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Putative mechanisms Underlying Myocardial infarction onset and Emotions (PUME): a randomised controlled study protocol

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ABSTRACT

Introduction The experience of negative emotions (eg, anger, anxiety and sadness) is associated with an increased short-term risk of incident cardiovascular disease (CVD) events, independent of traditional CVD risk factors. Impairment in endothelial function is one possible biological mechanism which may explain the association between negative emotions and incident CVD events. This laboratory-based, single-blind, randomised controlled experimental study aims to investigate the impact of induced negative emotions including anger, anxiety and sadness on endothelial function.

Methods and analysis In a between-subjects design, 280 healthy participants are randomised to one of four experimental negative emotion inductions: anger, anxiety, sadness or a neutral condition. Endothelium-dependent vasodilation, circulating levels of endothelial cell-derived microparticles and bone marrow-derived endothelial progenitor cells, and indices of nitric oxide inhibition are assessed before and 3, 40, 70 and 100 min after negative emotion induction. Finally, in a subsample of 84 participants, the potential moderating effects of cardiorespiratory fitness and habitual physical activity on the adverse effects of an acute negative emotion on endothelial function are investigated.

Ethics and dissemination This study is conducted in compliance with the Helsinki Declaration and the Columbia University Medical Center Institutional Review Board. The results of the study will be disseminated at several research conferences and as published articles in peer reviewed journals. The study will be implemented and reported in line with the SPIRIT statement.

Trial registration number NCT01909895; Pre-results.

Strengths and limitations of this study

- This is the first randomised controlled experiment designed to assess the acute effects of induced anger, anxiety and sadness on endothelial function; a potential biological pathway underlying cardiovascular disease onset.
- The study uses specific negative emotion induction tasks instead of a non-specific mental stress task. Further, this study includes an emotionally neutral comparison condition.
- In addition to functional measures (ie, flow-mediated dilation), this study is also assessing cellular measures of endothelial function including circulating endothelial cell-derived microparticles and endothelial progenitor cells.
- A substudy ('PUME-FIT') is investigating the possible moderating effects of cardiorespiratory fitness and habitual physical activity levels on the adverse effects of an acute negative emotion on endothelial function.
- Given its between-subjects design, a potential limitation of the study is the inability to compare the effects of the negative emotion induction versus the neutral conditions in each individual.

INTRODUCTION

Cardiovascular disease (CVD), including coronary heart disease, myocardial infarction (MI), heart failure and stroke, is the leading global cause of death, accounting for >17.3 million deaths (ie, 31% of all deaths) in 2013.[1 2] CVD is further associated with decreased quality of life, reduced work productivity and high economic burden on the healthcare system with an estimated global cost of US$863 billion (ie, 17% of overall national health expenditures).[3–5] These collectively underscore the immense health and economic burdens CVD produces in the USA and globally, and the need for further research in its underlying biological mechanisms.

There has been recent interest in the association between the experience of negative emotions (eg, sadness, anger, anxiety) and an elevated short-term risk of incident CVD events.[6] Of these negative emotions, anger has been well studied in large cohort studies.[7–9] The experience of anger is not only linked to negative psychological consequences but

it also acutely increases one's short-term vulnerability to CVD events.[7 9] For example, in the Determinants of Myocardial Infarction Onset Study[7] (n=1623), experiencing anger was associated with a significantly increased risk (relative risk of 2.3) for MI for a 2 hour period after the episode of anger. The Swedish Onset Study[8] involved a similar analysis of 699 patients, and here too, the results suggested that experiencing anger was associated with a significantly increased risk of MI within a 2 hour period, a risk that was highest in the first hour (relative risks of 9.0 in the first hour, and 2.3 between the first and the second hour). Similarly, the acute experience of sadness, and also anxiety, increases the risk of a CVD event, although there is less evidence of these associations compared with anger.[7 10] The biological mechanism(s) by which these negative emotions contribute to incident CVD risk remain(s) to be fully characterised.

One promising mechanism that may explain the link between negative emotional experiences and CVD events is impaired endothelial cell function. Vascular endothelial cells play an essential role in maintaining vascular tone and the integrity of blood vessels. Impaired endothelial cell function is an early pathogenic process underlying atherosclerosis development and CVD event onset.[7 8 11] Impaired flow-mediated vasodilation, as represented by endothelium-dependent vasodilation (EDV), endothelial cell injury, as represented by elevated levels of circulating endothelial cell-derived microparticles (EMPs), and reduced endothelial cell reparative capacity, also as represented by lower levels of circulating bone marrow-derived endothelial progenitor cells (EPCs), are all measures of impaired endothelial function and are associated with increased CVD risk.[12 13] In an earlier exploratory study of 14 apparently healthy individuals, we found that an anger recall task in comparison to a neutral control condition acutely induced impaired endothelial function by impairing EDV.[12] In another study of 30 apparently healthy participants,[13] we observed a reduction of EDV, an increase in circulating EMPs, phenotypic for endothelial cell activation and a decrease in circulating EPCs, a sign of reduced reparative capacity, subsequent to an anger recall task. These findings in two small samples suggest that impaired endothelial function is a mechanism underlying the link between anger provocation and CVD risk. While suggestive, it remains to be demonstrated whether this effect of anger induction on endothelial cell integrity is distinct from the effect of other negative emotions; for example, whether induction of anxiety and sadness also impair endothelial cell integrity (ie, reduce EDV, increase EMPs and reduce EPCs).

In addition to questions concerning the specificity of anger versus other negative emotions on endothelial function are questions concerning how these emotions might provoke damage to endothelial cells. Asymmetric dimethylarginine (ADMA), a competitive inhibitor of nitric oxide (NO) synthase that reduces NO bioavailability,[14 15] impairs EDV, induces EC injury and also inhibits the mobilisation, differentiation and survival of EPCs.[16 17] Similarly, oxidative stress (OS), which also reduces NO bioavailability, has been implicated in the reduction in EDV, in the formation of EMPs and in the inhibition of EPCs.[18 19] Therefore, the experience of negative emotions might inhibit endothelial function due to NO inhibition.[20] Other markers of the stress response, including blood pressure (BP), epinephrine and norepinephrine (indices of autonomic nervous system (ANS)), cortisol (index of hypothalamic–pituitary–adrenal (HPA)) and endothelin-1 have also been reported to be involved in endothelial function.[21–23] Accordingly, these biological pathways might potentially be mediators of negative emotion-provoked impairment in endothelial function.[14 15 21 23]

Potential methods for alleviating the deleterious cardiovascular consequences of negative emotions have also received limited attention in the literature. One such proposed approach might be through increasing physical activity (PA) and cardiorespiratory fitness (CRF). Experimental studies indicate that physically active individuals show an attenuated physiological response (eg, reduced heart rate (HR) and BP) to psychological stressors.[24–26] These findings have led to the derivation of the 'cross-stressor adaptation' hypothesis of PA,[27] which postulates that regular exposure to a physical stressor, such as engaging in regular PA, induces adaptations in the stress response systems of the body (eg, the HPA axis, ANS), which can then help buffer the adverse responses when exposed to other similarly taxing conditions such as anger-inducing, anxiety-inducing or sadness-inducing stimuli.[24 28] As such, it is possible that PA confers protection against impaired endothelial function induced by the experience of a negative emotion and thereby mitigates the CVD risk it incurs.

In summary, anger, anxiety and sadness are commonly experienced emotions[29–32] that are associated with increased incident CVD event risk.[33] Investigation into a unifying biological pathway linking the experience of negative emotions to CVD incidence is novel and may help identify effective preventive strategies for individuals at increased risk for CVD events. Accordingly, the Putative mechanisms Underlying Myocardial infarction onset and Emotions (PUME) study seeks to elucidate the pathophysiological mechanisms (and the respective mediators and moderators) underlying the link between the acute experience of negative emotions and CVD risk.

OBJECTIVES

The overall objective of the PUME study is to examine the acute effects of provoked negative emotions—anger, anxiety and sadness versus a neutral condition—on endothelial function. We hypothesise that an anger recall task, an anxiety recall task and a sadness induction task compared with the neutral condition will acutely induce impaired endothelial function characterised by impaired EDV, increased EMPs and reduced EPCs. Based on the role of NO bioavailability in endothelial function,[21–23]

we will also explore whether endogenous NO inhibition accounts, at least in part, for any observed adverse effects of these induced negative emotions on endothelial function. We will further explore the contributions of the ANS and HPA activity, and endothelin-1 on any observed adverse effects of induced negative emotions on endothelial function. In an ancillary study (ie, 'PUME-FIT'), we will investigate whether CRF and habitual PA moderate any observed effect of induced negative emotions on impairments in endothelial function.

METHODS AND ANALYSES

Brief study overview

This is a single-blind, between-subjects (ie, parallel arm) randomised study design in which 280 participants are randomised to one of the four negative emotion induction (including neutral/control) conditions, yielding 70 participants per condition. Except for those involved in administering the negative emotion induction procedure via allocation using sealed envelopes, the investigation team and research staff are blinded to the condition assignment. Condition allocation sequence is generated using block randomisation by the study statistician (WFC), who is not involved in the data collection process.

Participants

Eligible participants are ≥18 years of age and speak fluent English. Exclusions are (a) any chronic medical condition including prevalent CVD (defined as physician-diagnosed coronary artery disease, coronary revascularisation (eg, stent, angioplasty, coronary bypass surgery), stroke, transient ischaemic attack, peripheral arterial disease or heart failure) and traditional risk factors including history of hypertension, diabetes, dyslipidemia; (b) active smoking; (c) any medication use including over-the-counter drugs and herbal medications; or (d) self-reported history of psychosis, mood disorders or personality disorder diagnoses.

Recruitment and enrolment

Potential participants are recruited via flyers throughout the community, campus-wide emails and newsletters at Columbia University, and additionally through online and local newspaper advertisements. Those interested in participating access a secure web page (URL) to read the complete study description, and after providing consent, are asked to complete the online screening process. Those who are deemed eligible are contacted via phone to schedule a laboratory screening visit. At this visit, participants are checked for viable antecubital vein access (needed for the blood draws) by the research nurse, and those with viable access are asked to complete study consent and a questionnaire battery (see section 'Outcome measures'). They are then scheduled for the experimental study visit. Collected data are entered into a password-protected database and checked by the study coordinators. Data managers conduct quality assurance and maintain the security and storage of the data.

Procedures

Laboratory visit

A timeline of assessments during the laboratory visit is presented in figure 1. Participants are asked to arrive at the research laboratory at 08:30 and instructed to fast from the previous midnight onwards and refrain from any strenuous exercise (to maintain their hydration levels with the 64 oz of water they are asked to drink in the 24 hours prior to their visit) in the previous 12 hours. They are escorted to a temperature-controlled study room and seated in a comfortable chair for the entire visit. An appropriately sized BP cuff is placed on the non-dominant upper arm for BP measurement using a validated device (BpTru; model BPM-200). After 30 min of an initial rest period, BP measurements are taken twice, 1 min apart. A 20-gauge intravenous catheter is inserted into the antecubital vein of the dominant arm. Afterwards, a finger probe for the EndoPAT2000 device is placed on the first digit of each hand for the assessment of EDV. Computerised tracings from the probes are examined for proper placement and function. A BP cuff is placed on the non-dominant forearm for inducing reactive hyperaemia for EDV testing, which is initially deflated. Finally, a chest strap that wirelessly connects to a watch-based receiver (Polar V800)

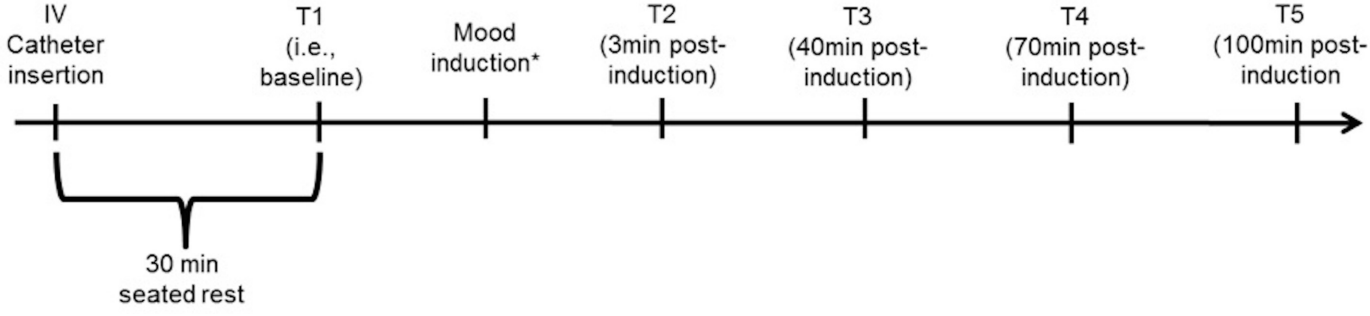

Figure 1 Timeline of events and outcome assessments during the experimental laboratory visit. *Negative emotion induction consists of one of the following emotion inductions: anger, anxious or sadness, or neutral. At each time point (ie, T1 through T5), the following outcomes measures are assessed: (1) endothelium-dependent vasodilation, endothelial-derived microparticles and early progenitor cells. IV, intravenous.

is fitted to the participant for continuous HR data collection. After this set-up, the participant is instructed to relax for 30 min. After this resting period, two BP readings are recorded 1 min apart (ie, time point 1/'baseline'), and EDV assessment is completed. Blood is then drawn into serum tubes, EDTA tubes and citrate tubes. The first tube of the withdrawn blood is discarded (ie, 'discard tube'). One citrated tube is used to measure circulating EMPs. One EDTA tube is used to measure EPCs. The rest of the blood (serum, plasma EDTA, plasma citrate) is centrifuged, divided into aliquots and stored at −80°C. Once ready to be assayed, the aliquots are thawed, and ADMA, measures of OS (see below) and circulating measures of the stress response (plasma cortisol, epinephrine, norepinephrine, endothelin-1) are performed. Likert scale (0=not at all to 10=extremely so) ratings of anger, anxiety and sadness are sequentially performed.

After the baseline measurements, the negative emotion induction task or neutral condition is administered (8 min in duration). This is followed by the same set of measurements (ie, endothelial, BP, HR, self-reported ratings) which are repeated at 3 (time point 2), 40 (time point 3), 70 (time point 4) and 100 min (time point 5) after the negative emotion induction/neutral task is completed (see figure 1).

Negative emotion induction and neutral/control tasks

A trained member of the research investigator team conducts the negative emotion recall and neutral control tasks. To induce the desired negative emotion, a recall technique is used for anger and anxiety, and the Velten mood induction technique is used for sadness, as described previously.[34 35] Briefly, the recall technique involves recalling relevant personal memories to evoke the emotion (ie, anger or anxiety) suggested by the memory. The Velten mood induction technique involves the participant reading descriptors of the target emotional experience to evoke the emotional state (ie, sadness) suggested by the sentence. Participants who are randomised to the neutral task—which controls for the potential effects of speech—are asked to count aloud by ones,[36] starting with 1 and ending with 100, over and over, until 8 min have elapsed. The participant is told that speed is not important when she/he counts aloud—she/he chooses the pace of counting. Likert scale assessment is used as a manipulation check at the end of baseline, at the end of negative emotion induction and at each assessment point during recovery (see section 'State negative affect measures'). As a form of quality control of the method, mood validators are observed using two-way mirror against a checklist of critical components of mood induction.

Outcome measures

Main outcomes

Endothelium-dependent vasodilation

EDV is defined as the reactive hyperaemia index (RHI; transient increase in blood flow following a brief period of arterial occlusion), which correlates with endothelial vasodilator function in the coronary arteries[37] and with brachial flow-mediated dilation.[38] RHI is assessed using EndoPAT2000, a peripheral arterial tonometry (PAT) device, which has been validated for endothelial function testing.[37 39–41] The PAT probes placed on each index finger are attached to a pressure transducer, and through it to the central processing unit, which records the amplitude of each pulse wave as a continuous tracing, providing a measure of the micro-arterial smooth muscle tone in the fingertip (ie, 'RHI'). To induce reactive hyperaemia, the BP cuff located on the non-dominant forearm is inflated for 5 min to 200 mm Hg or 60 mm Hg plus systolic BP (ie, whichever occlusion pressure is higher), and the cuff is then deflated.[37 40 41] The primary EDV outcome is defined as RHI, which is calculated as the ratio of the average amplitude of the PAT signal over a 90–120 s period post deflation divided by the average amplitude of the PAT signal of a 2 min period before cuff inflation (ie, resting period).[42] RHI values are then normalised to the control arm,[37 38 40] which controls for fluctuations in sympathetic nerve outflow that may induce changes in peripheral arterial tone that are superimposed on the hyperaemic response.[43]

Endothelial cell-derived microparticles

EC injury is assessed by measuring circulating EMPs, which are markers of activated or apoptotic ECs.[44] Previous studies indicate that peripheral EMPs expressing CD62E+ are phenotypic for EC activation, and EMPs expressing CD31+ and Annexin V+ are indicative of EC apoptosis.[45–47] Citrated blood is centrifuged at 160×*g* for 10 min to prepare platelet-rich plasma (PRP) and the PRP is further centrifuged for 6 min at 1500×*g* to obtain platelet-poor plasma (PPP). Fifty microlitres of PPP is incubated with three sets; (a) 4 µL of phycoerythrin (PE)-conjugated monoclonal antibody to CD31 (BD) and 4 µL of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to CD42b (BD), (b) 5 µL of PE-conjugated monoclonal antibody to CD62E (BD), and separately, (c) 4 µL of PE-conjugated monoclonal antibody to CD31 and 4 µL of FITC-conjugated Annexin V (BD). EMPs are defined as the number of particles with size <1.5 µm, which are positively labelled by CD62E+ (EMPs expressing CD62E; primary outcome); positively labelled by CD31 and negatively labelled by CD42 (CD31+/CD42 EMPs; secondary outcome); and positively labelled by FITC-conjugated Annexin V (CD31+/Annexin V+ EMPs; secondary outcome). Appropriate FITC-labelled and PE-labelled isotype-matched IgG are used as negative controls. Using standard beads (Bang Laboratories), total flow cytometry counts for each experiment are converted to the number of EMPs per microlitre.

Endothelial progenitor cells

The EC reparative capacity is assessed by measuring circulating EPCs, which are bone-marrow-derived haematopoietic progenitor cells that differentiate into mature ECs and contribute to EC repair after ischaemic injury.

A reduced number of EPCs expressing CD34+/CD133+/KDR+ and CD34+/KDR+ have been associated with increased risk of subclinical atherosclerosis, ischaemic stroke and future vascular events.[48–53] Blood samples are prepared and processed using flow cytometry (BD FACS Calibur) and analysed using previously published protocols.[13 19 52–54] Mononuclear cells in EDTA-anticoagulated blood are isolated by density-gradient centrifugation with Ficoll (Sigma) and counted using a Coulter Counter (Abx Pentra 60, Horiba). One million mononuclear cells are first aliquoted and incubated with 15 µL mouse serum (Sigma) to block non-specific binding of antibodies, followed by an incubation with monoclonal antibodies against human KDR (PE-labelled) (10 µL; R&D Systems), CD34 (FITC-labelled) (20 µL; BD) and CD133 (APC-labelled) (20 µL; Miltenyi Biotec). Isotype-identical antibodies IgG1-PE (BD), IgG-FITC (BD) and IgG2b-APC (eBioscience) serve as negative controls. Data are gated on the mononuclear lymphocytic population, and 500 000 events are collected in the gated region for each sample. Data for the two EPCs measures are expressed as percentages of the mononuclear lymphocytic populations that consist of CD34+/CD133+/KDR+ cells, the primary EPC outcome and CD34+/KDR+ cells, a secondary EPC outcome.

Exploratory outcomes

NO inhibition

Plasma ADMA and OS measures including 8-epi-PGF2 (an F2-isoprostane)[55] and oxidised low-density lipoprotein (oxLDL)[56 57] are NO inhibitors that have been implicated in the impairment of EDV,[18 58–60] formation of EMPs,[61 62] as well as the inhibition of EPCs.[16 18 19] ADMA and OS measures (8-epi-PGF2[55] and oxLDL[63]) will be assessed in plasma samples (EDTA) using commercially available ELISA kits.[64 65]

Stress response measures

Three types of measures are assessed as indices of stress response: ANS measures (ie, systolic and diastolic BP, high-frequency HR variability (hfHRV), epinephrine, norepinephrine), HPA axis measures (ie, cortisol) and endothelin-1. These measures have been selected based on the previous findings indicating their involvement in endothelial function.[21–23] Epinephrine, norepinephrine, cortisol and endothelin-1 will be quantified in serum samples using commercially available ELISA kits. During the study visit, as described earlier, haemodynamic data (BP and HR) are collected using a BpTRU automated blood pressure device. A commercial HR monitor (Polar V800) is used for the assessment of hfHRV (an index of parasympathetic activity); beat-to-beat (RR) intervals are digitised at 1000 Hz and collected by a wrist-based receiver. Unfiltered RR data are exported from the Polar Flow web service as a space delimited .txt file and are then analysed using Kubios HRV Analysis Software.[66]

Self-reported measures of psychosocial factors and health behaviours

The questionnaire battery includes the following questionnaires related to psychosocial factors, health behaviours and relevant family history: the 15-item Spielberger Trait Anger Inventory,[67] which measures the propensity to experience anger[68]; the 27-item version of the Cook-Medley Hostility scale, which includes subscales of cynicism, hostile affect and hostile aggression that represent the cognitive, behavioural and emotional components of hostility, and has been previously found to predict CVD events[69]; the 20-item Trait Anxiety Inventory, which measures the propensity to experience anxiety; the 8-item depression subscale of the NEO Personality Inventory[70 71]; the 14-item Interpersonal Reactivity Index,[72 73] which is a two-dimensional measure of empathy (including perspective-taking and empathic concern); the 12-item Life Orientation Test,[74] which measures dispositional optimism; the 10-item revised UCLA loneliness scale[75]; the 7-item International Physical Activity Questionnaire[76]; the 7-item Tobacco in Your Environment Questionnaire, which asks about smoking by the participant and by others in their home, their exposure to smoke in other spaces and their use of nicotine gum and patches; the 36-item Experiences in Close Relationships – Relationship Structures Questionnaire,[77] which measures attachment across multiple contexts and intrapersonal and interpersonal outcomes; an 11-item Alcohol and Caffeine Use Questionnaire, which measures typical daily consumption of caffeine, daily and weekly consumption of alcohol and maximum consumption of alcohol in a 1-month period; and a 3-item Family Cardiac History Questionnaire that asks participants about whether any family members have died from heart disease before age 55 (males) or age 65 (females).

State negative affect measures

Separate visual analogue scale ratings for anger, anxiety and sadness are used to assess self-reported emotions at each time point during the experiment: before, 3-, 40-, 70-, and 100 min post-negative emotion induction. Participants are asked three questions: ‘How angry or irritated do you feel?’, ‘How depressed, sad or blue do you feel?’, and ‘How anxious, nervous, or jittery do you feel?’. To enhance the sensitivity of this measure to the levels of induced anger, sadness and anxiety, the scale ranges from 1 to 10 for each of the three questions, and the labels on the response options for these scales are scored so that the scale from 1 to 8 ranges from ‘not at all’ to ‘moderately’; and the scores of 9 and 10 correspond to ‘very’ and ‘extremely’, respectively.

PUME-FIT

Procedures

For the subsample of 84 individuals who participate in the ancillary study ‘PUME-FIT’, a separate visit is scheduled 7–14 days after the experimental laboratory visit to administer the graded exercise test (GXT) and provide

instructions for 7-day accelerometry. Participants are instructed to maintain their usual PA habits during this 7–14 day time period and are asked to visit the laboratory for the GXT and the accelerometry instructions after having fasted for 3 hours and refrained from exercise in the previous 24 hours. After completing the GXT, participants are fitted with the activPAL (PAL Technologies) and given instructions for the 7-day accelerometer protocol and wear-time log sheets. Participants return the activPAL device and the sleep/wear-time log sheet to the research facility after completing the 7-day accelerometry protocol.

Cardiorespiratory fitness

Maximal oxygen uptake (VO_{2max}), a measure of CRF, is assessed by GXT on an electronic-braked cycle ergometer (Lode Corival; Groningen, The Netherlands) that is connected to a metabolic measurement cart (MedGraphics, Ultima CPX; MedGraphics, St. Paul, Minnesota, USA). An individualised ramping protocol (ie, 10, 15, 20 or 25 W each 2 min) is selected according to each participant's perceived exercise capacity (ascertained by the Veterans Specific Activity Questionnaire)[78] to yield a test duration of approximately 10 min. Each participant begins exercising at a power output of 30 W for 2 min. The work rate is then linearly increased at the individualised ramp rate while pedal cadence is maintained at 55–65 rev/min, until volitional fatigue, which is defined as the cessation of pedalling despite verbal encouragement to continue. Expired gases are collected breath-by-breath by a pneumotachometer and analysed using the metabolic cart. Calibrations for airflow (3 L syringe) and gas concentrations relative to medical grade gases (12% O_2 and 5% CO_2) are conducted immediately before each test. Twelve-lead ECG is used to continuously monitor HR (Welch Allyn CardioPerfect Workstation, Welch Allyn, Skaneateles Falls, New York, USA), and BP and rating of perceived exertion (Modified Borg 0–10 scale)[79] are measured every 2 min throughout the test. In order to confirm that a maximal test was performed (and hence confirm attainment of VO_{2max}), a supramaximal verification protocol[80] is completed wherein on reaching volitional fatigue during the ramping protocol participants are given a 5 min active recovery period of light cycling at 0–25 W, a 2 min passive recovery of seated rest and thereafter cycle against a resistance 105% of the maximal resistance attained during the ramp test until they again reach volitional fatigue.

Physical activity

PA is assessed using the activPAL (V.3, Glasgow, UK), a thigh-worn triaxial accelerometer and inclinometer that has been validated for determining step counts, PA, activities of daily living, posture (sitting/lying, standing or stepping), sedentary time, and sit-to-stand and stand-to-sit transitions in healthy adults.[81–87]

The activPAL is covered with a nitrile sleeve and is worn by participants on the midline of their right thigh, one-third of the way between the hip and knee via a breathable adhesive dressing (Hypafix or Tegaderm) in accordance with manufacturer specifications. Participants are fitted with the activPAL device during the laboratory visit and are provided instructions regarding activPAL wear including the correct orientation in which to wear the monitor. Participants are instructed to wear the device continuously for 7 days and to not remove the monitor (to increase weartime compliance) unless it is to be fully submerged in water (eg, swimming, bath). Nitrile sleeves and dressings to reattach the monitor are provided along with a single-page, paper-based diary to record daily sleep (ie, time into bed, time lights off) and wake (ie, wakeup time, out of bed time) times and times when the device is removed (if any). Sleep/wake times are ascertained using these logs in order to distinguish sedentary time from sleep time (both are inferred as inactivity by the activPAL). The activPAL is initialised by the activPAL software (V.7.2.32) using the manufacturer default settings including a sampling frequency of 20 Hz. On completion of the 7-day accelerometer protocol, the time stamped 15 s 'epoch' data file from the activPAL software is exported as a .csv file for processing and analysis in SAS. Mean minutes per day of moderate-to-vigorous intensity PA is a secondary measure.

Patient and public involvement

Patients and the public are not involved in the development of the research questions or the outcome measures, recruitment or the conduct of the study.

Statistical analyses

Results of the primary and secondary outcomes of EDV, EMPs and EPCs will be tested via mixed-effects regression models in which each of the three measures will separately be regressed on the five time points (naturally coded as minutes) and a dummy-coded variable representing the group comparisons (ie, 0=neutral condition, 1=anger/sadness/anxiety recall). That is, separate 2×5 mixed regression models will be conducted for each negative emotion induction task compared with the neutral, control condition. The error term for the analysis will be based on an unstructured variance–covariance matrix ('MANOVA') across the five time points. We recognise that this set of analyses does not represent an orthogonal set of comparisons as each emotion condition is compared with the same (neutral) condition. However, this set of analyses provides the most direct test of our hypotheses. The partial dependency of these analyses will be clearly noted in our reports. The analyses of the exploratory outcome measures of ADMA, OS and stress response will follow the same procedures (ie, mixed-effect regression models). The relations of these stress response indices with measures of endothelial function after each negative emotion recall or neutral task will be explored. The amount of change from pre- to post-negative emotion induction in EDV, EMP and EPC that are explained by changes in ADMA and OS levels will

be determined. For this purpose, previously described methods for mediation and generalisation to multilevel models will be followed.[88 89] To account for person-level variances in trait measures, we will conduct additional analyses to assess the possible moderating effect of state–trait interactions (ie, anger, hostility, sadness and anxiety) on impaired endothelial function. We will include each trait measure as a continuous person-level variable in the mixed-effects regression analysis and will hierarchically test the three-way product of group × time × trait in the analyses with group and trait as fixed factors and time as a random factor. Trait–state interactions on endothelial function will be explored by testing these traits (ie, anger, hostility, sadness and anxiety) as moderators of the effect of each negative emotion recall task on endothelial function.

Sample size estimation

Sample size was estimated based on previously published guidelines[90 91] on power calculation for longitudinal experimental designs. Using data from our previous studies,[12 13 92] we estimated that our effect size would be 'moderate' (ie, a standardised effect size d=0.30). This effect size is based on the smallest effect obtained on the outcome measures (expressing CD62E) in our open-label trial, downwardly adjusted by 25% to account for the use of a control group in this randomised trial. The sample size to detect a statistically significant effect based on a two-tailed test and alpha level set at 0.05 is 70 participants in each condition for a total of 280 participants.

ETHICS AND DISSEMINATION

Informed consent

Informed consent and Health Insurance Portability and Accountability Act Authorization for Research form is signed at the time of enrolment. The research coordinator describes the study to the prospective participant and answers any questions. They are reminded of confidentiality and the freedom to withdraw at any time without explanation or effect on their future interactions with their healthcare provider or employer. If the individual wishes to participate, he/she signs the informed consent document, which the research coordinator co-signs.

Ethics review and dissemination

This study is conducted in compliance with the Helsinki Declaration and the Columbia University Medical Center Institutional Review Board. The results of the study will be disseminated at several research conferences and as published articles in a peer reviewed journal. The study is implemented and will be reported in accordance with the SPIRIT[93] guidelines.

DISCUSSION

To our knowledge, the PUME study is the first randomised-controlled experiment to examine acute effects on endothelial function of induced anger, anxiety and sadness compared with each other and to an emotionally neutral condition. The study will also examine whether any adverse effects of induced negative emotion on endothelial function are mediated by changes in ADMA and OS. Episodic anger, anxiety and sadness are commonly experienced and associated with increased incident CVD event risk, and it is possible that each of these negative emotions differentially influence endothelial function. If the results of this study indicate that NO inhibition and OS levels mediate this association, then a potential strategy in future interventions might be to target this biological pathway.[94] In PUME-FIT, investigation of CRF and habitual PA levels as possible moderators can further elucidate if these factors buffer the adverse consequences of negative emotions experienced in daily life.

There are several possible limitations. First, the study has a between-subjects rather than within-subjects design. This decision was made to avoid potential carryover effects between conditions, which we have found in our prior studies (principal investigator, Dr Shimbo, PUME Pilot Study) with a within-subjects design. Second, this study does not capture the possible effects of negative emotions on endothelial function beyond the 100 min post-induction task, which limits our ability to make inferences regarding how long these effects are maintained. The purpose of the study, however, is to examine the *acute* adverse effects of negative emotions, not determine the time course of these effects post-negative emotion induction. Participants are apparently healthy individuals without prevalent CVD or CVD risk factors, thereby potentially limiting the study's generalisability. Participants with prevalent CVD or CVD risk factors are excluded given that the study purpose is to examine the mechanisms underlying incident CVD risk. Further, the inclusion of participants with prevalent CVD or CVD risk factors, which are themselves associated with a substantial impairment in endothelial function,[95 96] might obscure the adverse effects of negative emotions on endothelial function. The study also excludes participants taking any medications due to their possible effects on endothelial function. Whether the study findings can be extended to those with prevalent CVD, CVD risk factors and/or taking medications remains unknown.

In summary, the PUME study is a laboratory-based, translational study that aims to elucidate the mechanistic link between core negative emotions and CVD events, and may ultimately have a substantive impact on reducing incident CVD events. PUME-FIT aims to investigate the potential moderating effects of PA and CRF levels on this biological link. Investigation into a unifying biological pathway linking the experience of negative emotions to CVD incidence is novel and may help identify effective preventive strategies for individuals at increased risk for CVD events. Future randomised-controlled trials could test the efficacy of different psychosocial, pharmacological and PA or

exercise interventions to examine if such intervention approaches can help attenuate the impairment in endothelial function for individuals who experience frequent episodes of negative emotions.

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