Title: Insulin Resistance, Cognitive Health, and Perfusion of the Adolescent Brain

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Contents

I. Abbreviations/Acronyms

ASL- Arterial Spin Labeling
AE- Adverse Event
CBF- Cerebral Blood Flow
CP- Cerebral Perfusion
CRF- Case Report Form
CRU- Clinical Research Unit
DEXA- Dual Energy X-Ray Absorptiometry
DMC- Data Monitoring Committee
ECG- Electrocardiogram
eCRF- Electronic Case Report Form
HERC- Human Exercise Research Core
IR- Insulin Resistance
IV- Intravenous
MR- Magnetic Resonance
MRI- Magnetic Resonance Imaging
NSAID- Non-Steroidal Anti-Inflammatory Drug
OGTT- Oral glucose tolerance test
PC VIPR- Phase-Contrast Vastly-undersampled Isotropic Projection Reconstruction
REDCap- Research Electronic Data Capture
RPM- Revolutions Per Minute
SAE- Serious Adverse Event
SOP- Standard Operating Procedure
VCO_2- Carbon Dioxide Production
VO_2- Oxygen Consumption
WIMR- Wisconsin Institute of Medical Research

II. Key Personnel

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1. PROJECT SUMMARY

One in five American adolescents are obese, and many of these patients exhibit some level of insulin resistance (IR). IR is associated with cerebrovascular disease, reduced memory, attention, and cognition, but how IR contributes to these in the course of adolescent brain development is unclear. The goal of this proposal is to investigate the extent and by which IR drives reductions in neurocognitive function and cerebral blood flow (CBF) in adolescents at elevated risk for poor brain and cerebrovascular health. We are testing the overall hypothesis that neurocognitive decline is linked to the severity of IR, as are reductions in brain perfusion, due to dysfunctional insulin signaling. We propose to study acute CBF control in healthy adolescents (12-18 years) across a range of IR (diabetes excluded) in collaboration with expert childhood neuropsychologists who lead a comprehensive cognitive testing protocol. Next, two complementary MRI methods (ASL and phase contrast MRI), along with a physiologic stressor to challenge CBF, will be used to test whether the impact of IR on brain macrovascular CBF and microvascular perfusion is regionally specific, with more negative effects in temporal and parietal lobes. These lobes are some of the initial brain areas to change in neurodegenerative diseases, and are involved in attention, memory, learning, and cognition. Finally, this study tests whether the degree of cognitive decline is linked to the CBF stress response and to severity of IR, and to what extent brain hypoperfusion mediates the IR-driven lower cognitive function. This study will provide the first comprehensive look at IR in adolescent brains, by utilizing extensive neuropsychological testing and state-of-the-art MR imaging, and drawing on interdisciplinary collaborations between developmental neuropsychologists, vascular physiologists, and pediatric endocrinologists. These studies are designed to uncover fundamental relationships between IR, neurocognitive function, and CBF in these blossoming adolescent brains. Exciting preliminary data support the aims—particularly uncovered by a physiologic insulin surge—signifying these findings will serve as a foundation for prospective, mechanistic studies to reduce the burden of IR and improve brain and cognitive health in this clinically important population.

2. SIGNIFICANCE

2.1. Synopsis

One in five American adolescents is obese. Up to half of those are already exhibiting insulin resistance (IR), a hallmark of metabolic syndrome and diabetes linked to serious life-altering health disorders, including cardiovascular and cerebrovascular disease. In adults, IR negatively affects brain structure and function and is reflected in lower regional brain volumes, perfusion, increased white matter hyperintensities and abnormal neuropsychological status, especially affecting memory and attention—all changes associated with accelerated cognitive and brain aging and increased risk of dementia. In an analogous fashion, a limited set of literature suggests adolescents with IR exhibit similar brain changes during maturation. We posit then that the brains of obese adolescents are more susceptible to insults of IR during rapid brain development, positioning them on an abnormal cognitive trajectory, and predisposing them to issues related to learning, behavioral stress responses, and depression.

While the metabolic consequences of IR are well described in adolescence, the impact of IR on their neurocognitive status (intelligence, memory, attention, executive function, processing speed) and cerebrovascular function and their interactions remains largely unexplored. This is important since in addition to its classic role as a metabolic hormone, insulin acts as a vasodilator and supports neurotrophic signaling in healthy humans. Therefore, dysfunctional insulin signaling may hold tremendous influence over brain health in adolescents during this vital period of brain development. New insight is required to understand where, when, and how IR negatively transforms brain health, including whether a dose-response exists between IR severity and anomalies in brain and cognition.

The **long-term** goal of this research program is to determine the influence of IR on brain development in adolescents through the relationships between neurocognition and cerebral blood supply. The **primary goal of the current project** is to quantify fundamental neurocognitive and cerebrovascular function in relation to the severity of IR. **Our central hypothesis** is that as IR worsens: **a)** subtle but meaningful neurocognitive declines emerge; **b)** regional brain perfusion is reduced primarily in areas linked to learning and memory *despite* preserved resting global cerebral blood flow (CBF); **c)** acute insulin surges exacerbate regional hypoperfusion, and **d)** cognitive scores will be lower, mediated in part by insulin-stimulated hypoperfusion.

2.2. Detailed Rationale

Approximately one in five U.S. adolescents (12-19 years old) suffer from obesity (15, 32) and as many as half of those may suffer from insulin resistance (IR). IR is associated with reduced regional brain volumes, connectivity, perfusion, as well as cognitive deficits (46, 49, 52). Adolescents with IR also exhibit additional cardiovascular risk factors including hyperglycemia and hypertension, which are known to increase the risk and severity of cerebrovascular and neurocognitive diseases in adults (3, 17, 30, 35, 43). Exactly how IR influences the relationship between brain perfusion and neuronal function in adolescence is undetermined. To limit the potential future social, financial and health burdens associated with IR, these children and their physicians need new options, from new research directions, to ensure healthy brain development as they grow into adults.

Adolescence is marked by a proliferation of synapses with subsequent synaptic elimination (i.e. pruning) along with increases in white matter formation and concomitant decreases in grey matter density (1, 6), primarily in the prefrontal and parietal cortices. Emerging evidence suggests adolescents with IR exhibit reduced brain volumes and connectivity, temporally associated with changes in cognitive abilities, particularly related to reductions in executive function (5). Insulin signaling pathways are important to neuron growth and development (2, 3, 7, 8, 18, 22, 31), and insulin transport into the brain is dysfunctional

in subjects with IR (30). Consistent with insulin's vital role in brain health, intranasal insulin increases brain perfusion (38), and in older adults with IR intranasal insulin improves cognitive outcomes (9). In the rapidly proliferating adolescent brain, disrupted insulin delivery or transport foretells of harmful lifelong impacts of IR on brain and cognitive development. In adolescents, a key question is whether worsening IR contributes to an abnormal neurocognitive trajectory.

Despite the associations of IR with poor brain health, the cerebral blood flow (CBF) response to acute insulin surges in humans remains largely unexplored. In healthy blood vessels, insulin evokes vasodilation via endothelial nitric oxide synthase (NOS) activation. However, insulin also induces the potent vasoconstrictor, endothelin-1, and IR shifts the balance between NOS and endothelin-1, promoting vasoconstriction (20, 21, 55). More importantly, NOS becomes "uncoupled", producing high levels of reactive oxygen species (ROS) (20, 21, 55). Excessive ROS damages the neurovascular unit coupling blood supply to metabolism, and drives neuronal dysfunction (19, 21). In adolescents with IR, a key question is whether cerebrovascular dysfunction exists at all, and its relationship to neurocognitive abnormalities at this vital stage of brain development.

In summary, insulin acts as an endocrine hormone, a neurotrophic signal, and a vasodilator, so it is logical that dysfunctional insulin signaling plays a pivotal part in linking cerebrovascular and cognitive complications in IR (10). These adolescents are particularly vulnerable, as IR occurs during critical stages of neurological development, and they present cardiovascular complications earlier in life and for a longer duration (29). Detecting neurocognitive and cerebrovascular dysfunction in its earliest stages in adolescents will provide the best opportunities to preserve or restore brain function. Addressing the questions posed above, by using dynamic testing, will begin to fill a vital knowledge gap, by concentrating on essential aspects of when, where, and how adolescent IR cultivates cerebrovascular and neurocognitive dysfunction.

2.3. Innovation

Many youth-focused brain studies emphasize a sole aspect of brain structure, cognitive function, or CBF. A limitation of intriguing, but associative studies is that they assess effects of IR on resting measures in adolescence, obscuring insight into the daily impact of insulin on brain health (26, 37). These resting associations lack high resolution in terms of mechanistic insight, and thus add fuel to a classic chicken or egg question: Does vascular dysfunction or neural dysfunction dominate the *earliest* brain changes in adolescents with IR? To address several limitations, this proposal combines three unique areas of innovation. First, this proposal combines collaborative expertise in pediatric neuropsychology, pediatric endocrinology, and vascular physiology to address the complexities and subtleties of the preclinical disease process. A second innovation stems from combining two powerful, complementary brain-imaging approaches that quantify brain perfusion and identify the regions most impacted by IR. **P**hase-**C**ontrast **V**astly-undersampled **I**sotropic **P**rojection **R**econstruction, (**PC VIPR)** is a high-resolution, 4D flow approach used to quantify macroscopic CBF in *all* major extracranial and intracranial arteries. Arterial Spin Labeling (**ASL**) measures provide insight into microvascular cerebral perfusion allowing identification of focused brain regions impacted by IR. No single measure of flow or perfusion can comprehensively assess cerebrovascular function in health or IR; the combination of both MRI approaches will greatly enhance insight into CBF regulation in adolescence. Third, clinically relevant insulin surges acutely stress brain blood flow regulation, highlighting cerebrovascular dysfunction that is not apparent at rest. Indeed, we present novel pilot data indicating significant neurocognitive decline along with remarkable cerebrovascular dysfunction, both of which are related to the severity of IR. When combined, the technical and conceptual innovations in this proposal will deliver novel insight into the severity and regional impact of IR on brain health and cognition in adolescents.

3. SPECIFIC AIMS AND PRELIMINARY EVIDENCE

Pilot studies in Aim 1 were conducted in 7 adolescents (mean age 14.7 years); 3 with low IR (HOMA-IR ~3.4) and 4 with high IR (HOMA-IR ~4.6). Five of these 7 were studied for Aims 2 and 3 (2 with low IR, 3 with high IR).

These data were collected from the awarding of a previous UW Graduate School Fall Competition Grant under IRB# 2014-0529.

3.1. Aim 1

To test the hypothesis that adolescents with higher IR will demonstrate subtle but clinically meaningful neurocognitive dysfunction, primarily in the domains intelligence, memory, attention, executive function, and processing speed.

3.1.1. Rationale

Associations between cognition and IR have not been extensively studied and reports are mixed on whether IR is associated with cognitive abnormality (24, 42). A handful of studies suggest youth with obesity and IR demonstrate deficits in executive control processes (e.g. attention, memory) and lower overall intelligence (46, 47). Although IR has been postulated as being a mediator for such cognitive deficits (46, 49), the association between systemic IR and cognition has not been well established in adolescents (47, 48).

3.1.2. Preliminary Data

Pilot data indicate similar brain and hippocampal volumes between groups (2.93 vs 2.84 mm^3). Cognitive testing results did not differ significantly between groups. However, episodic memory, executive function, and attention, as well as total composite cognition scores were all quantitatively lower in adolescents with high IR. Notably, these smoldering decreases in cognition are directly correlated to HOMA-IR. Collectively, these data suggest brain structure remains intact at this stage, yet fundamental memory and attention measures are declining in relation to worsening insulin resistance.

3.2. Aim 2

To test the hypothesis that adolescents with higher IR:

a) exhibit intact resting global macrovascular CBF but reduced microvascular perfusion in key brain areas involved in cognition, memory, and learning
b) will exhibit further perfusion reductions in response to an endogenous insulin surge in contrast to increased perfusion in healthy adolescents.

3.2.1. Rationale

Patients with IR *and* cerebrovascular disease make it difficult to tease out the timing and interactions between IR, poor perfusion and neurodegenerative disease (12, 14, 21, 55). Using an OGTT as a “stress test” for the brain, may evoke acute reductions in perfusion and elevations in ROS, consistent with the concept that a disrupted insulin response directly and indirectly promotes neurocognitive decline, as OGTT.

3.2.2. Preliminary Data

First, total CBF is remarkably similar between groups (PC VIPR). However, adolescents with high IR exhibit reduced basal microvascular CP in numerous brain regions, including those involved in cognitive processing (ASL). The combination of MRI approaches suggests an uncoupling of macro and microvascular flow (17) (34), consistent with early but subtle *basal* microvascular dysfunction in IR. Therefore, it is crucial to look at both CBF and CP measures, as regional regulation appears to be differently affected by IR.

Second, following OGTT, regional CBF and CP patterns change markedly. Despite similar increases in plasma glucose, insulin increases much more in adolescents with high IR. CBF through the Middle Cerebral Artery (MCA) increases in adolescents with low IR but *decreases* in adolescents with high IR. Similar patterns were observed in the Anterior Cerebral Artery (ACA, not shown). Third, 45 minutes into OGTT, select regions increased CP in adolescents with low IR, but *decreased* CP in adolescents with high IR. Notably, many of these regions are downstream of the MCA and ACA and are linked with executive function, memory, and learning abilities. These data strongly support the hypothesis that adolescents with high IR demonstrate overt brain endothelial dysfunction, which decreases brain perfusion under the physiologic stress of hyperinsulinemia and hyperglycemia.

In summary, the combination of PC VIPR and ASL MRI approaches allow us to quantify dynamic CBF/CP responses, identify brain regions most sensitive to insulin vasodilation, and most impacted by IR. Preliminary data for Aim 2 OGTT evokes hypoperfusion occurring primarily in anterior brain regions associated with executive function and memory. In the context of insulin-driven ROS production (20, 21), and lower cognition scores (Aim 1), these data are consistent with the hypothesis that acute brain hypoperfusion, via daily insulin surges, plays an essential role in the early pathology of cognitive dysfunction in adolescents with high IR.

3.3. Aim 3

To test the hypothesis that higher IR is associated with lower cognitive function (Aim 1) in such a way that perfusion at rest (Aim 2A), and perfusion changes during insulin surge in particular (Aim 2B), mediate the relationship**.**

3.3.1. Rationale

Lower CBF is linked to cognitive decline. OGTT actively reduces CBF/CP in adolescents with high IR, activates ROS (20, 21). These multiple daily insults may independently or collectively contribute to poor cognitive outcomes during this vital stage of brain development.

3.4. Study Duration

Expected start date: July 1, 2019
Expected end date: June 30, 2021

4. RESEARCH DESIGN AND METHODS

The procedures described herein are largely the same or similar as that of past procedures outlined in IRB 2014-0529. General procedures that are the same include:

- Pediatric population (healthy and with IR)
- Venipuncture, IV placement, and blood draws
- Cognitive testing
- MRI scans
- OGTT administration

Procedures that differ include:

- Some changes in inclusion and exclusion criteria
- Addition of DEXA scan
- Addition of maximal exercise test
- More extensive cognitive tests

4.1. Subject Population

Fifty-five (55) otherwise healthy adolescents, post-puberty, between 12-18 years of age inclusive.

4.1.1. Inclusion Criteria

Subjects will be included if they meet all of the following criteria:

- Age 12-18 years inclusive
- Typically developing and cognitively intact

We do not plan to recruit subjects at specific levels of IR. By the variable clinical nature of IR, subjects may display similar glucose levels yet a broad range of insulin values. Subjects will have IR quantified directly via the OGTT. The range of IR increases clinical external validity and will provide important pilot data for future studies.

4.1.2. Exclusion Criteria

Subjects will be excluded if they meet any of the following criteria:

- Diabetes (≥126 mg dL^{-1} fasting glucose)
- Insulin treatment or sensitizing drugs
- Diagnosis of kidney, pulmonary, or heart disease
    - Note: Subjects with asthma who are taking or prescribed a daily inhaler medication (i.e., daily controller medication) will be excluded. Subjects with only as-needed albuterol prescriptions will not be excluded unless their asthma limits their ability to exercise or they require albuterol prior to any exercise.
- Current smoking*
- Pregnancy
- Neurological or developmental disorders (e.g., intellectual disability, autism)
- Significant head injury or medical conditions (e.g., concussion, encephalopathy, seizure disorder)
- Inability to undergo the MRI procedure
- Weight less than 94.5 lbs (42.9 kg) to adhere to safety guidelines regarding blood sampling and OGTT administration
- Tanner Stage <3

- Any other circumstance deemed by the PI not addressed above

*Current smoking will be defined as the use of tobacco or nicotine products >5 times in the past 30 days.

4.1.3. Subject Identification and Recruitment

Subjects will be recruited primarily from pediatric and pediatric endocrinology clinics via our collaborator, Dr. Aaron Carrel, and his staff in UWHC Pediatric Endocrinology. Additionally, subjects will be recruited from the greater Madison, WI community. Subjects may also be recruited from other studies in which our collaborating physician is an investigator:

- 2011-0214
- 2013-1647
- 2015-1172
- 2018-0974

These study consent forms included an authorization to be contacted for future research. Only subjects who signed the authorization will be contacted.

The following methods may also be used for recruitment:

- Mass email to UW-Madison students and staff
- Recruitment flyers
    - Locations may include:
        - UW-Madison campus and UW Hospital buildings
        - Local middle schools and high schools
            - Middleton/Cross Plains School District
            - Verona School District
            - Sun Prairie School District
        - Local businesses
        - Local physician offices
- Website postings (e.g. Craigslist, UW Student Job Center)

All recruitment material used will be IRB approved prior to posting.

Enrollment will continue until the target sample size (see section 4.1 Subject Population) completes all study procedures.

4.2. Research Sites

The following sites will be used for the following purposes:

- UW Medical Sciences Building
    - Maximal exercise testing
- UW Human Exercise Research Core (HERC) in the UW Nursing School*
    - Maximal exercise testing
    - DEXA Scan
- Clinical Research Unit (CRU)*
    - Screening visit blood draw
- Wisconsin Institute of Medical Research (WIMR)
    - MRI

  - IV placement
- Waisman Center
  - MRI
  - IV Placement
- UW Hospital
  - Cognitive testing

*This flexibility is requested to prevent potential space usage conflicts in the UW Natatorium. Firstly, other currently approved research in the PI's laboratory utilizes the limited space at the UW Natatorium. Secondly, in anticipation of the closing of the UW Natatorium circa summer 2020 and subsequent laboratory transition to the Medical Sciences Building. Thus, having the ability to conduct the necessary research at the UW HERC in the Nursing School or the CRU will allow the proposed research project to continue with minimal interference.

4.3. Experimental Procedures

4.3.1. Pre-Screening Subjects

Delegated study team members will screen patients for eligibility for the study based on medical history, medication use and evaluate general safety to undergo MRI (e.g. claustrophobia, metallic implants, etc.). Consent/permission will be obtained following a description of the study and prior to survey questions either:

- verbally for phone screen
- agreeing to the REDCap survey

The same script/information sheet will be used for both the phone screening and REDCap survey (i.e. the REDCap survey will be discussed with the subject verbally and constitutes the "phone screen").

Medical records of potential subjects will be reviewed by staff under the supervision of Dr. Aaron Carrel from Dr. Aaron Carrel's clinic(s) for fasting glucose and insulin to determine subject's eligibility for the study. This review will occur prior to pre-screening procedures.

These steps will be taken to minimize risk of participation. If an individual does not preliminarily meet all inclusion criteria or preliminarily meets any of the exclusion criteria, then the subject will be immediately eliminated from consideration for the study and their information will be destroyed (e.g. shredder or digital deletion).

4.3.2. Screening Subjects

Screening will be split into two (2) parts:

- Part 1 (virtual): Approximately 1 hour
- Part 2 (in-person): Approximately 0.5-1 hour

4.3.2.a. Virtual Screening Procedures

Procedures include:

- e-Consent:
  - Subjects and subject's parent(s) (if subject <18 years) will first be contacted regarding their preliminary eligibility and attainment of parental permission (if applicable). A REDCap link to the electronic version of the Informed Consent form and Informed Assent form (if

applicable) will be sent to the subject and subject's parent(s) (if applicable). Electronic verification and signature/initials will be obtained prior to other study activities.

- Questionnaires
    - Following attainment of consent, subjects and subject's parents (as applicable) will complete the following questionnaires in a virtual format via REDCap:
        - Health history (via questionnaire)
        - MRI safety (via questionnaire)

For participants <18 years old, the pre-screening survey answers will be reviewed with the parent of the potential subject to ensure accuracy. Any discrepancies will be documented. Verbal verification of accuracy by the parent will be documented.

4.3.2.b. In-Person Screening Procedures

A visit reminder will be provided to potential subjects via phone or email at least 24 hours prior reminding them of the following (see Sections 4.2. Research Sites and 4.3.4.b. Fasting, and Caffeine, NSAID and Exercise Restriction):

- Location of visit (with general campus directions)
- Fasting directions
- Caffeine, NSAIDs, and exercise restriction
- Inform the study team if any albuterol, inhaled steroids, or systemic steroids were taken within 48 hours

Subjects will arrive fasted.

The subject will be screened in-person for the following:

- Pregnancy*
- Height and weight
- Hip and waist circumference
- Blood draw
- Body composition via DEXA†
- Maximal exercise test†

*Female subjects will be asked whether they might be pregnant in a private setting away from their parents, and the information will not be shared with their parents.

†For scheduling reasons and the fact that the data obtained from these procedures do not affect eligibility, the DEXA scan and the maximal exercise test can be performed on any of the study visits or on independent visits.

The results of the screening procedure will be valid for study inclusion for 180 days. If the subject does not complete all study visits within 180 days of screening, another screening procedure will take place.

Other studies in the PI's lab that investigate the same subject population collect similar screening data and utilize many of the same procedures. To reduce subject burden, we will use all applicable screening data from the other study's screening procedure and perform only those procedures that were not previously performed. The use of previous data will be documented. The results of the screening procedure will be valid for study inclusion for 180 days from date of collection. If the subject does not complete all study visits within 180 days of screening, another screening procedure will take place.

4.3.3. Study Visits

Duration: Approximately 6.5-8 hours total.

The study involves 2-3 visits to the WIMR, Waisman Center, or UW Hospital (see section 4.2 Research Sites). Because the data collected in each visit is not dependent on the other visit in a temporal manner, the study visits can occur in any order (i.e. one visit does not need to occur before another). Additionally, the flexibility of conducting study visits in any order allows enhanced scheduling opportunities for subjects and staff.

The "Cognitive Testing" and "MRI Structural Scanning" study visits (see section 4.3.3.a Cognitive Testing Visit and 4.3.3.b MRI Structural Scanning Visit) may occur together or independently as best suited for the subject and research staff. This flexibility allows the potential for the reduction in the number of study visits or the reduction in the time spent at a single visit.

A visit reminder will be provided to potential subjects via phone or email at least 24 hours prior reminding them of the following (see Sections 4.2. Research Sites and 4.3.4.b. Fasting, and Caffeine, NSAID and Exercise Restriction):

- Location of visit (with general campus directions)
- Fasting directions
- Caffeine, NSAIDs, and exercise restriction

Subjects will arrive fasted prior to study visits. Parent(s)/guardian(s) will be encouraged but not required to be present during the study visits (exception: cognitive testing visit).

4.3.3.a. Cognitive Testing Visit

Duration: Approximately 2-3 hours.

Subjects will arrive fasted.

Subjects will undergo formal neuropsychological evaluations led by collaborators Drs. Bruce Hermann and Jana Jones and administered by trained personnel**.** Selected tests (see section 4.3.4.e. Cognitive Tests) reflect a broad set of core cognitive domains and related tests, which retain the same item pool across the age range to be investigated to facilitate examination of factors that influence cognitive development in youth with IR (25, 40, 41, 46, 47). This comprehensive approach aims to maximize our ability to detect and interpret subtle functional changes.

There is potential for confounders, such as history of academic problems, depression, ADHD, or anxiety. These factors will be assessed through a structured interview with the child's parent(s)/guardian(s) by an interviewer blinded to all cognitive and psychiatric information, and will assist in interpreting cognitive results. No cognitive information about the parent(s)/guardian(s) will be obtained; they will solely act as informants.

4.3.3.b. MRI Structural Scanning Visit

Duration: Approximately 1 hour.

Subjects will arrive fasted.

We do not expect differences in brain structure (but will compare statistically), but comprehensive imaging will examine potential neuroanatomical differences, and control for brain volume differences between subjects. Subjects will lie supine for approximately 1 hour while various MRI neuroanatomical, CBF, and CP scans are performed. Scans may include T1-weighted imaging, Diffusion Tensor Imaging, T2, T2FLAIR, MPnRAGE, ASL, and PC VIPR. These scans are well-established methods used by the PI's collaborators in the Wisconsin Alzheimer's Disease Research Center (4, 17, 43, 44).

4.3.3.c. OGTT and MRI Scanning Visit

Duration: Approximately 2 hours.

Subjects will arrive fasted.

Subjects will have one (1) IV catheter placed (see section 4.3.4.i. Intravenous Catheter for details) and then lie supine in the MRI machine. Subjects undergo baseline MRI scans lasting approximately 20 minutes, following which subjects will be removed from the MRI bore to sit to drink 75g glucose (OGTT; see section 4.3.4.g. Oral Glucose Tolerance Test (OGTT) for details) within 5 minutes followed by return to MRI bore. Finally, paired MRI scans (PC VIPR and ASL) will be repeated over the next 60 min, with IV blood sampling to quantify insulin and glucose changes. MRI scan time will end approximately 60 min post-OGTT:

1) for subject comfort in MRI
2) as pilot studies indicate robust perfusion changes occur within 60 minutes

ASL scans will be taken at the following time points (note times are approximate due to variance in scan times):

- Baseline (pre-OGTT)
- 10 minutes after OGTT
- 25 minutes after OGTT
- 40 minutes after OGTT
- 55 minutes after OGTT

PC VIPR scans will be taken at the following time points (note times are approximate due to variance in scan times):

- Baseline (pre-OGTT)
- 15 minutes after OGTT
- 30 minutes after OGTT
- 45 minutes after OGTT
- 60 minutes after OGTT

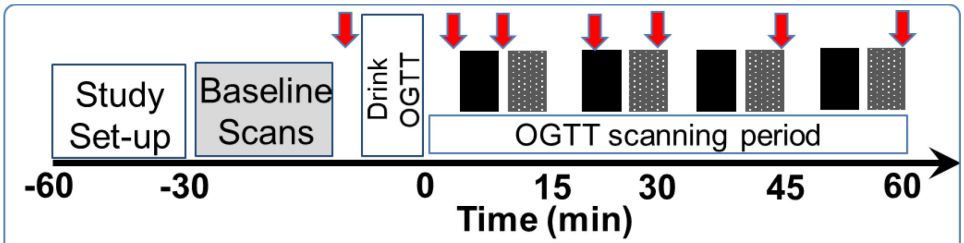

Figure 1. OGTT-MRI Experimental Timeline. ASL (black box) and PC VIPR (stippled box) scans at baseline and repeated during OGTT. Downward arrows indicates blood sampling and vital sign measurement.

4.3.4. Details of Procedures

4.3.4.a. Informed Consent/Assent

Informed consent/assent will be obtained from each participant outlining the potential risks. Consent/assent will be obtained at the initial screening visit and will take place prior to any other procedures being performed. If written consent/assent is refused, the subject will no longer be considered for the study.

4.3.4.b. Fasting, and Caffeine, NSAID and Exercise Restriction

Each subject will be asked to:

- fast for a minimum of 4 hours
- refrain from using NSAIDs (e.g. Ibuprofen) for a minimum of 24 hours
- refrain from caffeine for a minimum of 24 hours
- refrain from vigorous exercise for a minimum of 24 hours
- inform the study team if any albuterol, inhaled steroids, or systemic steroids were taken within 48 hours

4.3.4.c. MRI Screening Document

Each subject will be screened for MRI safety first during the pre-screening process (see section 4.3.1 Pre-Screening Subjects). At the initial screening visit, the subject will fill out an MRI screening document provided by the research team to ensure their safety and comfort in the MRI scanner.

4.3.4.d. Tanner Stage Questionnaire

Each subject will complete a sex-specific pubertal self-assessment questionnaire to assess pubertal development (Tanner Stage; 46). Subjects will complete this in private without parental assistance and will be asked to complete the assessment from memory and to avoid examining themselves.

4.3.4.e. Maximal Exercise Test

Each subject will complete a maximal exercise test on an upright cycle ergometer. Subjects will be fitted with a mask or mouthpiece to measure oxygen consumption (VO_2) and carbon dioxide production (VCO_2), and a 3-lead electrocardiogram (ECG) to measure heart rate. Following a 2-minute warm-up at 60-70

revolutions per minute (RPM) at 25 Watts, exercise intensity will increase 25 Watts every 2 minutes until the subject can no longer maintain RPM≥55 for more than 5 seconds despite strong verbal encouragement. Average duration of maximal exercise testing in adolescents is approximately 8-12 minutes (45, 48).

4.3.4.f. Cognitive Tests

Table 1 details the cognitive domains being assessed, the specific cognitive abilities, and the tests being performed.

Table 1. Neuropsychological Evaluation.

Domain	Ability	Tests
Intelligence	Verbal Functioning	WASI (Verbal IQ)
Intelligence	Nonverbal Functioning	WASI (Performance IQ)
Memory	Verbal memory	NIH Toolbox (List Learning)
Speed Function	Psychomotor speed	NIH Toolbox (Oral Symbol Digit Test)
Executive Function	Inattentiveness/inhibition	NIH Toolbox (Flanker Inhibitory Control and Attention)
Speed Function	Psychomotor speed	NIH Toolbox (Pattern Completion)
Memory	Visual memory	NIH Toolbox (Picture Sequence)
Memory	Visual memory	WRAML (Picture Memory)
Executive Function	Problem solving	D-KEFS (Color-Word Interference)
Executive Function	Divided attention	D-KEFS (Trail Making Test)
Psychiatric Quality of Life	Social-Emotional Functioning	PedsQL - (Child 8-12/Teen 13-18)
Psychiatric Quality of Life	Social-Emotional Functioning	Parents: -CBCL (Child 6-18) -BRIEF-II (Child 6-18) -PedsQL (Child 8-12/Teen 13-18)

These tests will be administered by trained personnel and/or pediatric neuropsychologists via computer, paper forms, and interview as appropriate.

4.3.4.g. Magnetic Resonance Imaging (MRI)

Images will be acquired on a GE 750 3T MRI system located in the WIMR or the Waisman Center. The scanner is located in the WIMR within the UW Department of Radiology or at the Waisman Center Imaging Core. The research team will be administering the scans, as well as processing and analysis of

the data. No contrast agent will be used. While in the scanner, subjects will be monitored for vital signs (see section 4.3.4.m Subject Monitoring). All MR scanners currently used at the University of Wisconsin stay within FDA guidelines and have received FDA approval for use in humans. All pulse sequences used in this study are designed to stay within the current guidelines for dB/dt established by the FDA.

The software and head coil used in this study are not intended for use in the diagnosis or treatment of subjects, nor are they being assessed for safety or efficacy as part of this study. Study data will not be submitted to the Food and Drug Administration (FDA) in support of labeling changes for these devices.

PC VIPR will be utilized to acquire volumetric data sets with three-directional velocity encoding and high spatial resolution in fairly short scan times. This technique has been developed over the last 10 years in the Departments of Medical Physics and Radiology, and has been used and validated in several hundred subjects and have been used with regularity by the medical imaging researchers collaborating on this IRB proposal (Oliver Wieben). We will also conduct ASL perfusion scans. These methods are well-characterized and used routinely during collaborative studies between Drs. Oliver Wieben and Schrage.

4.3.4.h. Oral Glucose Tolerance Test (OGTT)

OGTTs are clinically safe and a minimally invasive procedure to quantify IR. After a minimum 4-hour fast, subjects consume, within 5 min, a beverage containing 75 grams glucose (similar to a can of soda). This is the standard administration of OGTT (Poomthavorn, Nantarakchaikul, Mahachoklertwattana, Chailurkit, & Khlairit, 2013). Blood sampling occurs for 60 minutes (see section 4.3.4.j Blood Sampling). The Schrage lab has conducted multiple OGTT studies in IR adults and found the OGTT with MRI measures is well tolerated.

4.3.4.i. Venipuncture

Only during screening visit. Venipuncture will usually occur in the antecubital fossa or hand by trained personnel using standard aseptic technique.

4.3.4.j. Intravenous Catheter

Only during MRI-OGTT visit. An IV catheter will be placed by trained personnel in the antecubital fossa or hand using standard aseptic technique. Study staff trained and delegated in phlebotomy/IV placement, WIMR staff, research nurse, or higher study-associated clinical authority (e.g. physician) will insert an IV using standard medical procedures. The IV catheter will remain in place for approximately 2 hours.

4.3.4.k. Blood sampling

Screening visit:
Approximately 20 mL of blood will be obtained for blood chemistry values.

OGTT-MRI study visit:
Blood draws of approximately 10-15 mL per draw to a cumulative maximum of 100 mL will be obtained via the IV catheter at specific time points to measure blood chemistry values (e.g. insulin, glucose). The specific time points are as follows:

- Baseline
- 5 minutes after OGTT
- 10 minutes after OGTT
- 20 minutes after OGTT

- 30 minutes after OGTT
- 45 minutes after OGTT
- 60 minutes after OGTT

4.3.4.l. Subject Monitoring

During the screening visit, specifically the maximal exercise test, subjects will be monitored for:

- Heart rate (via electrocardiogram or heart rate monitor)
- Oxygen consumption (via mouthpiece or face mask)
- Adverse signs and symptoms

Throughout the OGTT-MRI visit, subjects will be monitored for:

- Blood glucose (via glucometer)
- Heart rate (via electrocardiogram or pulse oximeter)
- Blood pressure (via automated brachial artery auscultation)
- Blood oxygen saturation (via pulse oximeter)
- End-tidal carbon dioxide (via nasal cannula or mask)
- Adverse signs and symptoms

4.3.4.m DEXA Scan

This is for comparison of body composition (amount of body fat and bone density) between subjects. The scanner is a clinical scanner like many hospitals have for measuring body fat and bone density and is operated by a trained researcher.

4.3.5 Summary of Study Visits and Procedures

	Screening Visit	Cognitive Testing Visit	1-hour MRI Visit	OGTT-MRI Visit
Fasting	X	X	X	X
Questionnaires	X	X		
Body Measurements	X			
Blood Draw/IV	X			X
DEXA Scan	X	X (if needed)	X (if needed)	X (if needed)
Exercise Test	X	X (if needed)	X (if needed)	X (if needed)
Cognitive Tests		X		
MRI Scans			X	X
OGTT				X
Vital Signs	X		X	X

4.4. Confidentiality Protections

Risks to confidentiality will be minimized by keeping copies of the documents linking study assignment number and the participant's unique identifiers with the participant's informed consent in locked cabinets of the offices of Dr. Bill Schrage. Only subject numbers will be used for group assignment, data processing, and analyses. All data will be stored in locked cabinets in a secure lab, and electronic files are all stored on password-protected databases and computers.

Personal information such as name, gender, date of birth, and medication history will be stored in a locked file cabinet in a locked office in the PI's laboratory. Subject information will be coded to remove any personal identifiers during data analysis or research publications. Research oversight and regulatory groups may review study records.

Please see section 7.9.4. Protecting the Confidentiality of Participant Data for more details.

4.5. Remuneration

Subjects will be paid in the following manner:

- Up to $60 for the screening visit
  - $20 for completion of the virtual screening procedures
  - $10 for the blood draw
  - $10 for the DEXA scan
  - $20 for the maximal exercise test
- $60-$90 for the cognitive visit, prorated at $30/hr rounded to the nearest half hour
- $30 for the 1-hour MRI visit, prorated at $30/hr rounded to the nearest half hour
- $60 for the OGTT-MRI visit, prorated at $30/hr rounded to the nearest half hour

The total compensation is estimated to be $210-$240 for completing all study visits. Payment will be given to the participant following each visit or sent to the subject's provided address.

5. BANKING AND SHARING OF DATA AND BIOSPECIMENS

5.1. Data

Blood analysis results and MRI images will be stored for future research use. Banked data will be stored on secure computer servers that are password-protected.

5.2. Biospecimens

Blood samples will be stored for future research use. Samples will be stored in temperature controlled locations (e.g. freezer) in a secure location within the Department of Kinesiology.

5.3. Assurances

Any data or samples shared with personnel outside of the study team will be coded with a study ID number. Unless the staff is listed as key personnel on this study, they will not be given access to the key linking subject identifiers with their data, samples, or study ID number. The study PI will be responsible for the oversight of the data and sample banking, and will review all requests to utilize the data and samples. The PI will be responsible for confirming that IRB approval or exemption has been granted prior to the release of any data or samples. Future study results obtained from banked data or samples will not be reported to subjects.

As new information about insulin resistance or brain health becomes known, researchers may want to go back and analyze existing data or samples with a different aim. Additionally, combining similar information from multiple studies into a larger set could potentially provide more meaningful information than what was collected in any one study. All new analyses that do not fall under this project's aims or that combine more than one dataset will be approved through the IRB as separate protocols.

6. RISKS ASSOCIATED WITH PROCEDURES

6.1 Risk Summary

This protocol is modestly invasive with only one (1) IV catheter for blood sampling, therefore overall risk appears modest for IV catheter and mild for OGTT and MRI.

6.2 Detailed Risks

6.2.1. Fasting, and Caffeine, NSAID and Exercise Restriction

Risks include:

- feelings of hunger
- irritability
- fatigue
- light-headedness
- dizziness

There are no risks of NSAID restriction if NSAIDs are not prescribed by a physician for clinical care. There are no risks of acute abstention from exercise. These risks are considered minimal. See Letters of Support from physicians who regularly require fasting procedures in pediatric populations (section 11. Supplemental Information).

6.2.2. Maximal Exercise Test

Risks include:

- Feelings of exertion, fatigue, and breathlessness
- Discomfort due to VO_2 monitoring equipment (i.e. mouthpiece or mask)
- Serious complications including orthopedic injury, myocardial infarction, arrhythmia, hemodynamic instability, and death are rare (Takken 2016; van Brussel 2019)

These risks are considered minimal, resolving quickly (seconds to minutes) after test termination. See Letters of Support from pediatric physicians who regularly perform maximal exercise testing in pediatric populations (section 11. Supplemental Information).

6.2.3. Intravenous Catheter and Venipuncture

Risks include:

- bruise or clot formation
- infection
- pain at the site of catheter insertion/venipuncture
- hematoma after withdrawal
- soreness over the site

These should all be transient and resolve after several days.

6.2.4. Blood sampling

Risks include:

- Pain at site of blood draw
- Bleeding
- Infection
- Dizziness

Poses no further risk than IV catheterization.

6.2.5. OGTT

Risks include:

- Nausea
- Vomiting
- Abdominal bloating
- Headache
- Hypoglycemia

These risks are uncommon or rare.

6.2.6. MRI

MRI uses a powerful magnetic field, not ionizing radiation, to create an image of the scanned area of the body. No contrast agent will be used.

Risks include:

- There are no known risks of MRI, aside from the standard risks associated with persons with certain metallic implants. If potential participants have a metallic implant, cardiac pacemaker, or are pregnant, they will be disqualified.
- Sensation of claustrophobia or anxiety, particularly in individuals susceptible to it.
- People with metallic implants, such as prostheses or aneurysm clips, or persons with electronic implants, such as cardiac pacemakers, the magnetic field generated by the magnetic resonance machine can cause a displacement or malfunctioning of these devices.
- We know of no risks or adverse effects from the radio signals used in this study.
- Some people have also reported tingling or tapping sensations, or muscle twitches in different parts of their body during the imaging procedure.
    - These sensations are not hazardous and should not cause discomfort to the participants.
- A small increase in risk may be associated with rapid gradient waveform switching times associated with fast MR imaging. In certain situations, the rapid switching of gradient waveforms has caused peripheral nerve stimulation in subjects.
    - Significant nerve stimulation, however, has not occurred as long as the imaging system has been programmed to stay within certain limitations of gradient strength and switching time (dB/dt).
- Occasionally, people who have clasped their hands tightly together during the study have reported a feeling of electrical shock in their hands and arms.

    - This is also not hazardous; however, to avoid any possible discomfort, participants will be instructed to not clasp their hands together during the study.
- Women who are pregnant must not participate in this study. The potential risks to a fetus from the MRI scan are not definitely known.
- The MR scanner produces loud tapping sounds during operation, which may reach somewhat objectionable levels.
- Anatomical abnormalities
    - Images will not be reviewed by neuroradiology
    - Any obvious abnormalities will be reported to the IRB upon discovery for assistance in determining how to proceed with potential reporting

All MR scanners currently used at the University of Wisconsin stay within these guidelines and have received FDA approval for use in humans. All pulse sequences used in this study are designed to stay within the current guidelines for dB/dt established by the FDA.

6.2.7. Subject Monitoring

An automatic blood pressure cuff around the upper arm may feel uncomfortable while inflated, but this is temporary (30-60 seconds). Blood pressure measures are considered very safe.

6.2.8. Cognitive Testing

Sensitive information may be collected during the cognitive testing visit during the structured parental interview assessing the child's quality of life. The one measure with questions that may indicate illegal behaviors and suicidal ideation is the Child Behavior Checklist (CBCL).

The following items on the CBCL are notable:

- Item 2: drinks alcohol without parental approval
- Item 18: Deliberately harms self or attempts suicide
- Item 28: breaks rules at home, school, or elsewhere
- Item 57: physically attacks people
- Item 59: plays with sex parts in public
- Item 82: steals outside of the home
- Item 91: talks about killing self
- Item 99: smokes, chews, or sniffs tobacco
- Item 101: truancy, skips school
- Item 105: uses drugs for nonmedical purposes
- Item 106: vandalism

The answers to these questions may indicate illegal behaviors or psychiatric distress (e.g. suicidal ideation) which may be socially stigmatizing. Answers to sensitive questions will be safeguarded (see section 7.9.4 Protecting the Confidentiality of Participant Data).

6.2.9. DEXA Scan

The x-ray exposure during this test is very small (~30% of a typical chest X-ray) and is well below the levels that result in a high risk of harmful effects. The radiation dose for a whole-body scan is approximately 7 microSieverts. Using a background radiation dose of 2.4 mSv, this equates to a BERT value of about 24 hours (1 day).

7. DATA AND SAFETY MONITORING PLAN

7.1. Monitoring study safety

The study team and PI will monitor study data and safety through two different mechanisms:

1. During study visits, trained Schrage lab staff will be present to address any immediate safety concerns. Schrage lab staff will document all expected and unanticipated problems as described in this protocol.
2. We will complete internal audits of all subject files on a quarterly basis (see section 7.8 Internal Audits). These internal audits will identify any unanticipated outcomes or trends, and Schrage lab staff can then notify the IRB and the DMC of any new, common, or unanticipated adverse events.

The PI and the research team will consult if there are subject complaints or an adverse event is experienced (anticipated or unanticipated). The PI and the research team will review the data to ensure the subject populations are safely handling the study conditions and experimental conditions, that all procedures are being followed per IRB approved protocol, and that all forms are completed to ensure compliance with IRB regulations. Further, study staff will discuss concerns or subject safety issues, including the potential need to modify study procedures to reduce risk.

In addition to the monitoring of the study by the PI and study team, the PI has contracted with the Institute for Clinical and Translational Research Data Monitoring Committee (ICTR DMC) to provide independent, ongoing data and safety monitoring.

7.2. Data Monitoring Committee (DMC)

The ICTR DMC provides investigators with independent services to ensure appropriate measures are in place to promote subject safety, research integrity and compliance with federal regulations and local policies for individual clinical research protocols in need of DMC review as determined by the PI, the funding agency, the local Scientific Review Committee, or the local IRB, and for which no DMC exists. For these studies, the UW ICTR DMC will be the primary data and safety advisory group for the PI.

The DMC is supported in its mission of safety and compliance by experienced ICTR staff to provide administrative assistance, experienced members representing a diversity of backgrounds, skills and knowledge, and the use of REDCap which allows more efficient tracking of protocols and protocol subjects. In providing oversight for the conduct of this study, the ICTR DMC will meet on a biannual basis throughout the study. Additional meetings may be scheduled as determined by the DMC or as requested by the PI. The DMC members will review protocol-specific reports created by statisticians that serve a non-voting member role on the DMC using data pulled from the REDCap. These standard reports will include an overview of study objectives, a review of actual and projected accrual rates, an evaluation of patient demographics for balance of randomization (if applicable), and a summary of the number and seriousness of adverse events. An interim analysis of study results may be performed and source documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the PI. The DMC will make recommendations to the PI that could include actions of continuation, modification, suspension, or termination.

Data Type	Frequency of Review	Reviewers
Subject accrual (including compliance with protocol enrollment criteria)	Every 6 months	ICTR Data Monitoring Committee, PI
Subject demographics	Every 6 months	ICTR Data Monitoring Committee, PI
Status of all enrolled subjects as of date of reporting	Every 6 months	ICTR Data Monitoring Committee, PI
Primary outcome analysis	Every 6 months	ICTR Data Monitoring Committee
AEs and rates	Every 6 months	ICTR Data Monitoring Committee, PI
SAEs	Per occurrence	ICTR Data Monitoring Committee, PI

7.3. Adverse Event (AE) Reporting

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) v5.0 for adverse event monitoring and reporting. This information can be downloaded from the CTEP home page (http://ctep.info.nih.gov/CTC4/ctc_ind_term.htm).

The severity of the event will be graded using the CTCAE. In addition, for comparison to the CTCAE, adverse events will be tabulated using a 3-level schema as defined below:

- Mild
  - Event may be noticeable to patient
  - Does not influence daily activities
  - Usually does not require intervention.

- Moderate
  - Event may be of sufficient severity to make patient uncomfortable
  - Performance of daily activities may be influenced
  - Intervention may be needed.

- Severe
  - Event may cause severe discomfort
  - Usually interferes with daily activities; patient may not be able to continue in the study
  - Treatment or other intervention usually needed.

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily an SAE. For example, a headache may be severe (i.e. interferes significantly with patient's usual function) but would not be classified as serious unless it met one of the criteria for SAEs.

Next, it will be determined by the PI and study physician or clinical neuropsychologists whether the event is expected or unexpected and if the AE is related to the intervention or study procedures. With this information, it will be determined whether an AE should be reported as an expedited report in addition to submission via routine clinical data.

Expedited AE reporting may require e-mail notification, phone, or fax submission of a written report as directed below. All expedited AE reports will be submitted to the IRB of record, the UW Health Sciences Institutional Review Board (HS IRB) in the case of this study.

7.3.1. Assessment of Attribution

Attribution of AEs will be assessed in relation to the study procedures. When assessing whether an adverse event is related to a study procedure, the following attribution categories are utilized:

- Definitely Related
    - An AE categorized as definite is clearly related to study procedures. If the timing of the AE is definitely consistent with the exposure to the study related procedures and it is most likely that the AE was caused by the study procedures such as because a high occurrence of the AE was expected based on the study intervention materials. In this case, the PI may categorize the AE as definitely related.

- Probably Related
    - An AE that is likely related to study procedures. If the timing of the AE is consistent with the exposure to the study intervention and it is more likely that the AE was caused by the study procedures than not, the PI may categorize the AE as Probable.

- Possibly Related
    - An AE that may be related to study procedure. If the timing of the AE is reasonably consistent with the exposure to the study intervention, and there is another cause of the AE that could be equally likely, the PI may categorize the AE as Possible.

- Unlikely Related
    - An Unlikely AE is one that is doubtfully related to study procedures. The coincidence of the AE with the exposure of the investigational product or intervention should be assessed. An AE that continued while the intervention was interrupted or stopped, or if the AE resolved while the intervention continued, may be categorized as Unlikely. If there is another more likely cause of the AE, the PI may determine that the AE was unlikely related to the study intervention.

- Not Related/Unrelated
    - An Unrelated AE is one that is clearly NOT related to study procedures. An AE may be considered Unrelated if the subject did not receive the study intervention or if there is another obvious cause of the AE (for example, a car accident or other disease/condition).

7.4. Serious Adverse Event (SAE)

A serious adverse event (SAE) is an AE occurring during any phase of the study (i.e., screening, admission, treatment, or follow-up) that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization (more than 24 hours)

- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above

7.5. Identifying, Reviewing, and Reporting Adverse Events and Unanticipated Problems to the IRB

Identifying

- To effectively identify all AEs (anticipated or unanticipated), Schrage lab staff will follow laboratory and protocol specific SOPs, complete AE reporting forms for each subject as they arise, and maintain a database of all reported adverse events for each protocol by entering all AEs into REDCap within a timely manner. All stopping events or subject reported complaints/concerns will be documented via study notes during the study. Participants will be encouraged to contact us with any questions or concerns following the study visit.

Reviewing

- The PI, study physician, and study team will also review AEs during internal audits of study documents in a cumulative fashion to identify any trends of safety concerns (see section 7.8 Internal Audits). In the event of an SAE, the PI, study physician, DMC, and IRB will review the event as soon as possible.

Reporting

- All adverse events will be recorded in REDCap in a timely manner consistent with reporting requirements. Schrage lab staff will log all AEs in REDCap, and report AEs and unanticipated problems to the DMC and to the IRB as indicated by IRB guidance on Knowledge Base.

7.6. Expedited Adverse Event Reporting Requirements

Serious Adverse Event— Reported Within 24 Hours

- Serious Adverse Events requiring expedited reporting within 24 hours will be reported to both the UW Health Sciences IRB and the ICTR DMC Manager within one working day as directed below. Confirmation that all appropriate parties were notified will be done at this time.

Serious Adverse Event – Reported within 15 Days

- Serious Adverse Events requiring expedited reports in writing within 15 working days will be sent by the PI and study team.

7.7. Protocol Deviations

To effectively identify deviations from the IRB approved protocol, Schrage Lab staff will maintain protocol checklists that are designed to not only minimize protocol deviations but to identify deviations when they do occur. Additionally, Schrage Lab staff will conduct regular internal audits (see section 7.8 Internal Audits) to identify deviations that may have been overlooked. All deviations will be logged on a Deviation Tracking Log and a database of deviations will be maintained in REDCap. The PI will review deviations as they are identified, and oversight committees will be notified according to their guidelines. The study team will implement corrective action plans when appropriate to address deviations.

7.8. Internal Audits

Regular internal audits will be conducted to ensure compliance and safety. Internal audits will occur on a quarterly basis. These audits could consist of full or partial review of study records, depending on

observed past compliance. Audits will be documented on Internal Compliance Review forms modified specifically for the study and will include review of the following:

- Personnel (e.g., ensure all personnel have the appropriate training)
- Documentation of IRB submissions and correspondence
- Informed Consent/HIPAA Authorization Forms
- Laboratory Aspects of the Trial
- Equipment Maintenance
- Communication and Correspondence with oversight committees
- Study Conduct
- Completion of Case Report Forms
- Adverse Events
- Protocol deviations

These internal audits will identify any unanticipated outcomes or trends, and Schrage Lab staff can then notify the IRB of any new, common, or unanticipated adverse events, if applicable.

The results from internal audits (e.g., noncompliance, adverse event trends) will be discussed during regular lab meetings where team members can establish plans to prevent future issues.

7.9. Minimizing Research-Associated Risk

First and foremost, risks will be minimized by the prescreening procedures to determine preliminary eligibility, which includes thorough health history and MRI safety questionnaires (see section 4.3.1. Pre-Screening Subjects) which will be verified by the subject's parent/guardian (if <18 years of age). Secondly, in-person anthropometric measurements and survey of pregnancy (see section 4.3.2. In-Person Screening) will further establish safety for subject participation. This two-stage process should minimize risk prior to any study conditions being imposed on the participants.

Once enrolled as a subject and participation in the trials begins, we will monitor heart rate, blood oxygen saturation, blood pressure, end-tidal carbon dioxide, and subject comfort throughout the study visit (if applicable; see section 4.3.4.m. Subject Monitoring). This data will be used to monitor the health of our participants and for research purposes.

Having only trained personnel performing the IV catheter placement under aseptic conditions will minimize IV catheter placement risk.

Screening each subject for cardiovascular disease and any blood clotting disorders will minimize intravenous catheter placement risk.

If a participant reports positive pregnancy, the study team will immediately end further screening procedures and will only state that the subject is not eligible without details as to the reasoning. This is done to protect the confidentiality of the pregnancy screening procedure (see section 4.3.2. In-Person Screening).

Although we are extensively screening out subjects that have overt diabetes, the results of the experimental OGTT may produce results identifying that a subject may be diabetic which may indicate a clinical concern. The fact that insulin analysis will occur towards the end of the study (due to cost limitations with assay) means diabetic subjects will have completed all Aims of study. A diabetes finding does not pose further risk to the patient.

Using the standard exclusionary procedures to prevent subjects with hazardous metallic implants, cardiac pacemakers, or subjects who are pregnant will minimize the risks of MRI scanning. A standard safety screening form used for clinical MRI scanning will be used to exclude patients with hazardous metallic implants, cardiac pacemakers, or patients who are pregnant. In addition, all operators of the MRI equipment are trained in the proper use of hearing protection, padding, and subject monitoring. To minimize any discomfort, participants will be provided with disposable earplugs or headphones to wear during the procedure. These earplugs will protect their ears but still allow communication with the technician running the scan.

With constant monitoring by the study team under the supervision of Drs. Schrage and Carrel, we will record and identify trends and report any significant problems to the IRB. We will compile an electronic record of any adverse events associated with each subject and will review these events as they occur. We will also review cumulative events to identify trends as well as approaches to stop trends and approach the IRB with appropriate protocol modifications.

7.9.1. Initial screening

All subjects will be asked to complete an initial pre-screening, documenting physical activity, medical history, medications, and MRI safety (see section 4.3.1. Pre-Screening Subjects). The questions are designed to immediately eliminate those subjects meeting exclusion criteria. A detailed initial screening script and survey is included in the formal IRB proposal. Potential subjects who are ineligible, or who decline to participate, will have their screening information deleted or destroyed.

During the in-person screening, if the subject is ostensibly ineligible (e.g. weight<94.5 lbs), we will stop the screening process immediately. The reason for stoppage will be documented. This will not be considered a deviation to the protocol.

Other studies in the PI's lab investigate the same subject population and utilize similar screening procedures. A subject recruited from another study can have their screening information from that study used for the purposes of this study to minimize subject burden. Any procedures that were not included in the other studies' screening process that are required for this study will be conducted. Screening information will be valid for 180 days.

7.9.2. Subject Monitoring

Testing may include subject monitoring of heart rate, oxygen saturation, end tidal carbon dioxide, and blood pressure (if applicable; see section 4.3.4.m. Subject Monitoring). With continuous monitoring, we can readily observe any adverse events in real time and move to stop procedures immediately and return to baseline conditions. In case of serious cardiovascular event, a defibrillator is located nearby all testing areas, and CPR certified staff is present during procedures. In the case of emergency, 911 will be contacted. Further, in the event of a "code" for procedures at the WIMR, the WIMR is now part of the UWHC Code response area such that emergency team response time is excellent.

7.9.3. Blood Sampling

Blood sampling (not venipuncture or IV placement) will be performed only by research personnel that have had phlebotomy training. This training has encompassed the insertion, maintenance, and use of intravenous catheters. These personnel all have at least a bachelor's degree or higher health and/or physiology-related education in terms of academic preparation. We feel education and training of

personnel, and our positive track record over 10 years at UW performing similar invasive procedures, provide high confidence IV blood draws present a very low risk to subjects.

7.9.4. Protecting the Confidentiality of Participant Data

Risks to confidentiality will be minimized by keeping copies of the documents linking study assignment number and the participant's unique identifiers with the participant's informed consent in locked cabinets of the offices of Dr. Bill Schrage. Only subject numbers will be used for group assignment, data processing, and analyses. All data will be stored in locked cabinets in a secure lab, and electronic files are all stored on password-protected databases and computers.

Personal information such as name, gender, date of birth, and medication history will be stored in a locked file cabinet in a locked office in the PI's laboratory. Subject information will be coded to remove any personal identifiers during data analysis or research publications. Research oversight and regulatory groups may review study records.

Data will be stored as both hard copies and electronic files. Hard copy data collection forms will be locked in a file cabinet in the restricted-access laboratory area or in the Department of Neurology (cognitive testing files only). Electronic files that contain potential Protected Health Information (PHI) will be stored in REDCap, a secure web-based data management system, and/or a secure Box folder configured with the involvement of the UW-Madison HIPAA security officer. Electronic files will be HIPAA compliant and will use a subject ID instead of the subject's name. The raw MRI data will be stored on servers assigned to Dr. Wieben and managed by the Departments of Medical Physics and Radiology and/or on encrypted external hard drives.

Blood samples may be analyzed for insulin and inflammatory markers externally to the study team (e.g. UW Primate Research Center). Samples analyzed at the UW Health Clinical Labs will be placed in the medical record of the subjects per UW Health policy. Samples analyzed at research labs (e.g. UW Primate Research Center) will remain coded such that the outside blood analysis team will not be provided any identifiers or means by which they could connect the blood samples to any individuals that have participated in the study.

7.10. Stopping Guidelines

7.10.1. Study Stopping Guidelines

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension. Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable

The study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the applicable regulatory authorities.

7.10.2 Subject Stopping Guidelines

Each intervention (i.e. study visit) will be terminated based on the following specific guidelines:

- If the subject wishes to stop
- If the subject complains of serious pain or discomfort
- If the subject is admitted to hospital for psychiatric distress
- If the subject complains of (related to MRI):
    - Discomfort
    - Anxiety
    - Claustrophobia
- If baseline glucose is ≥126 mg/dL and is not the result of non-compliance with fasting requirement (see 4.1.2. Exclusion Criteria)
- Any SAE occurring during study procedures

8. STATISTICAL CONSIDERATIONS

Covariates for all analyses include sex, age, adiposity, aerobic fitness, and education unless noted otherwise.

The overarching statistical approach uses multiple regression to determine whether IR is inversely associated with outcome measures in Aims 1 and 2 and to assess mediation in Aim 3. Mean/summed outcomes (total cognition, total CBF or CP) will be predicted by HOMA-IR, for example. Not only is a composite measure calculated as a mean more reliable than the subdomain scores, but averaging multiple measures of a single construct yields a more powerful hypothesis test than using variables individually (40), under conditions we expect to hold here. This approach avoids inflation of Type I error rate as each hypothesis test examines the relationship among single variables or composites, not among multiple subdomain measures. If the test of the mean or composite score is significant, then there is no increased Type I error rate incurred in subsequent subdomain/regional tests (41).

8.1. MRI Data Processing

Standard and validated tools will be used for image processing. The research team is expert in analyzing brain imaging data and have published several studies focused on MRI-derived blood flow-based measures.

Maps representing gray matter probability and cerebral blood flow will be generated using SPM, FSL, or similar software. Scans will be processed using the most up to date and recommended processing scheme, including methods previously used by our research group and collaborators.

We expect to use standard viewing software (Advanced Workstation 4.3, GE Healthcare) for magnetic resonance angiography and phase contrast data, in addition to

1) MIMICS and EnSight, commercial software packages commonly used in 4D flow visualization and
2) MatLab-based software for quantitative analysis of 4D data sets

Quantitative data from two 4D multi venc flow acquisitions will be available. The data includes:

1) Quantitative blood flow values (peak and mean flow, peak and mean velocity, pulsatility and resistance index) of 4D PC
2) Quantitative morphological data (vessel diameter) of:
    a. Standard magnetic resonance angiography techniques
    b. 4D PC

8.2. Aim 1

Composite scores will be computed by calculating and averaging z-scores within each cognitive domain. Composite total cognitive scores will be predicted from HOMA-IR and the covariates. Pilot data yielded a partial correlation of –0.6 between HOMA-IR and the total cognitive score. A one-tail test of the population correlation coefficient with a Type I error rate of 0.05 requires a sample size of **N=13** to yield power of 0.80. If this composite score test is significant, similar analyses will be performed to examine the relationship between HOMA-IR and individual cognitive ability subdomain scores.

8.3. Aim 2

8.3.1. Aim 2 Data Expression

For conceptual clarity, pilot data are expressed as CBF or CP. For statistics, the key dependent variable in Aim 2 is cerebrovascular conductance (CVC), where CVC is CBF or CP normalized to mean arterial pressure (i.e. blood pressure), where

$$\text{CVC}=\text{CBF}/\text{mean arterial pressure}$$

CVC is critical, as:

1) it normalizes CBF/CP for differences in mean arterial pressure (up to 15 mmHg across IR spectrum)
2) it accounts for mean arterial pressure changes *with* OGTT
3) changes in CVC (ΔCVC) have a predictable relationship *independent* of baseline differences, where $+\Delta$CVC indicates dilation and $-\Delta$CVC indicates constriction (24, 53)

CVC_{MACRO} refers to macrocirculation (i.e. CBF) and CVC_{MICRO} to microcirculation (i.e. CP). While CVC_{MACRO} and CVC_{MICRO} are physiologically related, the two levels are conceptually independent. Testing both is crucial as preliminary data indicate resting CBF is normal, yet CP is lower as IR increases.

8.3.2. Aim 2A

Total CVC will be predicted from HOMA-IR and the covariates (intracranial volume is an additional covariate for CVC analyses). Resting CP data indicate reduced perfusion in several anterior brain regions. Pilot data yielded a correlation between HOMA-IR and resting CVC_{MICRO} of –0.324, such that a sample of size **N=55** will yield the power of 0.8 in a one-tailed test of the correlation, with Type I error rate of 0.05. In contrast, we will test, but do not expect, resting CVC_{MACRO} to be related to HOMA-IR, since resting CVC_{MACRO} is similar in low and high IR.

8.3.3. Aim 2B

The change in CVC (ΔCVC) will be predicted from HOMA-IR and the covariates, where

$$\Delta\text{CVC}=(\textit{peak}\ \text{CVC during OGTT})-(\textit{resting}\ \text{CVC})$$

Pilot data indicate the average correlation between HOMA-IR and ΔCVC_{MICRO} of -0.42, so a one-tailed test of the partial correlation will yield power of 0.80 with a sample size of **N=31**, with Type I error rate of 0.05. We plan to assess numerous regions of interest (ROIs) linked to executive functions and memory, including frontal, temporal, and parietal regions (e.g. middle, medial, and superior frontal gyri, anterior cingulate, hippocampus, middle and superior temporal gyri, posterior cingulate, precuneus), as well as ROIs *with less involvement* in memory or executive functions (e.g. precentral and postcentral gyri, occipital cortex). ΔCVC_{MICRO} is only predicted from HOMA-IR within an ROI, not between, to limit the number of comparisons and therefore limit the false discovery rate.

For ΔCVC_{MACRO}, up to 11 extra- and intra cranial arteries will be studied (basilar, 2 ACA, 2 MCA, 2 PCA, 2 vertebral, and 2 ICA; dependent on individual anatomy). Total CBF will be calculated, where

$$CBF_{TOTAL} = CBF_{BASILAR} + CBF_{ICA\text{-}Right} + CBF_{ICA\text{-}LEFT}$$

Pilot data indicate that the average correlation between HOMA-IR and MCA perfusion during OGTT is -0.796, so that a one-tailed test of the partial correlation will yield the power of 0.80 with a sample size N=6, with Type I error rate of 0.05. For all of Aim 2, if either total CVC test is significant (micro or macro), similar regression analyses will be performed to examine the mediating effects of ΔCVC between HOMA-IR and individual ROI (micro) or artery (macro) ΔCVC values.

8.4. Aim 3

Aim 3 tests the mediation effects of CVC on the relationship between HOMA-IR and the composite cognition scores assessed in Aim 1. Mediation will be assessed using the test of joint significance (23). The path between HOMA-IR and perfusion will have been examined in Aim 2. At rest (Aim 3A), mean CVC_{MICRO} and CVC_{MACRO} will be examined as the primary mediating variables. Pilot data indicate the correlation between resting CVC_{MICRO} and cognition is 0.37, so that a sample size of **N=41** would yield 0.8 power in a one-tailed test and Type I error rate of 0.05. During OGTT (Aim 3B), mean peak ΔCVC will be the primary mediator variable. Pilot data indicate that the partial correlation between post-OGTT ΔCVC_{MICRO} and the total cognitive score was 0.4, so that a directional test of this path requires a sample size of **N=34** to yield the power of 0.8 with Type I error rate of 0.05. A similar power is achieved for CVC_{MACRO}. If either test (rest or OGTT challenge) is significant, analyses will be performed to examine the mediating effects of CVC between HOMA-IR and individual cognitive ability domain scores.

8.5. Statistical Summary

With **N=55** for the overall grant, power in Aim 1 increases to near unity, power in Aim 2A is 0.8, power in Aim 2B increases to 0.95, power in Aim 3A increases to 0.9, and that in Aim 3B increases to 0.94, providing this study with sufficient to outstanding power to test the hypotheses of interest.

9. DATA AND RECORD KEEPING

9.1. Data Management

Delegated and appropriately trained study team members will be involved in data collection. All personnel who will be involved in data collection have completed appropriate Human Subjects Protection training or will before they engage in contact with subjects and subject data.

The Research Electronic Data Capture (REDCap) system is used to manage the data for this study. REDCap is a largely self-service, secure, web-based application for building and managing data collection forms. REDCap provides data management functionality by allowing the development of instruments and surveys to support data capture for research studies.

The REDCAP system used for this study is managed by ICTR. The ICTR REDCap support team will work with the investigator, statistician, and study team to ensure the relevant, applicable study data are collected and managed with restricted access using the software electronic data collection form instruments.

9.2. Assured Confidentiality

The raw MRI data will be stored on servers assigned to Dr. Wieben and managed by the Departments of Medical Physics and Radiology. The UW-Madison School of Education houses the Schrage Lab server and all electronic files will be coded to maintain HIPPA compliance. Subject information and study tracking information will also be stored in REDCap, which is a secure site used for data management.

Any forms with PHI will be stored in locked cabinets inside the PI's secure laboratory. All data collected electronically will be password protected. Only coded data will be used in data analysis. Any publications arising from this protocol will not include any personal identifying information or study code, thereby making the reported data completely de-identified.

9.3. Data Collection Methods

Data will be collected using REDCap, MRI, offline data collection software (e.g. LabChart), data analysis software, and data collection forms. These files will consist of electronic DICOM, .dat, LabChart, and Excel files, as well as hard copies of data collection forms for each subject.

All other study data referenced above will be collected using study visit checklists and data collection forms, most of which constitute both the Case Report Form (CRF) and the original source (i.e., the first and only place the data is manually written/recorded). These study specific forms will be developed and maintained with the assistance of the ICTR DMC for recording all necessary data for each subject. It is the PI's responsibility to ensure that these are properly, legibly, and fully completed and signed where appropriate. The CRFs are used to record study data and are an integral part of the study and subsequent reports. Therefore, the CRFs must be completed for each subject screened or enrolled according to the subject's source data on a per-visit basis. All study visit checklist and data collection forms will be retained in the subject research chart/file. All applicable data collected using the study visit checklists and data collection forms will be entered in to electronic Case Report Forms (eCRFs) within REDCap.

When the study is complete, the applicable CRFs must be signed by the investigator to attest that it is an accurate and complete record.

9.4. Retention of Study Records

In compliance with UW-Madison regulations, study records will be for a minimum retained for seven years following final project close-out.

10. DATA INTEGRITY

Integrity of data is vital for successful completion of the study. To that end, we will consider data to be valid if:

- all cognitive testing is performed
- three (3) of five (5) ASL scans are performed on the OGTT-MRI visit
- three (3) of seven (7) blood draws are performed on the OGTT-MRI visit
- maximal exercise testing meets a minimum of 2 of the following criteria:
    - plateau in oxygen uptake evidenced by <2 mL/kg/min increase in the last 60 seconds of the test
    - heart rate ≥90% of age-predicted maximal heart rate (i.e. 182-187 beats per minute) (45, 48)
    - respiratory exchange ratio (VCO_2:VO_2) ≥1.0 (45, 48)

These criteria are chosen based on the specific aims of the study (see section 3. Specific Aims and Preliminary Evidence) and for outcome measures to be appropriately assessed (see section 8. Statistical Considerations). Any procedures not performed will be documented with an explanation as to why it was not performed. Protocol deviations will only be filed if the aforementioned data are not collected or valid.

We will invite subjects to return to complete the specific testing that was not valid as stated previously if the reason for not obtaining that data was not due to reaching a stopping guideline (see section 7.10. Stopping Guidelines). The number of re-invitations to perform a specific procedure will be limited to one (1) to reduce repeated exposure to procedures. Such instances will likely be due to:

- technical difficulties which temporarily impede data collection
- delays and subsequent time constraints
- failure of attaining a valid maximal exercise testing response

Regarding the maximal exercise testing, on re-invitation, subjects will only need to undergo the maximal exercise test but not the other screening procedures performed previously.

Having this flexibility will allow the study team to attempt the data collection again on a subject that has experienced other procedures (i.e. may be farther along in the study) without needing to expose a new subject to these procedures. This will hopefully limit the number of subjects experiencing these procedures and assist the study team in attaining the target sample size.

We will continue enrollment until our target sample size (see section 4.1 Subject Population) is studied in full.

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12. SUPP**LEMENTAL INFORMATION**

University of Wisconsin
SCHOOL OF MEDICINE
AND PUBLIC HEALTH

Department of Pediatrics

February 20, 2019

William Schrage, PhD
Professor of Kinesiology
1149 Natatorium
University of Wisconsin
Madison WI 53706

Dear Bill:

As a Pediatric Endocrinologist active in clinical studies with children and adolescents, I have been asked to comment on the safety for: 1) fasting (4-8 hrs), with emphasis of blood glucose levels; and 2) VO2max testing in otherwise healthy adolescents (e.g. no cardiovascular disease).

In routine clinical settings including children with obesity, significant insulin resistance or overt Type 2 diabetes, we customarily ask children to fast for >8 hours for "routine fasting bloodwork". This duration is safe and well tolerated in children.

I have also been actively involved in exercise testing with children, including maximal VO_2 testing in children with obesity, significant insulin resistance, and/or overt Type 2 diabetes. Some UW protocols that I have been involved in include:

- 2002-090 (Carrel)
- 2011-0214 (Carrel)
- 2018-0715 (Watson).

Maximal VO_2 testing and other cardiopulmonary exercise testing is felt to be an important test in health assessment (VanBrussel et al. A systemic approach to interpreting cardiopulmonary exercise testing in pediatrics. Pediatr Exerc Sci 2019. Jan 28:1-10).

This type of testing is also done in children with more significant health concerns including:

- Type 1 diabetes (Singhvi A et al. Aerobic Fitness and glycemic variability in adolescents with Type 1 diabetes. Endocr Pract. 2014 Jun; 20(6): 566-70)
- Children and adolescents with traumatic brain injury (Cordingley DM et al. Graded aerobic treadmill testing in adolescent traumatic brain injury patients. Can J Neurol Sci. 2017, 44(6): 684-691)
- Children with pulmonary hypertension (Abumendi. Cardiol Young 2016).

Overall, the pediatric literature supports that graded aerobic exercise testing is a safe, well tolerated, and clinically useful tool for children. Please do not hesitate to contact me if there are additional questions.

Sincerely,

Aaron Carrel, MD
Professor of Pediatric Endocrinology, Diabetes and Fitness
Medical Director, UW Pediatric Fitness Clinic

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608/263-9558

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University of Wisconsin
Department of Orthopedics & Rehabilitation

March 7, 2019

Dear Members of the Institutional Review Board,

I am writing to express my support for the Institutional Review Board application of Dr. Bill Schrage entitled "Insulin Resistance, Cognitive Health, and Perfusion of the Adolescent Brain". Specifically, he has asked me to provide my input regarding the safety of maximal aerobic testing as part of this experimental protocol. As a pediatrician, sports medicine physician, and exercise physiologist, I am involved with the use of exercise in health promotion in children every day as well as exercise testing in research. I have conducted thousands of maximal exercise tests in children and adults with a wide range of health and fitness levels, including children formerly born preterm, children with insulin resistance, obesity, low cardiorespiratory fitness, cardiac dysfunction, and pulmonary disease. Not only does this testing modality offer information that is not attainable through other means, but I have never had a significant adverse event beyond the expected and transient symptoms associated with strenuous exercise such as muscle fatigue, shortness of breath, and feeling tired.

The literature also supports the safety of maximal exercise testing in children. Given the lack of a national registry, there is no current agreement on the incidence of sudden cardiac arrest in children and adolescents during exercise, although all studies in this age group suggest that the occurrence is very rare. The American College of Sports Medicine suggests an annual incidence of around 1 in 185,000 male and 1 in 1.5 million female young adult athletes. Although estimates are inconsistent, it is widely accepted that children and adolescents have an even lower incidence, making the likelihood of an adverse cardiac event during exercise extremely low. I am aware of no evidence that suggests that this incidence rate is influenced by differences in insulin resistance, body composition, or fitness level, as cardiac events during exercise appear to be almost exclusively due to congenital and hereditary cardiovascular abnormalities. The incidence of falls or other musculoskeletal injury during testing is unknown, but I have never witnessed or even heard of this happening in a laboratory setting. Any risk is even further reduced through the use of seated exercise on a cycle ergometer and vigilance of the nearby testing personnel, such that the likelihood of injury during testing is also extremely low.

In reality, if provided an unstructured play environment children will naturally seek out strenuous, if not maximal, exercise on their own. Studies of movement patterns of children regularly document that they prefer to exercise in bouts of strenuous exercise separated by periods of rest, and regularly exert themselves maximally. Based on this and the well-documented, wide-ranging health benefits of higher cardiorespiratory fitness, the American Academy of Pediatrics recommends at least 60 minutes of moderate to vigorous physical activity, where vigorous is defined as "activity that makes children's and adolescent's hearts beats much faster than normal and makes them breathe much harder than normal."

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