psychological variables warrant further investigation into the role of personality in the pathogenesis and/or symptom exacerbation in IBD. Perhaps there exists a distinct personality profile among IBD patients, which predispose them to their condition, determine the severity and frequency of symptom relapses, and predict future health outcomes and health-related quality of life.

1.10 Aims of Study & Hypotheses

The primary purpose of this study was to employ salivary cortisol as a biomarker to assess the functioning of the HPA axis in IBD patients in clinical remission under basal and stress-reactive conditions. These measurements were compared with those obtained from healthy control subjects to determine whether there exist any differences in the functioning of the HPA axis between these two groups of participants.

Apart from physiological parameters, several psychological variables were also measured, namely personality, self-esteem, perceived stress levels and coping style. Given the growing number of studies that indicate a role of psychological variables in the quality of life and symptom severity in IBD patients,[18, 134, 135] this study aimed to more clearly define any distinct personality characteristics in this patient population, as well as to better understand the role that personality plays in stress reactivity in IBD.

The two aforementioned hypotheses on basal cortisol in IBD patients were examined in this study: the former postulating a hypercortisolemic state, while the latter positing a hypocortisolemic state in IBD patients under basal conditions when compared to healthy control subjects. As well, the hypothesis of IBD patients having a blunted HPA response when they encounter an acute psychosocial stressor was tested.

2. GENERAL METHODOLOGY

2.1 Study Participants

This study was formally approved by the Faculty of Medicine Institutional Review Board of McGill University (see Appendix B for Research Ethics Board Certificate); all study participants provided written consent and all received monetary compensation for the time and inconvenience caused. All experimental procedures adhered strictly to human research guidelines. Ten IBD patients in clinical remission, defined as having a score of less than 150 in the Crohn's Disease Activity Index (CDAI) or a score of less than 5 in the Rachmilewitz Index for Ulcerative Colitis,[3, 4] were recruited from the McGill University Health Centre (MUHC) Gastroenterology Clinic at the Montreal General Hospital in Montreal, Quebec, Canada. Eligible IBD patients had to have a confirmed diagnosis for at least six months, between 18 and 50 years of age, and be fluent in either English or French. Patients were approached about this study during their regular medical visit to the clinic.

Each consenting IBD patient was asked to identify a healthy friend (HF) matched for age and gender to participate in the study to act as a paired control subject. We have chosen to use friends of IBD patients as control subjects because of the ease with which patients could find an age- and gender-matched friend who was willing to participate in the study. Moreover, friends are more likely to share similar environments, thus reducing the levels of environmental heterogeneity and ensuring a better pair-controlled study. In fact, patients' friends and family members are often used as control subjects in many IBD clinical and genetics studies, thus using friends in this study would allow for better and easier comparisons with the majority of the existing research on IBD. However, we later realized that using friends as control subjects might introduce new biases into the study, as friends might be more likely to share similar interests, attitudes, and personalities. Therefore, we have recruited a second group of healthy control subjects (NC) through the McGill Classifieds website (www.mcgill.ca/classified) – each was age- and gender-matched for a particular IBD participant in the study.

All participating subjects were free of psychotropic medication for the past one year, and exogenous glucocorticoids for at least six months, as recent use of these medications may affect the stress response.[136] As well, none of the participants had a history of cardiovascular, respiratory, renal and psychiatric disorders. Pregnant women were excluded from the study. All subjects underwent an initial medical evaluation at the MUHC Gastroenterology Clinic by Dr. Gary Wild to ensure remission status in IBD patients, as well as the general well-being and good health in all participants.

2.2 Psychological Measures

2.2.1 NEO Personality Five Factor Inventory (Short-Form)

This 60-item questionnaire measures the degree of each of the five personality dimensions: Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.[137] Neuroticism refers to an individual's propensity to experience negative emotional states; extraversion describes an individual's preferences for social settings; openness is the dimension that represents such characteristics as tolerance to novel experiences and broadmindedness; agreeableness is the dimension that describes interpersonal attitudes and behaviours; conscientiousness refers to the tendency to be reliable, organized and self-disciplined.[138] Subscores were generated for each of the five dimensions. Each item probes the subject's agreement on statements relating to aspects of personality, and is scored on a five-point Likert Scale, ranging from "strongly agree" to "strongly disagree". Cronbach's alpha values for the subscales range from 0.86 to 0.90.[139]

2.2.2 Rosenberg Self-Esteem Scale (RSE)

Self-esteem can be broadly defined as the value that one places on oneself; it is the answer that one provides when answering the question, "Am I any good?" This 10-item uni-dimensional scale allows for the assessment of an individual's personal worth, self-confidence, self-respect and self-depreciation.[140] Items in this questionnaire probe the subject's level of agreement to statements concerning

self-worth and self-evaluation. Each item is scored on a four-point Likert scale, ranging from "strongly agree" to "strongly disagree". High final scores indicate high levels of self-esteem. This scale achieved a Cronbach's alpha value of 0.80.[141]

2.2.3 Ten-Item Perceived Stress Scale (PSS)

This is a well-established measure of the degree to which one perceives one's life situations as stressful in the past month.[142] The items probe the frequency in which subjects had to handle specific stressful situations or feelings in the last month. The reliability of this scale has been tested and validated in community populations, and has been shown to achieve a Cronbach's alpha value of 0.86.[143] Each item is scored on a four-point Likert Scale, ranging from "never" to "very often". The total sum of this scale provides a reliable indication of the subjects' perceived stress level; the higher the score, the greater the level of perceived stress.

2.2.4 Coping Inventory for Stressful Situations (CISS)

This 48-item questionnaire measures three aspects of one's coping style: tasking-focused coping, which has to do with dealing with the problem at hand; emotion-focused coping, which is when one concentrates on the emotions that the problem at hand generates rather than dealing with the problem itself; and avoidance coping, which refers to evading the problem altogether.[144] Each item in the questionnaire presents a coping strategy whereby subjects are to rate on a five-point Likert scale (ranging from "not at all" to "very much") the frequency in which they employ each of the coping strategies. A score is generated for each of the three aspects of coping. The Cronbach's alpha value for the three coping style scores range from 0.82 to 0.90.[145] Incidentally, neuroticism has been found to be negatively associated with task-focused coping and positively associated with emotion-focused coping, while conscientiousness is negatively associated with avoidance coping.[144] Task-focused coping is generally related to adaptation and good health.

2.3 Saliva Sampling

As mentioned before, cortisol, being the end-product of the HPA axis, can be a used as a biomarker to gauge the level of activity of the HPA axis and to determine the stress reactivity in subjects. In this study, saliva samples were taken in order to assess cortisol levels. In contrast to painful, stressful and cumbersome blood sampling techniques, saliva sampling is a reliable, convenient and non-invasive method of assessing circulating levels of cortisol that are not bound to carrier proteins (i.e. bio-available cortisol).[146] Due to recent reports of lot-to-lot variation found in salivettes, which are devices that contain a piece of polyester material for subjects to chew on so that their saliva could be absorbed into the material, collection of pure spit was used.[147] For each sampling, subjects were asked to deposit approximately $1cm^3$ of saliva, excluding foam, via a small section of plastic straw (1cm) into a 107x25mm saliva sampling tube (Sarstedt, Ville St-Laurent).

2.4 Salivary Cortisol Concentration Determination

All saliva samples were maintained at -20°C until the time of cortisol concentration determination. All samples were assayed in duplicates. Basal saliva samples of IBD patients and control friends were analyzed using radioimmunoassay (RIA) in Dr. Michael Meaney's laboratory at the Douglas Hospital Research Centre (DHRC) using kits from DSL (Diagnostic System Laboratories, INC, Texas, USA) with small modifications. Total binding and non-specific binding typically range between 47-63% and 0.5-1.5%, respectively. The intra-assay and inter-assay coefficient of variation for these studies are 4.6% and 5%, respectively. The limit of detection of the assay is 0.01μg/dL.

Basal saliva samples of control non-friends, as well as stress-reactive saliva samples of IBD patients, control friends and control non-friends, however, were analyzed using enzyme immunoassay (EIA) in Dr. Gary Wild's laboratory at the Montreal General Hospital using kits from Salimetrics LLC (State College, PA, USA). The intra-assay and inter-assay coefficient of variation for these studies are 3.5% and 5.1%, respectively. The limit of detection is 0.003μg/dL.

Although samples were first analyzed using RIA, it was later realized that EIA is a high throughput system that is less cumbersome and less expensive than RIA. Hence, after careful consideration, it was decided that EIA should be used to analyze the rest of the saliva samples.

2.5 Calculation of an EIA/RIA Correction Quotient

As mentioned in the previous section, EIA was used to determine the cortisol concentration of all saliva samples taken from participants in all three groups (IBD, HF and NC) during the TSST protocol. However, for the home saliva samples (basal salivary cortisol), RIA was used for the samples of IBD patients and HF, whereas EIA was used for the samples of NC subjects.

In order to account for the use of two different assay techniques to quantify basal salivary cortisol (RIA for IBD patients and control friends; EIA for control non-friends), an EIA/RIA correction quotient was calculated. Even though such a calculation would vary as a function of the dose assayed and the position in the respective standard curve, it would nonetheless provide a rough indication of the level of discrepancy (and comparability) between the two assays.

Seven basal saliva samples were randomly selected from both the IBD and control friends groups, and were re-analyzed using EIA. For each of the samples, the salivary cortisol concentration obtained by EIA was divided by the salivary cortisol concentration obtained by RIA, thus giving an EIA/RIA correction quotient. The mean correction quotient (average of all 14 samples: 7 from IBD patients and 7 from control friends) was used to correct for all of the basal cortisol samples analyzed by RIA (IBD patients and control friends) by multiplying the mean correction quotient by the results obtained by RIA.

2.6 Basal Salivary Cortisol & Home Saliva Sampling

In order to discern subjects' diurnal cortisol secretion pattern under everyday (basal) conditions, they were asked to sample their saliva at home for three consecutive working days. Subjects were provided with proper instructions on saliva sampling, as well as saliva sampling tubes during the initial medical

evaluation at the MUHC. Subjects were also provided with a Saliva Sampling Record Sheet to chronicle the exact times and dates of each saliva sampling done at home. Subjects were instructed to keep all of their saliva samples in their home freezer until the day of their scheduled testing appointment at the DHRC, when they were to bring with them their home saliva samples and completed Saliva Sampling Record Sheet.

For each of the three consecutive working days, subjects sampled their saliva five times. Subjects were instructed not to sample their saliva on non-working days in order to prevent unusual late awakening times, which could have an impact on the normal circadian pattern. Samples were taken at the time of awakening, 30 minutes after awakening, 2pm, 4pm, and just before bedtime. Studies have shown that these sampling times give a reliable indication of subjects' diurnal cortisol secretion pattern.[148-150]

All subjects were instructed to refrain from eating, drinking (except water), and teeth brushing at least 30 minutes prior to sampling in order to prevent contamination of samples with food, drinks, and blood from micro-abrasions in the oral cavity, respectively. Subjects were also instructed to refrain from smoking prior to sampling, as nicotine has been found to activate the HPA axis under basal conditions.[151]

2.7 Trier Social Stress Test (TSST)

The Trier Social Stress Test (TSST) is a well-validated mild acute psychosocial stress paradigm under laboratory conditions that has been shown to reliably induce endocrine and cardiovascular stress response.[152] In fact, the TSST has been shown to reliably induce salivary cortisol levels by one- to three-fold.[146] As reviewed in Dickerson and Kemeny's (2004) meta-analysis of 208 studies on the effects of various acute stressors on cortisol response, the unpredictable and social evaluative elements of the TSST resulted in a greater cortisol response compared to other stress paradigms, such as noise exposure.[14]

The entire TSST protocol commenced with a Resting Phase, which is the phase before subjects were actually exposed to any psychosocial stress. Subjects

arrived for the testing session and were greeted by a research assistant. They sat in a testing room and remained calm during this phase, which lasted 50 minutes. In the last five minutes of the Resting Phase, subjects were told by the research assistant that they would have to give a speech in front of a "panel of experts" in a mock job interview scenario, lasting five minutes, during which the "panel of experts" would assess their verbal and behavioural skills by taking notes. Subjects were also told by the research assistant that they would be videotaped for later voice and behavioural analyses. Subjects were then allowed five minutes to mentally prepare for their speech; this constitutes the anticipation period in which subjects anticipated undergoing this psychologically stressful task (i.e. giving the speech). Subjects were then taken to another room by the research assistant and were greeted by the "panel of experts" dressed in white laboratory coats. The panel was always composed of one male and one female. The research assistant then leaves the room, thus signalling the start of the TSST/Psychosocial Stress Phase of the protocol.

During the speech (mock job interview) component of the TSST, subjects stood in front of the panel and had five minutes to explain to the panel why they thought they would be the ideal candidate for a particular job of their choice. The mental arithmetic component of the TSST then followed in which subjects were asked by one of the panel members to count aloud backwards from 2023 in 17-step sequences until they were told to stop. Subjects had to start from the beginning (i.e. 2023) should they miscalculate. Just as the speech component, the mental arithmetic component of the TSST lasted five minutes. Throughout the entire TSST, the panellists maintained a neutral demeanour in order to further accentuate the social evaluative threat element of the task. Subjects were then asked by the panel to leave the room; they were greeted by the receptionist outside of the TSST room, who escorted subjects back to the room that they were in during the Resting Phase. It is important to note that in reality, subjects were not videotaped and their performance during the TSST was not assessed by the panel members. These were merely staged in order to create an environment that would most likely induce a mild psychological stress response in subjects.

The Recovery Phase followed the TSST, which lasted 60 minutes, in which subjects remained calm in a room by themselves and were provided with landscape and nature magazines. Once the Recovery Phase was over, subjects were debriefed and compensated. Altogether, the testing appointment lasted approximately 130 minutes (Figure 1 in Appendix A shows the entire timeline of the TSST testing protocol at the DHRC).

Due to diurnal fluctuations of cortisol secretion, all TSST testing appointments took place on weekday afternoons (at 1:30pm) when baseline HPA activity was expected to be low, thus any stimulation of the HPA axis would be distinct from basal levels. All subjects were instructed to refrain from eating, drinking and smoking at least one hour prior to the testing session, which took place at the DHRC. IBD patients and control friends arrived to the DHRC for their testing session together on the same day at the same time. However, IBD patients and their friends were separated after their arrival and were tested in separate rooms by different research assistants. They were only reunited after the testing session was completed. Non-friend controls arrived to the DHRC for testing by themselves.

2.8 Stress-Reactive Cortisol

Ten saliva samples were obtained throughout the TSST protocol. Samples were obtained at 40, 25, 5 minutes, and immediately before the speech component of the TSST, as well as immediately, 10, 20, 30, 40, and 55 minutes after the TSST. Altogether, three saliva samples were collected during the Resting Phase (before the TSST), one saliva sample was taken immediately after the anticipation period and just prior to the speech component of the TSST, and six saliva samples were obtained during the Recovery Phase (after the TSST). This, therefore, allowed us to examine subjects' cortisol secretion pattern both before and after psychosocial stress exposure.

2.9 Hemodynamic Measurements

Systolic and diastolic blood pressure (sBP and dBP), and heart rate (HR), were measured using a digital blood pressure machine (LifeSource, Mulpitas, CA, USA) during the TSST testing appointment at the DHRC following the collection of each of the 10 saliva samples in order to obtain a crude indication of the sympathetic nervous system (SNS) activity in response to the TSST protocol. The SNS is activated rapidly but briefly in response to stress perception, followed by the slower but more sustained activation of the HPA axis. Studies have demonstrated the ability of the TSST to significantly alter these hemodynamic variables.[153, 154]

2.10 Experimental Protocol

Eligible quiescent IBD patients were approached by a research assistant during their scheduled medical visit to the MUHC Gastroenterology Clinic to participate in this study. Those who were interested were later contacted by another research assistant at the Douglas Hospital Research Centre (DHRC) by telephone, who gave further information about the study to the patients. Patients were asked to identify a healthy age- and gender-matched friend to participate in the study with them to act as a control subject. After the matched-control friend was identified, both friend and IBD patient were scheduled for an appointment at the MUHC to have a medical evaluation done to ensure the remission status of IBD patients and the general well-being and good health of all participants. During the medical evaluation, subjects were provided with home saliva sampling tubes, the Saliva Sampling Record Sheet, as well as the four psychological questionnaires to complete at home. Subjects were provided with detailed instructions on saliva sampling and storage techniques at home.

Both IBD patients and control friends were then scheduled to go to the DHRC for the TSST. Subjects were instructed to bring back their home saliva samples, their completed Saliva Sampling Record Sheet and questionnaires to their scheduled testing appointment at the DHRC. Although all subjects were provided with a copy of the Consent Form to read at home during the initial

medical evaluation, subjects did not sign the Consent Form until after their arrival at the DHRC for their scheduled testing appointment at the DHRC. The signing of the Consent Form was witnessed by a research assistant. Both IBD patients and their friends were asked to arrive to the DHRC together and were tested on the same day at the same time in separate rooms by different research assistants. IBD patients and their friends were reunited, debriefed and compensated at the end of the testing session.

Healthy control non-friend subjects were recruited through the McGill Classifieds website (www.mcgill.ca/classified); each was matched for the age and gender of one of the IBD patients in the study. Non-friend controls underwent the same experimental procedures as IBD patients and control friends. However, non-friend controls arrived to the DHRC for testing by themselves.

2.11 Statistical Analyses

Demographic information, including age and gender, were analyzed using chi-square analyses and one-way analyses of variance (ANOVA), as appropriate. Group (IBD vs. control friends [HF] vs. control non-friends [NC]) was used as the grouping variable for analyses of demographic information. Group differences in the total scores on the Rosenberg Self-Esteem Scale and the Perceived Stress Scale were analyzed with simple one-way ANOVAs, whereas total scores on the NEO Personality Five Factor Inventory (Short Form) and the Coping Inventory for Stressful Situations were analyzed using multivariate analyses of variance (MANOVA). Pairwise comparison tests were subsequently performed for significant results.

Results for basal salivary cortisol, stress-reactive salivary cortisol, and hemodynamic measures throughout the TSST protocol were each analyzed by two-way mixed design repeated measures ANOVA using Group (IBD vs. HF vs. NC) as the between-subjects variable and Sampling (saliva samples, blood pressure measurements or heart rate measurements) as the within-subjects variable. Significant interactions and/or main effects were subsequently analyzed using Tukey's Honestly Significant Difference post-hoc tests.

Post-hoc power analyses were performed for all statistically non-significant data, using $\alpha = 0.05$ and $\beta = 0.95$. In addition, fold stimulation was calculated for stress reactive cortisol to determine the magnitude of increase in salivary cortisol levels as a result of undergoing the TSST. Previous studies have shown that the TSST can reliably induce a one- to three-fold increase in salivary cortisol levels.[146] Fold stimulation was determined by taking the difference between the highest level of cortisol after the TSST (saliva sample 6; taken 10 minutes after the TSST) and the level of cortisol just before the TSST (saliva sample 4; taken immediately before the speech component of the TSST), and then dividing this difference by the level of cortisol just before the TSST (saliva 4). Hence,

$$\textbf{Fold Stimulation} = \frac{\textbf{(Peak Cortisol After TSST – Cortisol Before TSST)}}{\textbf{Cortisol Before TSST}}$$

$$= \frac{\textbf{(Sample 6 – Sample 4)}}{\textbf{Sample 4}}$$

3. RESULTS

3.1 PART I

3.1.1 *Stress Responsiveness*

Results concerning stress-reactive cortisol, fold stimulation, hemodynamic measures (systolic and diastolic blood pressures, and heart rate), as well as questionnaire scores are reported in a manuscript titled, "*Choosing your friends: Blunted cortisol reactivity to psychological stress in Inflammatory Bowel Disorder patients and their friends when compared to non-friend controls*", which was submitted to the Journal, **Psychoneuroendocrinology**, on January 31, 2007. The authors of the manuscript are: Benjamin W.B. Lai, Mehereen Wadiwalla, Kamala Pilgrim, Sonia J. Lupien, and Gary E. Wild. Please refer to the Preface for a description of author contributions to the manuscript and the proof of manuscript submission.

Since statistical non-significance (ps > 0.05) was found for hemodynamic measurements, Rosenberg Self-Esteem Scale (RSE) scores (Figure 2 in Appendix A), 10-Item Perceived Stress Scale (PSS) scores (Figure 3 in Appendix A), and Coping Inventory for Stressful Situations (CISS) scores (Figure 4 in Appendix A) post-hoc power analyses were performed on these data. For systolic blood pressure, a post-hoc power analysis for the between-subjects (Group) main effect revealed an effect size (f) of 0.184 and a power of 0.110. For diastolic blood pressure, the between-subjects (Group) main effect had an f = 0.265 and power = 0.187. In terms of the between-subjects (Group) main effect for heart rate, f = 0.192 and power = 0.116.

The effect size and the power for the RSE were 0.395 and 0.404, respectively; that of the PSS were 0.340 and 0.310, respectively; that of the CISS task-focused coping were 0.206 and 0.137, respectively; that of the CISS emotion-focused coping were 0.090 and 0.066, respectively; and that of CISS avoidance coping were 0.040 and 0.053, respectively.

Demographic information of all three groups of participants (IBD patients, healthy friend controls, and non-friend controls) is presented in Table 1 of Appendix A.

3.1.2 *Manuscript*

Choosing Your Friends: Blunted Cortisol Reactivity to Psychological Stress in Patients with Inflammatory Bowel Disease and Their Friends When Compared to Non-Friends

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This study was conducted in accordance with the Declaration of Helsinki for human subjects and was approved by the McGill University Faculty of Medicine Institutional Review Board. All study participants gave informed written consent prior to participation.

SUMMARY

Inflammatory Bowel Disease (IBD) is characterized by chronic relapsing and remitting of gastrointestinal inflammation and ulcerations. Given the unpredictable and uncontrollable nature of IBD, patients are likely to be under continual stress. It is, therefore, possible that acute psychological stressors could elicit a unique response of the hypothalamic-pituitary-adrenal (HPA) axis when compared to healthy individuals. Consequently, the goal of this study was to investigate the stress-reactive cortisol response of IBD patients, their healthy friends (HF), and healthy non-friend controls (NC) by using the Trier Social Stress Test (TSST), and to examine the association between stress response and personality. Ten quiescent IBD patients, 10 HF, and 10 NC subjects underwent the TSST, and salivary cortisol as well as blood pressures and heart rate measures were obtained before and after exposure to the TSST. All participants also filled out the NEO Personality Five Factor Inventory (Short Form), Rosenberg Self-Esteem Scale, Perceived Stress Scale, and Coping Inventory for Stress Situations. The results show that IBD patients and their friends present a blunted cortisol response to stress when compared to NC. Both IBD and HF showed less than a one-fold increase in salivary cortisol in response to TSST, compared to 1.75-fold in NC. Also, IBD and their friends scored significantly higher than NC in the Agreeableness factor of the NEO. These results suggest that the blunted cortisol response to stress observed in IBD patients might be more closely associated with their personality than with a true neuroendocrine disorder, and that IBD patients tend to choose their friends according to this personality pattern. The implications of these results for future studies on gastrointestinal disorders and stress are discussed.

Keywords: IBD, HPA axis, psychological stress, personality, agreeableness, social support

INTRODUCTION

The Inflammatory Bowel Diseases (IBD), comprising Crohn's Disease (CD) and Ulcerative Colitis (UC), are incurable chronic relapsing and remitting inflammatory diseases of the gastrointestinal (GI) tract.[1] The pathogenesis of IBD represents a complex interplay among genetic, immune and environmental factors.[2, 3] The normal microbial flora represents the key elements that define gene-environment interactions central to the pathogenesis of IBD. Far less is known about the manner in which other important environmental factors (e.g. smoking, diet, psychological stress) modify the expression of genes in susceptible individuals and modulate the intestinal immune system – both of which are key in determining the onset or relapse of IBD despite appropriate medical therapy.[4]

Psychological stress, as defined as any perceived threat to the homeostasis of an organism,[5] has long been suspected by clinicians and patients to exacerbate the symptoms of IBD and lead to clinical relapse.[6, 7] Conclusions from these studies are concordant with those employing experimentally induced forms of IBD in murine models, which have demonstrated that environmental stressors contribute to the reactivation of gut inflammation.[8-11]

Given the chronic, unpredictable and debilitating nature of IBD, patients are likely to be under continual stress. This may result in alterations of the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, whose end product in humans, cortisol, has potent anti-inflammatory and immunosuppressive properties.[12-16] Moreover, the inflammatory milieu of IBD patients could further promote the activity of the HPA axis, as inflammatory cytokines, such as IL-6 and TNF-α, have been shown to upregulate HPA activity and can subsequently increase circulating cortisol levels to suppress the ongoing inflammation in the GI tract.[17] It is plausible that the stress response in IBD in the setting of an acute psychosocial stressor might be different than that seen in healthy individuals. Support for this contention is derived from an earlier study which suggests that there might be an uncoupling of the sympathetic nervous system and the HPA axis in IBD patients.[18] As well, the chronically elevated levels of circulating inflammatory cytokines seen in IBD patients may lead to a blunting of the HPA

response to both inflammation and acute stress. Presumably, this could be due, at least in part, to the downregulation of CRF and adrenocorticotropic hormone (ACTH) receptors at the level of the HPA axis, since many inflammatory cytokines are potent CRF and ACTH secretagogues.[19, 20]

Moreover, dysfunctional regulations of the HPA axis has also been reported in chronic stress-related disorders, such as fibromyalgia and chronic fatigue syndrome,[21, 22] as well as other autoimmune inflammatory disease states, such as rheumatoid arthritis.[23, 24] It is, therefore, possible that IBD may be one of a group of disorders which individuals who experience chronic stress and exhibit a blunted HPA activity may be more susceptible to developing. Given the link between personality variables and some of the above mentioned conditions,[25-27] perhaps personality also plays a role in contributing to the onset and relapse of IBD.

Interestingly, personality variables appear to have an influence on stress reactivity in humans. For example, neuroticism has been previously found to be related to increased stress reactivity.[28, 29] Several studies have also demonstrated that individuals with low self-esteem secrete greater levels of cortisol when confronted with a stressor.[30, 31] More recently, Oswald et al. (2006) found that a higher level of neuroticism in women and a lower level of extraversion in men, as well as a lower level of openness in both sexes are associated with blunted cortisol response to the laboratory psychosocial stress paradigm, Trier Social Stress Test (TSST).[32] It has even been suggested that personality traits, such as neuroticism in CD, may be a more important determinant of quality of life than the extent or activity of the disease itself.[33]

The aim of this study was to employ salivary cortisol as a biomarker to directly investigate the stress-reactive HPA response of IBD patients, as well as to examine the association between HPA functioning and personality in this patient population. To our knowledge, no study has yet examined this association in IBD patients. There were two parts to this study. The first involved testing IBD patients and age- and gender-matched healthy control subjects who were friends

of IBD patients. The second part of this study involved testing age- and gender-matched healthy control subjects who were not friends with the patients.

PART ONE: METHODS

Inflammatory Bowel Disease Patients (IBD) & Healthy Friend Controls (HF)

Quiescent CD and UC patients (CDAI < 150; Rachmilewitz Index < 5) were recruited from the McGill University Health Centre Gastroenterology clinic at the Montreal General Hospital (MUHC) in Montreal, Quebec, Canada. Eligible patients had to have a confirmed diagnosis of CD or UC for at least six months, between 18 and 50 years of age, and be fluent in either English or French. Eligible IBD patients were approached by a research assistant about the participation of this study during their scheduled medical visit to the Gastroenterology clinic.

Each consenting IBD patient was asked to identify a healthy friend matched for their age and gender to participate in the study to act as control subjects. We have chosen to use healthy friends (HF) of IBD patients as control subjects in Part One of the study because of the ease with which patients could find an age- and gender-matched friend who was willing to participate in the study, thus ensuring a pair-controlled study. All recruitment and experimental procedures of this study adhered strictly to human research guidelines and approval was obtained from the McGill University Faculty of Medicine Institutional Review Board. Written informed consent was obtained from all participating IBD patients and control subjects.

A complete medical evaluation was performed on all subjects at the MUHC to ensure remission status for participating patients and general good health for all subjects. Both patients and controls were free of corticosteroids for at least six months, as recent use of corticosteroids may affect stress response.[34] All subjects were free of psychotropic medication for at least one year, and had no history of cardiovascular and respiratory diseases, as well as psychiatric disorders. Pregnant women were excluded from the study.

Personality Measures

NEO Personality Five Factor Inventory (Short-Form):

This 60-item questionnaire on personality is based on a five-point Likert Scale for each item ("strongly agree" to "strongly disagree"), and yields a score for each of the five personality subscales: Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness.[35] Cronbach's α for the subscales range from 0.86 to 0.92.[36]

Rosenberg Self-Esteem Scale (RSE):

This is a widely tested and validated 10-item uni-dimensional scale assessing personal worth, self-confidence, self-respect and self-depreciation of an individual.[37] Questions are scored on a four-point Likert Scale for each of the 10 statements ("strongly agree" to "strongly disagree"). This scale achieved a Cronbach's α of 0.80.[38]

Ten-Item Perceived Stress Scale (PSS):

This scale is a measure of the degree to which one perceives one's life situations as stressful in the past month.[39] The items probe the frequency in which subjects had to handle specific stressful situations or feelings in the last month. The Cronbach's α has been found to be 0.86.[40] Each item is scored on a four-point Likert Scale ("never" to "very often"). The higher the total score, the greater the level of perceived stress.

Coping Inventory for Stressful Situations (CISS):

This 48-item questionnaire measures three aspects of coping style: task-focused coping (i.e. dealing with the problem at hand), emotion-focused coping (i.e. concentrating on resultant emotions), and avoidance coping (i.e. trying to avoid the problem at hand).[41] A score is obtained for each of the three coping styles. Each item presents a coping strategy whereby subjects are to rate, on a five-point Likert scale ("not at all" to "very much"), the frequency in which they

employ each coping strategies. Cronbach's α for the three coping style scores range from 0.82 to 0.90.[42]

Trier Social Stress Test (TSST) and Stress-Reactive Salivary Cortisol Measures

In order to assess stress-reactive cortisol levels, subjects underwent the Trier Social Stress Test (TSST), which is a well-validated stress paradigm that can reliably induce endocrine and cardiovascular stress response.[43] In fact, the TSST has been shown to reliably induce salivary cortisol levels by one to three-fold.[44] As reviewed in Dickerson and Kemeny's (2004) meta-analysis of 208 studies on the effects of various acute stressors on cortisol response, the unpredictable and social evaluative elements of the TSST resulted in a greater cortisol response compared to other stress paradigms, such as noise exposure.[45] In brief, subjects were asked to give a five-minute speech about themselves in a mock job interview scenario where they were told that their verbal and paraverbal skills were assessed by a "panel of experts". Subjects were given five minutes to prepare for their speech (constituting the anticipation period). After the speech component, subjects performed a mental arithmetic task for another five minutes. Saliva samples were obtained 40, 25, five minutes, and immediately before the TSST, as well as immediately, 10, 20, 30, 40, and 55 minutes after the TSST. Due to the diurnal fluctuation of cortisol secretion, all testing sessions took place in the afternoon (at 1330h) when baseline HPA activity was expected to be low, thus any stimulation of the HPA axis (eliciting reactive cortisol secretion) would be distinct from basal levels. All subjects were instructed to refrain from eating, drinking (except water), and smoking one hour prior to the testing session.

Salivary samples were taken to assess cortisol levels. In contrast to painful and stressful blood sampling techniques, saliva sampling is a reliable, convenient and non-invasive method of assessing circulating levels of cortisol that are not bound to carrier proteins.[44] Each sample was taken using a 107x25mm saliva sampling tube (Sarstedt, Ville St-Laurent), whereby participants deposited 1.0 cm^3 of saliva, excluding foam, via a small section of plastic straw (1.0 cm).

Treatment of Salivery Samples & Salivary Cortisol Concentration Determination

All saliva samples were maintained at -20°C until the time of cortisol concentration determination, which was done via enzyme immunoassay (EIA) using kits from Salimetrics LLC (State College, PA, USA). This particular assay is specifically designed to quantify cortisol levels in saliva using only 25μL of saliva per test and has a built-in pH indicator to indicate extreme acidity ($pH \leq 3.5$) and basicity ($pH \geq 9.0$) of saliva samples. The intra-assay and inter-assay coefficient of variation for these studies are 3.5% and 5.1%, respectively. The limit of detection is 0.003μg/dL and all samples were assayed in duplicates.

Assessment of Hemodynamic Variables

Systolic and diastolic blood pressures and heart rate were measured using a digital blood pressure machine during the testing session following the collection of each of the 10 saliva samples in order to obtain a crude indication of the sympathetic nervous system (SNS) activity in response to the experimental protocol. The SNS is activated rapidly but briefly in response to stress perception, followed by the slower but more sustained activation of the HPA axis. Studies have demonstrated the ability of the TSST to significantly alter these hemodynamic variables.[46, 47]

Procedure

Eligible IBD patients were approached by a research assistant during their scheduled medical visit to the MUHC Gastroenterology Clinic to participate in this study. Those who were interested were later contacted by another research assistant at the Douglas Hospital Research Centre (DHRC) by telephone, who gave further information about the study to the patients. Patients were asked to identify a healthy age- and gender-matched friend to participate in the study with them. After the matched-control friend was identified, both the friend (HF) and the patient (IBD) were scheduled for an appointment at the MUHC to have a complete medical evaluation to ensure the remission status of IBD and the general

good health of HF. During this medical visit, subjects were provided with the four psychological questionnaires (NEO, RSE, PSS and CISS) to fill out at home. Both IBD and HF were then scheduled to go to the DHRC for the TSST. Both IBD and HF were asked to arrive to the DHRC together with their completed questionnaires; they were tested on the same day in different rooms by different research assistants. IBD and HF were only reunited after the testing session was finished. All subjects received a monetary compensation at the end of the TSST testing session.

Statistical Analysis

Demographic information was analyzed by t-tests and χ^2 analyses, as appropriate, using Group (IBD vs. HF) as the independent variable. In order to investigate possible differences in cortisol secretion and hemodynamic variables throughout the TSST paradigm between IBD and HF, two-way mixed design repeated measures analyses of variance were performed for stress-reactive salivary cortisol levels, systolic blood pressure, diastolic blood pressure, and heart rate using Group (IBD vs. HF) as the between-subjects variable and Samples (the 10 salivary cortisol and hemodynamic variable measurements taken throughout the TSST paradigm) as the within-subjects variable. Salivary cortisol fold stimulation was also calculated for IBD and HF by taking the difference between the peak cortisol sample taken after the TSST (Sample 6) and the sample taken just before the TSST (baseline; Sample 4), and then dividing this difference by the baseline sample (Sample 4; see Figure 1). Finally, t-tests (two group analysis) and ANOVA (three-group analysis) were used to assess group differences in scores on the personality questionnaires.

PART 1: RESULTS

Participants

Ten IBD patients in clinical remission were recruited. Eight patients had CD and two had UC; four of the patients were males and six were females. Mean age of IBD patients was 32.8 ± 6.3 years (ranging from 24 to 42 years). The 10 HF subjects recruited also consisted of four males and six females and had a mean age of 35.0 ± 8.3 years (ranging from 23 to 48 years). There were no significant differences in age or any other demographic variables between IBD and HF.

Stress-Reactive Cortisol

One female IBD and one female HF subject had mean cortisol levels throughout the entire visit to the DHRC (including the pre-TSST exposure) that were higher than two standard deviations of the mean cortisol levels of their respective groups. These two subjects were treated as outliers and were subsequently excluded from further statistical analyses. Two-way mixed design repeated measures ANOVA using Group (IBD vs. HF) as between-subjects variable and Samples (the 10 saliva samples throughout the TSST paradigm) as the within-subjects variable revealed a marginally significant interaction, $F(9, 8) = 2.967$, $p = 0.07$, with IBD exhibiting a higher cortisol response to TSST when compared to HF (Figure 1). Significant difference was found in Sample 4, which was the sample taken after the anticipation period and immediately before the TSST, as well as the sample taken 10 minutes after TSST (Sample 6). IBD had a 0.87-fold increase to the TSST, whereas that of HF was 0.58-fold.

Hemodynamic Measures

Two-way mixed design repeated measures ANOVA performed on systolic blood pressure, diastolic blood pressure, and heart rate throughout the TSST protocol using Group (IBD vs. HF) as between-subjects variable and Measurements (the 10 measurements taken throughout the TSST protocol) as within-subjects variable did not reveal any significant interactions or main effects for the three hemodynamic measures, $ps > 0.05$ (Figure 2).

Taken together, IBD patients demonstrated a greater reactivity to the TSST than their friends. However, using friends of IBD patients as controls might introduce a new bias, as IBD patients might be more likely to select friends who naturally react less to psychosocial stressors than other healthy individuals in the general population. Consequently, we recruited an additional 10 healthy individuals, each of them matched for the age and gender of a particular IBD patient, but who were not friends with the patients. This group of study participants constituted a second control group, the healthy non-friend controls (NC).

PART 2: METHODS

Healthy Non-Friend Control Subjects (NC)

NC subjects were recruited through the McGill University online classifieds website, www.mcgill.ca/classified. Each NC subject was matched for the age and gender of one of the IBD patients in the study. Recruitment and exclusion criteria were exactly the same as those employed for IBD patients and NF subjects.

Procedures

All NC subjects underwent the same medical evaluation and TSST protocol as IBD and NF subjects. All NC subjects arrived for the TSST testing session by themselves and were compensated with the same amount as IBD and NF at the end of the TSST testing session.

PART 2: RESULTS

Participants

The 10 NC subjects consisted of four males and six females with a mean age of 31.9 ± 8.2 years (ranging from 20 to 45 years). There were no significant differences in age and other demographic variables among the three groups of participants (IBD, NF and NC).

Stress-Reactive Cortisol

Two-way mixed design repeated measures analysis of variance (ANOVA) using Group (IBD vs. HF vs. NC) as between-subjects variable and Samples (the 10 saliva samples throughout the TSST paradigm) as within-subjects variable revealed a significant interaction between Group and Samples, $F(18, 34) = 2.671$, $p = 0.007$. Tukey's honestly significant difference post-hoc analyses on the Group effect revealed a significant difference between IBD patients and NC, $p = 0.051$, and a significant difference between HF and NC, $p = 0.002$. NC showed a significantly greater response to the TSST compared to IBD and HF. Figure 3 shows that significant difference between IBD and NC, as well as between HF and NC, was found in Sample 5 (immediately after TSST), Sample 6 (10 minutes after TSST) and Sample 8 (30 minutes after TSST). Significant difference between HF and NC was found in Sample 7 (20 minutes after TSST), Sample 9 (40 minutes after TSST) and Sample 10 (55 minutes after TSST). NC showed a 1.72-fold increase in cortisol in response to the TSST.

Hemodynamic Measures

Two-way mixed design repeated measures ANOVA were performed for systolic blood pressure, diastolic blood pressure, and heart rate throughout the TSST protocol using Group (IBD vs. HF vs. NC) as between-subjects variable and Measurements (the 10 measurements taken throughout the TSST protocol) as within-subjects variable. The analyses did not reveal any significant interactions or main effects for the three hemodynamic measures, $ps > 0.05$ (Figure 4).

NEO Personality Five Factor Inventory (Short Form)

Multivariate analysis of variance (MANOVA) performed on the five subscales of the NEO Personality Five Factor Inventory (Short Form) revealed significant Group difference (IBD vs. HF vs. NC; Figure 5), Wilks = 2.798, $p = 0.01$. Follow-up univariate analysis revealed that significant difference was found only in the Agreeableness subscale, $p < 0.001$. Pairwise comparisons revealed that a significant difference in Agreeableness exists between IBD and NC, as well as between HF and NC, $ps < 0.001$. IBD and HF scored significantly higher in Agreeableness than NC.

Rosenberg Self-Esteem Scale (RSE) & Perceived Stress Scale (PSS)

One-way analyses of variance (ANOVA) performed on scores for the Rosenberg Self-Esteem Scale and Perceived Stress Scale did not reveal significant Group differences.

Coping Inventory for Stressful Situations (CISS)

Multivariate analysis of variance (MANOVA) performed on the three coping style subscales did not reveal significant Group differences.

DISCUSSION

Our results show that IBD patients were more reactive to stress than their healthy friends (HF). However, both IBD and HF demonstrated less reactivity to the TSST when compared to healthy non-friend controls (NC). IBD and HF showed a 0.87-fold and 0.58-fold increase, respectively, which is lower than the established one- to three-fold increase shown in previous studies.[44] By comparison, NC subjects demonstrated a 1.72-fold increase, which is closer to the established fold increase values in response to the TSST reported in the literature. Therefore, it appears that IBD and HF exhibited a blunted cortisol response to the TSST. Interestingly, both IBD and HF scored significantly higher in the Agreeableness subscale of the NEO Personality Five Factor Index (Short Form) than the NC. All this indicates that IBD and HF are more similar in both personality and stress response.

Previous studies brought to light that IBD patients may display a distinct personality profile when compared to healthy individuals. For example, evidence suggesting that patients have slightly greater obsessional symptoms and neuroticism, when compared to healthy controls, have been found.[33, 48, 49] IBD patients also tend to be more dependent, conscientious, and conforming to the expectation of others in comparison to their healthy sibling.[50]

Interestingly, Schmitt (1970) asserted over three decades ago that IBD patients commonly come across as agreeable and often report *fewer* stressful life events than healthy individuals – a consequence of their inhibited personality and their inability to articulate their emotions.[51] McMahon et al. (1973) later noted that it is precisely due to IBD patients' inhibition that they often score well in life-stress indices because most of them are unable to openly acknowledge problems in their lives.[50] Studies have shown that agreeableness is associated with a long history of effortful control, self-regulation and prosocial motivation, thus resulting in decreased aggression and increased self-control in times of interpersonal conflict.[52-55] Given the chronic remitting-relapsing nature of IBD, patients might find it necessary to exert heightened self-regulation in their lifestyle and emotions in order to adequately adapt to the physical symptoms and social constraints in

their lives. Being agreeable may also allow patients to more effectively cope with the psychological impacts caused by IBD, and to help alleviate major stressors, which patients often believe have significant influence in disease activity.[56] It is important to underscore, however, that the IBD personality profile found in our study may only be specific to those who seek medical care.

Several studies have suggested that increasing social support can enhance IBD patients' quality of life and reduce their levels of distress.[57, 58] Research on patients with other chronic illnesses often conclude that a sound social support network could act to buffer stress and offer emotional support.[59-61] As IBD patients are more likely to conceal their disease in public, they would greatly benefit from the support of close friends to whom they could talk more openly about their illness.[62] It is well established that individuals with similar attitudes and personalities are more attracted to each other, as interacting with those who share a larger degree of similarity allow for the validation of one's views and the subsequent enhancement of self-esteem.[63, 64] This "likeness-leads-to-liking" phenomenon has been demonstrated in real-life situations, whereby the extent of similarity between individuals often predicts the degree of attraction.[65-67] Hence, IBD patients, being overtly agreeable, might befriend and confide in those with similar personality and attitudes, thus likely explaining the personality similarity observed in our study between IBD and HF. These friends might be better able to identify with and provide social support for patients. In addition, the personality similarities and differences in our three subject groups could also partially explain the differential stress reactivity observed, since personality is a potent predictor of stress reactivity.[28-31]

Kirschbaum et al. (1995) found decreased cortisol response to the TSST in males when a source of social support was present.[68] In fact, cortisol response was lowest when the male subject's girlfriend was present (providing the closest support), followed by the presence of a stranger, and finally when no support was present at all. Even though this study did not reveal a similar trend in female subjects, it nevertheless alluded to the possibility that the presence of social support, or at least the knowledge of the presence of a source of support, could

reduce cortisol responsiveness to the TSST. In our study, the fact that IBD and HF arrived together for testing, thus knowing of each other's presence during the TSST, could result in the blunting of their stress response. In order to examine the effects of social support in our study, a logical extension to take in the future would be to administer the same TSST paradigm on another group of healthy control subjects age- and gender-matched for the IBD patients of this study, and then ask these new subjects to identify an age- and gender-matched healthy friend to act as a new friend group. Results from these two new groups of subjects could be compared with the results found in the NC group of this study, who are not friends with these two new groups.

The results of our study require validation, as the preliminary nature of our investigation precluded us from recruiting a larger sample for each of our three subject groups, which resulted in low statistical power. In addition, we did not separate CD and UC patients in our IBD group, nor did we fully account for gender, smoking, menstrual cycle and use of oral contraception – all of which have been found to affect HPA activity.[69, 70]

To our knowledge, this is the first study to use cortisol as a biomarker to study HPA activity in IBD patients under stress-reactive conditions. The study of disease processes involved in IBD within a Biopsychosocial Model offers a clearer and a more complete understanding of the role of stress in IBD. It is our hope that results from this and subsequent studies could eventually allow physicians to devise treatments and disease management strategies that will be better suited for IBD patients, including psychotherapeutic interventions to reduce psychological stress, so that a greater quality of life could be achieved while making more effective use of health care resources.

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Figure 1

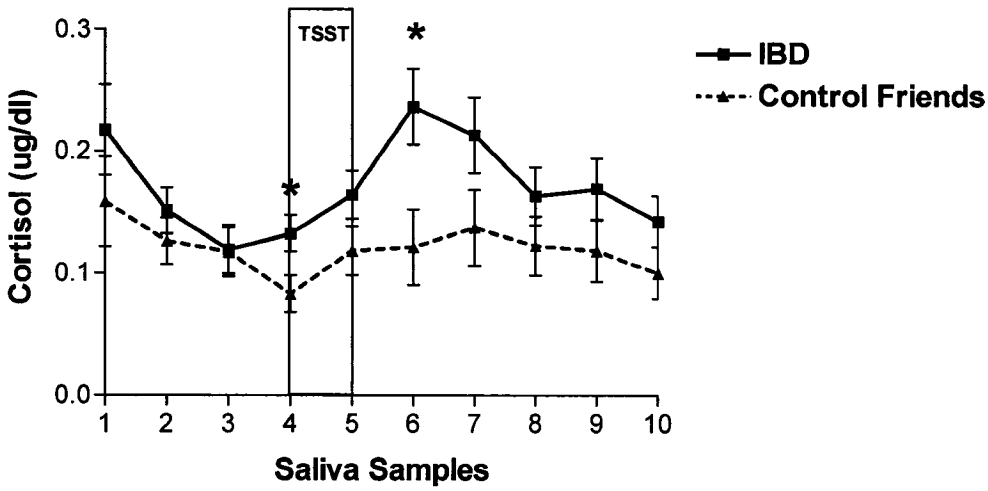
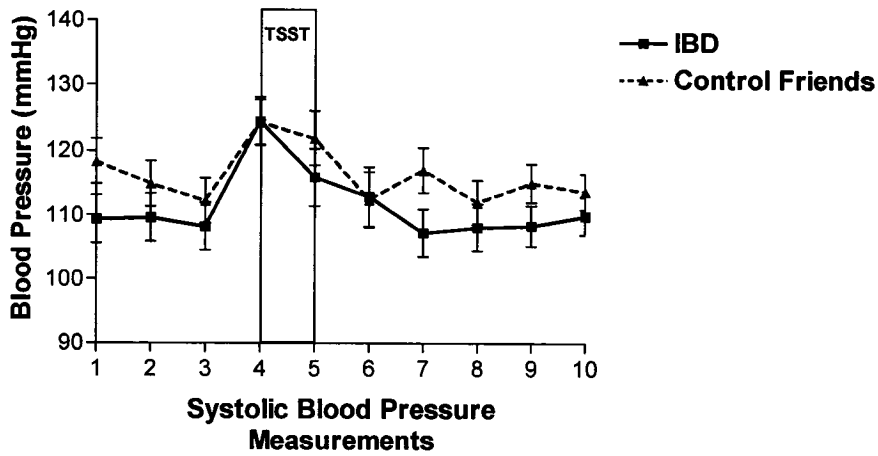
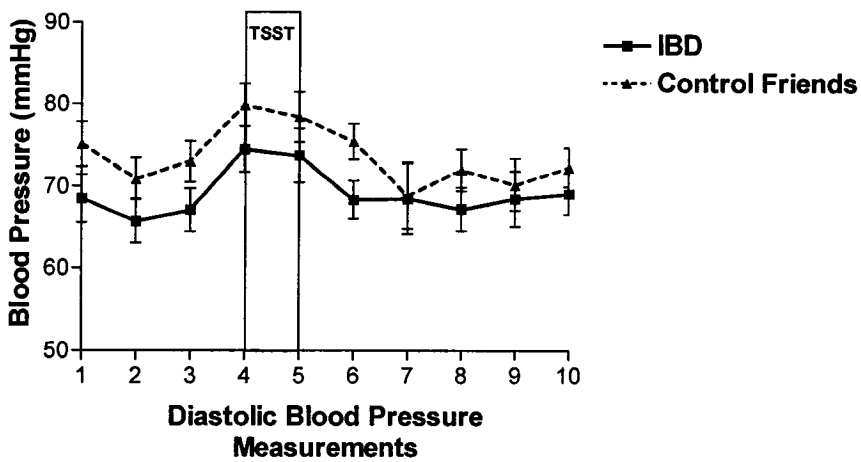

TSST
*
*
IBD
Control Friends
Cortisol (ug/dl)
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
Saliva Samples

Figure 2

(A)


Blood Pressure (mmHg)
140
130
120
110
100
90
TSST
1 2 3 4 5 6 7 8 9 10
Systolic Blood Pressure Measurements
IBD
Control Friends

(B)


Blood Pressure (mmHg)
90
80
70
60
50
TSST
1 2 3 4 5 6 7 8 9 10
Diastolic Blood Pressure Measurements
IBD
Control Friends

(C)

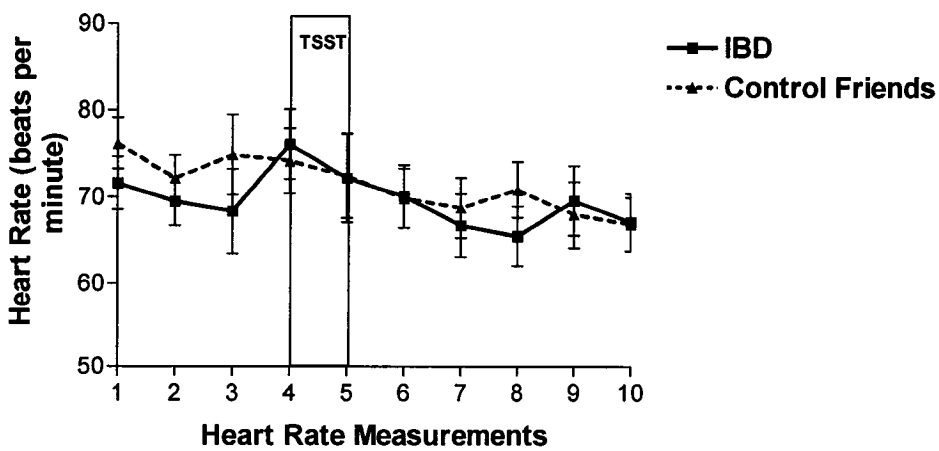

Heart Rate (beats per minute)
90
80
70
60
50
TSST
1 2 3 4 5 6 7 8 9 10
Heart Rate Measurements
IBD
Control Friends

Figure 3

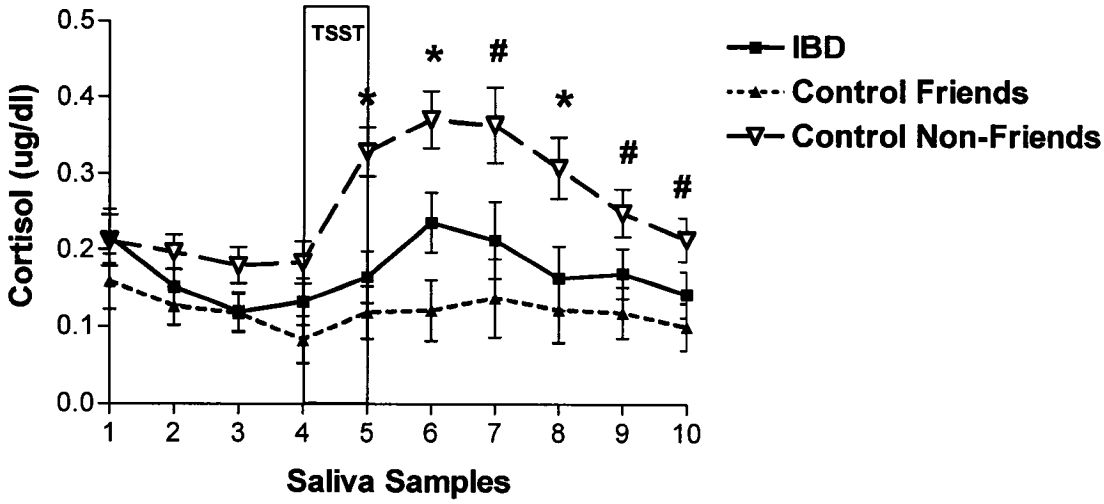
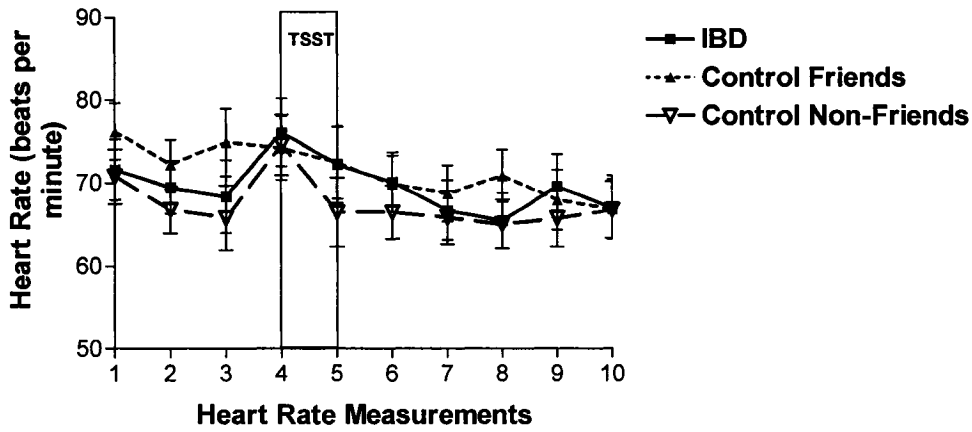
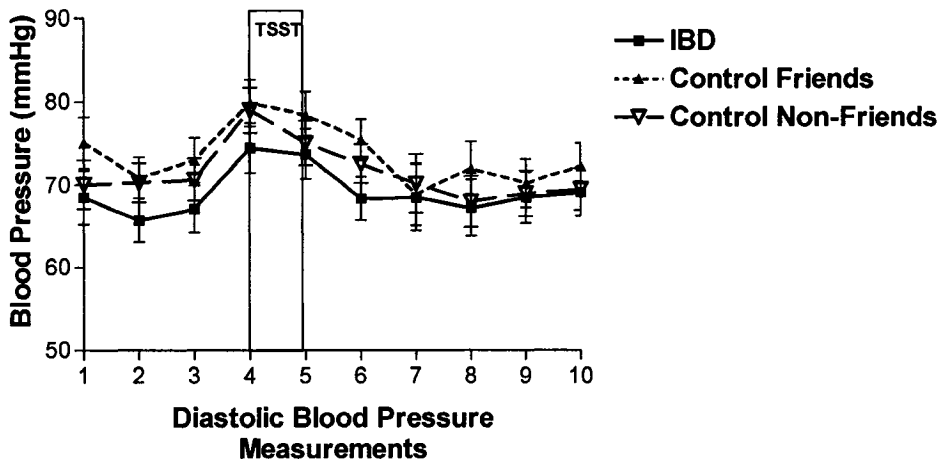

0.5
0.4
0.3
0.2
0.1
0.0
Cortisol (ug/dl)
TSST
*
#
*
#
#
1
2
3
4
5
6
7
8
9
10
Saliva Samples
IBD
Control Friends
Control Non-Friends

Figure 4

(A)


Heart Rate (beats per minute)
90
80
70
60
50
TSST
1 2 3 4 5 6 7 8 9 10
Heart Rate Measurements
IBD
Control Friends
Control Non-Friends

(B)


Blood Pressure (mmHg)
90
80
70
60
50
TSST
1 2 3 4 5 6 7 8 9 10
Diastolic Blood Pressure Measurements
IBD
Control Friends
Control Non-Friends

(C)

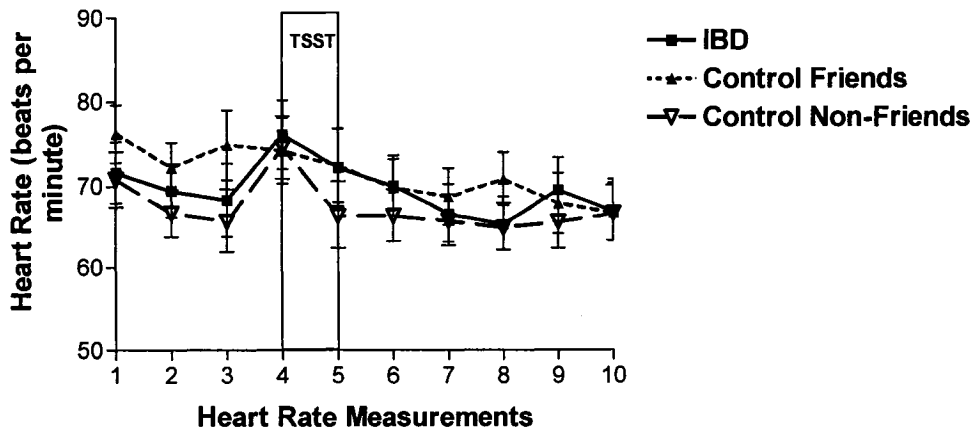

Heart Rate (beats per minute)
90
80
70
60
50
TSST
1 2 3 4 5 6 7 8 9 10
Heart Rate Measurements
IBD
Control Friends
Control Non-Friends

Figure 5

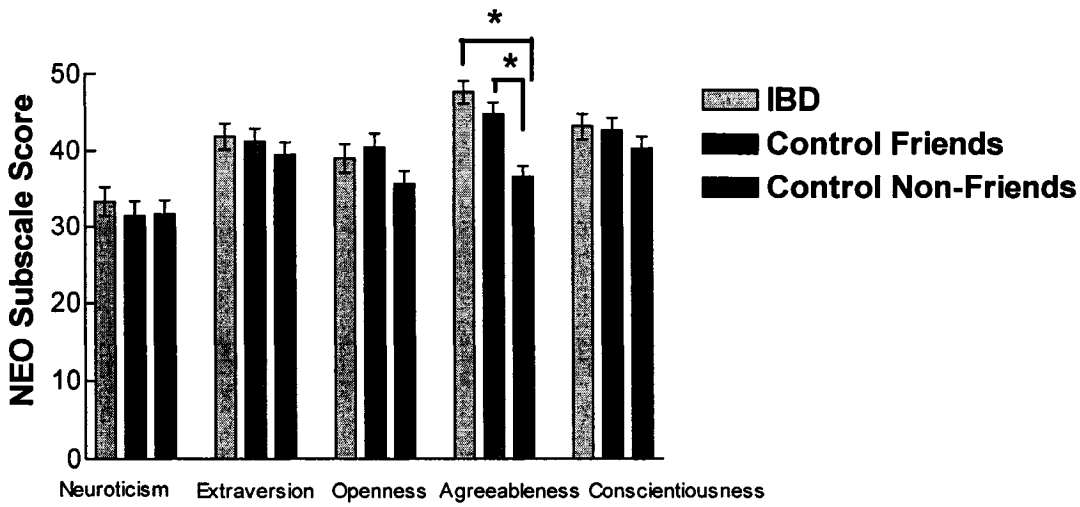

*
*
NEO Subscale Score
50
40
30
20
10
0
IBD
Control Friends
Control Non-Friends
Neuroticism
Extraversion
Openness
Agreeableness
Conscientiousness

FIGURE CAPTIONS

Figure 1: Salivary cortisol levels in response to the TSST in IBD patients and healthy friend controls (HF). Error bars on each data point represent standard error. Asterisks (*) indicate significant difference between IBD and HF.

Figure 2: (a) Systolic blood pressure, (b) diastolic blood pressure, and (c) heart rate in response to the TSST in IBD patients and healthy friend controls (HF). Error bars on each data point represent standard error.

Figure 3: Salivary cortisol levels in response to the TSST in IBD patients, healthy friend controls (HF), and healthy non-friend controls (NC). Error bars on each data point represent standard error. Asterisks (*) indicate significant difference between IBD and NC, and between HF and NC. Pound signs (#) indiciate significant difference between HF and NC only.

Figure 4: (a) Systolic blood pressure, (b) diastolic blood pressure, and (c) heart rate in response to the TSST in IBD patients, healthy friend controls (HF), and healthy non-friend controls (NC). Error bars on each data point represent standard error.

Figure 5: Subscores of the NEO Personality Five Factor Inventory (Short Form) in IBD patients, healthy friend controls (HF), and healthy non-friend controls (NC). Error bars represent standard error. Asterisks (*) indicate significant difference.

3.2 PART II

3.2.1 Basal Salivary Cortisol

As mentioned in the manuscript in Part I of the Results section, one female IBD patient with Crohn's Disease and one female friend control subject had stress-reactive cortisol levels that were consistently at least two standard deviations higher than their respective group means for all sampling times, including the pre-stress, baseline samples. We excluded these two subjects from all statistical analyses, including the analysis of basal salivary cortisol. Therefore, there were 9 IBD patients, 9 control friends (HF) and 10 control non-friends (NC) in our analysis for basal salivary cortisol.

Home saliva samples (basal salivary cortisol) of IBD patients and control friends were analyzed using radioimmunassay (RIA), whereas those for control non-friends were analyzed using enzyme immunoassay (EIA). However, we realized after the assays were completed, that the results obtained from RIA and EIA were not comparable, as there appeared to be large discrepancies in the results even after correction procedures were taken. EIA/RIA correction quotients ranged from 0.188 to 0.769. The average EIA/RIA correction quotient was 0.394 (standard deviation = 0.193) – indicating a wide variation in the discrepancy between EIA and RIA and a generally large difference in the results obtained by the two assaying techniques.

Consequently, we were only able to confidently compare basal salivary cortisol levels of IBD patients and control friends, as they were both analyzed using RIA, but we were unable to incorporate basal salivary cortisol levels of control non-friends into our analysis because non-friend control samples were analyzed using EIA (see Figure 5 in Appendix A). Although Figure 5 displays results of all three subject groups in the same graph, it is important to emphasize that basal salivary cortisol results of control non-friend subjects cannot be compared with those of IBD patients and control friend subjects. However, it is important to re-emphasize that for the reactive salivary cortisol samples (those taken during the TSST protocol), the three groups were analyzed using EIA; thus they can be compared with each other.

For the basal cortisol of IBD patients and control friends, a two-way mixed design repeated measures ANOVA was performed using Groups (IBD vs. HF) as the between-subjects variable and Samples (the five saliva samples taken throughout the day) as the within-subjects variable. The analysis did not reveal any significant Group by Samples interactions, nor was there a significant Group main effect, ps > 0.05 (Figure 5). Post-hoc power analysis revealed an observed power for the Group main effect as 0.050 (f = 0.00), and that of Group by Samples interaction as 0.255 (f = 0.504).

4. GENERAL DISCUSSION

Results from this study indicate that, in comparison to healthy non-friend control (NC) subjects, IBD patients and their healthy friends (HF) exhibited lower cortisol reactivity to the TSST and that the calculation of fold stimulation revealed a blunted HPA response to the TSST in IBD patients and their friends (with a fold stimulation below that of the general established range which the TSST has been known to elicit),[146] whereas non-friend controls showed a normal HPA response (within the general established range). In addition, IBD patients and their friends had a more agreeable personality than non-friend controls. The three groups did not differ in self-esteem, perceived stress levels and coping styles, nor were there any significant differences in the diurnal basal cortisol secretion between IBD patients and their friends. Post-hoc power analyses generally revealed small effect sizes and small statistical power for all non-significant data. Furthermore, results were inconclusive with regards to the comparison of diurnal basal cortisol secretion of non-friend controls with that of IBD patients and friend controls.

4.1 The Case for Personality & Social Support

Research dating back over three decades ago reported that IBD patients often have an agreeable personality and that they tend to have fewer stressful life experiences than healthy control subjects.[155] Incidentally, out of the five major personality dimensions – namely, neuroticism, extraversion, openness, agreeableness and conscientiousness – agreeableness is most concerned with interpersonal relationships and social evaluation.[156, 157] In addition, previous studies suggest that agreeableness is associated with a long history of effortful control, self-regulation and prosocial behaviours, especially in times of interpersonal conflicts. As a result, agreeableness has been found to be negatively related to experiences of anger and trait aggression.[157, 158] In fact, aggression-related word primes have been demonstrated to elicit prosocial thoughts among highly agreeable individuals, but not among those low in this personality trait.[159] Taken together, IBD patients may find it necessary to exert greater levels of self-regulation and effortful control in order to adequately deal with the physical,

social and psychological constraints posed by the chronic remitting-relapsing nature of their condition. Perhaps this also helps elucidate earlier findings of improved relationship over time between IBD patients and their spouse.[102]

It may also be precisely due to this heightened level of self-control in IBD that patients are either unable or unwilling to openly admit their distress, thus explaining the low levels of stressful life events that IBD patients are reported to experience.[109, 155] Support for this contention comes from studies suggesting an association between IBD and alexithymia, which can be defined as difficulties in experiencing, processing and articulating emotions.[160, 161] For example, Porcelli et al. (1995) observed significantly higher levels of alexithymia among IBD patients than healthy control subjects,[162] while Verissimo et al. (1998) revealed that alexithymia, along with emotional control, are important predictors of health-related quality of life in IBD patients.[135]

Numerous studies performed in the 1960s and 1970s reported that individuals who are similar in a host of psychological factors are more likely to be attracted to each other. In fact, intimate friendships are often formed among those who share similar personalities,[163, 164] cognitive constructive systems,[165, 166] attitudes, values, and interests.[167-169] For example, in a study where previously unacquainted male participants were placed in a simulated fall-out shelter confinement setting for 10 days, attitude similarity was found to be significantly related to post-confinement attraction.[170] Perceived similarity has also been suggested to be a crucial determinant of attraction in conjunction to actual similarity.[171] Such similarities foster the enhancement of self-esteem through the validation of views and other aspects of personal worth.[172, 173] A recent longitudinal study on the long-term status of first-year college friendships also brought to light that personality and psychological similarities might allow for the enhancement of social identity support.[174]

In the context of the present study, IBD patients, who are highly agreeable, may be more likely to become close friends with those who are equally agreeable. Given that agreeable individuals are often characterized as helpful, sympathetic and trusting,[138] IBD patients might be more attracted to individuals

who possess these characteristics in order to seek social, emotional and psychological support. Such attraction and attachment to others might be especially important for IBD patients as George Engel, the first to propose a biopsychosocial approach to studying diseases, asserted over half a century ago that IBD patients often have a deep "life and death kind" of dependency on the presence of key figures in their lives.[175] Certainly, it is possible that the healthy friends recruited in this study are among the key figures in the lives of the IBD patients. As IBD patients are more likely to actively conceal their disease in public, such as in work or in school, they may only turn to their family or their closest friends to talk openly about their condition and to seek social support.[176] This contention is supported by evidence showing that IBD patients' quality of life is significantly correlated with satisfaction and degree of closeness with members within their social network.[177] The fact that IBD patients rely on major key figures in their lives for social support may also explain the general lack of interest in regular support group meetings for IBD,[178, 179] as patients may not feel comfortable confiding in those with whom they do not feel a close emotional connection.

Furthermore, several studies have suggested that increasing social support can enhance IBD patients' quality of life and reduce their levels of distress.[71, 180] Janke et al. (2005) also found that the strengthening of social support can promote general life satisfaction in patients with IBD.[181] Research on patients with other chronic illnesses often conclude that a sound social support network could act to buffer stress and offer emotional support.[182, 183] In a three-year study, for example, Thong et al. (2006) measured dialysis patients' ratings on social companionship, daily emotional support, as well as total support, and found that discrepancies between expected and actual social support received by patients was associated with increased mortality.[184] Lower levels of diurnal basal cortisol were also found among metastatic breast cancer patients who received greater quality of social support.[185] It may be that the support provided by the patient's social network can help foster optimism and personal control, while creating positive illusions in the patient's actual medical conditions – all of which have been

demonstrated to lead to better health outcomes, lower cardiovascular responses to stress, more rapid cardiovascular recovery after stress, and lower baseline cortisol levels.[186, 187]

Numerous laboratory studies have also demonstrated reduced cardiovascular reactivity to brief psychological stressors, in the form of decreased magnitude of change in blood pressure and heart rate, when subjects were accompanied by a friend during the testing session.[188-190] Hence, it would seem that social support provides not only a psychological buffer, but also protects against physiological changes due to chronic and acute psychological stress. In the context of the present study, social support effects might play a prominent role, as IBD patients and HF are friends, which could be a contributing factor to the blunted HPA reactivity observed in these subjects in response to the TSST. Since IBD and their friends arrived together to the DHRC for testing at the same time, it is possible that the knowledge that these subjects had of their friend being tested with them could have impacted their HPA reactivity to the TSST. Even though they were not together during the actual testing session, they might still have felt socially and psychologically supported by the knowledge that their friend was being tested with them. Conversely, this social support effect was not present when control non-friend subjects were being tested, as they arrived for the testing session by themselves.

Kirschbaum et al. (1995) performed an ingenious study whereby young healthy male and female subjects underwent the TSST in three different conditions: the first being that the subjects' boyfriend or girlfriend was present to provide social support during the anticipation period of the TSST; the second being that strangers were present to provide social support; and the third being the situation where no social support was provided at all.[191] The study revealed that among male subjects, the lowest cortisol response to the TSST was elicited when the girlfriends were present, followed by when strangers were present, and the highest cortisol response was found in male subjects when no support was provided. Although this trend was not found in female subjects, it nonetheless revealed the significance that social support can have on HPA reactivity in times

of acute psychological stress. Results from this study are concordant with those from an earlier study by Hennessy (1984) where squirrel monkeys reared on inanimate maternal surrogates were found to emit significantly more high-pitched vocalizations and had higher levels of plasma cortisol when placed in a novel environment alone, when compared to exposing them to the novel environment in the presence of a similarly-reared peer.[192]

Such a social support effect has been linked to oxytocin, which is a hypothalamic neuropeptide released by the neurohypophysis known to inhibit sympathetic nervous system and HPA axis activity after stress in order to bring about a "calm and connection" effect in the presence of positive social cues.[193-195] Incidentally, oxytocin has been found to mediate prosocial and caregiving behaviours, such as the "tend and befriend" phenomenon described in females which includes nurturing behaviours to protect oneself and one's offspring, as well as the establishment of social networks to promote safety and decrease levels of distress.[196-198] Despite the interesting findings, many studies on oxytocin tend to focus on plasma concentrations of this neuropeptide in relation to behavioural changes. However, it has been shown previously that plasma oxytocin does not readily cross the blood brain barrier,[199, 200] and that there is no relationship between the level of oxytocin in the blood and that in the cerebrospinal fluid (CSF).[201] In fact, CSF oxytocin has a relatively long half-life (28 minutes) and exhibit a circadian secretion pattern.[202, 203] In contrast, plasma oxytocin has a much shorter half-life (1-2 minutes) and no circadian rhythms have been found. Therefore, oxytocin in these two sources are very different, thus it may not be appropriate to associate plasma oxytocin with behaviours, which are presumably controlled by the brain; it may be more likely that behaviours are influenced by CSF oxytocin. In our study, it is possible that oxytocin (at least that present in the CSF) might play a role in mediating the potential social support effects observed in IBD patients and their friends. Such a premise, however, requires validation.

Altogether, since personality variables are potent predictors of stress reactivity,[97, 98, 104, 105] perhaps the similarity in personality (high agreeableness) in IBD patients and friend controls, together with the social support provided by

each other during the testing session, resulted in the similarly blunted HPA reactivity to the experimental psychosocial stressor observed in our study. Interestingly, agreeableness has been previously found to be inversely associated with circulating levels of epinephrine, as well as resting systolic and diastolic blood pressures in healthy adults.[118] Unlike earlier findings of neuroticism in IBD patients,[12, 102] results from this study did not reveal any significant differences in this personality trait between the patient group and the other two control groups. This could be due to the fact that the IBD sample in this study comprises both CD and UC, and neuroticism has been more strongly associated with CD only.

4.2 Possible Neuroendocrine & Immune Involvements in IBD Stress Reactivity

In addition to personality and social support differences, the blunted cortisol response in IBD patients and their healthy friends could have been caused by habituation effects over time. Both animal and human studies found evidence of habituation of HPA response to repeated stressors over time, although most findings showing habituation effects are generally associated with homotypic stressors. For example, rats exposed to three successive trials of white-noise stimulation demonstrated attenuated neuroendocrine and behavioural responses to the same stimulus after the first trial.[204] In another study, rats were immobilized for 20 minutes per day for five consecutive days.[205] On the sixth day, rats were either exposed to the same immobilization stressor or a different (heterotypic) stressor: exposure to ether vapours. Only those who were exposed to the ether vapours exhibited a maximal increase in noradrenaline and ACTH responses. Recently, Schommer et al. (2003) exposed healthy human subjects to the TSST for three times and found reduced responses in salivary and total plasma cortisol, as well as ACTH and heart rate across successive sessions of the TSST.[154]

Cole et al.'s (2000) study using repeated restraint stress on rats suggested that habituation over time may be mediated by the negative feedback effects of central mineralocorticoid receptors (Type I GRs) during acute stress.[206] Since this study did not find any differences in the corticosterone response to CRH challenge in either restraint-naïve or restraint-habituated rats, it is likely that the

habituation of the HPA response is caused by changes at the level of the hypothalamic paraventricular nucleus or elsewhere in the brain. Furthermore, long-term stress has been associated with the downregulation of hippocampal Type II GR expression in males and the upregulation of hippocampal Type I GR expression in females – both of which would lead to the reduction of the levels of Type II GR signalling in the hippocampus.[207]

With regards to IBD, it may be that patients are exposed to high levels of chronic repeated stressors due to the remitting-relapsing nature of the condition, along with the associated physical and social constraints. In turn, this could result in the habituation of the HPA axis response over time – a phenomenon that might be mediated by a change in the ratio of Type I and Type II GRs in the central nervous system. With regards to the healthy friend controls in this study, it is very possible that patients often confide in their friends. Given the healthy friend's agreeable personality, they would be more likely to sympathize with the patients' stressful experiences, which could subsequently result in repeated exposures of psychological stress for the healthy friends. Nevertheless, scores for the 10-Item Perceived Stress Scale from our study show no significant differences in the three subject groups – indicating that the healthy friend controls, and even IBD patients, do not seem to have higher perceived stress levels when compared to non-friend controls. Given the association found between agreeableness and self-regulation,[208, 209] perhaps IBD and their friends simply inhibit their feelings of perceived stress and consequently report perceived stress levels that are comparable to those of non-friend controls, despite demonstrating a habituated neuroendocrine response to the TSST. This possibility warrants further investigation in the future in the form of measuring levels of self-regulation and effortful control among IBD patients and their friends, as well as to assess the degree of closeness between each subject pair (IBD patients and their friends).

In terms of the neuroendocrine-immune interplay in IBD, the chronic stress associated with the disease, together with the inflammatory milieu of the gastrointestinal tract, could result in the eventual receptor downregulation at the level of the HPA, such as CRF and ACTH receptors, due to chronic upregulation

of the HPA axis by proinflammatory cytokines. Hence, further stimulation of the HPA axis (e.g. by acute psychological stress) might simply elicit a blunted response.

Recently, Munhoz et al. (2006) reported that repeated unpredictable stress in rats can potentiate NF-κB signalling, a proinflammatory transcription factor, in the brain via the binding of GCs to centrally expressed GRs in the hippocampus and the frontal cortex, and the subsequent activation of NMDA (N-methyl-D-aspartic acid) receptors.[210] This study demonstrates that rather than having anti-inflammatory properties in the brain, chronic elevations of GCs from long-term exposure to unpredictable stress may actually promote an inflammatory state in the central nervous system. The activation of NF-κB can augment the levels of proinflammatory cytokines in the brain.[211, 212] Although speculative at this point, it is not unreasonable to postulate that over time, it may be possible for the persistent inflammation and increased levels of centrally- and peripherally-derived proinflammatory cytokines to lead to neuronal damage and the downregulation of cytokine receptors in the brain. This may be especially true in regions with a high density of GRs, such as the hippocampus and the frontal cortex. Proinflammatory cytokine receptor downregulation in these brain regions could also lead to a reduction in proinflammatory cytokine signalling, which could attenuate the upregulatory effects of the HPA axis by proinflammatory cytokines, thus leading to a blunted HPA response.

In addition, events associated with chronic activation of NMDA receptors in the hippocampus and frontal cortex may lead to subsequent excitotoxicity and damage or death of neurons in these brain regions. Together, these events could affect the activity of the HPA axis since the HPA has important connections with both the hippocampus and the frontal cortex.[213] Hence, it is possible that the blunted HPA reactivity observed in IBD patients in our study may be a consequence of the downregulation of receptors in the HPA axis, as well as the inflammatory milieu of the brain. Clearly, further studies are required to determine whether or not these possible chains of events might occur as a result of chronic stress exposure.

4.3 Chronic Inflammatory Diseases & Stress-Related Functional Disorders: A Possible Link?

Although the aforementioned argument may be sufficient to address the blunted HPA reactivity in IBD patients of the present study, it does not explain the blunted response observed in healthy friend controls. Since the friend control subjects are healthy, one might assume that either an agreeable personality and/or social support could lead to a hyporeactive HPA response (as mentioned before), or there may exist other physiological and psychological differences between friend controls and non-friends controls that were simply not investigated in the present study.

A more satisfactory possibility is that individuals with dysfunctional regulations of HPA activity may be more likely to develop IBD. Hence, even though the healthy friend controls did not present any pathophysiologies and were assumed healthy in this study, they may nevertheless have varying degrees of HPA dysregulation. A useful method to assess the functioning of the HPA axis in the friend controls would be to compare the diurnal basal cortisol secretion pattern of these subjects with non-friend controls. It is clear from our analysis that IBD patients and friend controls did not show any significant differences in the diurnal cortisol secretion, but should significant differences arise between friend controls and non-friend controls, it could indicate possible HPA dysregulation in both IBD patients and their friends. Unfortunately, due to the incomparability of our basal cortisol results, we were unable to make any further conclusions with regards to HPA regulation and functioning. However, it has already been found that many patients with chronic stress-related functional disorders, such as fibromyalgia, chronic fatigue syndrome, and post-traumatic stress disorder, have a hypoactive HPA axis.[72] Further, HPA dysregulation has been identified in autoimmune inflammatory disease states, such as rheumatoid arthritis,[214, 215] systemic lupus erythromatosis,[216, 217] and multiple sclerosis.[218, 219]

A recent study by Varghese et al. (2006) demonstrated that neonatal maternal separation in mice resulted in depressive-like behaviour, along with enhanced intestinal permeability and increased risk of intestinal inflammation in

adulthood.[220] With the administration of antidepressants, however, there was a significant reduction in intestinal permeability, thus providing strong evidence that depression is likely to precede or coincide with increased GI permeability. These results appear to be in line with studies indicating that patients suffering from depression, who also have high levels of early life stress, have enhanced inflammatory responsiveness to psychological stress.[221, 222] Together, these studies suggest a link between early adversity, depression, and a proinflammatory state later on in life. However, results from our study do not seem to concur with this premise, as depression is usually associated with HPA hyperactivity and hypercortisolism,[223] whereas both the IBD patients and the friend controls in our study exhibited a blunted HPA response to the TSST.

Interestingly, patients with irritable bowel syndrome (IBS) have been shown to demonstrate HPA *hypo*reactivity.[224] Given that a significantly higher proportion of IBD patients experience IBS-like symptoms when compared to healthy individuals,[225] as well as recent suggestions that IBD and certain forms of IBS might be related,[114, 226] it would not be unreasonable to speculate that the blunted HPA response observed in our study might be related to that observed in IBS patients. Certainly, an association between a chronic stress-related functional disorder and an autoimmune inflammatory disorder has been reported before. For example, there is a high prevalence of fibromyalgia among patients suffering from systemic lupus erythromatosis (SLE).[227] In fact, it was recently found that the number of fibromyalgia tender points in SLE patients is strongly associated with health status.[228]

In sum, it may be that HPA dysregulation – either by chronic psychological stress earlier in life or by other physiological or psychological insults – is a necessary preceding component for the onset of IBD. Alternatively, rather than being a consequence of IBD, the presence of a hyporeactive HPA axis might be seen as an independent risk factor for the development of IBD later on in life. Given this premise, one might assume that the healthy friend controls in our study, because of their blunted HPA response, might also be at risk for developing IBD or other disease states associated with HPA dysregulation. Undoubtedly,

more needs to be done to clearly define the directionality of causality between HPA activity and disease onset, as well as to test the hypotheses proposed above.

4.4 Study Limitations

Due to the preliminary nature of this study, only a small sample of subjects was recruited, thus yielding small statistical power. As a result, it was not possible to separately analyze CD and UC within our IBD group. Although UC and CD both involve gut inflammation, they are distinct disease processes with different immune profiles and inflammatory patterns in the GI tract. UC involves inflammation of the mucosal lining of the GI tract and inflammation is usually confined to the rectum and the colon (up to the ileocecal region of the GI tract). Conversely, CD involves transmural inflammation, which can occur in any part of the GI tract.[229] With regards to immune physiology, UC is associated with an excessive T_{H2} cell phenotype, which leads to the production of such cytokines as IL-4, IL-5, and IL-13, while CD is associated with an excessive T_{H1} cell phenotype, which results in the production of such cytokines as IFN-γ, IL-2 and TNF.[230]

Differences in psychological and personality variables have also been found between the two conditions. As reviewed by Sainsbury and Heatley (2005), CD patients have higher levels of depression and anxiety than UC patients.[231] CD patients also tend to have a lower health-related quality of life than UC.[232] Taken together, grouping CD and UC together may not be appropriate, as important physiological and psychological differences may be overlooked. Future studies should take into consideration the differences between CD and UC by recruiting sufficient number of patients for each of the two conditions and keeping the patient groups separate in the statistical analysis. In addition, the small sample size precluded us from adequately addressing gender, menstrual phase and the use of oral contraception in female participants, as well as smoking habits in our sample – all of which have been previously shown to influence HPA activity.[151, 233]

It is also interesting to note the wide age range of the three groups of participants, as indicated by the large standard deviations (see Table 1 in Appendix A). Even though there were no significant differences in the mean age of these three participant groups, the IBD group and the non-friend control group have a slightly younger mean age than the friend control group. Incidentally, the IBD group and the non-friend control group also demonstrated a higher cortisol response to the TSST when compared to the older friend control group. Therefore, it is possible that the differential stress reactivity could, at least in part, be explained by the fact that older participants might not respond as much to the TSST. Perhaps these older participants had more interview experience in their lives and/or they might not have perceived the TSST panel as credible, since panel members were graduate students in the laboratory, thus were younger than these older participants. As a result, these factors could have lessened the novel and social evaluative nature of the task for these older participants. Certainly, further studies are required to determine whether or not age has a significant impact on the cortisol response to the TSST.

Another limitation to this study is the fact that we employed radioimmunoassay (RIA) to analyze the home saliva samples of IBD patients and healthy friend controls, and enzyme immunoassay (EIA) to analyze the home saliva samples of non-friend controls, as well as the stress-reactive saliva samples collected during the TSST session of all three subject groups. Based on the fact that EIA is a high throughput system with an even greater sensitivity to salivary cortisol when compared to RIA, we did not anticipate any major discrepancies of the results generated by the two techniques. Our rationale for changing assaying techniques was also based on a study by Raff et al. (2003), where it was found that Salimetrics EIA kits for salivary cortisol (the kits that we used for our EIA analyses) yielded results that were very similar to those obtained by RIA in healthy adults, Cushing patients, and patients with allergic rhinitis.[234] Moreover, the Salimetric EIA kits required less saliva per sample (25μL) than RIA (100μL), and contains a built-in pH indicator to signify extreme acidity ($pH \leq 3.5$) and basicity ($pH \geq 9.0$), which is extremely important because a pH below 4 has been

shown to artificially inflate salivary cortisol levels.[235] Given the aforementioned arguments, we did not initially anticipate problems of comparability between RIA and EIA. From our results, however, we realize that any future extensions to our study should employ only one of the two assay techniques.

As mentioned before, it is also worth exploring the social support effect that might have influenced the results in our study. The blunted cortisol response in IBD patients and their friends could have been a result of the two participants arriving for testing at the same time, thus each knowing that their friend was being tested with them even though they were tested separately. In order to see whether or not social support did affect the cortisol response, it might be useful to recruit a group of healthy control subjects to participate in the same experimental protocol as described in this study, and then to ask each of these healthy subjects to identify a healthy friend to participate with them. Results from these two new groups of subjects could then be compared with results obtained from the non-friend control subjects of this study to determine whether or not social support does affect stress reactivity. Alternatively, a new group of IBD patients with a similar medical history as those in the present study can be recruited and tested alone to see whether or not any differences exist between this new group of IBD patients and those in this study.

Furthermore, the degree of friendship between pairs of friends (IBD patients and their friends) can be assessed prior to the testing session in the future. Through this, pairs of friends with varying degrees of friendship/closeness can be recruited to see whether or not the degree of friendship can modulate stress response in both IBD patients and their healthy friends. Degree of friendship can be assessed by such questionnaires as *The McGill Friendship Questionnaire-Respondent's Affection*, which probes the respondents' feelings for a friend and friendship satisfaction, and *The McGill Friendship Questionnaire-Friend's Functions*, which measures the degree to which the respondent feels a particular friend fulfills six specific friendship functions: stimulating companionship, help, intimacy, reliable alliance, self-validation, and emotional security.[236] Indeed, such an assessment on friendship quality can truly tap into the role that social

support plays when faced with psychological stress in the context of a chronic inflammatory disease.

4.5 Future Directions

To further investigate the stress reactivity in IBD, the sympathetic response must be examined more closely in addition to HPA reactivity. In the present study, we did not reveal any significant differences in hemodynamic responses to the TSST in the three participant groups. However, periodic blood pressure and heart rate measurements taken at specific time points of the TSST protocol only provide a crude indication of autonomic activity, as autonomic responses to physical and psychological stressors, including changes of blood pressure and heart rate to an acute stressor, are generally fast-acting and short-lived. Therefore, the hemodynamic parameters measured in the present study may not reflect the true impact of the TSST to the three participant groups, as there was a delay between the end of the psychosocial stress phase of the TSST and the actual measurement of these hemodynamic parameters. By the time these parameters were measured, autonomic activity may have attenuated (when compared to immediately after the TSST) or even have returned to baseline levels. In addition, a hormonal dissociation between the sympathetic nervous system and the HPA axis has been found in both healthy subjects and IBD patients.[74, 237-239] Taken together, perhaps a more useful approach to better gauge the activities of the sympathetic nervous system would be to continuously monitor each participant's hemodynamic parameters throughout the TSST protocol using such equipment as digital continuous heart rate monitors.

Another approach to further examine the activities of the sympathetic nervous system throughout the TSST protocol would be to measure the levels of salivary α-amylase (sAA) under basal and stress-reactive conditions. sAA is an enzyme secreted by the salivary glands that catalyzes the hydrolysis of starch.[240] Recent studies indicate that the TSST can elicit an increased sAA response.[241, 242] Given earlier observations that sAA concentration increases with sympathetic stimulation and salivary flow rate increases with parasympathetic stimulation

(thus decreasing sAA concentration),[243] as well as the fact that significant correlations have been shown between sAA and catecholamine levels,[244] it has been proposed that sAA may be a powerful biomarker for assessing the activity of the sympathetic nervous system.[241, 242]

In addition, the role of the immune system in the context of psychological stress in IBD patients could be greatly clarified with the measurement of an immune biomarker during the TSST protocol. For example, the measurement of serum IL-6 (a proinflammatory cytokine) and C-reactive protein levels during the TSST protocol may yield important insights into the immune-stress reactivity in these patients. A recent study has shown that in healthy adult males, there appears to be a lack of habituation effects in serum IL-6 to repeated sessions of TSST, despite an unequivocal habituation in cortisol response.[245] It would, therefore, be enlightening to determine whether or not the same trend appears in IBD patients.

Furthermore, in order to discern the effects of chronic inflammation on stress reactivity in humans, it might be beneficial to employ the same experimental protocol described in this study in patients that suffer from other chronic autoimmune inflammatory diseases. To date, few studies have utilized cortisol as a biomarker to gauge the stress reactivity in patients with such conditions. Results from existing studies have yet to provide a clear illustration of the stress reactivity within the context of chronic inflammation. For example, Buske-Kirschbaum et al. (1998) examined the stress reactivity in steroid-naïve children with atopic dermatitis (AD), a chronic inflammatory skin disease, using a version of the TSST designed for children.[246] The authors found that these patients, like the IBD subjects in our study, showed a blunted HPA response when compared to healthy controls. Yet, in another study by some of the same authors, it was found that there is no significant difference in HPA responsiveness to the TSST between adult AD patients and healthy controls, but that patients with psoriasis (PSO), which is another chronic inflammatory skin disease, had a greater sympathetic responsiveness to the TSST when compared to healthy controls.[247] The most recent study by the same group found no significant

differences in ACTH and cortisol responses to the TSST between PSO patients and healthy individuals, although elevations of these two endocrine biomarkers were positively correlated with changes in the number of circulating granulocytes and NK cells, and negatively correlated with B-cell number.[248] In brief, much remains unknown about the nature of the neuroendocrine-immune interplay and its effects on stress reactivity.

Additionally, given cortisol's effects on the brain, especially the hippocampus, it would be enlightening to examine possible differences in hippocampal-related functions, such as declarative memory performance, as well as other aspects of cognitive functions in IBD patients and healthy control subjects.[150, 249-252] It has been found that the use of high doses of corticosteroids in IBD can lead to impaired short-term memory for details, slower speed, and problems with executive functions and mood.[253] Boyer et al. (2006) also found that young children suffering from recurrent abdominal pain showed non-conscious attention to, but conscious avoidance of, pain-related words.[254] In addition, a recent study found that IBD patients have a lower verbal IQ than the patient's own performance IQ, and the verbal IQ of healthy controls.[255] Thus, the experimental evidence to date provides strong support for a possible cognitive difference between IBD patients and healthy individuals. Whether or not this difference is a result of a dysregulated HPA axis, the use of exogenous glucocorticoids, and/or the proinflammatory milieu that is prevalent among patients, remains to be determined.

Studies in other chronic autoimmune inflammatory diseases, such as SLE and rheumatoid arthritis, have found cognitive deficits in these patients.[256, 257] Therefore, future studies employing neurocognitive testing paradigms that are more specifically targeted towards patients suffering from chronic illnesses can contribute greatly to the understanding of various cognitive effects caused by these medical conditions. The use of imaging techniques, such as functional magnetic resonance imaging (fMRI), on such patients might be another path to obtaining valuable knowledge into the cognitive functioning in this population to determine whether or not there exist any neuroanatomical and neurophysiological

differences in IBD patients, when compared to healthy individuals. Further, psychosocial stress paradigms, such as the Montreal Imaging Stress Task,[258] can be administered to patients during imaging sessions to determine patterns of brain activation and deactivation in these patients in response to neurocognitive tasks before and after psychological stress exposure.

4.6 Conclusion

Results from this study point to a highly agreeable personality type and blunted HPA reactivity to an acute psychosocial stressor in IBD patients, as well as their healthy friends, when compared to healthy non-friend controls. The differential endocrine response may be attributed to personality, social support, and/or neuroendocrine-immune dysregulation. To our knowledge, this is the first study to employ cortisol as a biomarker to study HPA activity in IBD under basal and stress-reactive conditions. This multidisciplinary translational study underscores the importance of the role of personality and social support in IBD. Hopefully, results from this study will stimulate future biopsychosocial research endeavours and eventually lead to the development of effective psychotherapeutic interventions to reduce psychological stress in IBD patients, improve their quality of life, and make more effective use of existing medical resources.

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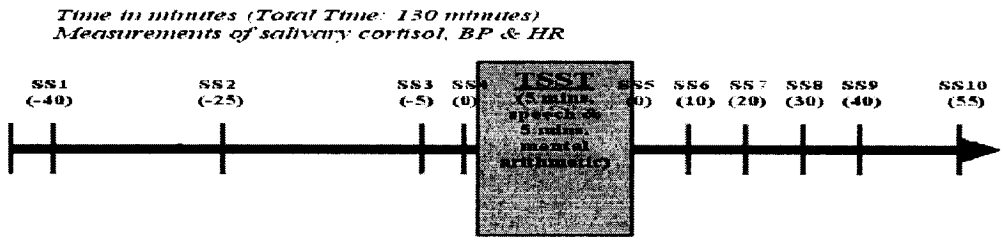
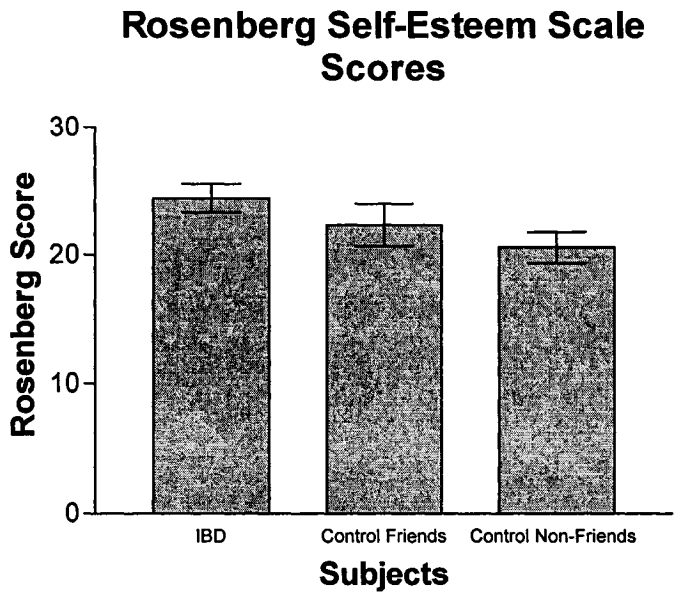
APPENDIX A
FIGURES & DEMOGRAPHIC TABLE

Figure 1



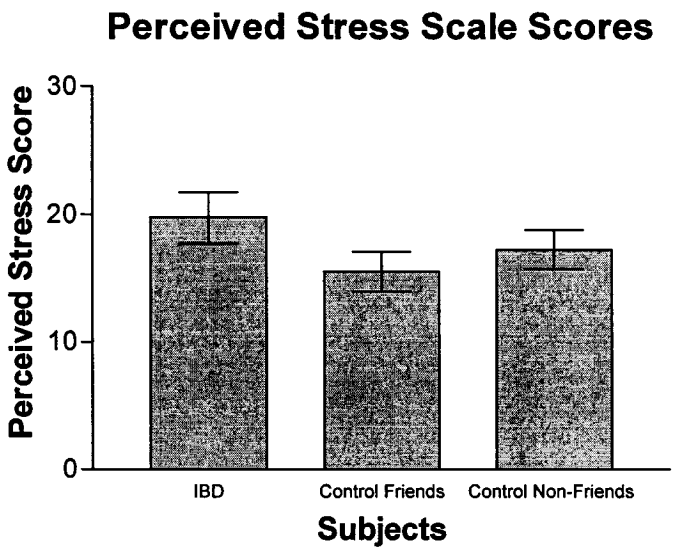
Timeline of the Trier Social Stress Test (TSST) protocol at the Douglas Hospital Research Centre. 3 saliva samples (SS) and blood pressure and heart rate measurements were taken before the TSST (at 40, 25, and 5 minutes before the TSST). One set of measurement was taken immediate before the speech component of the TSST, and 6 sets of measurements were taken after the TSST (immediately, 10, 20, 30, 40, and 55 minutes after the TSST).

Figure 2



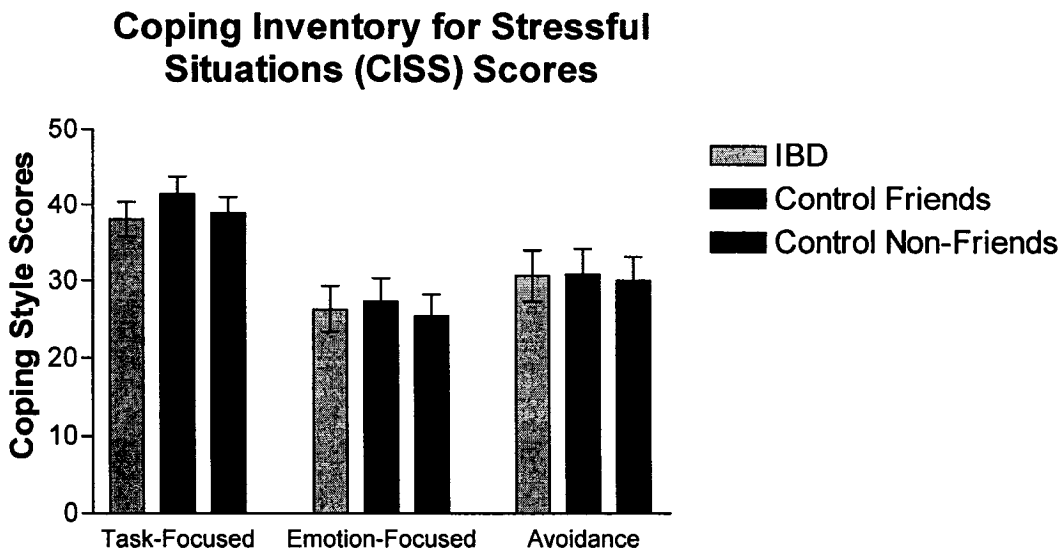
Rosenberg Self-Esteem Scale scores for IBD, control friends, and control non-friends. No statistical differences were found and error bars indicate standard error.

Figure 3



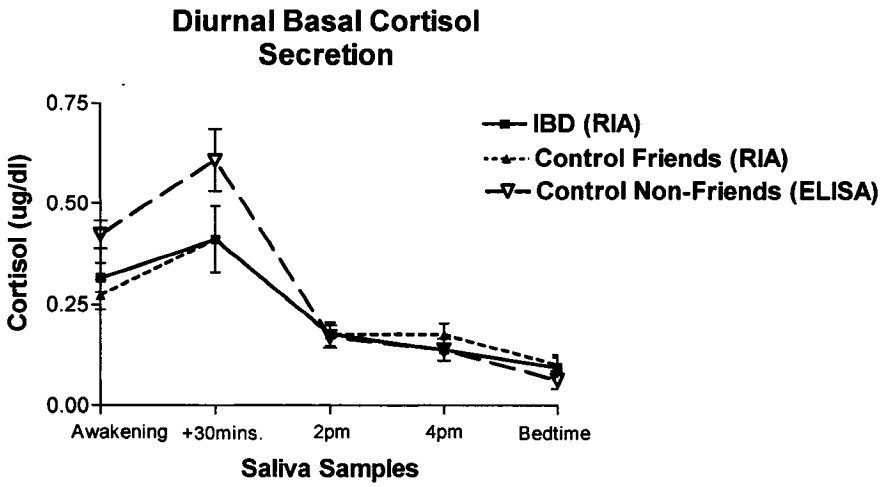
Scores for the 10-Item Perceived Stress Scale in IBD, control friends, and control non-friends. No statistical significance was found and error bars indicate standard error.

Figure 4



Subscores for task-focused coping, emotion-focused coping, and avoidance coping for IBD patients, control friends and control non-friends from the Coping Inventory for Stress Situations. No significant Group difference was found and error bars indicate standard error.

Figure 5



Basal saliva samples of IBD patients and control friends were analyzed using radioimmunoassay (RIA), whereas those of control non-friends were analyzed using enzyme immunoassay (EIA). Error bars on each data point represent stardard error. Due to the incomparability of RIA and EIA data, no conclusions can be made with regards to the basal salivary cortisol secretion pattern between IBD patients and friend controls, and non-friend controls.

Table 1

	IBD Patients	*Control Friends*	*Control Non-Friends*
Total Number (n)	**10 (8 CD; 2 UC)**	**10**	**10**
Males	**4**	**4**	**4**
Females	**6**	**6**	**6**
Mean Age (± standard deviation)	**32.8 (± 6.3)**	**35.0 (± 8.3)**	**31.9 (± 8.2)**

Demographic information of IBD patients, control friends, and control non-friends.

HÔPITAL DOUGLAS HOSPITAL

Centre universitaire de santé McGill
McGill University Health Centre

Title of Project:	A Biopsychosocial Approach to Chronic Inflammatory Bowel Diseases and Functional Gastrointestinal Disorders
Principal Researchers:	Sonia J. Lupien, Ph.D.; Gary E. Wild, M.D., Ph.D., FRCP(C)
Collaborators:	Lorne Cousins, Ph.D.; Benjamin Lai, Graduate Student
Institutions:	Research Center, Douglas Hospital Research Centre Gastroenterology Clinic, McGill University Health Centre
Project Sponsored by:	Crohn's and Colitis Foundation of Canada
Date:	March 17th, 2006

1. RESEARCH PROJECT DESCRIPTION

1.1 Justification

The goal of this research study is to compare various physical and psychological reactions to stress between patients with Inflammatory Bowel Diseases, Functional Gastrointestinal Disorders, and healthy individuals. Inflammatory Bowel Diseases (IBD) include Crohn's Disease and ulcerative colitis. Functional Gastrointestinal Disorders (FGID) comprise Irritable Bowel Syndrome and non-ulcer dyspepsia. Recent studies show that various personality and psychological factors, such as subjective feelings of control in a situation and levels of stress, can influence aspects of the endocrine and the immune systems. This includes a change in the levels of certain hormones in the body, such as those associated with stress. In turn, these changes can affect symptom severity in Inflammatory Bowel Diseases and in Functional Gastrointestinal Disorders. High levels of stress hormones are also associated with aspects of cognition, including memory.

This research project aims to assess the levels of stress hormones that we will measure in your saliva, as well as your memory performance, which we will measure using various tests on a computer. This study may provide important insights into the ways in which psychological factors and a chronic disease state, such as Inflammatory Bowel Diseases or Functional Gastrointestinal Disorders, may affect cognitive and physiological functioning.

You have been asked to participate in this research project because you are between 18 and 45 years of age, and you either have Inflammatory Bowel Diseases, Functional Gastrointestinal Disorders, or you

HÔPITAL DOUGLAS HOSPITAL

Centre universitaire de santé McGill
McGill University Health Centre

FORMULAIRE DE CONSENTEMENT

Titre du Projet:	Étude biopsychosociale des troubles inflammatoires intestinaux et des désordres fonctionnels gastrointestinaux
Chercheurs Principaux:	Sonia J. Lupien, Ph.D.; Gary E. Wild, M.D., Ph.D., FRCP(C)
Collaborateurs:	Lorne Cousins, Ph.D.; Benjamin Lai, Étudiant gradué
Institutions:	Centre de recherche, Hôpital Douglas de Montréal Clinique de Gastroentérologie, Centre Universitaire de Santé McGill
Projet subventionné par:	Fondation Crohn's and Colitis du Canada
Date:	19 Mai 2006

1. DESCRIPTION DU PROJET DE RECHERCHE

1.1 Justification

Le but de cette étude est de comparer diverses réactions physiques et psychologiques au stress chez des patients qui souffrent de maladies inflammatoires de l'intestin, ou de désordres fonctionnels gastrointestinaux et de comparer les résultats à ceux obtenus auprès d'individus ne souffrant pas de ces désordres.

Les maladies inflammatoires de l'intestin incluent la maladie de Crohn et les coliques ulcératives. Les désordres intestinaux fonctionnels incluent le Syndrome du côlon irritable et la dyspepsie non-ulcérative. Des études récentes démontrent que divers facteurs psychologiques et de personnalité, tels que le sentiment subjectif de contrôler une situation ou des niveaux de stress, peuvent influencer certains aspects du système endocrinien (sécrétion d'hormones) et immunitaire (système qui nous aide à combattre les maladies). Ces changements incluent des modifications dans les niveaux circulants de certaines hormones du corps, telles que celles associées au stress. En retour, ces modifications hormonales peuvent affecter la sévérité des symptômes de la maladie inflammatoire de l'intestin ou des désordres gastrointestinaux fonctionnels. De plus, des études ont démontré que des niveaux élevés d'hormones de stress peuvent influencer l'apprentissage et la mémoire.

HÔPITAL DOUGLAS HOSPITAL

DATE OF I.R.B. APPROVAL
MAY 26 2006
Faculty of Medicine
McGill University

Centre universitaire de santé McGill
McGill University Health Centre

Title of Project: A Biopsychosocial Approach to Chronic Inflammatory Bowel Diseases and Functional Gastrointestinal Disorders

Researchers: Sonia J. Lupien, Ph.D.; Gary E. Wild, M.D., Ph.D., FRCP(C)

Institutions: Centre for Studies on Human Stress, Douglas Hospital Research Centre
Gastroenterology Clinic, McGill University Health Centre

Project Sponsored by: Crohn's and Colitis Foundation of Canada

Date: May 19th, 2006

APPENDIX C
QUESTIONNAIRES USED IN THE STUDY

NEO Personality Five Factor Inventory

Read each statement carefully. For each statement fill in the circle with the response that best represents you opinion.

	Statements	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
1	I am not a worrier.	①	②	③	④	⑤
2	I like to have a lot of people around me.	①	②	③	④	⑤
3	I don't like to waste my time daydreaming.	①	②	③	④	⑤
4	I try to be courteous to everyone I met.	①	②	③	④	⑤
5	I keep my belongings neat and clean.	①	②	③	④	⑤
6	I often feel inferior to others.	①	②	③	④	⑤
7	I laugh easily.	①	②	③	④	⑤
8	Once I find the right way to do something, I stick to it.	①	②	③	④	⑤
9	I often get into arguments with my family and co-workers.	①	②	③	④	⑤
10	I'm pretty good about pacing myself so as to get things done on time.	①	②	③	④	⑤
11	When I'm under a great deal of stress, sometimes I feel like I'm going to pieces.	①	②	③	④	⑤
12	I don't consider myself especially "light-hearted".	①	②	③	④	⑤
13	I am intrigued by the patterns I find in art and nature.	①	②	③	④	⑤
14	Some people think I'm selfish and egotistical.	①	②	③	④	⑤
15	I am not a very methodical person.	①	②	③	④	⑤
16	I rarely feel lonely or blue.	①	②	③	④	⑤
17	I really enjoy talking to people.	①	②	③	④	⑤
18	I believe letting students hear controversial speakers can only confuse and mislead them.	①	②	③	④	⑤
19	I would rather cooperate with others than compete with them.	①	②	③	④	⑤
20	I try to perform all the tasks assigned to me conscientiously.	①	②	③	④	⑤
21	I often feel tense and jittery.	①	②	③	④	⑤
22	I like to be where the action is.	①	②	③	④	⑤
23	Poetry has little or no effect on me.	①	②	③	④	⑤
24	I tend to be cynical and skeptical of others' intentions.	①	②	③	④	⑤
25	I have a clear set of goals and work toward them in an orderly fashion.	①	②	③	④	⑤
26	Sometimes I feel completely worthless.	①	②	③	④	⑤
27	I usually prefer to do things alone.	①	②	③	④	⑤

28	I often try new and foreign foods.	①	②	③	④	⑤
29	I believe that most people will take advantage of you if you let them.	①	②	③	④	⑤
30	I waste a lot of time before settling down to work.	①	②	③	④	⑤
31	I rarely feel fearful or anxious.	①	②	③	④	⑤
32	I often feel as if I'm bursting with energy.	①	②	③	④	⑤
33	I seldom notice the moods or feelings that different environments produce.	①	②	③	④	⑤
34	Most people I know like me.	①	②	③	④	⑤
35	I work hard to accomplish my goals.	①	②	③	④	⑤
36	I often get angry at the way people treat me.	①	②	③	④	⑤
37	I am a cheerful, high-spirited person.	①	②	③	④	⑤
38	I believe we should look to our religious authorities for decisions on moral issues.	①	②	③	④	⑤
39	Some people think of me as cold and calculating.	①	②	③	④	⑤
40	When I make a commitment, I can always be counted on to follow through.	①	②	③	④	⑤
41	Too often, when things go wrong, I get discouraged and feel like giving up.	①	②	③	④	⑤
42	I am not a cheerful optimist.	①	②	③	④	⑤
43	Sometimes when I am reading poetry or looking at a work of art, I feel a chill or wave of excitement.	①	②	③	④	⑤
44	I'm hard-headed and tough-minded in my attitudes.	①	②	③	④	⑤
45	Sometimes I'm not as dependable or reliable as I should be.	①	②	③	④	⑤
46	I am seldom sad or depressed.	①	②	③	④	⑤
47	My life is fast-paced.	①	②	③	④	⑤
48	I have little interest in speculating on the nature of the universe or the human condition.	①	②	③	④	⑤
49	I generally try to be thoughtful and considerate.	①	②	③	④	⑤
50	I am a productive person who always gets the job done.	①	②	③	④	⑤
51	I often feel helpless and want someone else to solve my problems.	①	②	③	④	⑤
52	I am a very active person.	①	②	③	④	⑤
53	I have a lot of intellectual curiosity.	①	②	③	④	⑤
54	If I don't like people, I let them know it.	①	②	③	④	⑤
55	I never seem to be able to get organized.	①	②	③	④	⑤
56	At times I have been so ashamed I just wanted to hide.	①	②	③	④	⑤
57	I would rather go my own way than be a leader of others.	①	②	③	④	⑤
58	I often enjoy playing with theories or abstract ideas.	①	②	③	④	⑤
59	If necessary, I am willing to manipulate people to get what I want.	①	②	③	④	⑤
60	I strive for excellence in everything I do.	①	②	③	④	⑤

Rosenberg Self-Esteem Scale

FORM K

Please mark the appropriate answer for each of the statements below.

	Strongly Disagree	**Disagree**	**Agree**	**Strongly Agree**
I feel that I'm a person of worth, at least on an equal plane with others.				
I feel that I have a number of good qualities.				
All in all, I am inclined to feel that I am a failure.				
I am able to do things as well as most other people.				
I feel I do not have much to be proud of.				
I take a positive attitude toward myself.				
On the whole, I am satisfied with myself.				
I wish I could have more respect for myself.				
I certainly feel useless at times.				
At times I think I am no good at all.				

Ten-Item Perceived Stress Scale

Form H

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, please indicate with a check how often you felt or thought a certain way.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

3. In the last month, how often have you felt nervous and "stressed"?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

5. In the last month, how often have you felt that things were going your way?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

7. In the last month, how often have you been able to control irritations in your life?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

8. In the last month, how often have you felt that you were on top of things?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

9. In the last month, how often have you been angered because of things that were outside of your control?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

Coping Inventory for Stressful Situations

FORM O

Please indicate how much you engage in the following 48 activities during a stressful situation.

		Not at all	Rarely	Some-times	Quite a bit	Very much
1.	Use my time better					
2.	Focus on the problem					
3.	Think about good times					
4.	Be with others					
5.	Blame myself for wasting time					
6.	Take best course of action					
7.	Preoccupied with minor aches					
8.	Blame myself for the situation					
9.	Window shop					
10.	Determine priorities					
11.	Get some sleep					
12.	Treat myself to a nice snack					
13.	Worry about not being able to cope					
14.	Become tense					
15.	Consider similar problems					
16.	Tell myself "it's really not happening"					
17.	Blame myself for being too emotional					
18.	Go out for a meal					
19.	Get upset					

		Not at all	Rarely	Some-times	Quite a bit	Very much
20.	Go shopping					
21.	Decide course of action					
22.	Blame myself for not having a solution					
23.	Go to a party					
24.	Understand the situation					
25.	"Freeze"					
26.	Act immediately					
27.	Think about and learn from my mistakes					
28.	Wish that I could change things					
29.	Seek company					
30.	Worry about next step					
31.	Be with a special person					
32.	Go for a walk					
33.	Tell myself "it will never happen again"					
34.	Focus on myself					
35.	Talk to someone					
36.	Analyze the problem					
37.	Phone someone					
38.	Become angry					
39.	Change priorities					
40.	Catch a movie					
41.	Get control of things					

		Not at all	Rarely	Some-times	Quite a bit	Very much
42.	Make an extra effort					
43.	Consider different solutions to the problem					
44.	Take time off					
45.	Take it out on others					
46.	Use the situation to prove myself					
47.	Try to be organized					
48.	Watch TV					