**1. Protocol Title**: Exercise and Pharmacotherapy for Anxiety in Cardiac Patients

**2. Purpose of the Study:**

Coronary heart disease (CHD) is the leading cause of death in the United States; more than 600,000 Americans suffer a fatal cardiac event each year. Traditional CHD risk factors such as high blood pressure, smoking, and elevated cholesterol do not fully account for the timing and occurrence of CHD events. The term "cardiovascular vulnerable patient" has been used to describe patients susceptible to acute coronary events based upon plaque, blood, or myocardial characteristics. Psychosocial factors also have been shown to be associated with increased adverse health outcomes and increased cardiovascular vulnerability. For example, clinical depression and elevated depressive symptoms are associated with increased morbidity and mortality, and as a result, the American Heart Association has recommended that clinicians should routinely assess depression in CHD patients. Although much research and clinical recommendations have focused on depression, the significance of anxiety has been largely ignored, despite the fact that anxiety disorders are as prevalent as depression in the general population and are associated with similar levels of disability.

Despite the prevalence and prognostic significance of anxiety in CHD populations, there have been few randomized clinical trials (RCTs) specifically targeting anxious CHD patients. Anxiolytic medications, including selective serotonin reuptake inhibitors (SSRIs), have been shown to be effective in treating anxiety. SSRIs have been evaluated for the treatment of clinical depression in cardiac patients, with equivocal results. Surprisingly, to our knowledge, there have been no RCTs examining the efficacy of medications for treating anxiety in CHD patients. Moreover, because many cardiac patients are reluctant to take additional medications and psychotropic medications may not be effective for everyone or may produce unwanted side effects, there continue to be a need to identify alternative approaches for treating anxiety in cardiac patients. We believe that exercise may be one such approach.

The purpose of this study is to evaluate the following hypotheses in a population of CHD patients with elevated symptoms of anxiety. The present study will examine the impact of a 3-month intervention of either exercise, Lexapro, or placebo on anxiety symptoms and CHD biomarkers among individuals with cardiac disease and elevated anxiety. We hypothesize that: (1) Both exercise training and medication will reduce anxiety symptoms to a greater extent than placebo; (2) Exercise training will improve CHD biomarkers of risk including autonomic regulation, vascular endothelial function, and inflammation more than either medication or placebo; and (3) Improvements in CHD biomarkers will be mediated by reductions in symptoms of anxiety. We also will explore potential moderators of treatment (e.g., anxiety diagnoses, CHD severity) as well as the longer-term benefits of treatment by documenting medical events and health care costs over a follow-up period of up to 4 years.

**3. Background & Significance:** Over $100 billion is spent on CHD each year in direct medical costs, disability payments, and lost productivity.[61] Mental illness also is a major health problem in this country, with estimated direct costs of $57.5 billion in 2006.[62] Mental disorders are associated with significant impairment of function that may, at times, be worse than that of chronic medical disorders. Anxiety disorders are the most commonly diagnosed forms of mental illness in the U.S. and are responsible for one-third of the total expenditures of the federal government for mental illness.[63] Approximately half of those costs are due to the repeated use of health care services since people with anxiety disorders often solicit medical evaluation for symptoms that resemble physical illnesses.[64] Nationally representative surveys indicate that as many as 30% of patients will suffer from some kind of anxiety disorder during their lifetimes,[65] a figure that has increased significantly over the past 2 decades. Anxiety symptoms have been correlated with the presence of one or more chronic diseases,[66] as well as impaired work performance, increased use of medical services, decreased well-being, and lowered functioning.[31,67] Although the actual prevalence of anxiety disorders among cardiac patients is not known, Tully and Cosh reported an 11-14% prevalence of generalized anxiety disorder (GAD) across 12 studies (N=3485) and a pooled lifetime prevalence of 26%.[68] Frasure-Smith[35] noted that 5.3% of a sample of 804 patients with stable CHD had GAD and 41.4% had elevated anxiety symptoms measured by the Hospital Anxiety and Depression Scale-Anxiety (HADS-A). Thus, anxiety is common in CHD patients. However, the prior set of reviews raised questions about the significance of anxiety in CHD patients and of the novelty of exercise to treat anxiety. Our team took up this challenge by revisiting and expanding our previous review of the relevant literature and conducting new analyses of pilot data from our laboratory, both of which proved helpful in further articulating a working conceptual model and providing a rationale for our proposed RCT.

Anxiety and CHD Risk. Findings from a number of prospective epidemiological studies report a strong association of anxiety with mortality in healthy individuals [32,69,70] and in CHD patients.[35,39,71-75] Our own studies

57  have shown that elevated scores on the anxiety subscale of the HADS were associated with increased risk of
58  mortality after accounting for established risk factors in 934 men and women with CHD (Hazard Ratio  HR ,
59  2.27; 95% CI, 1.55 to 3.33, p<.001).[45]  Elevated anxiety symptoms have been shown to be associated with a 2-
60  fold increased risk of mortality in CABG patients[72,74] and in outpatients with CHD.[35,42,44]  Frasure-Smith and
61  colleagues reported that CHD patients with GAD assessed two months following hospital discharge showed a
62  2.3-fold increased risk of adverse cardiac events, and Strik et al.[44] reported a 2.8-fold increased risk of adverse
63  events in acute post-MI patients in which anxiety was measured one month following hospital discharge.
64  Similarly, a 2-fold increased risk of adverse events was observed in stable CHD patients[76] and in patients with
65  elevated anxiety during annual clinic visits.[43]  Similar to the depression literature,[14] not all studies have found a
66  prognostic relationship, especially when anxiety was measured in-hospital following an acute coronary event or
67  during diagnostic exercise stress testing.[26,27,29-31,77]  In the proposed study, anxiety symptoms will be evaluated
68  outside of the clinic and hospital environment in individuals with stable CHD with high levels of anxiety.
69          Anxiety and depression share high comorbidity.[35,78,79]  Findings from several recent prospective studies
70  suggest that anxiety predicts increased risk independently of depression and that the presence of both anxiety
71  and depression identifies individuals at greater risk of mortality than those with either prognostic factor
72  alone.[38,45]  We observed a 3-fold increased risk of mortality in CHD patients with comorbid anxiety and
73  depression, compared with an approximate 2-fold risk in patients with either anxiety or depression alone.[45]
74  The additive effects of anxiety and depressive symptoms have been noted by other investigators,[38,80] and we
75  will explore this issue in our proposed trial.
76          Treatment of Anxiety Disorders in Cardiac Patients.  Despite compelling reasons for treating anxiety in
77  CHD patients, there have been few trials that have examined the effects of treating anxiety symptoms in
78  cardiac patients, and no studies reported the effects of treating anxiety on clinical outcomes.  Several studies,
79  including the ENRICHD trial, have examined the effects of treating depressed post-MI patients,[81] and several
80  pharmacologic studies have examined the effects of SSRIs on depressive symptoms and outcomes in cardiac
81  patients.[48-50,82,83]  The SADHART-CHF trial found no advantage for sertraline over placebo in either reducing
82  depression or in improving clinical outcomes.[48]  The SADHART study[49] also reported no differences between
83  CHD patients receiving sertraline compared to placebo, although greater reductions in depressive symptoms
84  were observed in the subset of patients with more severe depression.   We will consider more severe anxiety
85  (i.e., diagnosed anxiety disorders) as a potential moderator of treatment in our proposed RCT.
86     Exercise and Anxiety.   The use of aerobic exercise has been widely used in many secondary prevention
87  programs[84] and may reduce risk of fatal CHD events.[85]  The mechanisms for this benefit remain uncertain,
88  although exercise has been shown to reduce traditional risk factors such as hypertension and hyperlipidemia,[84]
89  attenuate cardiovascular responses to mental stress and reduce myocardial ischemia,[86-89] and reduce
90  depressive symptoms in patients with MDD,[55] heart failure,[90] and in CHD patients with elevated depressive
91  symptoms.[91]  Epidemiological studies have observed an inverse relationship between exercise and anxiety.  In
92  a study of 8,098 adults, Goodwin[92] reported that persons who indicated that they exercise "regularly" were at
93  reduced risk for being diagnosed with an anxiety disorder compared to their sedentary counterparts.  As was
94  noted by a previous reviewer, there have been many exercise trials that have reported anxiety as an outcome.
95  For example, Wipfli and colleagues[93] found that exercise was associated with an overall effect size of 0.48,
96  indicating greater reductions in anxiety symptoms compared to no-treatment controls, and Herring and
97  colleagues[59] reported that exercise was associated with an overall effect size of .29, compared to control
98  conditions.  In a review of 8 RCTs of patients with a broad range of anxiety disorders, Jayakody[94] noted that
99  exercise seems to be effective as an adjunctive treatment for most anxiety disorders, but there were too few
100 studies to provide meaningful conclusions.  In response to the prior review, we re-examined the exercise
101 literature and identified only 12 RCTs specifically targeting patients with high anxiety, most of which had
102 serious methodological shortcomings, including small sample sizes, lack of blinding of assessors, confounding
103 of exercise with other treatments, and no adherence to intent-to-treat analytic principles.[95]  In one of the few
104 studies of cardiac patients, Lavie and Milani[23] reported more than a 69% reduction in anxiety among highly
105 anxious participants in an exercise-based cardiac rehabilitation (CR) program; however, there was no control
106 group and exercise was only one component of the intervention.  In the one RCT that targeted cardiac patients
107 with elevated anxiety, Oldridge and colleagues reported greater reductions in symptoms of anxiety assessed
108 by the STAI and POMS after 8 weeks of CR compared to community care controls.[60,96]  However, the CR
109 group also received concurrent weekly 90-minute group counseling sessions, including training in progressive
110 relaxation, so that the benefits of exercise training could not be determined.  Thus, while results from previous
111 exercise studies are encouraging, there remains an important gap in understanding the potential therapeutic
112 benefits of exercise, especially among anxious cardiac patients who are vulnerable to adverse cardiac events.

Biomarkers of CHD Risk. Reliance on "hard" clinical endpoints (MI and death) that occur infrequently and require large sample sizes over extended follow-up intervals has proven to be a major challenge to furthering our understanding of the optimal ways to treat vulnerable cardiac patients because of the obvious logistical and financial obstacles such investigations present. One solution to this problem is to first study intermediate markers of risk in patients who are vulnerable to untoward cardiac events.[5] Examination of changes in intermediate endpoints can provide important insights into the mechanisms and potential value of clinical interventions and has a number of important advantages over "hard endpoints," in that fewer patients are required to detect treatment effects and changes in cardiac risk can be reliably and objectively measured over short follow-up intervals. Following the guidelines advanced by a panel of prominent cardiovascular scientists,[2,3] we propose to examine intervention effects on autonomic, vascular, and inflammatory biomarkers of cardiovascular risk (i.e., "intermediate endpoints") in "vulnerable" CHD patients with high anxiety:

*Heart rate variability* (HRV) is widely recognized as an important index of autonomic regulation of the heart and prognostic indicator of risk and will serve as our primary biomarker of interest. Reduced 24-hour HRV independently predicts mortality in community samples[97-99] and patients with stable CHD,[100] with a recent MI, [101] or with heart failure (HF).[102]

*Baroreceptor reflex sensitivity* (BRS) is also an index of cardiac regulation by the parasympathetic nervous system (PSNS), with experimental models showing that low levels of BRS predict sudden cardiac death (SCD) due to ventricular fibrillation.[103] Anxiety has been linked to reduced PSNS control of heart rate in several different populations, including patients with anxiety disorders,[104-108] MDD, [109,110] and CHD,[111] as well as healthy volunteers.[112] Elevated sympathetic nervous system (SNS) activity is another pathophysiological aspect of autonomic dysregulation contributing to the development of CHD[113] that is thought to contribute to increased cardiovascular risk associated with anxiety.[18,114]

Although no study has compared the effects of an SSRI with exercise training on HRV in anxious cardiac patients, it is well documented that SSRIs that inhibit the reuptake of norepinephrine reduce HRV.[115,116] Several RCTs have provided evidence that regular exercise improves HRV, as well as BRS, in middle-aged and elderly sedentary subjects[117,118] and patients with CHD. In the present proposal, 24-hour HRV will be our primary biomarker in examining the impact of exercise and anxiolytic medication on cardiovascular risk in anxious CHD patients, with SNS activity also assessed in order to provide a comprehensive assessment of intervention effects on autonomic regulation.

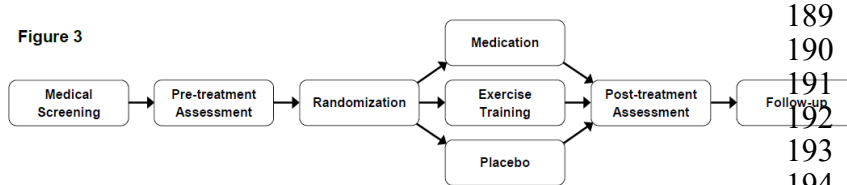*Endothelial dysfunction* plays a vital role in the development, progression, and clinical manifestations of atherosclerosis.[119,120] It can be assessed non-invasively using standardized vascular ultrasound techniques to determine flow-mediated dilatation (FMD) of the brachial artery,[121] has been related to a wide range of CHD risk factors,[122] and has been shown to be prognostic in cardiac patients. Impaired FMD has been linked to elevated depression symptoms in stable CHD patients,[123] and to elevated anxiety symptoms in CHD patients following a percutaneous coronary intervention.[124] Several studies also have shown that acute mental stress is associated with impaired FMD.[125-127] There is preliminary evidence that exercise may improve endothelial function in CHD patients. Several cross-sectional studies have found that self-reported physical activity is associated with preserved endothelial function.[128,129] Several small interventional studies also have shown that FMD may be improved by exercise training in cardiac patients.[130-133] These observations suggest that reducing anxiety symptoms may result in improved FMD and that anxious CHD patients randomized to the exercise intervention may demonstrate the most marked improvements in FMD.

*Inflammation* is widely considered to play a central role in the development and progression of CHD. C-reactive protein (CRP), an acute-phase reactant primarily produced in hepatocytes, is a highly sensitive marker of underlying systemic inflammation.[134] An elevated level of CRP is an independent risk factor for MI and stroke.[135,136] Interleukin-6 (IL-6) is an inflammatory cytokine that may be the initial event leading to an increase in CRP levels, and recent meta-analyses indicate that IL-6 may be more strongly related than CRP to the promotion of atherosclerosis.[137,138] Elevated levels of both CRP and IL-6 have been observed in patients with depression,[139-141] but few studies have examined the relationship with anxiety.[142] In a subsample of healthy participants in the ATTICA study, Pitsavos et al.[143] observed a significant dose-response relationship between the severity of anxiety symptoms and CRP and IL-6. In a cross-sectional study of 120 stable CHD outpatients, Bankier et al.[144] also found GAD to be associated with increased CRP. In a sample of 682 post-ACS patients, Frasure-Smith et al.[145] reported that HRV was correlated with inflammatory markers and suggested that interventions targeting regulation of both autonomic control and inflammation may be especially worthwhile. The interventions proposed in this RCT are designed to accomplish this goal.

Summary. We propose to examine the effects of exercise and anxiolytic medication on both symptoms of anxiety and on "intermediate endpoints" that are associated with adverse outcomes in CHD

169 patients. In this application, we propose a RCT to compare the effects of exercise training and anxiolytic
170 medication (escitalopram Lexapro ) to a placebo in anxious patients with CHD. Patients will be assessed on
171 clinical, behavioral, and physiological dimensions at baseline, 3 months (post-treatment), and 6-month follow-
172 up. Additional measures of quality of life also will be obtained along with annual follow-ups to document
173 clinical events and medical costs. The findings from this RCT will have directly translational implications for
174 anxiety assessment and management in CHD patients.
175

176
177 **4. Design & Procedures:** This will be a single-site, randomized clinical trial of exercise training and anxiolytic
178 medication in the treatment of anxiety in anxious CHD patients without MDD. One hundred fifty men and
179 women aged >39 years with stable CHD and elevated anxiety symptoms (HADS-A scores >10) will be
180 randomly assigned to Exercise Training, Medication (escitalopram), or Placebo. Patients will be evaluated for
181 anxiety at baseline, after 3 months of treatment, and at 6-month and annual follow-ups. Three months is
182 ample time to observe cardiovascular conditioning effects, even among older cardiac patients, whose
183 responses are often quite variable, and is also adequate to assess the efficacy of pharmacologic treatment.
184 Comprehensive assessments will include clinical evaluations of anxiety and measures of intermediate
185 surrogate endpoints including autonomic, vascular, and blood markers of risk. Following the completion of the
186 3-month treatment program, subjects will undergo a post-intervention repeat assessment. Patients will then
187 undergo a 6-month follow-up evaluation for anxiety and CHD risk status and will be followed annually for
188 anxiety and clinical events (see Figure 3) . All assessors will be blinded to patients' treatment group



Figure 3

189 assignment; groups receiving a pill will be
190 double-blind. Following completion of the
191 intervention, participants will be advised to
192 contact their referring physicians, if
193 necessary, for further treatment, or they
194 will be referred to appropriate mental
195 health care providers.

196
197 Methodological Considerations: As we noted in our original application, we carefully considered several
198 alternative experimental designs for the proposed study. A 2-group design (e.g., Exercise vs. Medication or
199 Medication with or without Exercise) was not adopted because it did not allow for a rigorous examination of the
200 separate effects of exercise and medication. If both groups were to improve, it would be impossible to
201 determine if this were true improvement or a placebo response. We also considered the merits of the placebo
202 condition and questioned whether the inclusion of a placebo condition was of sufficient scientific value to offset
203 the possible negative consequences of providing a "treatment" known to be of limited effectiveness. The use
204 of placebo control groups has been questioned on scientific and ethical grounds,[155] but we believe that a
205 placebo condition is crucial to accomplishing the aims of the study and that we can provide appropriate
206 safeguards to assure the safety of subjects randomized to receive this treatment. Because escitalopram
207 already has been proven superior to placebo in double-blind clinical trials, it could be argued that a positive
208 study finding that exercise therapy is equivalent in clinical effectiveness to escitalopram would be tantamount
209 to demonstrating that exercise therapy is superior to placebo. However, this argument assumes the rate of
210 placebo responding to be relatively low and fairly consistent across study situations and patient populations;[156]
211 in actuality, placebo response rates in pharmacological studies of anxiety disorders are high and variable.[157,158]
212 This variability greatly limits our freedom to make assumptions about the placebo response rate in any new
213 study context. Unless a superiority of medication to pill-placebo can be demonstrated in the current study, it
214 could be maintained that these patients were not medication-responsive and that any observed treatment
215 effects would be open to the parsimonious explanation of a placebo response. Thus, it is not adequate merely
216 to compare the exercise intervention to an FDA-approved and widely-utilized anxiolytic medication
217 (escitalopram); a pill-placebo condition is necessary to claim therapeutic equivalence.[159]
218 Alternatives to placebo-controlled studies also have significant limitations[160] that cannot be adequately
219 addressed by even the most sophisticated statistical procedures. Klerman[161] has identified several scientific
220 reasons for the use of placebo conditions, including controlling for "package effects" such as passage of time,
221 increased staff attention, and hope. We acknowledge that expectations for patients receiving pill and exercise
222 may be different, and we will obtain ratings of patient expectations prior to and following randomization, which
223 we plan to incorporate into our analyses and interpretation. We also recognize that the amount of contact
224 between patients taking pills may be less than those receiving exercise. However, the conventional wisdom
225 that an attention control group automatically makes a trial more scientifically rigorous has been challenged.[162-

226 [164] Unlike pharmacologic trials, it also is impossible to keep patients blinded to their treatment condition. So,
227 while recognizing the inherent limitations of studies comparing pharmacologic and behavioral treatments, we
228 propose exploratory analyses to assess potential confounding. We will consider the effects of patient
229 expectations on outcomes, as well as different elements of the interventions that might be responsible for any
230 treatment group differences. We also emphasize that our proposed RCT is not intended to be a "head-to-
231 head" comparison of exercise and medication on anxiety. It would be considerably more difficult to distinguish
232 between active therapies than it would be to find a difference between active therapies and placebo, and such
233 a trial would require a very large sample that would be prohibitively expensive.
234     In response to the prior review, we also considered alternative medications. All SSRI drugs have been
235 approved for one or more anxiety disorder and could therefore be potential candidates for the proposed study.
236 Escitalopram is one of the more widely used anxiolytic medications and is a drug with which we have worked
237 extensively, both in cardiac patients[83] and in populations diagnosed with GAD[165,166] and specific phobias.[167]
238 The drug has been approved by the FDA for treatment of GAD, and its efficacy has been demonstrated relative
239 to placebo in social anxiety disorder,[168] panic disorder (PD),[169] specific phobias,[167] and OCD.[170] Among the
240 SSRI group of drugs, escitalopram is least likely to induce reactions with other drugs through the cytochrome
241 (CYP) P450 pathway.[171] Other drugs besides SSRIs that could theoretically be studied in this population
242 include the benzodiazepines, which have significant abuse potential, and no single benzodiazepine has been
243 shown to have efficacy across the spectrum of anxiety disorders that will be included in this study. The SNRI
244 drug, venlafaxine, might also be a candidate, but it has been demonstrated to reduce HRV in depression,[172]
245 and its dose titration is more complicated than that of escitalopram. Therefore, we submit that escitalopram is
246 the optimal choice for achieving the objectives of this study. The *Human Subjects* section includes further
247 discussion of the use of escitalopram.
248     The value of treating subclinical anxiety disorders with SSRIs also was carefully considered by our
249 research team. In view of the growing evidence that elevated anxiety, and not simply the diagnosis of an
250 anxiety disorder, is associated with increased CHD risk,[69,173,174] we believe that treating patients with elevated
251 symptoms of anxiety is justified. Subclinical anxiety disorders are highly prevalent. Haller et al.[175] reported the
252 prevalence of subthreshold GAD was twice that for the full syndrome, with an average rate of 4.4% in the
253 general population, which is likely even higher in cardiac populations. Subthreshold GAD is typically
254 persistent, causing considerably more suffering and impairment in psychosocial and occupational functioning
255 and greater health care utilization, compared to non-anxious individuals. In addition, subclinical anxiety
256 increases the risk of onset and worsens the course of a wide range of comorbid mental health disorders, pain,
257 and somatic disorders, further increasing medical costs.[176-178] This evidence supports the value of effectively
258 treating subclinical anxiety disorders as well as treating anxiety disorders that meet DSM-5 criteria.

263 **II. <u>Assessment of Anxiety</u>**

265     Patients will undergo a comprehensive assessment of anxiety. The primary outcome measure will be
266 scores on the <u>Hospital Anxiety and Depression Scale – Anxiety (HADS-A)</u>[181]. The HADS-A is a commonly
267 used psychiatric self-report questionnaire designed to identify anxiety and depression among patients in
268 nonpsychiatric medical settings[182] and has been shown to detect independent constructs of anxiety and
269 depression.[181,183,184] It has excellent psychometric properties[181,183-187] and has been widely used in outpatient
270 RCTs in patients with clinical anxiety.[188,189] Published clinical cutoffs[190] are 0-7 (normal), 8-10 (mild), 11-14
271 (moderate), and 15-21 (severe). Based on reviewer comments, we have revised our eligibility criteria to
272 include patients with HADS-A scores >10, which represents a more conservative cut-point for identifying
273 patients with significant anxiety who are at increased risk for adverse CHD events and who may receive
274 greater benefit from treatment.
275     A clinical psychologist will administer modules from the <u>Structured Clinical Interview for DSM-5 Disorders</u>
276 <u>(SCID)</u>,[191] including the anxiety disorder section, the mood disorder section (to rule out MDD), the somatization
277 section, and the alcohol abuse and/or dependence section. The psychologist also will administer the 14-item
278 <u>Hamilton Anxiety Rating Scale</u> (HAMA)[192] to obtain a clinical rating of symptom severity.
279     To assess ongoing treatment response, participants will complete the 20-item <u>Spielberger State-Trait</u>
280 <u>Anxiety Inventory (State)</u> (STAI)[193] at weekly intervals throughout the duration of the 3-month intervention by a
281 clinician who will be blinded to patients' treatment group status. The STAI will be used to evaluate the process
282 of change and to assess at-risk patients (i.e., worsening anxiety) regularly and systematically. Suicidal
283 potential also will be carefully assessed. Contact time will be documented and content limited (to avoid
284 simulating psychotherapy, which would seriously confound data interpretation).

285    Importantly, patients with MDD will be excluded from this trial.  However, because symptoms of depression
286 are likely to co-occur with anxiety, we also will assess depressive symptoms with the <u>Beck Depression</u>
287 <u>Inventory-II </u>(BDI-II).[194]  The BDI-II is a widely used measure of depressive symptomatology, consisting of 21
288 items, each corresponding to a specific category of symptoms and attitudes.  It has been shown to be both a
289 reliable and valid measure of depression severity[195] and has been used in many major interventional trials of
290 cardiac patients.[81,196,197]
291

292    **III.  <u>Intermediate Endpoint Assessments</u>**
293

294    1. <u>Heart Rate Variability</u>.   For the 24-hour HRV measurement, patients will be instrumented with a
295 DelMar-Reynolds Holter ambulatory ECG monitor.  Following instrumentation, patients will be reminded to
296 engage in their normal pattern of activity and to wear the monitor for 24 hours.   A Laser scanner (DelMar
297 Medical, Irvine, California, USA) will be used to scan the recordings using standard Holter analysis procedures.
298 The labeled beat-to-beat file will then be processed using the DelMar time domain HRV analysis software and
299 the DelMar enhanced 24-hour spectral heart rate variability analysis software.  Heart rate variability will be
300 estimated from the standard deviation of all normal R-R intervals (SDNN) and from spectral power summed
301 across each of the following bands: high frequency (0.14 to 0.5 Hz), low frequency (0.05 to 0.139 Hz), very low
302 frequency (0.003 to 0.049 Hz), and ultra-low frequency (0.000015 to 0.0029 Hz).  For the purposes of the
303 proposed study, the primary hypotheses will be tested using the SDNN measure of HRV, because it is less
304 affected by artifact and nonstationarities found in the ambulatory environment and is most strongly predictive of
305 increased risk of mortality.[101]  Certain medications may affect HRV through effects on the autonomic nervous
306 system.  Beta-blockers, for example, are known to increase the tonic levels of several measures of HRV, such
307 as RSA.  However, the primary outcome HRV measure in the proposed study is the standard deviation of all
308 normal R-R intervals over a 24-hr period (SDNN), which is relatively resistant to the effects of beta-blockade.[198]
309    2. <u>Baroreflex Sensitivity</u>.  BRS studies will be performed between 0800 and 1000 hours under fasting
310 conditions.  In the supine posture, following 5 minutes of quiet rest, 10 minutes of beat-to-beat BP data will be
311 collected using the Finometer PRO Model-1 (Finapres Medical System, Amsterdam, the Netherlands), and 10
312 minutes of beat-to-beat R-R interval will be collected from an electrocardiogram (ECG) recorded digitally at
313 1000 Hz.  The Finometer instrument utilizes the vascular unloading technique to measure SBP, DBP, and MAP
314 on a beat-to-beat basis and has been validated against intra-arterial measures under various conditions.[199]
315 Power spectra will be estimated using the Welch algorithm.[200]  Power spectra will be derived as the average of
316 60-second data segments, overlapping by half.  BRS will be estimated from the modulus of the cross spectrum
317 of R-R interval and SBP for frequencies ranging from 0.070 - 0.129 Hz.  This method produces reliable
318 measures that are comparable to estimates of BRS obtained using the invasive phenylephrine injection
319 technique, widely considered to be the "gold standard" measurement method.[201]
320    3. <u>Vascular Endothelial Function</u>.  Our approach for assessing endothelial function conforms to the
321 recently published guidelines for assessment of flow-mediated arterial vasodilatation.[202]  Longitudinal B-mode
322 ultrasound images of the brachial artery, 4-6 cm proximal to the antecubital crease, will be obtained at end-
323 diastole (ECG R-wave gated digital image capture) using a dedicated Acuson Aspen ultrasound platform.  All
324 images will be acquired with participants supine, utilizing an 11 MHz linear array probe with stereotactic holder
325 in our temperature-controlled clinical research laboratory, by Michael Ellis, RDMS, RVT, who has over 15
326 years of experience performing the standardized image acquisition protocols for our ultrasound FMD
327 assessments.  In an unpublished evaluation of 20 healthy men and women who underwent our FMD
328 assessment protocol on two consecutive days, repeat FMD values showed a correlation of r=0.81, p<.001, a
329 mean absolute difference of 0.64%.  Images will be obtained and stored digitally at resting baseline, as well as
330 during and following inflation to 250 mm Hg of an occlusion cuff placed around the forearm, 2 cm below the
331 elbow.  All arterial diameter measurements will be performed by the same experienced member of the
332 research team (AS), blinded to participant identity and treatment condition, using edge detection software
333 (Brachial Analyzer, MIA-LLC, Coralville, IA).  FMD response will be assessed from 10-120 seconds post-
334 deflation of the forearm cuff, with peak arterial diameter quantified using polynomial curve fitting, and FMD
335 thereby defined as the maximum percent change in arterial diameter relative to pre-inflation resting baseline.
336 As others have reported, using this rigorous standardization of FMD methodology, our FMD assessments will
337 be obtained with optimal reproducibility, reflected in a coefficient of variation of approximately 10% or less.[203-
338 205]  Peak hyperemic flow and shear stress will be derived by standard formulae based upon Doppler velocity
339 measurements during the first 10 seconds following deflation of the occlusion cuff.[206,207]  In participants for
340 whom there is no contraindication (e.g., history of migraine), we also will assess brachial artery response to the
341 administration of 0.4 mg sublingual glyceryl trinitrate (GTN), which is the standard approach to confirming
342 vascular endothelial specificity of FMD findings.[121]

343    4. <u>Measures of chronic inflammation</u>. Plasma inflammatory biomarkers will be measured by ELISA
344    using commercially available kits.  We will examine C-Reactive Protein (hsCRP) using a high-sensitivity assay
345    obtained from American Diagnostica, Inc. (Stamford, CT).  We will also measure interleukin-6 (IL-6), an
346    inflammatory cytokine that promotes myocardial hypertrophy and may be elevated prior to increases in CRP
347    levels.   On the day of blood work, we will ask participants to hold off taking nonsteroidal anti-inflammatory
348    medications (including aspirin) and antihistamines until after their morning (0700-0800 hours) blood draw.
349    5. <u>Urinary catecholamines</u>.  Patients will be asked to collect urine over a 24-hour period, with samples kept
350    cold by storage in a portable cooler.  Samples will be assayed for norepinephrine, epinephrine, and creatinine.
351    Catecholamine levels will be expressed as urine concentration ($\mu$g/ml) per urine concentration of creatinine
352    (mg/ml), yielding norepinephrine and epinephrine values in units of $\mu$g per mg creatinine for each sample.  This
353    provides catecholamine excretion indices that are corrected for individual differences in body size and urine
354    volume.[208]  In prior studies, urinary catecholamine data have proven informative, with low subject burden and
355    excellent compliance.[114,209,210]   As in these prior studies, excellent compliance with urine collection is achieved
356    by emphasizing to participants the importance of a <u>complete</u> 24-hour collection and by providing detailed
357    instructions on how this can be achieved.  The completeness of 24-hour urine collections will be assessed by
358    ascertaining whether 24-hour urinary creatinine excretion falls within boundaries based upon body size, race
359    and gender.[211]  Incomplete collections will be repeated.
360    6. <u>Lipids</u>.  Total cholesterol, HDL- and LDL-cholesterol, VLDL-cholesterol, and triglycerides will be assayed
361    from fasting blood samples drawn between 0700 and 0800 hours.
362

### IV. <u>Assessment of Aerobic Capacity</u>

365    Graded treadmill exercise testing will be conducted at baseline, prior to randomization, and at the
366    conclusion of treatment using a protocol developed at Duke and Wake Forest Universities.[212]  Fasting subjects
367    will exercise to exhaustion or other standard endpoints (e.g., chest pain, ST-segment depression, etc.) under
368    continuous electrocardiographic monitoring.  Expired gases will be analyzed continuously by a Parvo Medics
369    True One measurement system (Parvo Medics, Sandy, Utah).  Peak VO$_2$ will serve as the primary measure of
370    aerobic capacity.

### V. <u>Assessment of Quality of Life</u>

374    The proposed interventions may not only reduce anxiety, but also may impact a number of other areas
375    of functioning that are considered to reflect "quality of life."  We propose to use a questionnaire battery that
376    includes a measure of functional status,[213] anger,[214] general distress,[215] and social/role functioning (and
377    employment status).  We also will use the Short Form 36 (SF-36) generic quality of life instrument developed
378    by the Rand group from their longer instrument used in the Health Insurance Experiment and the Medical
379    Outcomes Study.[216]  In addition to including the traditional quality of life domains, we will measure self-
380    esteem,[217] life satisfaction,[218] perceived stress,[219] coping,[220] dysfunctional attitudes,[221] and perceived social
381    support.[222]  These are clearly exploratory measures and are peripheral to our main hypotheses.  However, this
382    information is of interest and can be obtained at minimal additional cost.

### VI. <u>Post-treatment Assessments at 3-Months and at 9-Months (Six-month follow-up)</u>

385    At the conclusion of the 3-month intervention (post-treatment), or at the time a participant may drop out
386    of the study (or in the rare instance of requiring additional or different anxiolytic treatment outside of our
387    protocol), patients will complete the same clinical, biomarker, and psychometric test battery that they
388    completed at baseline.  At the 6-month follow-up, we will assess anxiety, patients' exercise habits, any
389    psychiatric treatment, psychopharmacologic medications, and cardiac status including revascularization
390    procedures and CHD events.  This will be a naturalistic follow-up that we have used in previous trials,[223,224]
391    which will allow us to determine patients' anxiety status 6 months after the conclusion of study-related
392    treatment, along with changes in health habits and measures of quality of life.   Table 1 presents an overview
393    of the assessment schedule.  As suggested by the prior review, we will carefully document cardiac-related
394    hospitalizations and emergency room visits, revascularizations, and any deaths for up to 4 years post-
395    treatment.  Medical costs also will be documented (see below).

### <u>Annual Follow up for Clinical Events and Medical Expenses</u>

As requested by the prior review, we now propose to track medical outcomes and costs. Our main medical outcome is combined all-cause mortality and CHD hospitalizations, emergency department visits, and unscheduled physician office visits because of worsening angina. The study staff will collect these data via medical records review, which will be coordinated by Dr. Alan Hinderliter at UNC. We have conducted these types of follow-up assessments in our prior work.[148,225] Based upon unpublished data from our UPBEAT trial, it is estimated that there will be a 30%

#### Table 1. Assessment Schedule

| Measure | Initial Screen | Pre-Treatment Assessment | Interim Assessments | Post-Treatment Assessment | 6 months Post-Treatment | Annual Follow-Up |
|---|---|---|---|---|---|---|
| Physical Exam | X | | | | | |
| **Anxiety Assessments** | | | | | | |
| ADD | X | | | | | |
| HADS-A | | X | | X | X | X |
| MINI | | X | | X | X | |
| HAM-A | | X | | X | X | |
| STAI-S | | | X | | | |
| **Intermediate Endpoints** | | | | | | |
| HRV | | X | | X | | |
| BRC | | X | | X | | |
| FMD | | X | | X | | |
| CRP/IL-6 | | X | | X | | |
| Catecholamines | | X | | X | | |
| BP | | X | | X | | |
| Lipids | | X | | X | | |
| Aerobic Fitness | | X | | X | | |
| BDI-II and Ancillary Questionnaires | | X | | X | X | |
| Clinical Events | | | | | X | X |
| Medical Expenses | | | | | X | X |

annual event rate in this vulnerable population of anxious CHD patients. As suggested by a prior reviewer, we also now propose to measure health care costs and resource consumption as accurately as possible using empirical cost data collected on each study participant.[226,227] This approach reflects the "state of the art" in cost studies and is preferable to an approach where counts of selected "big ticket" items (e.g., CABG, PTCA, etc.) are multiplied by arbitrarily selected unit prices to estimate costs. At annual follow-ups, patients will be interviewed to determine interval medical care consumption. Any indication of hospitalization or physician visits will prompt requests for relevant medical bills. Outpatient medical care will be carefully enumerated, including medical care visits to a spectrum of health care providers (including those providing non-traditional medical care), follow-up medication consumption, and out-of-pocket payments for medication and other health care costs.

**5. Selection of Subjects:** This will be an outpatient study of 150 cardiac patients with stable CHD and elevated symptoms of anxiety. To accomplish our recruitment and treatment goals, we have established a referral network of community hospitals including Duke University Medical Center, the Durham Veterans Affairs (VA) Hospital, Duke Raleigh Hospital, Alamance Regional Hospital, and the UNC Health Care system in Chapel Hill. Patients will be recruited through a variety of sources including IRB-approved advertising, self-referral, and by referral from physicians, psychologists, and other mental health care professionals. This is a racially and socioeconomically diverse region, which will be reflected in our sample of anxious CHD patients. Patients from Duke, UNC, and outside hospitals will be considered for participation. Non-Duke patients will be provided with Duke's Notice of Privacy Practices.

Inclusion criteria. Men and women with documented CHD (i.e., a prior MI, coronary revascularization procedure, or >70% stenosis in at least one coronary artery) age >39 years will be selected for study. Patients also will have an anxiety symptom severity score of at least 11 on the Hospital Anxiety and Depression-Anxiety scale (HADS-A); a subgroup of patients will also have a DSM-5 diagnosis of an Anxiety Disorder. We plan to actively recruit women and minorities, with at least 50% women and 25% minorities.

Exclusion criteria. Medical exclusions will include an MI or coronary revascularization procedure (i.e., CABG or percutaneous coronary intervention) within the last 3 months, unstable angina, severe left ventricular dysfunction (ejection fraction <30%) or decompensated heart failure, unrevascularized left main coronary artery stenosis >50%, pacemaker dependence, resting BP >200/120 mm Hg, and conditions that would preclude randomization to either the drug (e.g., prolonged QT interval, known allergy to or intolerance of escitalopram) or exercise (e.g., musculoskeletal problems or abnormal cardiac response to exercise). Patients with a primary psychiatric diagnosis other than Anxiety Disorder will be excluded, including patients with MDD, PTSD, OCD, or any of the following DSM-5 diagnoses: 1) Dementia, delirium; 2) Schizophrenia, Schizoaffective, or other psychotic disorder; 3) Psychotic features including any delusions or hallucinations; or 4) Current alcohol or other substance abuse disorder. Similarly, patients who pose an acute suicide or homicide risk or who, during the course of the study, would likely require treatment with additional psychopharmacologic agents will not be enrolled. Patients will also be excluded if they are taking other medications that would preclude assignment to either drug or exercise conditions (e.g., clonidine, dicumarol, anticonvulsants, and MAO inhibitors) or are taking herbal supplements with purported mood effects (e.g., St. John's Wort, valerian, ginkgo). Patients already engaged in regular exercise (at least 30 minutes >1x/week) will not be enrolled. Finally, pregnant women will be excluded from participation.

## I. Screening procedures

1. <u>Psychiatric Screen</u>. All potential candidates will be screened using the Anxiety and Depression Detector (ADD).[179] This 5-item instrument has been used to detect anxiety (and depression) in medical settings and is an effective screening tool for anxiety disorders (e.g., panic disorder, social phobia, and GAD). It is a good overall measure of distress and is likely to reflect a diagnosis of at least one of these conditions or at least significantly elevated levels of anxiety. Patients must obtain a score of $\geq 1$; the ADD is correlated with the Overall Anxiety Severity and Impairment Scale (OASIS),[180] which also has been used to detect the presence of significant anxiety.

2. <u>Medical Screen</u>. Each participant will receive a screening physical examination. Blood pressure (BP) will be determined by standard sphygmomanometry. Subjects will undergo routine blood tests including creatinine, electrolytes, liver function tests, complete blood count, B12, and thyroid profile, which will be performed at the Duke Clinical Research Unit (DCRU). If a subject is found to have any significant medical illness during the medical screen that would contraindicate safe participation in this study, he/she will be excluded. Because older cardiac patients are likely to have at least one additional chronic disease, the presence of a comorbid medical condition is not, in itself, a reason for exclusion. Smoking and alcohol use also will be documented at screening, as well as during follow-up.

**6. Subject Recruitment and Compensation:** One hundred fifty anxious CHD patients (approximately 50% women and 25% minority) will be recruited from the North Carolina Piedmont region. Because children are unlikely to suffer a cardiac event, and age (and CHD risk factors) is strongly related to our intermediate endpoints, children will not be included in our proposed investigation. The catchment area from which we draw our research subjects encompasses the counties of Durham (pop. 192,566; 50% minority), Orange (pop. 104,186; 25% minority), Chatham (pop. 42,985; 21% minority), Alamance (pop. 115,278; 20% minority), and Wake (pop. 518,206; 23% minority). We believe that recruitment from this geographic area will allow us to enroll sufficient minorities and patients from diverse socioeconomic backgrounds. It also should be noted that approximately 4,000 patients with suspected CHD are catheterized each year at Duke, with 70% (2,800 patients) having stenoses of >75% in at least 1 artery. At the Durham VA, 600 patients are catheterized annually, with 90% having CHD. More than 1,200 patients undergo cardiac catheterizations at UNC, and more than 2,000 patients with stable CHD are in the UNC Health Care Network. Thus, there is an ample recruitment base for potential patients for this study.

**7. Consent Process:** Informed consent will be obtained during interviews between potential subjects and a member of the investigational team familiar with all aspects of the project. In addition to receiving free evaluations and treatment programs, subjects will be compensated with $100 for completing the study.

**8. Subject's Capacity to Give Legally Effective Consent:** Subjects who are <18 years of age or with diminished capacity will be excluded.

**9. Study Interventions:**

<u>Randomization</u>: All eligible subjects will be randomly assigned to one of the 3 treatment conditions. Randomization will occur after each subject has completed the assessment protocol and will adhere to standard procedures for randomized clinical trials.[228] We will employ a conditional randomization procedure such that equal proportion of participants with anxiety disorders will be assigned to the respective treatment groups. We also will stratify by gender and age (<59/$\geq$59). In order to increase the power to detect between group differences, we increased our sample size to 150 patients and propose to randomize 60 patients to Aerobic Exercise, 60 patients to Medication, and 30 patients to Placebo control.

**1. Supervised aerobic exercise**. Patients will exercise three times per week, under medical supervision, at a level of 70-85% of their $VO_{2peak}$ as determined at the time of their baseline exercise stress test. Patients' exercise will consist of 10 minutes of gradual warm-up exercises followed by 35 minutes of continuous walking, biking, or jogging, and 5 minutes of cool down exercises for a total a 50 minutes per session. Patients will be instructed to monitor their radial pulses and will be checked at least three times per session to ensure that they are within their prescribed exercise training ranges.

**2/3. Medication/placebo**. Treatment in the medication and placebo pill arms will be supervised by Dr. Wei Jiang. Drug dispensing will be done by licensed pharmacists at the Duke Investigational Pharmacy Service, who have extensive experience in clinical trials. They will maintain the blind and will dispense enough

medication to last until the next visit. Each bottle will have a label displaying the dose and the subject number of the patient who is to receive the medication. We will use the SSRI escitalopram (Lexapro), which has received FDA approval for the treatment of anxiety, in 5 mg tablets or matched placebo. Medication will be dispensed as tablets of escitalopram or placebo in individually coded bottles. A new bottle of medications will be dispensed at each medication dispensing visit to study staff. Medication will be taken once daily in the morning but can be switched to once daily in the evening if deemed necessary. Medication adherence will be assessed using electronic monitors and pill count at each study visit. We will use the Medication Event Monitoring Systems (MEMS) to document medication adherence. The MEMS bottle cap incorporates a microelectronic circuit that registers and stores data when the closure is opened and when it is closed. Each study participant will receive a MEMS monitor and will be asked to use the monitor as the cap on the bottle of their medication (drug or placebo). Medication adherence, defined as the percentage of days on which the medication was taken, will be compared between the two pill arms to determine whether group differences in HADS-A scores could be attributed to differences in medication adherence.

Patients will see Dr. Jiang (who will be blinded to pill condition) at week 0 (baseline), week 2, week 4, week 8, and week 12. Patients will be contacted weekly by telephone between regularly scheduled visits. Dr. Jiang will make all medication adjustments based primarily upon STAI scores and safety/tolerability assessments. Patients will be randomly assigned to receive escitalopram (5 mg/d) or placebo. Depending on symptoms, daily escitalopram (or placebo) doses will be titrated to 10 mg after week 2 and to 15 mg or placebo equivalent at week 3 if patients show no change or only minimal improvement. 5mg was selected as the starting dose in order to optimize patient safety and minimize side effects (i.e. "low and slow"). At week 4, if patients show no change or only minimal improvement, they will receive a maximum daily dose of 20 mg or placebo equivalent. The dose can be reduced in the event of side effects. Dr. Jiang is an experienced clinical investigator in pharmacological RCTs and will serve as the treating psychiatrist for this trial. In all cases, the treating physician will use supportive measures to help manage medication side effects. In cases of severe side effects or patient discomfort, the physician may elect to decrease the medication dosage at any time or permit unscheduled visits during the study. At each medication dispensing visit, the patient will see the physician's assistant and treating physician before visiting the on-site investigational pharmacy. Patients will return any unused medication and the pharmacist will then dispense enough medication to last just beyond the next dispensing visit. The pharmacy will be responsible for medication storage and verifying expiration, maintaining the blind, storing codes in a sealed envelope accessible at emergencies, and for dispensing medication.

Patients and raters will be kept blinded to drug therapy assignments both during the treatment phase (3 months) and until the patient has completed the post-treatment assessments. Dr. Jiang, who will perform the ongoing medication monitoring and safety ratings, will be blinded until the completion of the trial. Limited use of sleep medications, but not other anxiolytic agents, will be permitted (see Human Subjects).

## 10. Risk / Benefit Assessment:
Potential risks and minimizing potential risks: Procedures for protecting subjects against potential risks include the careful screening procedure outlined in the application, weekly anxiety monitoring of all patients by a trained clinical psychologist, and the attendance of qualified personnel at all exercise testing and training sessions. The treatment duration is sufficiently brief (3 months) to permit a placebo-controlled trial and yet is of adequate duration to produce therapeutic benefits.

The favorable efficacy profile with low incidence of side effects has led to SSRIs being the most frequently prescribed class of medications for treating anxiety disorders. After consultation with a number of experts in the psychopharmacological treatment of anxiety disorders, including our collaborator, Dr. Jonathan Davidson, we ultimately selected the FDA-approved SSRI anxiolytic agent escitalopram for this study because it is among the more selective serotonin re-uptake inhibitors, has an optimal profile for safety and effectiveness in the treatment of anxiety disorders, and it has been used safely in cardiac patients in a variety of studies. Escitalopram also has been investigated across a range of anxiety disorders and has been found to be safe for elderly persons with CHD and central nervous system (CNS) disorders (e.g. dementia, stroke). Multiple studies have demonstrated that SSRIs are effective in improving various anxiety symptoms in animal models and individuals who do not have formally diagnosed anxiety disorders based on DSM criteria. The effects of escitalopram on GAD and other anxiety disorders have been well-demonstrated in a variety of RCTs. Owing to multiple metabolic degrading pathways, the clinically relevant interactions of escitalopram with other drugs are minimal, which is especially important for our proposed study since these cardiac patients are likely to be on multiple cardiac medications. Compared with other anxiolytic medications, escitalopram is generally better

tolerated, its onset of action is relatively fast, it requires no dose adjustment in renal dysfunction and older age, and its use may have cost–effectiveness and cost–utility advantages. Therefore, we believe that escitalopram is the optimal choice as a safe and effective first-line option in the management of patients with high anxiety and with anxiety disorders. It should be noted that we also considered other medications, including different classes of medications for which anxiety is an indication (e.g., benzodiazepines), but rejected the use of medications such as diazepam because of potential adverse side effects (e.g., falls, cognitive impairment) and because such medications are addictive and promote drug dependence. Because of the relatively short (i.e., 12-weeks) intervention, patients randomized to placebo will have limited exposure to a "treatment" that may have minimal benefit. Moreover, only 30 patients (20%) will actually receive the placebo pill. The fact that 80% of patients will receive an active treatment (exercise or escitalopram) should also facilitate patient recruitment into the trial.

  The value of treating subclinical anxiety disorders with SSRIs also was carefully considered by our research team. In view of the growing evidence that elevated anxiety, and not simply the diagnosis of an anxiety disorder, is associated with impaired quality of life and increased CHD risk,[69,173,174] we believe that treating cardiac patients with elevated symptoms of anxiety is justified. Escitalopram is considered the first line of psychotropic medications for treating elevated anxiety symptoms and not just for treating an anxiety disorder, and it is regarded as one of the safest and most widely prescribed anxiolytic medications in the US. The risks of receiving escitalopram include common side effects such as nausea, insomnia, headache, tremor, diarrhea, nervousness, dry mouth, increased sweating, and sexual dysfunction. Less common side effects include rash, bruising, agitation, and hyponatremia. We are also aware of recent FDA alerts regarding the possibility of SSRIs being associated with worsening of suicidal ideation, agitation, and depression, and we plan to include these warnings in the informed consent, and all patients will be counseled by the study psychiatrist about these potential risks. Importantly, we will exclude patients with MDD or active suicidal ideation or those who are judged to not be appropriate candidates for escitalopram or placebo therapy (e.g., prior non-responders, psychotic depression, active suicidal ideation, severe agitation, etc.). If such adverse events should develop during the course of the study, we will refer them for appropriate care immediately. We estimate that less than 10% of the patients may drop out due to such side effects.


**11. Costs to the Subject:** Subjects will not incur any costs related to study-related assessments or interventions.


**12. Data Management and Statistical Considerations**

 Data Analytic Approach. The basic analytic strategy is a linear model carried out in the MPlus modeling software,[229] with treatment group contrasts as factors, and ethnicity, age, gender, and pre-treatment measure of the outcome variable as the adjustment covariables. The intent-to-treat principle (ITT) will be followed in all models, using full information maximum likelihood available in MPlus to manage missing data. For the case of the primary outcome, HADS-A, the model will include post-treatment HADS-A score as the response, with the predictors including two planned contrast variables representing 1) the two active treatments (exercise and escitalopram) vs. placebo and 2) exercise vs. escitalopram, with ethnicity, gender, age, and pre-treatment level of the HADS-A as the adjustment covariables. The contrast tests among treatment groups will be examined directly, i.e., no omnibus or "gateway" test will be required before interpreting them. In support of the HADS-A outcome, we also will compare the pattern of weekly changes in the STAI scores over time using a repeated measures model with Proc Mixed in SAS (SAS Institute, Cary, NC). The primary biomarker outcome, HRV, will be evaluated similarly. It is possible that a small number of participants may require off-protocol psychotropic medication during the course of the trial. These participants will be included in the ITT analysis and will not be replaced. We also will perform a sensitivity analysis with change in medication included as an adjustment variable in the statistical model. Finally, we will compare the treatments on the number of clinical events using a generalized linear model with the appropriate distributional form (e.g., negative binomial), using the same contrasts and covariates as in the linear model described above.

  We also will examine treatment effects for a number of additional supportive outcome measures (e.g., BDI-II, QoL, inflammatory markers, BRS, urinary catecholamines, etc.). Significant treatment effects for these variables will help us interpret our primary outcomes and may inform further study and clinical application. For example, if the additional quality of life variables or CHD biomarker measures are also responsive to exercise

treatment, it may indicate that exercise has a more global salutary effect above and beyond reducing anxiety. Despite their importance, we recognize that conducting these additional comparisons raises the potential for an inflated Type I error rate. We will therefore note in publications that these additional analyses represent exploratory analyses. We shall otherwise carry these analyses out using the same modeling approach as we do for the primary treatment analyses, attending in particular to the distribution of the residuals for each model and modifying our approach accordingly. The above models can be easily extended to include the 6-month follow-up measurement occasion.

Prior to conducting these analyses, preliminary examination of the assumptions of the model will be conducted to examine the homogeneity of regression assumption. This assumption requires that the relationship between treatment and the response variable be homogeneous for each level of the adjustment covariables. Should the data indicate that this assumption is violated, we will model the corresponding multiplicative (interaction) term(s). In addition to assessing the adequacy of the model with respect to the homogeneity of slopes assumption, we also will evaluate the model for violations of heteroscedasticity of errors and non-linearity using standard graphical methods. Should these assumptions be violated, appropriate transformations (e.g., Box-Cox method) will be made. All analyses of treatment effects for secondary hypotheses concerning continuous or binary variables will be conducted using the modeling capabilities available in MPlus.

In addition to treatment effects, we also will test the hypothesis that pre- to post-treatment change in HADS-A scores will mediate the improvements in intermediate CHD biomarkers. M-Plus allows a direct, formal test of this hypothesis via path analysis modeling. For example, a model will be specified so that both active treatment groups will be contrasted against the placebo arm, with a variable representing the change in anxiety intervening between the treatment contrast variables and post-treatment HRV (with the covariates, pre-treatment HRV, age, ethnicity, and gender). Mediation is tested by examining the product of the two component path coefficients (treatment ->mediator->outcome) and its standard error, which yields the point estimate of the mediated effect along with the statistical significance of treatment on post-treatment biomarker (e.g., HRV) via change in HADS-A. [230] We also will explore possible treatment-specific mediators (e.g., change in $VO_2$ mediating change in HRV for the Exercise group). In addition to the above tests, we will examine the impact of dropout on the estimates of treatment effects using mixture modeling available in MPlus. Specifically, we will examine the primary outcomes using Rubin's Complier-Average Causal Effect Estimation (CACE) model,[231] which estimates the effect of treatment for all participants who adhere to the protocol irrespective of treatment group assignment. We will explore possible moderators of the treatment effect using interaction terms. Specifically, we will examine the possible moderating effects of gender, race, patient expectations, and initial severity of anxiety (e.g., DSM-5 diagnosis for an anxiety disorder); if sufficient numbers, we also plan to explore individual diagnoses by adding treatment interaction terms for these variables simultaneously with the primary model, and using a pooled test of the terms to determine whether the individual terms should be interpreted. Participants who voluntarily drop out of treatment or who are unable to complete our protocol will not be replaced but will undergo follow-up assessment.

Power Analysis. Our study is powered with a focus on the primary hypothesis test: the treatment effect on anxiety as measured by HADS-A scores. We estimated power and sample size under the following assumptions: an alpha of .05, a linear model with age, gender, ethnicity, and baseline HADS-A score as covariates, a conservative estimate of the R-squared of .20 for the full model predicting post-treatment HADS-A, a 15% attrition rate, and two planned contrasts: active treatment vs. placebo and exercise vs. escitalopram. The comparison of primary interest will be active treatments vs. placebo. We estimate that a sample size of 150 (127 after attrition) will yield .80 power to detect at least a .45 SD difference between the active treatments and Placebo. In the general linear model, the exercise vs. escitalopram test will be only slightly less powered in being able to detect a .50 SD difference. In response to a previous reviewer comment, we believe that achieving this effect size is feasible. For example, in individuals with high anxiety sensitivity, Broman-Fulks[232] reported a .61 SD difference between exercisers and controls. We also re-examined data from a subset of highly anxious patients (STAI$\geq$45; N=32) participating in our recently completed UPBEAT study[91] and found an effect size of .85 SDs on the STAI for both active treatments vs. placebo controls. We note that our power estimates are conservative in that the R-squared for the model is likely to be greater than .20 and dropout may be lower than 15%. With respect to the secondary outcome of HRV, our prior work showed that the R-squared between the baseline covariates and HRV was .73, greatly increasing the power of the tests of treatment on HRV. For the test of exercise vs. escitalopram on HRV, our primary CHD biomarker of interest, we will have a power of .80 to detect a .25 SD effect at alpha of .05. Finally, for our ancillary outcomes, including FMD, lipids, urinary catecholamines, and inflammatory markers, we have sufficient pilot data for FMD, which we use as an

exemplar for power estimates: our earlier work showed that for FMD, the multiple R-squared between the baseline covariates (age, gender, ethnicity, and baseline FMD) and FMD was .50.  Thus, we will have a power of .80 to detect a .34 SD effect for FMD with an alpha of .05.  We estimate the power for the secondary analysis of treatment on time-to-clinical-event, assuming an event rate of 50% in the placebo group (based on unpublished data from our prior work[91]), 42 months for patient accrual, median follow-up time of 30 months and alpha =.05, is .80 to detect a reduction in event rate of 50% between both treatment groups and placebo, as well as .80 to detect a reduction in event rate of 56% between either intervention group individually and placebo.  Unpublished data from our recently completed ENHANCED trial[233] revealed a 54% reduction in major adverse cardiac events among patients participating in CR compared to a matched control group of patients who did not participate in exercise-based CR.

## 13. Data Safety and Monitoring

Study participants will be closely monitored for side effects, level of anxiety, and suicide risk. Those subjects who are unable to complete our protocol (e.g., if the anxiety  or other psychiatric condition  requires hospitalization) will be referred to their referring physician or to the Duke Outpatient Affective Disorders Program, the Duke Psychology Clinic, or a local mental health center. If patients become suicidal, they will be evaluated by the treating psychiatrist and appropriate referral or admission procedures will be initiated. Our study coordinator will contact the patient within 24 hours to make sure that he or she followed through with the referral. Furthermore, at the conclusion of the interventions, patients will be followed bimonthly via telephone and referred to their local physicians for further treatment, if necessary, or to the Outpatient Treatment Program at Duke University Medical Center or to the Duke Psychology Clinic. The Duke University Psychiatry Outpatient Clinic is staffed by Duke psychiatrists and psychologists who treat varying levels of severity of anxiety. The Duke University Psychology Clinic is staffed by the Duke clinical psychology faculty, postdoctoral fellows trained specifically in the treatment of anxiety disorders, and advanced clinical graduate students who see patients on a sliding fee schedule and who function under the supervision of licensed psychologists. We believe that these facilities, in addition to private practitioners in the community, can serve as appropriate referral sources for patients needing further treatment. We believe that careful monitoring of all patients entered into our protocol and tracking of all participants following the completion of the interventions will minimize risk to study participants. The study personnel, including physicians, psychologists, the study coordinator, and the physician's assistant, have the training and experience to provide the necessary safeguards for participation.

Suicidality will be assessed at each visit and by interim telephone contacts as needed. Subjects may experience no response to treatment or a worsening of symptoms; however, all subjects will be carefully monitored and withdrawn from the study if their symptoms worsen. Clinical assessors will have experience in clinical psychiatric assessment and will make every effort to implement protocol procedures in a sensitive and supportive manner. Other measures to minimize risks include the careful assessment of each subject at the time of enrollment and close clinical scrutiny through participation in the study. There are no physical risks of diagnostic procedures or ratings. The study clinicians all have extensive experience in the treatment of anxiety disorders. The study psychiatrist (Dr. Wei Jiang) has served as a PI or co-PI on a number of clinical trials including many patients with CHD (e.g., REMIT, SADHART, MIIT, SADHART-CHF).

**14. Privacy, Data Storage, and Confidentiality:** All participant data will be kept in locked offices and electronic data in password-protected folders on the shared drive that are only accessible to key personnel.