Neuroinflammation in Obesity

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SCHEMA

Title: Neuroinflammation in Obesity

Total N = 20 (10 non-obese and 10 obese), nondiabetic male and female adults

Screening
- Medical history
- Current psychiatric disorders as assessed by Structured Clinical Interview for DSM-IV[1]
- Current binge eating disorder as assessed by Binge Eating Scale[2]

Inclusion criteria:
- Non-obese (BMI = 18 -25 kg/m^2) or Obese (BMI ≥ 30 kg/m^2)
- Age 18-75 years
- Any race or ethnicity
- Male or Female
- Native English Speaker

Exclusion criteria:
- Current or past diabetes
- Psychotropic medications
- Previous or current neurologic illness including but not limited to stroke, seizure, generalized dystonia, parkinsonism
- History of or current drug abuse
- Current mental illness
- Binge eating disorder
- Non-native speaker of English
- MRI contraindications
- Pregnancy (confirmed with negative urine pregnancy test unless postmenopausal or surgically sterilized)
- Currently lactating
- Tobacco use within the past month
- >350 lb

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1.0 BACKGROUND AND RATIONALE

More than a third of individuals in the United States are obese[3]. Obesity has recently been associated with increased risk for cognitive impairment and dementia such as Alzheimer's disease (AD)[4-10]. In young and middle-aged people, higher BMI relates to diminished learning, memory, and executive function[11-14]. Cournot et al. found that higher BMI during middle age is associated with impaired delayed recall at baseline and at several years follow-up[11]. Long-term obesity (from age 25 through late midlife,mean age = 61 yrs) predicts lower Mini-Mental State Examination (MMSE) scores and impairments in memory and executive function in late midlife independent of age and education, according to the Whitehall II Cohort Study[15]. Although previous human studies have investigated structural and functional differences between obese and non-obese individuals, strong relationships between these differences and cognitive performance have not been found[16]. It is likely that a key specific component to obesity-related brain effects on cognition has yet to be studied.

Obesity is a disease characterized by chronic low-grade systemic inflammation[17]. We propose that **neuroinflammation** is a major mechanism by which young and mid-life overweight or obesity contributes to cognitive impairment. Reduced blood brain barrier integrity induced by chronic high fat diet is thought to be a major way in which pro-inflammatory elements cross over into the brain and induce neuroinflammation[18]. Indeed, recent studies in rodents show that diet-induced obesity induces neuroinflammation[19-23] accompanied by cognitive impairment[19,23]. However, only a few studies have addressed these questions in obese humans[22,24].

The current proposal investigates whether indicators of neuroinflammation are present in **obese humans** and whether these indicators are associated with cognitive performance. We will use MR-based DBSI, an extension of diffusion weighted imaging (DWI) developed at Washington University School of Medicine (WUSM) by Dr. Sheng-Kwei Song that differentiates between axon/myelin integrity, axonal density, and extra-fiber components including inflammation and edema in white matter[25]. In addition, we will use a complimentary MR-based multimodal approach that includes functional connectivity MRI (fcMRI) and quantitative gradient recalled echo (qGRE, GEPCI, SMART), which distinguishes neuronal, synaptic and glial components of the MR signal[26].

2.0 OBJECTIVES

The objective of this proposal is to use MRI imaging to identify indicators of neuroinflammation in obese individuals. Further, greater neuroinflammation should correlate with greater cognitive impairment in obese individuals.

Aim 1: To determine if obesity is associated with increased cellularity and glial levels in the brain using MR-based Diffusion Basis Spectrum Imaging (DBSI) and qGRE (GEPCI/SMART).
Aim 2: To determine if indicators of neuroinflammation, including cellularity and glial levels are associated with cognitive performance in obesity.

3.0 METHODS

3.1.1 Research Subjects:

We will study 10 non-obese and 10 obese adults, both male and female, who are non-diabetic, aged 18-75 years.

Inclusion criteria: Non-obese (18-25 kg/m^2) or obese (BMI ≥ 30 kg/m^2), male or female, any race or ethnicity, aged 18-75 years.

Exclusion criteria: Exclusion criteria include the following: history of diabetes, >350 lb (scanner weight limit), binge eating disorder, history of or current serious neurological illness, current mental illness, not a native speaker of English, history of drug abuse or currently taking psychotropic medication, MRI contraindications. Additionally, participants will be excluded if they are currently pregnant (positive urine pregnancy test) or breastfeeding. Finally, any participant with tobacco use in the past month will also be excluded.

Sample size justification: We received funding to study 20 individuals. This is a pilot and feasibility study.

3.1.2. Recruitment:

We will call or email previous participants from a previous study of obesity (IRB#201104109; PI: Hershey). Subjects will be screened on a private line in a private suite using the phone script. They will be informed of the nature of the experiment and, if interested, will be questioned about inclusion and exclusion criteria. Those subjects meeting these criteria, and expressing further interest after this screening will be scheduled for study visits.

We will also recruit through Volunteers for Health and make use of their services including Facebook, Centerwatch, and Twitter posts, and flyers (for email blasts and posting at various sites).

Upon arrival into the laboratory for the first session, if the investigators are satisfied that the subject meets all of the inclusion and exclusion criteria, and the subject agrees to participate, the Human Research Protection Office (HRPO) approved informed consent will be signed.

3.2. Experimental Design:

3.2.1. Facilities.

The MRIs will take place in a Siemens 3T scanner in the Center for Clinical Imaging Research (CCIR) at Barnes-Jewish Hospital or in the East Building at WUSM. Screening and cognitive testing will take place in a private testing room. Oral glucose tolerance tests (OGTTs) will take place at the Clinical Research Unit (CTRU) at WUSM.

3.2.2. Overview of Procedures.

Initially, potential subjects will be screened by a phone interview that assesses medical history. In addition, subjects will be screened for psychiatric disorders and Binge Eating Disorder using the SCID for DSM-IV[1]. If they remain eligible for the study, they will be emailed a link to a REDCap survey on binge eating habits (https://redcap.wustl.edu/redcap/surveys/?s=8WWXCWFLJR; adapted from the Binge Eating Scale[2]). If their score is below the threshold for binge eating, they will remain eligible for the study. An informed consent document will be emailed to eligible volunteers. Obtainment of informed consent will occur via email prior to the on-site study visit day. The OGTT, 90 min MRI scan and cognitive testing will occur during one on-site study visit day.

3.2.3. Imaging Methods:

3.2.3.2 MRI

MRI-based scans will be performed in the Siemens 3T Prisma in the Center for Clinical Imaging Research (CCIR) at Barnes-Jewish Hospital or in the East Building at WUSM. Scans will be performed over 1.5 hr.

High-resolution structural images will be acquired using a sagittal MP-RAGE 3D T1-weighted sequence (sagittal orientation, TR=2500 ms, TE=2.9 ms, TI=1070 ms, voxel res=1x1x1mm, frames=176, Flip=8, Time=6:09-8:42 min.) and T2-weighted anatomical images (sagittal orientation, TR=3200 ms, TE=564 ms, voxel res=1x1x1mm, frames=176, Flip=120, Time=4:42-6:51 min). These structural images will be used for between-subject registration and anatomic localization.

We will acquire a DWI sequence optimized for DBSI analyses with 2 echo planar sequences with a multiband factor of 3 and 102 volumes each, one with b-values ranging from 0-1500 and the other 0-3000 s/mm^2 (transverse orientation, TR=3500 ms, TE=83 ms, voxel res=2x2x2 mm, Time=6:14 min).

Three 6 min blood oxygenation level dependent (BOLD) functional MRI (fMRI) scans will be acquired for analyses of functional connectivity of networks at rest. Sequence parameters: 64 slices; transverse orientation; TR=1230 ms; TE=33 ms; FA=63 degrees; FOV=216 x 216; voxel resolution=2.4 x 2.4 x 2.4 mm.

Gradient Echo Plural Contrast Imaging (GEPCI)/SMART. Used to quantify tissue alterations that reflect integrity of cellular brain structure. (1) GEPCI: TR=50 ms, 10 echo times equally spaced from 4-40ms, voxel res=1x1x2mm, FA=30 deg, Time=6:05 min. (2) SMART: TR=20 ms, TE 1,2,3=3,7,11 ms, voxel res=1x1x2mm, Time=2:01 min x 5 variations of flip angle (5, 10, 20, 40, 60 deg). This novel technique, which includes the more advanced GEPCI-based sequences qGRE and SMART, should provide quantitative in vivo high-resolution 3D measurements of several brain-tissue-specific relaxation properties (GEPCI metrics) of the gradient recalled echo (GRE) MRI signal. Since GEPCI metrics depend on the molecular constituents present in the brain, the GEPCI metrics can serve as surrogate markers of tissue alterations that reflect the integrity of brain cellular structure and disease-related tissue damage. These techniques are based on a standard Siemens GRE-based sequence and do not require contrast.

3.2.4 Oral Glucose Tolerance Test (OGTT)

For the OGTT patients will be admitted to the CTRU in the a.m. after at least 8 hours of fasting. An IV will be put in place for blood draws. Patient will be asked to drink 75 grams of glucola after drawing time 0' sample of OGTT. Samples for blood glucose levels by glucose oxidase method and insulin levels and C- peptide by immulite will be measured at -5, 0, 10, 20, 30, 60 and 120 min. Insulin secretion and sensitivity will be calculated from the OGTT glucose, insulin and C-peptide levels using the homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI) and modeling of insulin secretion. Blood samples will be saved and sent to the Core Lab for Clinical Studies for measuring of peripheral immune function.

3.2.5 Hemoglobin A1c testing

Approximately 2 tsp of blood will be drawn from your arm to conduct blood tests that include blood glucose levels and hemoglobin Alc

3.2.6 Cognitive testing.

Participants will be assessed for cognitive function using well-validated computer-based tests available from the NIH Toolbox[27]. Episodic memory[28], executive function, and processing speed[29] test scores are expected to be lower in obese relative to non-obese individuals. The testing should take approximately 30 min.

3.2.7. Study Visit

3.2.7.1 Anticipated duration of entire research activity per patient

Participant involvement will last for approximately 6 hours over the span of 1-3 days including phone screening and electronic survey completion. If there is a technical problem with the MR data collection, participants may be asked to repeat the MR session. In this case, total participation would last up to 8 hours. Participants will be provided with lunch after the OGTT.

3.3. Statistical Analysis:

Aim 1: To determine if obesity is associated with increased cellularity and glial levels in the brain using MR-based Diffusion Basis Spectrum Imaging (DBSI) and qGRE (GEPCI/SMART). We will use analyses of covariance (ANCOVA) to compare MRI-based metrics between obese and non-obese controlling for age, sex, gender, and insulin resistance . **Aim 2**: **To determine if indicators of neuroinflammation, including cellularity and glial levels are associated with cognitive performance in obesity.** We will use Pearson's *r* correlational analyses to determine whether MRI metrics relate to impaired cognition in obesity.

4.0. DATA SAFETY MONITORING

These are not clinical trials as defined by NIH. The independent data and safety monitor is Dr. Ana Maria Arbelaez, M.D. She will monitor the study for unanticipated problems, life-threatening events or deaths.

The Data and Safety Monitoring Plan (DSMP) for this project includes monitoring any adverse events and reporting by Dr. Arbelaez and the PI for this protocol. Dr. Arbelaez will meet quarterly with the PI and other study personnel to discuss the study. Any serious adverse events or other adverse events are reported to the Washington University's Human Research Protection Office (HRPO) and to the Radioactive Drug Research Committee (RDRC).

Serious adverse events (SAEs) will be reported to HRPO within 7 calendar days using the eIRB Notification System. In the case of an SAE, the study is not resumed until authorized by HRPO. Stopping criteria for an individual participant, in addition to the participant's wish to

stop for any reason, include evidence of harmful side-effects of the research procedures. In the absence of adverse events, summary reports are provided annually to HRPO.

4.1. Human Subjects Education Certification

All key personnel involved in the design or conduct of research involving human subjects will receive the required education on the protection of human research participants prior to the performance of these studies.

4.2. Procedures for Maintaining Confidentiality and Data Security

All staff present will be HIPAA certified and essential to conduct the study. Any potential observers for educational purposes will be allowed only at the discretion of the subject. After consent, we will assure the subjects that no information regarding third parties will be asked or recorded. We will collect information as described in the protocol but if subjects decide that they no longer want to participate, we will not collect any more information. We will assure all subjects that their participation in this study will be held in the strictest of confidence.

Paper data will be stored in a locked file drawer in the East Building, which is a secured building. All data will be de-identified utilizing a code number. The material obtained from the research participants is specifically for research purposes and will only be shared among the study team members. Electronic data is stored in a private, firewall protected, password protected manner on a drive specified for research data. All research data will only be available to members of the research team.

Saliva obtained for genotyping purposes will not be saved. Blood obtained for OGTT and peripheral immune measures will not be saved after analyses are complete.

The following privacy protections will be enacted for all e-mail communications involving PHI:
1) a test e-mail will be sent to the participant to verify their identity (confirm correct recipient) and that this e-mail will be sent in a secure manner (i.e., [secure] in subject line);
2) the body of the e-mail will instruct the participant to send all information as a response to this thread and to not remove the "[secure]" from the subject line;
3) We will document in our research records the participant's agreement to provide information over e-mail.

4.3. Risks and Benefits

We see no psychosocial, social or legal risks beyond those of participating in non-therapeutic research *per se*. The physical risks are those associated with intravenous lines (bruising and very low risk of infection), discomfort from lying flat (including lying flat for 1.5 hour for the MRI), and possible risk of claustrophobia. All research carries some risk of loss of confidentiality.

4.3.1. Likely Risks:

There are no known physical risks to subjects receiving MRI scans who meet our inclusion and exclusion criteria.

4.3.2. Less Likely Risks:

Risks of i.v. placement, including pain, bleeding, bruising, and infection.

Participants may develop mild muscle aches and pains due to immobility and exposure to the acoustic noise of the MRI scanner. The development of muscle aches and pains is minimized by providing appropriate cushions at pressure points and beneath the knees as desired by the subject. Ear phones or earplugs will be used to dampen the sound of the MRI procedure. Participants may feel anxious when in the MR scanner. However, participants will be able to communicate with us throughout the scan and can tell us whenever they want the scan to be stopped or interrupted. Subjects may experience claustrophobia when placed in a MRI scanner. This can occur without a prior history or claustrophobia.

The risk of having IVs placed and blood samples taken include soreness or slight bruising at the site of the blood draw. Further risks of the OGTT are nausea and lightheadedness.

The risks of performing cognitive testing include frustration when tasks are difficult and fatigue.

4.3.3. Rare Risks:

Loss of confidentiality.

There is a potential risk of heart rhythm disturbances in individuals who have previous rhythm abnormalities or in patients with certain types of heart pacemakers. Because individuals with heart rhythm disturbances or pacemakers might be harmed by being in the MRI scanner, they should not participate in the study. There is a substantial risk to individuals who have metallic objects inside their bodies because the MRI scanner uses a high strength magnet. This risk involves the metallic objects being pulled by the magnetic field, which would cause physical harm. The following items may interfere with the MRI scans and some can potentially be hazardous: Cardiac pacemaker, aneurysm clip(s), implanted insulin/drug pump, neurostimulator (TENS unit), biostimulator/bone growth stimulator, hearing aid/cochlear implant, Gianturco coil (embolus coil), vascular clip(s), surgical clip or staple(s), heart valve prosthesis, Greenfield vena cava filter, middle ear implant, penile prosthesis, shrapnel or bullet, wire sutures, tattooed eye liner, any type of dental item held in place by a magnet, any other implanted item not mentioned, diaphragm/IUD, intraventricular shunt, wire mesh, artificial limb or joint, any orthopedic item (i.e., pins, rods, screws, nails, clips, plates, wire, etc.), dentures, dental braces, any type of removable dental items, or pregnancy. Participants complete an MR screening form to screen for these items and will not be consented if they meet any of the above rare risk factors. Thus, if participants have any of the above, they will be excluded from the study.

4.3.4. Benefits:

Direct benefits to the participating subjects are not anticipated. To the extent of societal benefit, it leads to a better understanding of the occurrence of neuroinflammation in obesity and whether it contributes to cognitive impairment in obesity. Given these potential benefits and our efforts to minimize risks, the risks of this study are reasonable in relation to the benefits.

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