Reduced Opioid Analgesic Requirements Via Improved Endogenous Opioid Function

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1.0 Background

Chronic pain (CP) affects 100 million individuals in the U.S. CP management has increasingly utilized long-term opioid analgesic therapy, a change associated with increased opioid abuse (via greater exposure in vulnerable individuals), non-pain health consequences (hormone changes, falls), and a dramatic rise in opioid-related overdoses and deaths. Treatment strategies that minimize the need for chronic high-dose opioids are sorely needed. This project will test the novel hypothesis that effective pain relief can be achieved at lower opioid analgesic doses by increasing levels of endogenous opioids (EOs). This hypothesis follows from our prior work demonstrating that analgesic responses to opioid medications are significantly influenced by individual differences in EO activity[18,19]. Results indicated that for individuals exhibiting higher natural EO levels, morphine produced little incremental analgesic benefit. In contrast, for those displaying less natural EO analgesia, morphine produced significant analgesia. Findings suggested that morphine in effect supplemented lower levels of EO analgesia, making pain responses of individuals lower in EO function who had received morphine look statistically similar to individuals higher in EO function not receiving morphine[18]. An Opioid Supplement Model was suggested, in which opioid analgesics supplement EO function, making up the difference necessary to maximize opioid receptor-mediated pain inhibition. Our previous work looked only at natural variations in EO function, and only at a single opioid analgesic dosage. Therefore, the key clinical implication of the Opioid Supplement Model has not been tested: can effective pain relief be achieved at lower opioid analgesic dosages by enhancing EO function? This is one primary aim of the current proposal. While several controlled trials indicate that aerobic exercise is effective for reducing CP intensity, and it is widely assumed these benefits are related to enhanced EO analgesia, this assumption has received surprisingly little empirical testing. Therefore, a second aim of this project is for the first time to directly test whether aerobic exercise training enhances EO function in individuals with chronic low back pain (CLBP), and whether resulting reductions in CP intensity are attributable to these EO changes. Results will provide important information on mechanisms underlying a common CP therapy and have potentially important implications for public health, as results would highlight the potential for strategic combination of lower dose opioid analgesic therapy with nonpharmacological EO-enhancing interventions, potentially reducing negative side effects and mitigating opioid therapy risks.

2.0 Rationale and Specific Aims

Daily use of high-dose opioid analgesics for CP management has increased dramatically, and is associated with increasing numbers of patients experiencing opioid-related negative health effects, abuse, overdose, and even death. Ways of providing effective chronic pain relief with less reliance on high-dose opioid analgesics are sorely needed. Based on recent work indicating that responses to opioid analgesics are influenced by functioning in endogenous opioid systems[18,19], this project will determine whether enhancing endogenous opioids (via aerobic exercise training) permits achieving desired levels of analgesia with lower dosages of opioid analgesics, and fewer side effects and abuse-relevant drug effects. This 4 year project will test study hypotheses in a sample of 116 chronic low back pain patients. The study will have two key elements: 1) a randomized, controlled aerobic exercise manipulation in CP patients completing daily

electronic pain diaries and 2) laboratory evoked thermal pain protocols pre- and post-exercise permitting direct examination of changes in both opioid analgesic effects (in response to a series of incremental morphine doses) and EO activity (indexed by comparing pain responses after placebo vs. opioid blockade). This design will permit testing the novel hypothesis that aerobic exercise can facilitate desired analgesia levels at lower dosages of opioid analgesics via enhanced EO function.

1) *To determine whether an aerobic exercise manipulation increases EO activity in CLBP patients, and whether these EO changes mediate manipulation-induced reductions in evoked pain responsiveness and daily CP intensity.* If hypotheses are supported, a) participants randomized to exercise will exhibit greater decreases in CP intensity and evoked pain responsiveness and greater increases in EO function (indexed by laboratory responses to opioid blockade) than those assigned to the control condition, and b) the indirect path linking the exercise condition to reduced CP intensity via increased EO function will be significant.

2) *To determine whether the exercise manipulation is related to achieving analgesic targets at lower opioid analgesic dosages, and whether these changes in analgesic requirements are mediated by pre-post EO changes*. If hypotheses are supported, a) participants assigned to exercise will exhibit a 25% reduction in acute pain responses (relative to pre-manipulation placebo responses) at a significantly lower total morphine dose (based on incremental dosing) than controls, and b) the indirect path linking the exercise condition to reduced morphine requirements via increased EO function will be significant.

3) *To determine whether the exercise manipulation is related to achieving a 25% morphine-induced reduction in evoked pain responsiveness with fewer negative side effects and abuse-relevant effects, and whether these changes are mediated by pre- to post-manipulation EO changes*. If hypotheses are supported, a) participants assigned to the exercise condition will exhibit a 25% reduction in acute pain responses (relative to pre-manipulation placebo responses) during morphine administration with significantly fewer negative side effects (e.g., nausea, sedation) and less abuse-relevant subjective drug effects (e.g., drug liking, euphoria) than those in the control condition, and b) the indirect path linking the exercise condition to reduced side effects and abuse-relevant effects via increased EO function will be significant.

3.0 Animal Studies and Previous Human Studies

Both EOs and opioid medications produce analgesia through activating the same opioid receptors. This common mechanism of action between EO analgesia and responses to opioid-based medications suggests that the former may be related to the latter. Surprisingly, until recently this question had never been directly addressed in humans, although indirect evidence did support these hypothesized associations. For example, a systematic review of randomized acupuncture trials found that acupuncture not only significantly reduces postoperative acute pain, but also reduces opioid analgesic consumption and opioid side effects[112]. To the extent that the effects of acupuncture are EO-mediated, as suggested by several studies indicating its analgesic effects can be blocked by opioid antagonists[57,58,113], these findings imply that enhancing EO function

might supplement analgesia gained from opioid-based medications and thus permit reduced dosages.

The Co-PIs recently reported the first human work directly examining associations between EO function and opioid analgesic responses. Using controlled laboratory methods, associations were examined between spontaneous EO function (indexed by differences in acute pain responses between opioid blockade [naloxone] and placebo conditions) and analgesic responses to morphine, the prototypic mu opioid agonist[18]. In a sample of 45 chronic low back pain patients and 31 pain-free healthy controls (combined for analyses given no significant main or interaction effects of subject type), significant associations were observed between EO function and morphine analgesic responses on all 7 measures of pain responsiveness examined across two different evoked pain tasks (ischemic pain, thermal pain)[18]. We recently extended these findings to clinical chronic pain[19]. Individuals exhibiting less EO inhibition of their chronic back pain (less effect of opioid blockade on back pain intensity) experienced greater relief of their back pain with morphine administration relative to higher EO individuals[19]. To isolate the source of the effects noted in Bruehl et al.[18] and provide a clear means of portraying them, median splits were conducted on EO function variables derived for each pain measure, with placebo condition and morphine condition responses for each measure then compared across the resulting groups. The pattern of results indicated an intriguing source of these observed associations. First, as expected, when EO systems were intact (placebo condition), individuals exhibiting relatively lower spontaneous EO activity were significantly more responsive to both evoked pain tasks on all measures. Second, individuals with lower EO function exhibited substantial morphine analgesic responses whereas those with higher EO function did not.

A key finding in Bruehl et al.[18] was that morphine reduced evoked pain responsiveness among individuals lower in EO function to levels statistically comparable to those observed in participants with higher spontaneous EO function *in the absence of any morphine*. We propose that these findings fit what we term an "opioid supplement model," in which opioid analgesics supplement EO function, making up the difference necessary to maximize opioid receptor-mediated pain inhibition. If the opioid supplement model is valid, then the desired degree of pain relief might be obtained by a combination of lower EO activity and higher amounts of opioid analgesics, or conversely – and in most cases preferably - higher EO activity and lower amounts of opioid analgesics. In this latter context, we propose that aerobic exercise may be used to enhance EO function, with subsequent beneficial effects on the dose of opioid analgesic needed to achieve desired pain relief.

Rationale for use of an aerobic exercise manipulation to enhance EO function derives from several sources. First, aerobic exercise is used clinically in CP management, and results of a number of controlled studies document that it significantly reduces evoked pain responses and clinical pain intensity in CP patients[46,72,94,103,107]. Second, a review by Co-Inv Dr. Koltyn documents the analgesic effects of exercise with regard to laboratory evoked pain responses[76], with several opioid blockade studies conducted in individuals free of CP supporting a role for enhanced EO activity in these effects[55,68,74,98,99]. These latter studies demonstrated that reductions in evoked pain responsiveness induced by acute exercise when healthy subjects are administered placebo do not occur when EO function is blocked by pharmacological opioid antagonists. Third, recent brain imaging research also supports these findings,

indicating that an acute exercise manipulation reduced evoked pain responses and enhanced pain-related brain activations in an important EO pain modulatory brain region (periaqueductal gray), with parallel increases in circulating levels of beta-endorphin, a key EO analgesic[104]. Fourth, potential importance of EO mechanisms in reducing CP via regular aerobic exercise training is suggested by opioid blockade work in animals. Regular exercise (from 5 days to 5 weeks) was found to significantly reduce, via EO mechanisms, behavioral indicators of CP in animal models of chronic muscle pain and neuropathic pain[6,110]. Interestingly, significantly increased brain levels of beta-endorphin were noted during the exercise period, with exercise-related analgesia persisting for 5 days following exercise discontinuation[110]. The latter finding is particularly intriguing, as it suggests that regular aerobic exercise training may enhance EO function not only immediately after acute exercise, but for an extended period of time after acute exercise has ceased. Recently published work in humans further indicates that self-reports of greater physical activity, including exercise, is associated with enhanced conditioned pain modulation[96], a marker of descending pain inhibitory function which some work suggests has an EO-mediated component[75,122].

Plasma endocannabinoid levels will be assayed in vitro from blood samples obtained in the study. Endocannabinoids can produce analgesia, may potentially interact with and influence opioid responses (thus serving as a potential confound to tests of primary study hypotheses), and may represent a previously unexplored mechanisms of change for analgesic effects of exercise.[73] .

4.0 Inclusion/Exclusion Criteria

Inclusion criteria for study participation will be:

- Intact cognitive status and ability to provide informed consent
- Ability to read and write in English sufficiently to understand and complete study questionnaires
- Age 18-55 inclusive
- Presence of persistent daily low back pain of at least three months duration and of at least a 3/10 in average intensity

Exclusion criteria will be:

- Engagement in > 2 days/wk and > 60 min/wk of moderate or vigorous intensity activity based on responses to 6 validated survey questions at screening (CDC BRFSS), and confirmed by exercise-specific questions in the electronic diary obtained prior to the exercise intervention period
- History of renal or hepatic dysfunction
- Current or past alcohol or substance dependence
- A history of PTSD, psychotic, or bipolar disorders
- Chronic pain due to malignancy (e.g., cancer), autoimmune disorders (e.g., rheumatoid arthritis, lupus), or fibromyalgia
- Recent daily opiate use
- Use of any opioid analgesic medications within 72 hours of study participation (confirmed through rapid urine screening conducted prior to study participation)
- Females who are pregnant
- History of cardiovascular disease (including myocardial infarction)
- History of seizure disorder

- Prior allergic reaction/intolerance to morphine or its analogs
- Presence of cardiac disease or any other medical condition that would make engaging in the aerobic exercise manipulation unsafe

5.0 Enrollment/Randomization

Participants will be recruited from a list of potentially qualifying participants from past studies in our lab who had previously indicated a desire to be contacted about future studies, as well as via local newspaper advertising, posted flyers (including at the Vanderbilt Interventional Pain Center and the Osher Center for Integrative Medicine at Vanderbilt),and e-mail advertisements distributed throughout campus via the Vanderbilt mass e-mail system. Interested individuals will be able to contact the designated study research assistant by phone or e-mail. Random assignment to both study conditions will be stratified by community vs. clinic population recruitment source to control for possible influence of this variable on results. Approximately equal numbers of males and females of all races will be recruited for the study. Individuals expressing an interest will first be provided verbally with information on the study procedures, risks, and benefits, and if interested, will be given the opportunity to read the IRB-approved informed consent form. All questions from potential subjects will be answered by the study representative as accurately as possible. All individuals agreeing to participate will provide written informed consent prior to beginning any study procedures. Subject recruitment and consent procedures will be carried out by individuals designated and trained by the PIs and the IRB to carry out these procedures (i.e., the research assistant).

The study will employ a mixed between/within-subjects design using double-blind, counterbalanced, placebo-controlled administration of both an opioid antagonist (naloxone) and an opioid agonist (morphine). The study will use a 6 week supervised aerobic exercise manipulation, with subjects randomly assigned to the exercise protocol or a no exercise control condition. All participants will undergo three identical laboratory pain-induction sessions (each ≈5 days apart) prior to randomization to experimental condition, and again at the end of the 6 week exercise manipulation period (regardless of exercise group assignment) during which they will receive the 3 study drugs and participate in controlled laboratory evaluation of evoked thermal pain responsiveness.

6.0 Study Procedures

Screening/Consent visit (Day 1 of Study Participation): Potential participants will be screened to determine eligibility for participation in the study. This screening, supervised by the study physician, will include evaluation to characterize the nature of the chronic back pain and to confirm eligibility to engage in the exercise manipulation. Potential participants will also be screened for the exercise exclusion criteria by answering questions related to moderate-vigorous physical activity from the CDC Behavioral Risk Factor Surveillance Survey to classify potential subjects as active or inactive. Following informed consent, participants will complete study questionnaires. Questionnaires will include assessment of occupational status (Employed, Not Working, Disabled), and for working individuals, number of hours worked per week and nature of the job for use as an additional measure of activity. Participants will also complete the International Physical Activity Questionnaire (IPAQ) to gather additional relevant physical activity

information prior to beginning participation in the study, to supplement diary-based data regarding exercise activity. Questionnaires assessing factors related to back pain, anger, depression, anxiety, pain coping, and pain medication responses will be completed. Participants will then complete the 5-day pre-manipulation electronic diary assessment procedures.

Pre-Manipulation 5 day Electronic Diary Monitoring Period (Days 2-6): Daily chronic back pain intensity will be assessed for 5 days pre-manipulation via an electronic pain diary using handheld Palm® Zire 22 PDAs, running the Palm OS platform. To provide a representative sampling of daily back pain intensity, participants will be prompted by an audible alert at 9am and again at 6pm daily. At each prompting, participants will be asked to electronically record ratings of their current back pain, recent sleep, activity levels, and mood using 9 point numeric scales. Participants will begin the laboratory portion of the study following completion of this first diary monitoring period.

Lab Visit 1 (≈Day 7): Participants will undergo a urine screening for opiate use (females will also be screened for pregnancy); complete psychometric instruments; training for heat pain stimulus (Visit 1 only); 5 minutes of seated rest; participants rate their current low back pain intensity; nurse inserts i.v. cannula; nurse obtains a 4ml blood sample via cannula to assess endocannabinoid levels (Visits 1 and 4 only); nurse administers randomized drug dose #1. Administration of randomized drug dose #1 (specific drug protocol is summarized below) will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #1 (described in detail below); assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #2. Administration of drug dose #2 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #2; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #3 . Administration of drug dose #3 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #3; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #4. Administration of drug dose #4 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #4; assess thermal pain intensity; assess drug side effects and psychoactive effects; seated rest until side effects remit.

(each session ≈ 5 days apart)

Lab Visit 2: Participants will undergo a urine screening for opiate use (females will also be screened for pregnancy); 5 minutes of seated rest; participants rate their current low back pain intensity; nurse inserts i.v. cannula; nurse administers randomized drug dose #1. Administration of randomized drug dose #1 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #1; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #2. Administration of drug dose #2 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #2; assess thermal pain

intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #3 . Administration of drug dose #3 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #3; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #4. Administration of drug dose #4 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #4; assess thermal pain intensity; assess drug side effects and psychoactive effects; seated rest until side effects remit.

(each session ≈ 5 days apart)

Lab Visit 3: Participants will undergo a urine screening for opiate use (females will also be screened for pregnancy); 5 minutes of seated rest; participants rate their current low back pain intensity; nurse inserts i.v. cannula; nurse administers randomized drug dose #1. Administration of randomized drug dose #1 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #1; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #2. Administration of drug dose #2 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #2; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #3 . Administration of drug dose #3 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #3; assess thermal pain intensity; assess drug side effects and psychoactive effects; nurse administers randomized drug dose #4. Administration of drug dose #4 will be followed by 10 minutes of seated rest to achieve peak drug levels; participant rating of chronic pain intensity; thermal pain task #4; assess thermal pain intensity; assess drug side effects and psychoactive effects; seated rest until side effects remit.

Exercise Manipulation (Weeks 4 – 10 of study participation): Following the third laboratory visit, participants will be randomly assigned to study condition, stratified by recruitment source (clinic vs. community). Participants randomly assigned to the exercise condition will then complete the 18 session aerobic exercise manipulation supervised by an ACSM-certified personal trainer at the Dayani Center (3 exercise sessions per week for 6 weeks). Participants assigned to the control condition will not undergo any manipulation during this 6 week period, and will be asked to continue their current activity levels and not engage in any additional exercise activity during the study period (confirmed via electronic diary). All subjects will complete brief pain and psychosocial questionnaires once weekly by phone.

Lab Visits 4 – 6 (≈Weeks 11-12): Identical procedures to those described above for Lab Visits 1-3. Drug order is again randomized and counterbalanced.

Post-Manipulation 5 day Electronic Diary Monitoring Period (≈Week 11, between Lab Visits 4-5): Identical to pre-manipulation electronic diary procedures described above.

Description of Laboratory Visit Procedures:
All laboratory procedures will be conducted with participants seated upright in a comfortable chair. Each participant's series of three laboratory sessions (placebo, naloxone, morphine) carried out both pre- and post-manipulation will be scheduled within an ≈10 day period, at the same time of day to control for variance due to circadian rhythms.

Study Drug Protocol: Study drugs will be administered (in double-blind fashion) in randomized, counter-balanced order. The assigned study drug will be administered within a given session over 4 infusions (2 min each) according to the following protocol, depending on the assigned drug for that visit: 1) 4 doses of saline placebo (20ml each), 2) an 8mg dose of naloxone (in 20ml saline vehicle), followed by saline, 4mg naloxone, and saline, or 3) morphine sulfate (0.03 mg/kg in 20ml saline vehicle initially, followed by 3 incremental doses of 0.02mg/kg each). To avoid excessive morphine doses (and increased side effect risks) for individuals who are obese, a modified weight adjustment will be employed for individuals more than 30% over ideal body weight based on subjects' observed weight in clinic compared to appropriate ideal body weight for males (50kg+2.3kg for each inch over 5 feet) or females (45.5kg +2.3kg for each inch over 5 feet) [from http://www.globalrph.com/]. For individuals meeting this obesity criterion, morphine dosage will be based on Adjusted Body Weight rather than actual body weight, as calculated by: Ideal Body Weight + 0.4 (actual weight – Ideal Body Weight).

Thermal Pain Task Procedures: The heat pain task will use a computerized Medoc TSAII NeuroSensory Analyzer to assess heat pain threshold and tolerance using an ascending method of limits protocol. Ten minutes after each drug infusion, three trials will be conducted for heat pain threshold and three trials for heat pain tolerance, with each trial conducted sequentially at one of three non-overlapping sites on the non-dominant ventral forearm. An interval of 30 seconds between successive stimuli will be employed. For pain threshold trials, the probe will start at an adaptation temperature of 32°C, with temperature increasing at a ramp rate of 0.5°C/sec until the participant indicates that the stimulus has begun to feel "painful." For each tolerance trial, the probe will start at an adaptation temperature of 40°C, with the temperature increasing at a ramp rate of 0.5°C/sec until the participant indicates maximum tolerance has been reached. Immediately upon completion of the final heat pain tolerance trial at each drug dosage, participants will be asked to rate the pain just experienced using the MPQ.

Patient instructions for this task: "During this next part of the experiment, I'll be testing your sensitivity to the heat pain stimulus I showed you earlier. First we will do the pain threshold test, which asks you to indicate when the heat FIRST begins to feel painful. The probe will start out slightly cool, and when we start the test, you will hear a beep and the probe will begin feeling warm and the temperature will steadily increase. During the test, keep your finger on the LEFT button of this mouse, and press this button the moment the heat first becomes painful. Keep in mind that this is not a test of how much pain you can tolerate, but rather, we want to know the instant at which the heat stimulus first feels painful. Please stay alert and concentrate throughout the test. Pressing the button will turn the heat off, so don't press it until the heat first feels painful to you. We will do this test several times. Do you have any questions about this test? The next test we will do is the heat pain tolerance test. Again, each trial will be at

a different spot on your forearm. During this test, the probe will start out slightly warm. The temperature will then begin slowly increasing, and I'd like you to go as long as you can, and then indicate when the heat pain has reached the maximum you can tolerate by clicking the LEFT button on this mouse. Pressing the LEFT button will turn the heat off, so don't press it until the heat becomes intolerable. We'll repeat this procedure several times. Do you have any questions?"

Exercise Manipulation Protocol: Participants randomly assigned to the exercise training group will undergo aerobic exercise training 3 times/wk for 6 wks. To enhance and monitor adherence, all of the exercise sessions will be conducted at the Dayani Center and supervised by ACSM certified personal trainers thoroughly trained in study procedures. Each exercise session will consist of a 5 minute warm-up, 30 minutes of aerobic exercise, followed by a 5 minute cool-down period. Aerobic exercise will consist of treadmill walking/running, stepping, elliptical, or cycling exercise as preferred by the participant. This will allow participants to choose the type of aerobic exercise most tolerable to them to minimize symptom exacerbation (i.e., acute increases in pain) while maximizing adherence to the training program. Effort levels across the different types of aerobic exercise will be standardized using heart rate (HR) and perceived exertion (RPE) monitoring as recommended by the American College of Sports Medicine (ACSM). Target heart rate zones will be established using the Karvonen formula and heart rate reserve (HRR). Duration of exercise will be standardized at 30 minutes with a target exercise intensity between 70-85% HRR (RPE = 15, hard). Because of the focus on de-conditioned individuals with CLBP, the duration and intensity of exercise will be progressively increased up to target during the first two weeks to avoid symptom exacerbation and minimize study drop-out. Participants will begin with 10-15 minutes of exercise between 40-55% HRR (RPE = 11-12, light) during the first week, 20-30 minutes of exercise between 55-70% HRR (RPE = 12-13, somewhat hard) during the second week, and then 30 minutes of exercise between 70-85% HRR (RPE= 14-16, hard) for the duration of the study. To ensure that participants are exercising within their prescribed workload during each session, HR and RPE will be assessed and recorded every 5 minutes during exercise using Polar HR monitors and Borg's 6-20 RPE scale. In addition, pain will be assessed every 5 minutes during exercise using a pain rating scale developed for this purpose to monitor back pain, with adjustments to the workload in the event of back pain during exercise.

Endocannabinoid Assay Protocol: Outside of the participants' laboratory sessions, we will also separately conduct in vitro assessment of plasma endocannabinoid levels from sampled blood. All samples will remain de-identified using only assigned study subject numbers. Assays will be carried out on plasma extracted from blood samples designated for this purpose obtained before and after the 6 week exercise intervention (sessions 1 and 4 only). Assays will be conducted under the supervision of Co-Investigator Dr. Kelli Koltyn (Univ. of Wisconsin), given her prior experience with these assays[73]. The endocannabinoids N-arachidonylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), key endogenous agonists of the CB1 receptor, as well as 4 structural lipid analogs (palmitoylethanolamide [PEA], oleoylethanolamide [OEA], N-docosahexaenoylethanolamine [DEA], and 2-oleoylglycerol [2-OG]), will be quantified using isotope-dilution, liquid chromatography–electrospray ionization–mass spectrometry based on published methods.

7.0 Risks

There are several potential risks to subjects. First, subjects will experience brief, moderate intensity acute pain upon application of the thermal pain stimuli used for evaluation of acute pain responsiveness. However, subjects have total control over the duration of their exposure to this pain because they terminate the task by indicating when they have reached their tolerance limit. Previous research indicates that this task is safe, but to further maximize safety, individuals experiencing cardiovascular problems will be excluded from this study. Because subjects have total control over the duration of the task, its psychological impact is expected to be minimal.

Participants will experience very brief, mild pain upon insertion of a cannula (for study drug administration) during each experimental session. Insertion will be performed by a trained nurse or physician to minimize discomfort associated with insertion of the cannula. There is a risk of infection and local inflammation at the site of cannula insertion. Precautions will be taken to insure that such risks are minimized.

The medication used for opioid blockade, naloxone, is not an experimental or new drug, and is FDA-approved. Previous studies indicate that it is safe for individuals who are not opiate dependent, do not have liver disease, and do not have cardiovascular problems. Potential subjects experiencing these types of problems will be excluded from this study to insure a maximal level of safety with drug administration. Subjects taking daily opiates, even if not dependent, will also be excluded from the study to avoid precipitating minor withdrawal symptoms. In some individuals, naloxone may increase pain sensitivity to the acute pain tasks somewhat, but again, participants may terminate these tasks if they've reached their tolerance limit. Based on previous studies, naloxone is expected to have limited if any direct effects on clinical pain intensity among pain patients, and therefore, there appears to be little risk of exacerbating patients' chronic pain conditions. Even if such changes do occur, the brief half-life of naloxone (on average, approximately 60-80 minutes) insures that any pain exacerbation will be of short duration. With the exception of possible effects on pain sensitivity, naloxone is not known to be associated with other clinically significant effects in healthy individuals who are not opiate dependent or using daily opiates.

The other study drug, morphine, is an FDA-approved opioid analgesic with a very long history of use in standard clinical practice. It can produce dose-dependent side effects including sedation, somnolence, nausea, brief vomiting, euphoria, and respiratory depression. The cumulative dose to be used in the proposed study (0.09 mg/kg) will be weight adjusted, and represents a relatively low dose within the normal range of dosages used in standard clinical practice, representing a dosage only slightly larger than in our recent work (0.08 mg/kg; Bruehl et al., 2013). This should help limit any negative side effects. The half-life of morphine is approximately 2 hours, and study participants will be kept under observation in each study session until any side effects remit to the point where it is safe for them to leave unassisted. If morphine side effects are severe, these effects will be immediately reversed by administration of naloxone as determined appropriate by the study physician.

As part of this study, half of the participants will be randomly assigned to undergo a 6-week supervised aerobic exercise manipulation as described above. To maximize safety with this type of exercise, individuals with a history of cardiovascular disease are excluded from participating in this study. The intensity of this aerobic exercise training will start low but progressively increase over the 6-week intervention period. Safety and possible discomfort associated with these increases in exercise intensity will be minimized via close monitoring by a certified personal trainer of participant's heart rate, perceived exertion levels, and back pain intensity during exercise. Exercise intensity will be adjusted as appropriate to maximize the participant's level of conditioning while minimizing exacerbation of back pain. In addition, participants will be able to choose your preferred form of exercise from several available options.

In the event new information becomes available that may affect the risks or benefits associated with this study, participants will be notified so that they can make an informed decision whether or not to continue their participation in this study. There may be unknown or unanticipated adverse effects in addition to those listed.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

The PI and co-investigators will carefully monitor adherence to the study protocol on a regular basis via direct supervision, monitoring of obtained study materials, and regular study team meetings. All adverse events and unanticipated problems will be immediately communicated across the two study sites. Deviations from protocol will be immediately corrected, and if appropriate, will be reported to the IRB and the program officer at NIH/NIDA (sponsoring agency).

9.0 Study Withdrawal/Discontinuation

Subjects will be informed that they can withdraw for any reason from the study at any time without penalty. Subjects may be withdrawn from the study if the study physician determines that their medical condition makes their participation in the study unsafe. Upon withdrawal from the study, subjects will be compensated for their participation (prorated as appropriate).

10.0 Statistical Considerations

Given the project focus on testing indirect (mediated) effects of the aerobic exercise manipulation via EO enhancement (mediator) on CP intensity (Aim 1), morphine analgesic requirements (Aim 2), and associated abuse relevant subjective effects (Aim 3), the project is powered specifically for the proposed bias-corrected bootstrap mediation analyses. Empirical power estimates for this mediation analysis approach are available[64]. The mediation analyses will test the mediation model presented in Figure 2 below. The power estimates below jointly take into consideration the effect sizes for the a and b paths in Figure 2. The effects of exercise on EO analgesia (a path) can be derived from 3 studies in healthy individuals reporting differences in pain sensitivity following acute exercise between placebo and EO blockade (naloxone) conditions[55,68,74].

Effect sizes ranged from d=.6 to d=1.2 in directions indicating that opioid blockade reduced post-exercise analgesia, suggesting that EO function partly mediated the effect of acute exercise on pain intensity. The mean effect size across these studies for the impact of EO mechanisms on exercise-related analgesia was d= .93 (equivalent to r=0.42). This effect size is key to initial analyses of Specific Aim 1. The proposed sample size will result in a power exceeding 0.90 for testing effects of the exercise manipulation on EO function (two-sided $p<.05$ significance criterion). Effect size estimates for the b path in the mediational model reflecting influences of EO function on morphine responses can be derived from our prior work, which indicated an average effect size of r=0.27 for associations between natural variations in EO function (opioid blockade effects) and analgesic responses to a single morphine dose across all pain measures and both laboratory evoked pain tasks[18]. Effect size for the b path reflecting influences of EO function on chronic back pain intensity can be estimated from Bruehl et al.[25], with an effect size of r=0.26 for associations between EO analgesic function in the laboratory and diary ratings of chronic back pain intensity (greater EO function was associated with lower daily back pain intensity). Given these effect sizes for the a and b paths in the mediation model, the total sample size for the proposed project was selected as n=116, resulting in a power of 0.80 (two-tailed $p<.05$ criterion for significance) for testing mediation effects hypothesized in Aims 1-3 using the proposed bootstrap approach (based on Fritz and MacKinnon[64]).

Data Reduction and Analyses

Blockade effect variables reflecting EO function will be derived by subtracting evoked pain responses during placebo from comparable responses during opioid blockade (separately for pre-manipulation baseline [BL] and post-manipulation). Given no theoretical rationale for expecting dose response effects with repeated dosing for placebo or naloxone conditions, we will average over the 4 pain stimuli within each of these drug conditions (separately for pre- and post-manipulation sessions) as a means of reducing random measurement error in deriving blockade effects. Blockade effects will be derived such that larger positive values reflect greater EO analgesia. To reduce number of variables, a mean value of the standardized EO function variables (blockade effects) will be derived and will be used initially in analyses (as in Bruehl et al.[18]). Chronic pain intensity pre- and post-manipulation will be defined as mean chronic pain ratings during the respective electronic diary sampling periods. As manipulation checks, we will determine whether degree of spontaneous EO function pre-manipulation is correlated significantly with evoked pain sensitivity and chronic pain intensity at BL. Total number of exercise minutes recorded during the post-intervention diary monitoring period (obtained in both groups) will be explored as a potential covariate for use in primary group analyses.

Primary Analyses.

Aim 1: To determine whether an aerobic exercise manipulation increases EO activity in CLBP patients, and whether these EO changes mediate manipulation-induced reductions in evoked pain responsiveness and daily CP intensity. We will conduct Group (exercise, control) x Time (BL, post-manipulation) mixed design ANOVAs to determine whether evoked pain sensitivity and CP intensity decreased significantly in the aerobic exercise group only. We will also conduct Group (exercise, control) x Time (BL, post-manipulation) mixed design ANOVAs on EO function (blockade effect variables pre- and

post-manipulation) to determine whether EO function increased in the aerobic exercise condition only. We will then test whether group differences in pre- to post-manipulation EO changes mediate group differences in pre- to post-manipulation reductions in evoked pain responsiveness and CP intensity using the bias-corrected bootstrap mediation testing approach of Preacher and Hayes100. This approach is statistically powerful and not dependent on any assumptions about the sampling distribution of the variables, and was used successfully in our recently published work[18]. Per Preacher & Hayes[100], the only precondition for testing mediation will be presence of an effect to be mediated (i.e., the exercise condition is associated with significantly greater reductions in evoked pain responses and CP intensity than the control condition). For any outcomes for which this precondition is met, bootstrap tests of mediation will proceed using the Indirect procedure for SPSS100,[101] (http://www.afhayes.com/spss-sas-and-mplus-macros-and-code.html#sobel) as in our recent work[18]. If the 95% CI for the indirect effect in a given model does not include zero, this will indicate that the hypothesized indirect (mediated) effect is significant at $p<.05$. In this case, a significant indirect path will indicate that the link between aerobic exercise condition and reduced evoked pain responsiveness or CP intensity is significantly mediated by exercise-related EO enhancements. Significant direct and indirect paths jointly for the same outcome will indicate partial mediation. A significant direct path in the absence of a significant indirect path will be interpreted as indicating that the effects of the exercise manipulation on that outcome are mediated via non-opioid mechanisms.

Aim 2: To determine whether the exercise manipulation is related to achieving analgesic targets at lower opioid analgesic dosages, and whether these changes in analgesic requirements are mediated by pre-post EO changes. We will calculate dose response curves (per Prentice[102]) based on morphine condition evoked pain responses at the four incremental morphine dose levels (separately for pre- and post-manipulation). Based on results of our prior work18, a 25% reduction in pain sensitivity relative to pre-exercise placebo pain responses will be the target. The total dosage of morphine needed at pre-manipulation BL to achieve a 25% reduction in pain responses (based on the dose response curve) will be compared to the dose of morphine needed to achieve a 25% pain reduction at post-exercise. We will conduct Group (exercise, control) x Time (BL, post-manipulation) mixed design ANOVAs to determine whether the total morphine dose required to achieve 25% pain relief decreased significantly in the exercise group only. We will then use mediation analysis procedures as described above to test whether group differences in pre- to post-manipulation EO changes mediate group differences in pre- to post-manipulation reductions in morphine dosage required to achieve 25% relief. The only precondition for conducting these mediation tests will be that there is an effect to be mediated, i.e., the exercise manipulation significantly alters the morphine dosage required to achieve 25% pain relief.

Aim 3: To determine whether the exercise manipulation is related to achieving a 25% morphine-induced reduction in evoked pain responsiveness with fewer negative side effects and abuse-relevant effects, and whether these changes are mediated by pre- to post-manipulation EO changes. Aim 3 analyses will parallel Aim 2 except that side effects and abuse-relevant effects at the 25% pain reduction target will be the DV.

Secondary Analyses.

Secondary exploratory analyses will examine potential moderator effects of sex and BL EO function on effects observed in primary analyses. These analyses will test whether the direction and magnitude of Group effects and/or Group x Time interaction effects for DVs depend on the sex of the participant and/or on degree of BL EO analgesia. To illustrate, GLM procedures will be used to test for a significant Group x Time x BL EO function interaction effect for morphine dosage outcomes in Aim 2. If significant, simple effects will be used to test whether Group was related to changes in required morphine dosages from BL to post-manipulation differently for those low vs high in BL EO function. Within the exercise group only, we will also examine associations between exercise intensity (i.e., average number of minutes ≥ target 70% HRR during sessions) and pre-post manipulation EO changes and changes in morphine responsiveness. Finally, we will test whether: a) BL depression and anxiety symptoms predict analgesic responses; b) the exercise manipulation reduces these symptoms; c) such changes contribute to manipulation-related changes in analgesic responses.

11.0 Privacy/Confidentiality Issues

All published data will be reported in a manner in which individual data for specific subjects are not identifiable. Data will be coded on paper and in the computer by subject number rather than name to help further insure confidentiality. All hardcopy study records from each site will be maintained in a filing cabinet in a locked office at each PI's office suite, and will be accessible only to the principal investigators and their designees. All other subject data records will be maintained on password-protected computers stored in the locked offices of each PI. Hardcopies of the data will be maintained for 6 years, after which they will be destroyed.

12.0 Follow-up and Record Retention

The expected duration of this study is 4 years. Hardcopies of the data will be maintained for 6 years after the study is completed, after which they will be destroyed.

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