Cardiovascular Effects of Adderall in Healthy Young Adults. A Randomized Clinical Trial

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Cardiovascular Effects of Adderall in Healthy Young Adults. A Randomized Clinical Trial.

Regulatory Sponsor:	*Anna Svatikova, M.D., Ph.D.* *Department of Cardiovascular Diseases* *Mayo Clinic*
Study Product:	*Dextroamphetamine and amphetamine* *Brand name: Adderall*
Protocol Number: (IRBe)	*16-004743*

Version 4.2 (25-February-2021)

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List of Abbreviations

LIST OF ABBREVIATIONS

BP	Blood Pressure
CRTU	Clinical Research and Trials Unit
DBP	Diastolic Blood Pressure
DSMP	Data and Safety Monitoring Plan
ECG	Electrocardiography
FMD	Flow-Mediated Vasodilation
FTP	File Transfer Protocol
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
SBP	Systolic Blood Pressure
UPIRTSO	Unanticipated Problems Involving Risk to Subjects or Others

Study Summary

Title	*Cardiovascular Effects of Adderall in Healthy Young Adults. A Randomized Clinical Trial.*
Running Title	*Cardiovascular Effects of Adderall in Healthy Young Adults.*
Protocol Number	*16-004743*
Phase	*Phase I*
Methodology	*Double blind, randomized, placebo controlled, cross-over study*
Overall Study Duration	*2016-2019*
Subject Participation Duration	*2 study day visits*
Single or Multi-Site	*Single-site*
Objectives	*To investigate the blood pressure and heart rate response to consumption of a dose of Adderall in healthy young subjects*
Number of Subjects	*30*
Diagnosis and Main Inclusion Criteria	*1) Adults 18 years of age and older* *2) Healthy subjects without known cardiovascular disease, thyroid disease or documented mental health illness* *3) Subjects who are on no medications that directly interact with the study drug* *4) Subjects with no prior history of regular amphetamine use, and non-prescription stimulants* *5) Nonsmokers*
Study Product, Dose, Route, Regimen	*Adderall 25 mg PO once*
Duration of Administration	*1 time dose of Adderall and 1 time dose of placebo drug*
Reference therapy	*Placebo drug*
Statistical Methodology	*Student t test will be used for between group comparisons. Data will be also analyzed using two-way analysis of variance for repeated-measures.*

1 Introduction

This document is a protocol for a human research study examining the acute cardiovascular effects that may result from ingesting a dose of a common, but frequently abused, prescription medication, Adderall. Adderall will be studied in healthy young adults for whom Adderall is not clinically indicated. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

Attention-deficit/hyperactivity disorder (ADHD) is a disorder commonly diagnosed in school-age children. As the name implies, it is characterized by limited attention span, hyperactivity, impulsivity, an inability to concentrate, leading to poor school performance. The diagnosis of ADHD among children and adolescence increased substantially over the last 2 decades. More recently "adult" ADHD has emerged as a new diagnosis. Adderall, containing dextroamphetamine and amphetamine salts, is an approved pharmacologic therapy for ADHD and belongs to a class of prescription drugs with high potential for abuse. An estimated 10% of school-age children and adolescents suffer from ADHD and are prescribed Adderall. ADHD medication prescriptions written for children and adolescents age 3-19 years old increased by 80% from 1998 to 2005, from 6.5 million prescriptions to almost 12 million over 8 years.[1]

Among the controversies associated with amphetamines, particularly Adderall, is the abuse in individuals who do not possess a prescription for the medication but who seek the stimulant effects of the drug, to improve their cognitive, academic performance or to experience euphoria. Adderall in such instances is used sporadically (i.e. Adderall-naïve individuals), sometimes in a reckless manner. The stimulating effects of amphetamines appear to be dose dependant. Young people often believe that "more is better", leading to the intermittent but frequent consumption of high doses of Adderall, often in combination with other stimulants, smoking or alcohol. The potential adverse effects of Adderall are frequently ignored.

Nonmedical users commonly obtain the stimulant medications from their friends and peers. The abuse of prescription stimulants among young adults has brought up serious public health concerns. The number of emergency department visits due to side effects from prescription stimulants more than doubled over the last 5 years. Case reports of serious cardiac events, and even cases of sudden death have been reported.

While significant research focus has been on the neurocognitive effects of Adderall in ADHD population, little is known about its cardiovascular consequences in those not carrying the ADHD diagnosis, who take Adderall intermittently, only as a "study-drug". The proposed research study is a randomized, double-blind, placebo-controlled clinical trial to investigate the acute cardiovascular effects of Adderall in a population of healthy, young people, in whom Adderall is not clinically indicated.

1.1 Background

Increasing number of healthy US students report nonmedical use of prescription stimulants, mainly amphetamines, such as Adderall, to improve their mental focus and academic

performance.[2-5] A University of Kentucky study found that 34% of students used the drugs illegally without prescription during times of high academic stress.[4] Students report almost immediate increase in attention span, calmer demeanor, greater emotional stability and improvement in school and academic performance.

Amphetamines act by stimulating both the central and autonomic nervous system, increasing the release of neurotransmitters dopamine and norepinephrine at the neuronal synapses, while simultaneously inhibiting their reuptake.[6] Amphetamines were implemented as a drug to treat ADHD on this basis, suspecting that increased dopamine in the brain would amplify the capacity of children with ADHD to focus on a given task.

The unsupervised, unauthorized abuse and black market sale of Adderall has raised serious public health concerns. The increasing number of young adults who consume a variety of stimulants parallels with an increasing number of emergency department visits due to side effects.[2-4] Case reports have linked prescription amphetamines with development of acute cardiac events such as myocardial infarction, coronary thrombosis, aortic dissection and sudden cardiac death.[7-9] Reports of new tachyarrhythmias, cardiomyopathies, and systemic and pulmonary hypertension have also been reported.[10,11] A joint study conducted by Columbia University and the New York State Psychiatric Institute (per request of the FDA) determined that the odds of sudden death in children between ages 7-19 years old were 7.4 times higher in individuals prescribed ADHD stimulants.[7] Researchers from the University of Texas Southwestern Medical Center noted a link between amphetamine abuse and occurrence of cardiac arrest, secondary to excessive stimulant consumption, potentially inducing acute inflammatory responses and arterial vasospasms, thus reducing blood supply to the heart muscle.[8] The mechanisms of these effects however remain unclear.

Stimulants such as amphetamines and methampethamies have been also increasing recognized to contribute to heat –related illness risk. Several case reports describing possible amphetamine related “heat stress” leading to complicated cardiovascular outcomes.[12-15] Limited literature is currently available on the effects of stimulants on body’s core temperature and possible risk for stroke.

It is speculated that the adverse events are due to the high sympathomimetic action of stimulants. However, to our knowledge, the mechanisms and magnitude of such hemodynamic responses that may occur with nonmedical use of Adderall in Adderall-naïve, healthy young adults have not been studied in humans in a randomized, double-blind, placebo-controlled fashion.

The acute cardiovascular effects of stimulants in healthy young people are likely to be significant. Considering the increased use of Adderall in adults, these risks may be even higher in people with preexisting cardiac conditions (unidentified hypertension, coronary disease, silent arrhythmias) or in those with a family history of premature heart disease. The goal of the proposed clinical trial is to investigate acute cardiovascular effects of Adderall, administered orally, in healthy, young people not used to amphetamines. We hypothesize that taking an oral dose of Adderall, compared to a placebo pill, increases blood pressure (BP) and heart rate (HR) in Adderall-naïve healthy adults at rest and in response to conditions of mental and physical stress. Furthermore, we hypothesize that these hemodynamic changes are associated with sympathetic activation and

impaired endothelial and cardiac function, which could itself predispose to increased cardiovascular risk. Lastly, we hypothesize that taking an oral dose of Adderall, compared to a placebo pill, alters thermoregulation and acutely suppresses appetite in Adderall-naïve healthy adults.

1.2 Investigational Agent

-Name of investigational drug: Dextroamphetamine and amphetamine
-Brand name: Adderall
- Oral Generic Adderall: total of 25 mg dose. This dose will be compounded by CRTU Pharmacy. The compounded dose will contain: [dextroamphetamine sulfate 6.25 mg, dextroamphetamine saccharate 6.25 mg, amphetamine aspartate monohydrate 6.25 mg, amphetamine sulfate 6.25 mg (equivalent to amphetamine base 15.73 mg)].

The mean elimination half-lives for d-amphetamine and l-amphetamine in adults are 10 and 13 hours, respectively. To ensure the drug is entirely out of the subject's system, we will separate the visits by at least 5 half-lives (65 hours, approx. 3 days). To be safe we will separate study days by at least 5 days.

The duration of effect for Adderall is suggested to be somewhere between 4-6 hours, with the peak effect at 3 hours.

There will be a study run-in phase to determine appropriate dose level for study drug, but there will not be any formal interim analysis. If the drug dose is deemed inadequate, the dose will be increased to 35 mg dose.

-Pharmacologic Category: Central Nervous System Stimulant

Amphetamines are sympathomimetic amines with central nervous system stimulant activity. Amphetamines promote release of catecholamines (primarily dopamine and norepinephrine) from their storage sites in the presynaptic nerve terminals. Amphetamines also block the reuptake of catecholamines by competitive inhibition.

We will study the cardiovascular effects of generic short acting Adderall, 25 mg, as this is the dose commonly misused by young adults. This dose is also reflective of frequent adult doses used in ADHD and in narcolepsy. In general, the drug may be administered without regard to meals. Our subjects will be fasting prior to initiation of the study. Pregnancy tests will be performed prior to each study day and subjects with positive test results will be excluded for the study. Adderall is a pregnancy class C drug, meaning that the risk cannot be ruled out. Studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal effects or other), and there are no controlled studies in women, or studies in women and animals are not available. The drug will be compounded/over encapsulated by the Research Pharmacy in lactose powder for blinding purposes, and thus we will exclude subjects with known history of lactose intolerance.

According to the drug information as described in UpToDate and Lexicomp, the initial recommended oral dosing for adults with ADHD is 5 mg once or twice daily; increasing daily dose in 5 mg increments at weekly intervals until optimal response is obtained; usual maximum dose is 40 mg daily given in 1 to 3 divided doses per day. The recommended initial oral dose of Adderall for adults with narcolepsy is 10 mg daily; increasing daily dose in 10 mg increments at weekly intervals until optimal response is obtained; maximum dose: 60 mg daily given in 1 to 3 divided doses per day.

The medication does not require renal or hepatic dosage adjustments. Since we are studying healthy young subjects, we expect them to have normal renal and hepatic function.

Relevant pharmacodynamics and pharmacokinetics of Adderall are as follows:

- Duration of action: Tablet: 4 to 6 hours
- Half-life elimination: Adults: d-amphetamine: 10 hours; l-amphetamine: 13 hours
- Metabolism: Hepatic oxidation via cytochrome P450 to 4-hydroxyamphetamine (active) norephedrine (active), and alpha-hydroxy-amphetamine with both active metabolites subsequently oxidized to 4-hydroxy-norephedrine. Cytochrome P450 2D6 is primarily responsible for the formation of 4-hydroxy-amphetamine.
- Time to peak: Adderall: 3 hours.
- Excretion: Urine (highly dependent on urinary pH); excreted as unchanged amphetamine (30%, may range from ~1% in alkaline urine to ~75% in acidic urine), and derivatives of alpha-hydroxyamphetamine (50%)

Adderall is Controlled Substance, C-II. The alert US Boxed Warning states the drug's potential of abuse, stating "Amphetamines have a high potential for abuse. Administration of amphetamines for prolonged periods of time may lead to drug dependence and must be avoided. Particular attention should be paid to the possibility of persons obtaining amphetamines for nontherapeutic use or distribution to others, and the drugs should be prescribed or dispensed sparingly. Cardiovascular events: Misuse of amphetamines may cause sudden death and serious cardiovascular adverse events."

Please see the documents which are attached to the IRB application for complete information on Adderall, as provided by Lexicomp (available via UptoDate).

1.3 Clinical Data to Date

Published data on BP and HR response to Adderall in ADHD patients has yielded conflicting results. While significant research focus has been on the neurocognitive influences of prescription stimulants in ADHD, inconclusive results are reported with regard to changes in vital signs, although they appear to be clinically insignificant. This is mainly due to differences in populations, methodology, dose and duration of administration of stimulants. Studies in children and adolescents suggest that the absolute risk of cardiac events is low, while increased risk for transient ischemic attack, ventricular arrhythmias and sudden death was evident in a few adult studies.[16]

Most current evidence is based on research in ADHD children that has not been specifically designed to investigate the cardiovascular effects of stimulants, thus it is difficult to draw definitive conclusions.[17] Furthermore, most studies comment on long-term effects of stimulants in the ADHD population. A 24-hr recording of BP and HR after intake of three different stimulant drugs in children with ADHD, Samuels et al showed statistically, but not clinically significant increase in DBP and HR.[18] Another study performed in ADHD patients, 4-17 years old, showed that only DBP and HR tended to increase with weekly up-titration of daily dose of Adderall from 5 mg to 15 mg. The DBP increased by 3 mmHg and HR increased by 1 beat per minute, while SBP did not change significantly. These changes were thought to be clinically insignificant.[19] Methylphenidate-induced short-long term mean systolic BP changes ranged from +21 to -4 mmHg, while HR ranged from +6 to -13 bpm while short term (around 4 weeks) effects of lisdexamfetamine on systolic BP ranged from +5.4mmHg to -0.8mmHg with HR increases by less than 5bpm [20] In another study of more than 220 adults with ADHD the extended release preparation of mixed amphetamine salts (20-60 mg/day) increased BP and HR insignificantly (SBP by 2.3mmHg; DBP by 1.3 mmHg and HR by 2.1 bpm) over 24 months of monitoring.[21] In adults with ADHD treated for several weeks with amphetamine compounds showed statistically significant increase in SBP (by >5 mmHg) and HR (by >7 bpm).[22] Their study also suggested possible inverse relationship between baseline BP and BP response to prescription amphetamine. Adults with relatively lower BP at baseline manifested more change with treatment, suggesting that adults with normal BP may be more susceptible to cardiovascular changes induced by amphetamines.[22] Overall, varied results in the ADHD population are likely due to varied lengths of treatment follow up, stimulant dose and type used, age of subjects and comorbidities present. No studies have examined the acute cardiovascular effects of Adderall in Adderall-naïve healthy, young adults who sporadically abuse the stimulant, taking it as a "study-aid".

We have recently conducted a randomized clinical trial investigating the acute effects of a commercially available energy drink, showing that BP and plasma norepinephrine levels increase strikingly after energy drink intake. The energy drink elicited almost 74% increase in plasma norepinephrine levels, and systolic BP increased by more than 6% after ingestion of one can of energy drink.[23] Amphetamines abused by healthy young people may similarly cause significant hemodynamic changes predisposing them to early cardiovascular events. The acute cardiovascular effects of Adderall when abused intermittently by young people are not known, but could potentially lead to serious health complications, whether used alone or in combination with other stimulants.

1.4 Dose Rationale and Risk/Benefits

We will study the cardiovascular effects of an oral 25 mg dose of generic, short acting, Adderall, as this is the dose commonly misused by young adults. Prior clinical studies in ADHD patients have used Adderall dose in range of 5-15 mg doses per day[19] while others investigating the immediate effects of Adderall did not cite the dose used.[18] Adderall XR has been used in doses ranging from 10-60 mg per day.[17]

According to the drug information provided by Lexicomp, the initial recommended oral dosing for adults with ADHD is 5 mg once or twice daily; increasing daily dose in 5 mg increments at weekly intervals until optimal response is obtained; usual maximum dose is 40 mg daily given in

1 to 3 divided doses per day. The recommended initial oral dose of Adderall for adults with narcolepsy is 10 mg daily; increasing daily dose in 10 mg increments at weekly intervals until optimal response is obtained; maximum dose: 60 mg daily given in 1 to 3 divided doses per day. Thus considering the prior clinical research data, we elect to study 25 mg dose of Adderall administered orally, one time dose.

Adderall will be administered orally. Although other forms of Adderall exist, our healthy research participants should have no difficulties with swallowing and oral administration of the medication would be easiest. Design of the placebo drug will also be more effective with oral medication. The drug may be administered without regard to meals. Our study subjects will be fasting prior to initiation of the study.

The following information is taken from Lexicomp, available at Mayo Clinic via UpToDate.

Adverse Reactions Significant: Frequency not always defined.

Cardiovascular: Systolic hypertension (extended release; adolescents: 12% to 35%; dose related; transient), tachycardia (extended release; adults: ≤6%), palpitations (extended release: 2% to 4%), increased blood pressure, myocardial infarction, Raynaud's phenomenon

Central nervous system: Insomnia (extended release: 12% to 27%), headache (extended release; adults: ≤26%), emotional lability (extended release: 2% to 9%), anxiety (extended release; adults: 8%), agitation (extended release; adults: ≤8%), dizziness (extended release: 2% to 7%), nervousness (extended release: 6%), drowsiness (extended release: 2% to 4%), speech disturbance (extended release: 2% to 4%), twitching (extended release: 2% to 4%), aggressive behavior, depression, dysphoria, euphoria, exacerbation of vocal tics, formication, irritability, outbursts of anger, overstimulation, paresthesia, psychosis, restlessness, talkativeness

Dermatologic: Diaphoresis (extended release: 2% to 4%), skin photosensitivity (extended release: 2% to 4%), alopecia, dermatillomania, skin rash, urticaria

Endocrine & metabolic: Weight loss (extended release: 4% to 10%), decreased libido (extended release: 2% to 4%), dysmenorrhea (extended release: 2% to 4%)

Gastrointestinal: Decreased appetite (extended release: 22% to 36%), xerostomia (extended release: 2% to 35%), abdominal pain (extended release: 11% to 14%), nausea (extended release: 2% to 8%), vomiting (extended release: 2% to 7%), diarrhea (extended release: 2% to 6%), constipation (extended release: 2% to 4%), dyspepsia (extended release: 2% to 4%), teeth clenching (extended release: ≤4%), tooth infection (extended release: ≤4%), anorexia (extended release: 2%), bruxism, unpleasant taste

Genitourinary: Urinary tract infection (extended release: 5%), impotence (extended release: 2% to 4%), frequent erections, prolonged erections

Hypersensitivity: Anaphylaxis, angioedema, hypersensitivity reaction

Infection: Infection (extended release: 2% to 4%)

Neuromuscular & skeletal: Dyskinesia, rhabdomyolysis, tremor

Ophthalmic: Blurred vision, mydriasis

Respiratory: Dyspnea (extended release: 2% to 4%)

Miscellaneous: Fever (extended release: 5%)

<1% (Limited to important or life-threatening): Cardiomyopathy, cerebrovascular accident, Gilles de la Tourette's syndrome (exacerbation), peripheral vascular disease, seizure, toxic epidermal necrolysis

Warnings/Precautions:
Concerns related to adverse effects:

• Cardiovascular events: [U.S. Boxed Warning]: Use has been associated with serious cardiovascular events including sudden death in patients with preexisting structural cardiac abnormalities or other serious heart problems (sudden death in children and adolescents; sudden death, stroke and MI in adults). These products should be avoided in the patients with known serious structural cardiac abnormalities, cardiomyopathy, serious heart rhythm abnormalities, or other serious cardiac problems that could increase the risk of sudden death that these conditions alone carry. Patients should be carefully evaluated for cardiac disease prior to initiation of therapy. Patients who develop symptoms such as exertional chest pain, unexplained syncope, or other symptoms suggestive of cardiac disease during treatment should undergo a prompt cardiac evaluation.

• CNS effects: Amphetamines may impair the ability to engage in potentially hazardous activities.

• Peripheral vasculopathy: Stimulants are associated with peripheral vasculopathy, including Raynaud’s phenomenon; signs/symptoms are usually mild and intermittent, and generally improve with dose reduction or discontinuation. Digital ulceration and/or soft tissue breakdown have been observed rarely; monitor for digital changes during therapy and seek further evaluation (eg, rheumatology) if necessary.

• Visual disturbance: Difficulty in accommodation and blurred vision has been reported with the use of stimulants.

Disease-related concerns:

• Abuse potential: [U.S. Boxed Warning]: Potential for drug dependency exists; prolonged use may lead to drug dependency. Use is contraindicated in patients with history of drug abuse.

Prescriptions should be written for the smallest quantity consistent with good patient care to minimize possibility of overdose.

• Hypertension: Use with caution in patients with hypertension and other cardiovascular conditions that might be exacerbated by increases in BP and HR. Use is contraindicated in patients with moderate to severe hypertension.

• Psychiatric disorders: Use with caution in patients with preexisting psychosis or bipolar disorder (may induce mixed/manic episode). May exacerbate symptoms of behavior and thought disorder in psychotic patients; new onset psychosis or mania may occur with stimulant use; observe for symptoms of aggression and/or hostility. Screen patients with comorbid depressive symptoms prior to initiating treatment to determine if they are at risk for bipolar disorder.

• Seizure disorder: Limited information exists regarding amphetamine use in seizure disorder (Cortese, 2013). The manufacturer recommends use with caution in patients with a history of seizure disorder; may lower seizure threshold leading to new onset or breakthrough seizure activity.

• Tourette syndrome: Use with caution in patients with Tourette syndrome; stimulants may unmask tics.

To minimize the risk of side effects, we elected to use a short acting form of Adderall. Participants will be closely monitored in the CRTU at St Mary's Hospital where medical personnel is available to assist if medical needs were to arise.

2 Study Objectives

Primary Objective

1) Determine the hemodynamic changes (magnitude of BP and HR response) in healthy adults after taking one dose of prescription medication Adderall 25 mg orally, one time, and compare these responses to those after a placebo pill

Secondary Objective

1) Determine ECG changes and incidence of arrhythmias after consumption of Adderall compared to baseline ECG
2) Determine if consumption of a dose of Adderall is associated with altered neurohormonal and cardiovascular activation, assessed by measurements of biomarkers, such as catecholamines before and after Adderall/placebo intake
3) Determine if taking Adderall induces orthostatic changes
4) Assess baroreflex sensitivity (pace breathing) after Adderall vs placebo intake
5) Determine the effect of Adderall on cardiovascular hemodynamics during stress conditions including mental stress (arrhythmics), physical stress (sustained hand grip) and pain stress (cold pressor stress) as compared to measurements at rest
6) Determine the changes in heart function and brachial artery reactivity after Adderall intake by using an ultrasound
7) Determine the changes in appetite and food consumption after Adderall vs placebo

8) Determine the prevalence of stimulant medication use, history of weight changes and dietary habits among healthy adults (utilizing standard questionnaires)

3 Study Design

3.1 General Design

The cardiovascular effects of Adderall, prescription medication often abused by students for non-medical purposes, are not well understood. This is a pilot study to investigate the cardiovascular and metabolic response to taking an oral dose of Adderall (25 mg) by healthy adults. Subjects will be studied using a randomized, double-blind, placebo-controlled, crossover design with two separate experimental sessions: Adderall and placebo pill; studies will be performed on 2 separate study days, each study lasting from 6-8 hours.

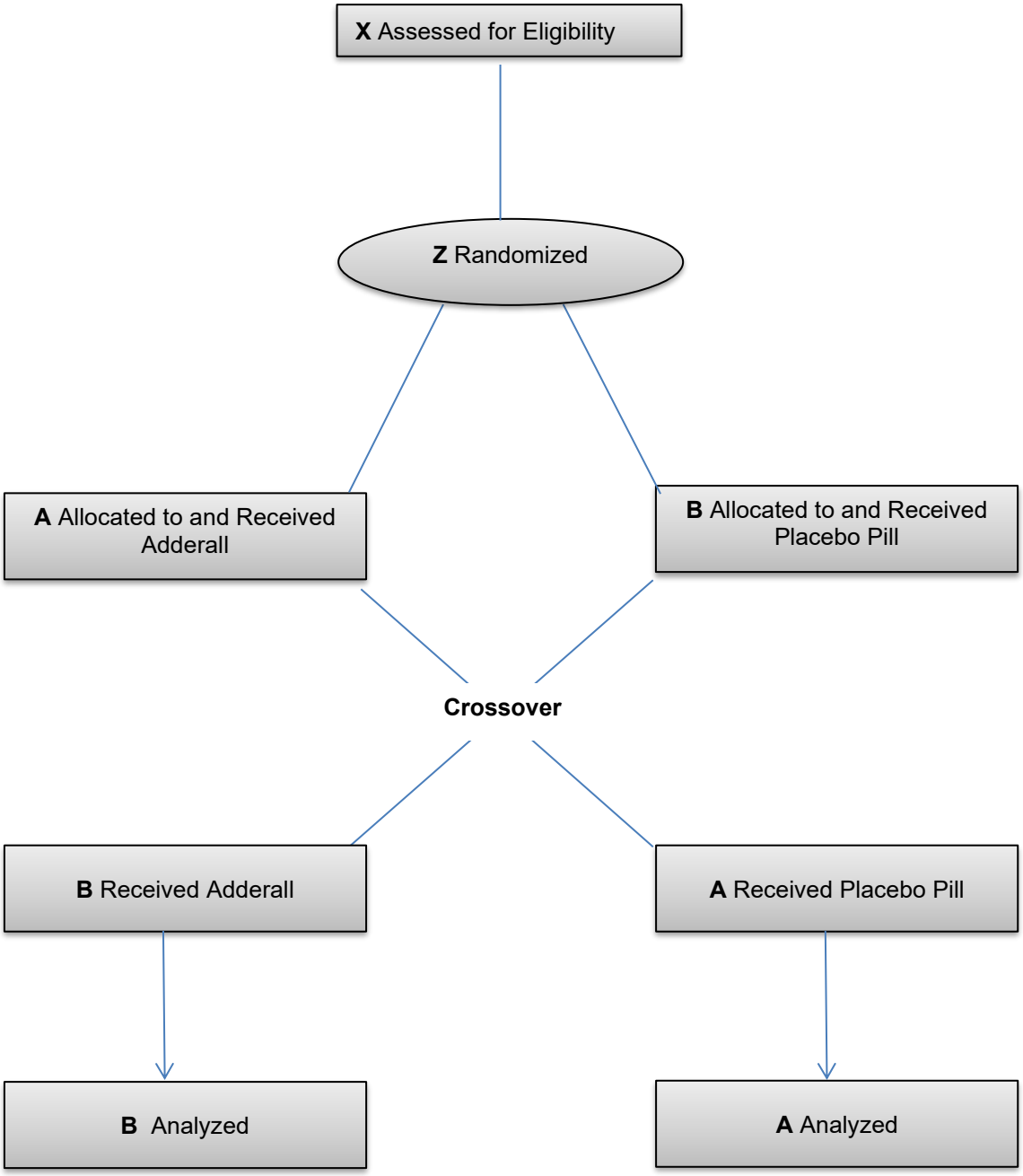
Active drug: Oral Generic Adderall: total of 25 mg dose. This dose will be compounded by Pharmacy. The compounded dose will contain: [dextroamphetamine sulfate 6.25 mg, dextroamphetamine saccharate 6.25 mg, amphetamine aspartate monohydrate 6.25 mg, amphetamine sulfate6.25 mg (equivalent to amphetamine base 15.73 mg)].
Placebo drug: Placebo designed by CRTU Research Pharmacist team

Adderall and placebo drug will be prepared by research pharmacists in similar capsule form. Placebo pill is carefully designed by our team of CRTU Research Pharmacists to match the prescription medication, Adderall. Both drugs will be given in an identical manner so that participants and study investigators are not aware of which drug is being administered. Subjects will remain unaware of the nature of the drug administered on each study day throughout the study. Each experimental session (Adderall and placebo pill) will be conducted using the same protocol, in a random order.

Study participants will be recruited and offered participation in this trial as described in other sections of this protocol. Interested qualified participants will be consented. The study will be performed at St Marys CRTU on two separate days minimum 5 days apart. Participants will be consented upon arrival to the CRTU and will be consented by the Research Staff. The start time of the studies will be the same. Participants will be fasting 4 hours prior to the study and will be asked to abstain from all stimulants, including caffeine and alcohol for at least 24 hours prior to initiation of this study. When presenting to the CRTU on the first day of the study, participant's height and weight will be obtained by the CRTU staff. On each study day, CRTU personnel will perform urine pregnancy test if needed. If positive pregnancy test, participant will not be able to participate in the study. The research staff will obtain waist and hip and bioimpedance measurements (described below); the participant, together with the research staff, will fill out several questionnaires regarding cardiovascular health and dietary habits (attached with application). Subsequently, the participants will take part in research studies as outlined below. Baseline measurements will be obtained at rest. After the end of baseline recordings, the subjects will be given 25 mg oral dose of Adderall vs placebo pill. The second set of measurements will be obtained starting 3 hours after taking the pill, using similar protocol as at baseline.

We expect to enroll approximately 25 healthy subjects.

Schematic diagram (flowchart) of study events:



3.2 Primary Study Endpoints

1) Determine the hemodynamic changes (magnitude of BP and HR response) in healthy adults after taking one dose of prescription medication Adderall 25 mg orally, one time, and compare these responses to those after a placebo pill

3.3 Secondary Study Endpoints

1) Determine ECG changes and incidence of arrhythmias after consumption of Adderall compared to baseline ECG
2) Determine if consumption of a dose of Adderall is associated with altered neurohormonal and cardiovascular activation, assessed by measurements of biomarkers, such as catecholamines before and after Adderall/placebo intake
3) Determine if taking Adderall induces orthostatic changes
4) Assess baroreflex sensitivity (paced breathing) before and after Adderall vs placebo intake
5) Determine the effect of Adderall on cardiovascular hemodynamics during stress conditions including mental stress (arrhythmics), physical stress (sustained hand grip) and pain stress (cold pressor stress) as compared to measurements at rest
6) Determine the changes in heart function and brachial artery reactivity after Adderall intake using ultrasound
7) Determine the changes in appetite and food consumption after Adderall vs placebo
8) Determine the prevalence of stimulant medication use, history of weight changes and dietary habits among healthy adults (utilizing standard questionnaires).

3.4 Primary Safety Endpoints

This research study does not measure primary safety endpoints.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Target accrual: 30 healthy adults will be recruited for this study.

Subject population: Healthy adults. Subjects will be able to provide written consent to be included in the research study. The inclusion criteria will be verified with the subjects and by review of the Mayo medical documents if available.

Inclusion Criteria:

1) Adults 18 years of age and older
2) Healthy subjects without known cardiovascular disease, thyroid disease or documented mental health illness
3) Subjects who are on no medications that have significant interaction with study drug.
4) Subjects with no prior history of regular amphetamine use, and non-prescription stimulants
5) Nonsmokers

4.2 Exclusion Criteria

Exclusion Criteria:

1) Subjects with known cardiovascular disease, thyroid disease
2) Subjects with history of psychotic disorders/mental health illness, including but not limited to anxiety, depression, bipolar disorder; history of substance abuse or dependence
3) Subjects currently taking medications that have significant interaction with study drug.
4) Prior history of regular amphetamine use, or non-prescription stimulants
5) Smokers
6) Pregnant subjects
7) Known lactose intolerance (due to presence of lactose in the prepared medication)
8) Family history of sudden cardiac death

4.3 Subject Recruitment, Enrollment and Screening

Study personnel will recruit subjects by word-of-mouth. Study personnel will review whether the subjects meet the inclusion criteria (Section 4.1 of this document), based on review of their available medical records.

Dr. [redacted]' lab also keeps a list of prior research participants who were recruited as healthy adults for other research studies and who agreed to be contacted for future studies. Study personnel will review this list and if subjects fulfill the inclusion criteria they will be contacted via the phone, to consider participation in the study (see the attachment: Verbal and Phone Recruitment Script).

Eligible subjects will be thus approached by the study personnel and study will be explained in detail. If subjects show interest in participation, their contact information will be collected and forwarded to study team/study coordinator.

Subjects will sign IRB approved consent form when presenting to the CRTU for the research study. Informed consent may also be obtained via Ptrax digital signature capture technology on Mayo devices.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subject participation in this study is completely voluntary. Subjects can withdraw their consent and discontinue participation in the study at any time without affecting their relationship with Mayo Clinic doctors or Mayo Clinic. Although we do not anticipate high withdrawal rates in our healthy study population, rare possible reasons for withdrawal from this protocol is if subject becomes suddenly concerned about the safety or side effects of the study drug. This type of

withdrawal could be prevented by clear disclosure of the potential side effects of Adderall to the subject prior to enrollment. However if such situation does occur, subject will be allowed to discontinue participation in the two day study protocol.

Subject participation in this study may be ended by the principal investigator if it is felt it is in the best interest of the subject, if subjects do not follow the study procedures, or if the study is stopped.

Subjects will not need to be replaced per-se; enrollment of subjects will continue until desired number of participants is completed. There is no long term follow up in this study protocol.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If subjects inform the study team of their voluntary withdrawal from the study protocol, data obtained up to the moment of withdrawal from the study may be used in the analysis, unless subject notifies us otherwise.

This study is not designed to answer any long-term follow up questions.

5 Study Drug

5.1 Description

-Name of investigational drug: Dextroamphetamine and amphetamine
-Brand name: Adderall
-Oral Generic Adderall: total of 25 mg dose. This dose will be compounded by Pharmacy. The compounded dose will contain: [dextroamphetamine sulfate 6.25 mg, dextroamphetamine saccharate 6.25 mg, amphetamine aspartate monohydrate 6.25 mg, amphetamine sulfate 6.25 mg (equivalent to amphetamine base 15.73 mg)].

The study drug and placebo drugs will be prepared by the CRTU Pharmacists. The drugs will be given in a capsule form.

5.2 Treatment Regimen

Adderall 25 mg given orally, one time dose.

5.3 Method for Assigning Subjects to Treatment Groups

The proposed research study is a randomized, double-blind, placebo-controlled clinical trial. The randomization list will be computer generated. We will utilize a randomized block design, with a block size of 6, considering enrolling 30 subjects, each completing two study days. This assures balance between the treatment arms after each group of 6 subjects. Subjects and study personnel will be blinded to the nature of the pill administered on each study day.

Randomization will be performed by CTSA statistician. De-identified coded spreadsheet will be provided to the research pharmacist who will be distributing the drug/placebo.

5.4 Preparation and Administration of Study Drug

Study drug and placebo will be prepared and dispensed by the research pharmacist, [redacted] Pharm.D., R.Ph., or [redacted] colleagues from SMH Research Pharmacy, [redacted]. The study drug will be administered by the research team (blinded to the pill being administered) in identical dispensing cups.

5.5 Subject Compliance Monitoring

The study protocol involves oral ingestion of one study pill (Adderall vs placebo pill), at one time. Study subjects will be personally observed by the study team when subjects take the drug, to assure their compliance with study protocol.

5.6 Prior and Concomitant Therapy

No prior or concomitant medical therapy will be utilized.

5.7 Packaging

The study drug and placebo drug will be packaged in a capsule form, delivering 25 mg of Adderall vs placebo. The study drug and placebo drug will be supplied by the Research Pharmacy at SMH.

5.8 Masking/Blinding of Study

Both drug preparations will be dispensed by an independent Research Pharmacist and will be given to the subject by research personnel in identical cups so that neither participants nor study personnel are aware of which drug is being administered. Study personnel blinded to the study will give the participant the active vs placebo drug, according to the randomized, double-blind, placebo-control crossover design of this study.

Blinding will be maintained until completion of the analysis of data.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Adderall will be obtained from Mayo Clinic/St. Marys Hospital Pharmacy, which will provide the drug to the Research Pharmacy at St. Marys Hospital. The drug will be verified by the pharmacist and drug receipt log will be kept by the pharmacy as well. Any discrepancies, damaged or unusable study drug (active drug or placebo) will be documented in the study files. The Principal Investigator of the study will be notified immediately of any discrepancies, damaged or unusable products.

5.9.2 Storage

Adderall and placebo drugs will be stored in a safe, dry place, protected from light, at room temperature (20-25 C), according to the recommendation published.

5.9.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed by the research pharmacist to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the research pharmacist.

5.9.4 Return or Destruction of Study Drug

Study drug will be supplied by the Mayo Clinic pharmacy on a one-to-one basis, rather than batches. In this respect we do not anticipate to have drugs remaining or drugs to return. At the completion of the study, there will be a final reconciliation of drug provided by the Mayo Clinic Pharmacy, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Visit 1

Two CRTU visits - minimum 5 days apart, maximum 3 months apart.

CRTU Day # 1

1. Subjects report to SMH CRTU at the already pre-scheduled appointment time. Participants should not eat at least 4 hours prior to coming to CRTU. Participant is reminded to avoid all stimulants including caffeine and alcohol 24 hours prior to the study.

2. CRTU personnel informs study personnel when the subject arrives at the CRTU.

3. Study personnel explain the research study to the participant and obtain written informed consent.

4. CRTU personnel will obtain height and weight of the participant.

5. CRTU personnel performs urine pregnancy test as needed (Urine collection #1).
   If the participant is pregnant, then the participant is unable to participate in the study, and will receive $25.00 for their time. In all participants at least 100 ml of urine may be collected for future biomarker analysis at baseline

6. Study personnel verbally ask and record the responses to Cardiovascular Research Data Sheet including anthropometrics and bioimpedance measurements.

7. Participant will be asked to fill out the following questionnaires on a Mayo laptop or on paper. Some of these questionnaires may be administered more than once: Stimulant Intake Questionnaire, Berlin Questionnaire, Breakfast Questionnaire, Food Scale, Food Intake Questionnaire, Adult ADHD Self Report Checklist, Symptoms Questionnaire, Sleepiness Scale.

8. CRTU Staff will insert peripheral intravenous line into the forearm and blood draw #1 will be obtained after at least 30 minutes of supine resting.

9. Study personnel will obtain BP and HR measurements in supine position. Measurements will be taken every 1-3 minutes throughout the study.

10. Study personnel will be obtaining 12-lead ECG recording throughout the study.

11. Study personnel will perform paced breathing test, details described in the IRB Protocol. Breathing will be measured by elastic bands around the chest and abdomen.

12. Baseline endothelial function test will be performed by the study team.

13. Study personnel will perform orthostatic BP and HR measurements.

14. Study personnel blinded to the study will give the participant the study drug vs placebo drug with a glass of water, according to the randomized, double-blind, placebo-control crossover design of this study.

15. Study subject will be asked to rest in sedentary or recumbent position for 3 hours.

16. CRTU Staff will do blood draw #2 after 30 minutes of supine resting,3 hours after intake of active drug/placebo drug.

17. The 12-lead ECG recording continues on.

18. Study personnel will perform post-intervention paced breathing test, details described in the IRB Protocol. Breathing will be measured by elastic bands around the chest and abdomen.

19. Post intervention endothelial function test will be performed by the study team.

20. Post intervention ultrasound of the heart may be performed by the study team or echo lab personnel.

21. Study personnel will perform orthostatic BP and HR measurements.

22. Study personnel will describe the series of stress tests that will follow and BP, HR, ECG measurements will continue through the stress tests.

23. Study personnel will perform series of stress tests (physical, mental, and cold stress), details described in the IRB Protocol. During the stress tests continuous BP, HR and 12-lead ECG data will be recorded and will continue to be recorded the recovery time periods.

Participants will be allowed to eat, selecting meals from a prepared menu.

24. Study subjects will be scheduled a follow up appointment in the CRTU to be done within 2 months (minimum 5 days apart) but at the same time of the day; and will be dismissed home for the day.

6.2 Visit 2

CRTU Day # 2

1. Subjects report to SMH CRTU at the already pre-scheduled appointment time. Participants should not eat at least 4 hours prior to coming to CRTU. Participant is reminded to avoid all stimulants including caffeine and alcohol 24 hours prior to the study.

2. CRTU personnel informs study personnel when the subject arrives at the CRTU.

3. Study personnel explain the research plan for day #2 and answers any questions the participant may have.

4. CRTU personnel will obtain height and weight of the participant.CRTU personnel performs urine pregnancy test as needed (Urine collection #2). If the participant is pregnant, then the participant is unable to participate in the study, and will receive $25.00 for their time. In all participants at least 100 ml of urine may be collected for future biomarker analysis at baseline and

5. Participant will be asked to fill out the following questionnaires on a Mayo laptop or on paper. Some of these questionnaires may be administered more than once: Food Scale, Symptoms Questionnaire, and Sleepiness Scales.

6. CRTU Staff will insert peripheral IV line into the forearm and blood draw #1 will be obtained after at least 30 minute rest.

7. Study personnel will obtain BP and HR measurements in supine position. Measurements will be taken every 1-3 minutes throughout the study.

8. Study personnel will be obtaining 12-lead ECG recording throughout the study.

9. Study personnel will perform paced breathing test, details described in the IRB Protocol. Breathing will be measured by elastic bands around the chest and abdomen.

10. Baseline endothelial function test will be performed by the study team.

11. Study personnel will perform orthostatic BP and HR measurements.

12. Study personnel blinded to the study will give the participant the study drug vs placebo drug (one not received on Day 1), according to the randomized, double-blind, placebo-control crossover design of this study.

13. Study subject will be asked to rest in sedentary or recumbent position for 3 hours.

14. CRTU Staff will do blood draw #2 after 30 minutes of supine resting at 3 hours after intake of active drug/placebo drug.

15. The 12-lead ECG recording continues on.

16. Study personnel will perform post-intervention paced breathing test, details described in the IRB Protocol. Breathing will be measured by a elastic bands around the chest and abdomen.

17. Post intervention endothelial function test will be performed by the study team.

18. Post intervention ultrasound of the heart may be performed by the study team or echo lab personnel..

19. Study personnel will perform orthostatic BP and HR measurements.

20. Study personnel will describe the series of stress tests that will follow and BP, HR, ECG measurements will continue through the stress tests.

21. Study personnel will perform series of stress tests (physical, mental, and cold stress), details described in the IRB Protocol. During the stress tests continuous BP, HR and 12-lead ECG data will be recorded and will continue to be recorded over the recovery time period.

22. Participants will be allowed to eat, selecting meals from a prepared menu.

23. Participant completes the study and will receive $200 if both study days are completed. If participants did only one day of this protocol, they will be paid $80

During this study, the participants will take part in the following studies:

1. Medical history interview and completion of questionnaires: This involves answering questions about past and present medical, family history, sleep habits, and social history including intake of stimulants. Questionnaires used are: Cardiovascular Research Data Sheet, Stimulant Intake Questionnaire, Berlin Questionnaire, Sleep Breakfast Questionnaire, Food Scale, Adult ADHD Self Report Checklist, Symptoms Questionnaire and Sleepiness Scales.

2. Baseline body composition measurements: This involves measurements of height, weight, neck, arm, waist and hip circumferences. Bioimpedance measurement- It involves attaching 2 pairs of electrodes – one on the foot and one on the hand. A small electrical current is used to determine resistance and reactance to evaluate body composition. Although there are no known harmful effects of the small electrical current, as a precaution, this measurement will not be done on those subjects that have an implanted device such as a pacemaker, defibrillator, or infusion pump, etc., or any other implanted device.

3. Blood pressure and heart rate measurements: This involves having a BP cuff inflated around the arm at periodic intervals in standing and lying positions. Heart rate will be measured at the time of BP measurement. Measurements will be taken at regular intervals.

4. 12-lead ECG recording: A set of electrodes will be attached to the skin on the chest and ECG tracing will be obtained in lying position throughout the study.

5. Blood samples: A peripheral IV catheter will be inserted into patient's forearm to obtain blood samples and will be kept until the end of the day study. Samples will be obtained at baseline and after study drug/placebo drug administration during supine rest. About 1/2 cup of blood will be drawn with each blood draw to measure cardiovascular markers. Blood samples will be stored safely for future studies of serum markers and will be given a code.
6. Paced Breathing: This involves measurements of HR variability and baroreflex sensitivity to enable better characterization of cardiometabolic changes done before and after drug intake. Subjects will undergo detailed training to perform spontaneous and controlled breathing tests in the supine position. Continuous non-invasive blood pressure will be monitored during this test using a small finger BP cuff. Breathing will be measured by elastic bands around the chest and abdomen.

7. Stress response: The effects of study drug/placebo drug on cardiovascular/circulatory response to stress stimuli will be also studied. Stress tests (sustained handgrip, mental stress, and the cold pressor test) will be conducted in a sequential fashion and continuous BP and HR measurements will be done. Mental stress will be conducted by asking the subjects to complete serial mathematical tasks as fast as possible for three minutes. Physical stress, isometric hand grip performed with a dynamometer and by asking he subjects to sustain a handgrip of one-third of their maximum voluntary hand grip contraction maintained for 2 minutes. The cold pressor test requires subjects to place one hand into an ice water for three minutes up to the level of the wrist. The cold pressor test will be performed always last because of sustained effects of the test. Throughout all tests, measurements of BP, HR,ECG and SpO2will be obtained.

8. Ultrasound of the heart and arm vessel: Ultrasound assessment of heart and flow-mediated vasodilation (FMD) of the brachial artery will be performed. B-mode images and Doppler flow

will be collected to estimate brachial artery diameter and blood flow velocity. After baseline measures, reactive hyperemia will be induced by the inflation of a BP cuff placed around the forearm to a pressure of 50 mmHg above SBP (to a maximum of 200 mmHg) for 5 minutes, followed by release. Brachial artery diameter and flow velocity will be measured after deflation to measure FMD and shear stress rate.

9. Food/Diet monitoring: At the end of the study day, subjects will be given a menu of food options and will be asked to select the foods they wish to eat. The menu will be consistent for all subjects and include low fat, high fiber foods as well as high fat, high sugar and high sodium foods. The food will be weighed by specially trained staff from the team of dieticians. Subjects will be allowed to order and consume as much food as desired. When done eating, the tray of food, including leftovers, will be removed. We will record their appetite level, selection of foods, and actual amount of consumption of food. Leftovers will be weighed and the nutrient analysis will be calculated for the actual food intake using ProNutra software. The analysis will include Calories, Protein, Fat, Carbohydrate, Fiber, and Sodium as well as total weight of the food and beverages served. A consistent menu will be offered and the same procedure will be followed after subjects receive placebo vs. Adderall.

10. Urine sample: At least 100 ml of urine will be collected for future biomarker analysis with each urine collection.

7 Statistical Plan

7.1 Sample Size Determination

Anticipating to study 30 subjects, we will have 80% power to detect a significant increase in the blood parameters (i.e. BP, HR etc.) for the study drug as compared to the placebo drug, with a 2-sided significance level of 0.05. Specifically, we are powered to detect an effect size of 0.80, which is a difference in the means between the different types of drugs (study vs. placebo) that is 80% of the standard deviation. For example, we are powered to detect an increase of 5% in the blood parameters for the study drug vs. only a 1% increase for the placebo drug (4% difference), assuming a standard deviation of 5% (effect size = 0.80).

Data Analysis Plan: Plan is to analyze data using two-way analysis of variance (ANOVA) for repeated-measures with time (before vs after drug intake) as the within factor and group (study drug vs placebo drug) as the between factor. The key variable will be the group-by-time interaction. Differences in hemodynamics (BP, HR) before and after consumption of a drug will be determined by repeated measures ANOVA. Student *t* test (paired or unpaired) will be used for between group comparisons with respect to change in given variable. $P < 0.05$ will be considered statistically significant. Data will be expressed as mean ± SEM.

Endpoints:

Primary Endpoint:

1) Determine the hemodynamic changes (magnitude of BP and HR response) in healthy adults after taking one dose of prescription medication Adderall 25 mg orally, one time, and compare these responses to those after a placebo pill

Secondary Endpoint:

1) Determine ECG changes and incidence of arrhythmias after consumption of Adderall compared to baseline ECG
2) Determine if consumption of a dose of Adderall is associated with altered neurohormonal and cardiovascular activation, assessed by measurements of biomarkers, such as catecholamines before and after Adderall/placebo intake
3) Determine if taking Adderall induces orthostatic changes
4) Assess baroreflex sensitivity (paced breathing) before and after Adderall vs placebo intake
5) Determine the effect of Adderall on cardiovascular hemodynamics during stress conditions including mental stress (arrhythmics), physical stress (sustained hand grip) and pain stress (cold pressor stress) as compared to measurements at rest
6) Determine the changes in heart function and brachial artery reactivity after Adderall intake using an ultrasound
7) Determine the changes in appetite and food consumption after Adderall vs placebo
8) Determine the prevalence of stimulant medication use, history of weight changes and dietary habits among healthy adults (utilizing standard questionnaires).

7.2 Statistical Methods

Descriptive Statistics

Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for both groups (i.e., study drug vs placebo drug). Data will be inspected before analysis for the need of transformations to reduce the possible impact of outliers in skewed distributions, and transformed accordingly. Distributions across subgroups used in randomization will also be compared to assess whether the randomization was successful in equalizing distributions of these prognostic variables across treatment groups. Putative prognostic variables that will be investigated through these descriptive analyses include variables such as BP, HR, age, gender, BMI, baroreflex sensitivity, catecholamine responses.

Handling of Missing Data

We expect missing data to be minimal. However, some subjects may drop out from the two study day protocol. The two-day longitudinal model is flexible to allow available data to be analyzed, for instance, if a subject completes the first study day, but not second study day. These models assume ignorable (MCAR/MAR) missingness. Sensitivity analyses may be performed to assess the robustness of these assumptions.

Multiplicity

There is no need to account for multiple comparisons in this study. With only 30 subjects, no correction for multiple comparisons using Bonferroni or Tukey's procedure will be necessary.

Primary Hypothesis: We hypothesize that BP and HR increase after intake of study drug compared to placebo.

Data will be analyzed using two-way analysis of variance (ANOVA) for repeated-measures with time (before vs after drug intake) as the within factor and group (study drug vs placebo drug) as the between factor. The key variable will be the group-by-time interaction. Differences in hemodynamics before and after consumption of a drug will be determined by repeated measures ANOVA. Student *t* test (paired or unpaired) will be used for between group comparisons with respect to change in given variable. $P < 0.05$ will be considered statistically significant. Data will be expressed as mean ± SEM.

Secondary Hypothesis 1: We hypothesize that study drug will induce more frequent and more severe electrocardiographic findings compared to intake of a placebo drug. We hypothesize that biochemical/metabolic responses to consumption of study drug will be altered after consumption of study drug as compared to placebo drug (ie: increased catecholaminergic response after study drug consumption).

Data will be analyzed using two-way analysis of variance (ANOVA) for repeated-measures with time (before vs after drug intake) as the within factor and group (study drug vs placebo drug) as the between factor. The key variable will be the group-by-time interaction. Differences in electrocardiographic and biochemical/metabolic responses before and after consumption of a drug will be determined by repeated measures ANOVA. Student *t* test (paired or unpaired) will be used for between group comparisons with respect to change in given variable. $P < 0.05$ will be considered statistically significant. Data will be expressed as mean ± SEM.

Interim Analysis

There will not be any interim analysis.

7.3 Subject Population(s) for Analysis

All-randomized population: Any subject randomized into the study.

8 Safety and Adverse Events

The overall study risk for this protocol is considered greater than minimal risk.

Consent: Consent will be obtained before initiating study specific procedures. A quiet, comfortable, and private setting for the informed consent process will be provided. The consent process will be explained to the subject, time will be provided to consider all options and when possible the subject will be provided a copy of the consent form well in advance for review at

home. Study staff will consider the subject's likely reading abilities and provide an impartial witness in the informed consent process if necessary. All questions will be answered. Coercion or other undue influences will be avoided through the use of a quiet, comfortable, and private setting away from areas involved in direct clinical practice will be used for the informed consent process. The subject will be told that informed consent can be revisited at any time and consent can be withdrawn at any time without affecting the subject's medical care. The informed consent process will continue throughout the subject's participation in the study and consent will be informally verified on a continuing basis. Significant new information will be given to the subject as it is available.

Safety: While the measurements and interventions proposed in these studies are used frequently and often on a routine basis in our laboratory, there are some risks to some of the measurements and interventions which will be described below:

- The major risk is that of possible side effects of Adderall. The side effects are outlined in the prior sections of this document. As with any medication, allergic reactions are also a possibility. We will enroll only healthy young subjects. This risk potentially induced by Adderall will be minimized by administering short acting form of Adderall and only one time dose. In the event that subjects develop sustained side effects such as elevated BP, elevated HR or other form of arrhythmia that may acutely compromised subject's health, emergent care will be provided by the medical staff.
- To control for behavioral risks, subjects with a history of psychiatric disorders will be excluded from participation.
- Occurrence of any symptoms and/or side effects will be closely monitored during the study period. Subjects will be informed to report any adverse events to the study team and if necessary they will be promptly referred for care.
- Blood draw: The risks of drawing blood via intravenously inserted catheter include pain, bruising, and rarely, infection at the site of the needle stick and at the side of IV catheter. Risks for blood draw may also include light-headedness. All blood samples will be taken with subjects in the supine position, while being very closely monitored for any light-headedness. The amount of blood taken will not exceed the allowed blood draw volume recommended by the Institutional Review Board (IRB) without obtaining hemoglobin level prior to the blood draw.
- Questionnaires: Some of the questions asked in the study questionnaires may make the subject feel uncomfortable. The subject may choose not to answer any questions that make them feel uncomfortable.
- Blood pressure measurements: There are no significant risks related to noninvasive BP measurements other than temporary discomfort when the cuff is inflated.
- Stress tests: When performing stress tests such as sustained handgrip or inserting hand into ice cold water subjects may experience brief periods of discomfort or pain. Some subjects may feel uncomfortable when asked to do mathematical tasks.
- Ultrasound of the heart and arm vessels: When performing ultrasound of the heart subject may feel temporary discomfort as the ultrasound probe is touching the chest wall. Ultrasound of the arm vessels are performed for endothelial function assessment, which entails inflation of BP cuff, to a pressure of 200 mmHg (or 50 mmHg greater than SBP) for 5 minutes. There are no significant risks related to such noninvasive measurements other than temporary discomfort when the ultrasound probe touches the body or when the

blood pressure cuff is inflated. Ultrasound probe will be pressing lightly on subject's body which may cause only temporary discomfort.

Toxicity: It is not anticipated that there will be any toxicologic issues. One time dose of short acting Adderall 25 mg will be administered orally.

Stopping Rules: Subject decision to withdraw from the study; or meeting of any of the exclusion criteria while participating in the study; or emergence of significant subject safety concers.

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as 4 hours following the last administration of study treatment. The mean elimination half-life for d-amphetamine and l-amphetamine in adults are 10 and 13 hours, respectively. We will continue to monitor the subject until the completion of study protocol each day after ingestion of the drug (anticipating for about 4 hours) in the CRTU. After this time, subjects will be instructed to present to the Emergency Department if within next 24 hours any side effects of Adderall occur. Subjects will also notify the investigators of this study of any side effects.

To ensure the drug is entirely out of the subject's system, we will separate the study visits by at least 5 half-lives (65 hours, approx. 3 days). We will lengthen this interval to be on the safe side to minimum of 5 days between the studies.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
The only laboratory value we will obtain prior to initiation of the study protocol is pregnancy test. If positive, subject will be informed of the results and will be excluded from participation in the study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

To follow the safety of our subjects, all subjects will be closely monitored throughout the study days. Any unanticipated medical problems involving risk to subjects or others and adverse events will be addressed by medical staff, will be recorded and reported as indicated.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required Investigational New Drug Application, IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Stopping Rules

Subject decision to withdraw from the study; or meeting of any of the exclusion criteria while participating in the study; or emergence of significant subject safety concerns.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Internal Data and Safety Monitoring Board

A Data and Safety Monitoring Plan (DSMP) is established to identify and document monitoring activities intended to protect the safety of the subjects, the validity of the data and the integrity of the research study.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

Not applicable.

Data Management

Study data will be collected at the source through electronic data streams generated by study devices and using the custom-designed MATLAB (MathWorks, Natick, Massachusetts) routines to extract data from our monitoring systems that we are using in ongoing studies.

Blood samples and urine samples will be stored in the 2915 Grohne Building on Valleyhigh Drive NW in Dr. Somers' -86C freezers and batched for analysis.

Data Security and Confidentiality

Patient confidentiality is one of the paramount concerns for Mayo Clinic. Its internal policies for safe guarding the confidentiality of the medical record are some of the most stringent in the health care industry. Institution-wide, it is the expectation that only individuals who need to access specific pieces of confidential data will access such data. Data sheets and other paper records will be kept in locked offices and locked drawers, and properly disposed of after use.

Direct identifiers will be removed from specimens or pieces of data whenever possible, and use of coding and anonymizing will be used where possible. When the results of this study will be made available, the participant's identity will remain confidential. All computers are password protected and there is no access to the LAN, Mainframe, or PC without a password. Password requirements vary by system but all generally use a six-attempt account lockout, passwords of at least six characters which change at least every 180 days with a strict reuse policy and prohibition against shared guest accounts. Individually identifiable or deducible data will not be transmitted by unsecured telecommunications, which include the Internet, email, and electronic File Transfer Protocol (FTP). Further, the data will not be physically moved or transmitted in any way from its location without written approval from appropriate personnel.
Another level of security is conveyed by the fact that the building in which the key personnel are located has limited electronic passkey access during non-business hours i.e., between 6 pm - 6 am and 24 hrs during weekends and national holidays. This facility is also guarded by security personnel who make frequent rounds throughout the building throughout the day. In addition, access to the data centers is severely restricted requiring an electronic passkey or a physical key. Server rooms are further access-controlled through swipe card access.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

To protect the subjects' confidentiality, subject-specific data and Case Report Forms will be coded. The materials and the subject identification code list will be stored in secure place. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted] whichever is longer

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

A Data and Safety Monitoring Plan (DSMP) is established to identify and document monitoring activities intended to protect the safety of the subjects, the validity of the data and the integrity of the research study. Data and Safety Monitoring Plan was completed and is attached to this IRBe application.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

Support for this study comes from CTSA Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH).

12.2 Conflict of Interest

Study team members have no conflict of interest with this study.

12.3 Subject Stipends or Payments

After completion of this research study (2 study days in the CRTU) subjects will receive $[redacted]; for one day they will receive $[redacted]. If the subject is unable to participate in the study because of being pregnant, subject will be compensated $[redacted] for their time and travel.

13 Publication Plan

The research study team holds the primary responsibility for publication of the results of this study. The research findings will be shared with medical research community through presentations at national and international scientific meetings and through publications.

The clinical trial will be registered with ClinicalTrials.gov prior to subject recruitment and enrollment.

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