Mindful Student Study: The physiology of mindfulness sub-study

Protocol

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Background

The human mind, brain and immune systems have much in common. All are complex, self-organising economies, recognising self from non-self, interacting with the environment with memory. The immune system alone is a highly complex network of effectors, regulators and signals which depend on dynamic balances to determine the best configuration for each environmental challenge. Recent decades have seen a growing interest in immune dysregulation as key determinant of chronic systemic inflammation, which leads to increased risk of chronic diseases such as cancer, cardiovascular disease, diabetes, dementia and depression (Tabas & Glass, 2013), the most important causes of morbidity and mortality, worldwide. Thus, interventions that balance immunological status in healthy and at risk populations are desirable to prevent a range of mental and physical disorders (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). Even small improvements at a population level could lead to large reductions in morbidity and mortality (Huppert, 2009).
Stress persisting for several weeks (chronic stress) has been shown to promote a pro-inflammatory state (Morey, Boggero, Scott, & Segerstrom, 2015). Healthy, young but stressed individuals may greatly benefit from learning lifelong skills to manage their stress and therefore prevent immune dysregulation which, if maintained throughout life, could lead to chronic disease.

Mindfulness training provides stress management skills (De Vibe, 2012). However, evidence that mindfulness training prevents immune dysregulation or pro-inflammatory states in healthy people is scarce and inconclusive. Few basic immunological outcomes have been tested in randomised trials (Barrett et al., 2012; Creswell et al., 2012; Davidson et al., 2003; Rosenkranz et al., 2013; Walsh, 2011), but no comprehensive and rigorous assessments of how mindfulness moderates the effects of stress on the normal immune system have been conducted. We aim to investigate this in a typically healthy but psychologically stressed population of university students (Macaskill, 2013).

Aims

Among undergraduates at the University of Cambridge stress peaks during the exam term, two months of revision and examinations which determine the outcome of their entire academic year; grades play a crucial role in defining their career paths. The University of Cambridge has recently funded the Mindful Student Study: a randomised, waiting-list controlled evaluation of an 8-week manualised mindfulness programme adapted to students and based on the book *Mindfulness: A Practical Guide to Finding Peace in a Frantic World*, by Mark Williams and Danny Penman. Outcomes are psychological distress up to a year later, academic performance, mental health services use, coping levels, and sleep and physical activity patterns during the exam term.
We propose to conduct a comprehensive, exploratory study on a sub-sample of the 550 evaluation participants to see whether and how mindfulness training could help mitigate immune dysregulation and pro-inflammatory states stemming from chronic stress during the exam term and how this relates to the other outcomes of the study. This is a unique opportunity to add a thorough exploratory study of high-impact biological outcomes to a large randomised evaluation of mindfulness training.

Methods

Immunophenotyping by multi-colour flow cytometry is the most powerful technology available for the analysis of population dynamics, cellular phenotype and function in the immune system (Maecker, McCoy, & Nussenblatt, 2012). It systematically evaluates the unique repertoires of cell subsets and activation markers. We will use state-of-the-art immunophenotyping to examine the proportions of over 80 different key peripheral immune cell subsets to explore the activation patterns that emerge from exposure to chronic stress, and how mindfulness training modifies these patterns.
In addition, we plan to measure key peripheral hormones, cytokines, inflammatory markers and latent virus antibodies to further assess immune system's functionality: cortisol, pro- and anti-inflammatory cytokines, CRP and Epstein Barr virus and cytomegalovirus antibodies.

After volunteer participants consent for the Mindful Student Study online or in person, they will be offered to optionally take part in a sub-study involving blood taking. A sub-sample of 50 consenting volunteers, 25 from each study arm (mindfulness and waiting list), will be recruited. Two blood samples will be obtained for each participant, one before and one two months after mindfulness training (during the exam period). As a token of appreciation £15 pounds will be offered to each participant per sampling session in the form of Amazon vouchers.

Eligibility criteria
Inclusion criteria:

- Mindful Student Study Lent volunteer participants (defined as those who have consented to take part in the Mindful Student Study in Lent)
- Having an exam, viva, 1st year PhD report, or dissertation deadline between 15 May & 15 July 2016

Exclusion criteria:

- Personal or family history of autoimmune disorders, severe allergy or asthma
- Being on regular steroid medication
- Illegal drug or alcohol dependence (addiction)
- Having meditated for more than 10 hours in total in the past or having done a mindfulness 8-week course.

Venous blood sampling

All participants will provide two samples of 22.5 mL venous blood. Sampling will be conducted at the Herchel Smith Building. Blood will be drawn from a butterfly placed in the forearm while participants are lying quietly on an examination couch in a clinical research facility with medical supervision. Samples for each individual will be given a unique, anonymised barcode and stored. Participants' contact details and blood samples will be stored in two separate files such that individual details will be only accessible to the clinical team. The researchers processing the samples will be unaware of the identities of the individuals who gave the samples. Samples will be processed by researchers in the Department of Medicine, Addenbrooke's Hospital.

The sample will be immediately subdivided as follows:

10 ml EDTA tube for immunophenotyping

10 ml EDTA tube for PBMC cryopreservation

2.5 ml serum tube for cytokine analysis

Blood samples will be immediately taken to BRC Cell Phenotyping hub for immunophenotyping. Briefly, peripheral Blood Mononuclear Cells (PBMC) will be isolated by centrifugation over ficoll. An aliquot (1x107 cells) of PBMC will be frozen in FCS/10% DMSO for subsequent analyses. PBMCs will be stored at the Cambridge Institute of Medical Research which has an appropriate licence for the human tissue authority. Serum will be obtained and transported to the Core Biochemical Assay Laboratory, Addenbrooke's Hospital, Cambridge, for cytokine analysis.

Immunophenotyping

Immunophenotyping will be performed by 11 colour flow cytometry of whole blood using an expanded panel of antibodies based on that described by the Human Immunophenotyping Consortium. The 12 antibody cocktails allow discrimination of the following T cell subsets; naive CD4 T cells (CD3+CD4+CD45RA+CCR7+), central memory CD4 T cells (CD3+CD4+CD45RA-CCR7+), effector memory CD4 T cells (CD3+CD4+CD45RA-CCR7-), naive CD8 Tcells (CD3+CD8+CD45RA+CCR7+), central memory CD8 T cells (CD3+CD8+CD45RA-CCR7+), effector memory CD8 T cells (CD3+CD8+CD45RA-CCR7-), effector memory RA CD8 T cells (CD3+CD8+CD45RA+CCR7-), and CD4 Treg cells (CD3+CD4+CCR4+CD127dimCD25+). In addition, additional antibodies allow the detection of activated T cells (CD38+HLA-DR+), Th1 cells (CXCR3+CCR6-), Th2 cells (CXCR3-CCR6-), Th17 cells (CXCR3-CCR6+CD161+IL23R+), and Tfh cells (CXCR5+PD1+). The panel discriminates the following B cell subsets; naive B cells (CD3-CD19+CD27-), transitional B cells (CD3-CD19+CD24hiCD38hi), non-switched memory B cells (CD3-CD19+CD27+IgD+IgG-), switched memory B cells (CD3-CD19+CD27+IgD-IgG+), and plasmablasts (CD3-CD19+CD20-CD38+). The panel discriminates the following monocyte, dendritic cell (DC), natural killer (NK) cell and NKT cell subsets; classical monocytes (CD3-CD19-CD14+CD16-) and inflammatory monocytes (CD3-CD19-CD14+CD16+), myeloid DC (CD3-CD19-CD14-CD20-HLA-DR+CD11c+), plasmacytoid DC (CD3-CD19-CD14-CD20-HLA-DR+CD123+), NK cell (CD3-CD19-CD14-CD20-CD56+CD16+/-), and NKT cell (CD3+CD19-CD14-CD20-CD56+). Flow cytometry will be performed on a Fortessa II Cell Analyzer (Becton Dickinson) and data analysis will be carried out using FlowJo software (TreeStar). Additional analysis of CD34+ will also be performed.

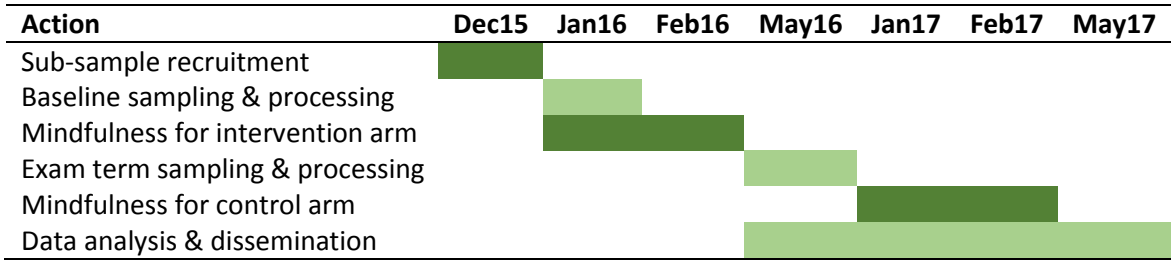
Cytokine Analysis (inflammatory markers)

From volunteers' plasma, 10 cytokines will be analyzed, using ELISA kits (following manufacturer's instructions) at CBAL. The list of cytokines includes: IL-1, IL-4, IL-6, IL-8, TGFβ, IL-12, IFN-a, TNFα, sIL-2R, CCL11 and SDF-1α.

Key outcomes & working hypotheses

This will be an exploratory study generating further biological hypotheses. However, previous research suggests the following basic working hypotheses: (a) Chronic stress will generate a dysregulated pro-inflammatory state, characterised by increased numbers of monocytes, macrophages, activated lymphocytes, and T regulatory cells, accompanied by increases in CRP and pro-inflammatory cytokines IFNγ and TNFα (Barnaba, Paroli, & Piconese, 2012; Morey et al., 2015); (b) Individuals trained in mindfulness will show less pro-inflammatory dysregulation (Creswell et al., 2012; Walsh, 2011); (c) Reduced cellular immunity generated by stress will increase the count of latent virus antibodies as a consequence of viral re-activation (Morey et al., 2015); (d) Mindfulness training will result in reduced viral re-activation and thus less circulating viral antibodies.

Timeline



Plans for dissemination

Crucially, this study will provide important parameters and effect sizes for future research in this area. Findings will be submitted to high impact peer review journals and a clear strategy for public engagement and dissemination will be outlined. It will mainly involve developing an online interactive social media presence, taking part in public engagement events, and using media channels to disseminate research results. It is expected that these activities will impact by increasing public awareness and understanding of mindfulness research and by promoting the adoption of evidence-based stress management techniques.

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Participant information sheet

Before you decide to take part in this study it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. If there is anything that is not clear or if you would like more information please contact a member of the study team (email them at mindfulstudentstudy@medschl.cam.ac.uk, phone them at 01223 746090 or talk to them at one of the information sessions <link to/attachment information session dates>). Take time to decide whether or not you wish to take part.

What is this study about?

This study will explore a key aspect of human physiology that may be affected by mindfulness training: immunity.

The immune system is a highly complex regulated network. Immune system dysregulation is a key determinant of chronic systemic inflammation, which leads to increased risk of chronic diseases such as cancer, cardiovascular disease, diabetes, dementia and depression, the most important causes of morbidity and mortality, worldwide. Thus, interventions that balance immunological status in healthy and at risk populations are desirable to prevent a range of mental and physical disorders.

Stress persisting for several weeks (chronic stress) has been shown to promote a pro-inflammatory state. Healthy, young but stressed individuals may greatly benefit from learning lifelong skills to manage their stress and therefore prevent immune dysregulation which, if maintained throughout life, could lead to chronic disease. Mindfulness training provides stress management skills. However, evidence that mindfulness training prevents immune dysregulation or pro-inflammatory states in healthy people is scarce and inconclusive.

We are conducting an exploratory study on a sub-sample of the Mindful Student Study participants to see whether and how mindfulness training could help mitigate immune dysregulation and pro-inflammatory states stemming from chronic stress during the exam term and how this relates to the other outcomes of the study. For this, we will use a technique called immunophenotyping, a powerful technology. In addition, we plan to measure key stress hormones, inflammatory markers and virus antibodies to further assess immune system's functionality.

How does it work?

All the participants who have consented for the main study Lent recruitment call will be contacted by email with an invitation to take part in this sub-study. Those who consent to take part will choose a blood-sampling slot. We will keep recruitment open until all the blood-taking slots are filled.

In addition to the Mindful Student Study activities, we will request participants in this sub-study to attend Addenbrookes' Hospital twice, one before/during Lent Term (December 2015-January 2016), and the other in Easter Term. Each visit will last half an hour or less.

Each time 22.5ml blood samples will be drawn from each participant. Blood will be drawn from a butterfly placed in the forearm while participants are lying quietly on an examination couch in a clinical research facility with medical supervision.

Who can take part?

In order to take part you need to:

- Be a Mindful Student Study Lent volunteer participant (defined as those who have consented to take part in the Mindful Student Study for Lent courses)

- Have an exam, viva, 1st year PhD report, or dissertation deadline between 15 May & 15 July 2016

However, you **cannot** take part in this study if:

- You or any members of your immediate family suffer from an autoimmune disorder or severe allergy or asthma.
- You are on regular steroid medication
- You suffer from illegal drug or alcohol dependence (addiction)
- You have meditated for more than 10 hours in total in the past or have done a mindfulness 8-week course.

Do I have to take part in this sub-study to continue being a participant in the Mindful Student Study?

No! Taking part is entirely voluntary. If you decide not to take part this will not affect your allocation to a mindfulness course or the way you are treated in any way.

If you take part, you will be able to withdraw from the sub-study at any time with no penalty or loss. You may even request that we destroy all or part of the data that you have contributed before withdrawing from the study (as long as this is requested before data have been aggregated and prepared for publication).

What will happen to me if I take part?

After consenting to take part, participants will be requested to choose a slot for their first visit. Testing will be conducted at the Herchel Smith Building. 22.5 ml of blood will be drawn by an experienced nurse. Blood will be drawn from a butterfly placed in the forearm while participants are lying quietly on an examination couch in a clinical research facility with medical supervision. The same procedure will be followed for the second sampling session in Easter term.

Are there possible disadvantages and/or risks in taking part?

The risks of having blood drawn include soreness and bruising at the puncture site, and sometimes there may be discomfort during the procedure. Occasionally people feel lightheaded or faint. There

is a small risk of infection whenever blood is drawn or when a catheter (tube) is placed in the vein. The amount of blood to be taken (22.5ml) is not considered to be a significant amount, and is therefore not expected to have any significant risk.

If you have any particular worries we strongly suggest you discuss them with us, your college nurse and/or your GP before enrolling in this particular sub-study.

What are the possible benefits of taking part?

Recompense for time and inconvenience associated with participation will be given in the form of a £15 Amazon voucher on each visit. We hope that you will also have the pleasure of knowing that you have made a contribution to our understanding of the physiological effects of mindfulness training.

What happens if you find something abnormal in my blood?

Rarely, we may find abnormal results. If this is the case, we will tell the individual concerned asking them to make an appointment with their GP to whom we will make the results available. The PI of the study (Prof Peter Jones) will be happy to discuss this with any affected subjects personally.

Will my taking part in this project be kept confidential?

All the information you give us will be kept strictly confidential. Records will be maintained in accordance with the terms of the Data Protection Act and the Freedom of Information Act.

Samples for each individual will be given a unique, anonymised barcode and stored. Participants' contact details and blood samples will be stored in two separate files such that individual details will be only accessible to the clinical team. The researchers processing the samples will be unaware of the identities of the individuals who gave the data. Samples will be processed by researchers in the Department of Medicine, Addenbrooke's Hospital. Personal details will be kept encrypted in a secure computer with access only by the data manager and some members of the study team. Reported findings will not have identifying information. Your mindfulness teacher will not have access to your physiological data.

What will happen to the results of the research project?

Results will be presented at conferences and written-up in journals, as well as to the University staff and students. Results are normally presented in terms of groups of individuals. If any individual data are presented, the data will be totally anonymous, without any means of identifying the individuals involved. You will be able to request the results of this study and view any reports which result from it by contacting the study team.

Who is organising and funding the research?

The University of Cambridge is the organiser and main funder of the Mindful Student Study. Research costs associated with this particular sub-study were funded by a Research Award from the Mindful Trust.

Ethical review of the study

This project has received ethical approval from the Psychology Research Ethics Committee of the University of Cambridge. The University has insurance cover for this study.

Contact for further information

For further information please contact the study team at:
mindfulstudentstudy@medschl.cam.ac.uk
01223 746090

Should you be concerned about any aspect of this study you can also contact the research associate:

Dr Julieta Galante
Department of Psychiatry
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Or the principal investigator:

Professor Peter B. Jones
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University of Cambridge
Herchel Smith Building
Cambridge, CB2 0SZ
pbj21@cam.ac.uk

If you are not satisfied with the response you receive, you can contact the Chair of Cambridge Psychology Research Ethics Committee:

Professor John Suckling
Department of Psychiatry
js369@cam.ac.uk

Consent form

Mindful Student Study:

The physiology of mindfulness sub-study

Consent form

By agreeing to take part you **declare** that:

Tick box to confirm you have read each statement:

• You have read and understand the **information webpages preceding this consent**	
• You have had the opportunity to **ask questions** and had them answered	
• You understand that personal details will remain strictly **confidential** and will be separated from all other information so that researchers work with anonymous data.	
• You understand that your participation is voluntary and that you are **free to withdraw** at any time without giving a reason.	
• You are a Lent **Mindful Student Study** participant	
• You **have** an exam, viva, 1st year PhD report, or dissertation deadline between 15 May & 15 July 2016	
• You **don't** suffer from an **autoimmune disorder** or **severe allergy** or **asthma**.	
• **No** members of your immediate family suffer from an **autoimmune disorder** or **severe allergy or asthma**.	
• You are **NOT** on regular steroid medication	
• You do **NOT** suffer from illegal drug or alcohol dependence (addiction)	
• You have **NOT** meditated for more than 10 hours in total in the past or have done a mindfulness 8-week course.	

Now that you understand what is involved, do you agree to take part in 'The physiology of mindfulness sub-study'?

(**download a copy of this consent for your own records)**

I agree

If you don't want to join this sub-study you don't have to do anything/please close this window now. Thank you for reading this far.