Title: Endogenous opioid activity and affective state in insulin resistant women

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Title: Endogenous opioid activity and affective state in insulin resistant women

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1. Objective

The objective of this study is to examine the role of the endogenous μ-opioid system in mediating the relationship between metabolic dysfunction and depressive symptoms in reproductive aged women. This study is planned in anticipation of an NIH K01 mentored research scientist career development award, with funds likely to be awarded in the fall of 2012.

2. Specific Aims

As the frequency of metabolic disorders increases (including type II diabetes, the metabolic syndrome, and insulin resistance), associated co-morbidities are also becoming more prevalent. A relationship between metabolic dysfunction and mood disorders, including depression, is well-documented, and individuals with an underlying metabolic disorder represent a population with high risk of developing depression. While the mechanisms underlying the relationship between insulin metabolism and mood dysregulation remain largely unexplored, the endogenous opioid system, acting through μ-opioid receptors, has separately documented regulatory roles in both insulin metabolism and mood regulation, and our preliminary data suggests a role in mediating their relationship. We propose to evaluate central μ-opioid system function (in the amygdala and nucleus accumbens, motivation and affect-regulating regions) and mood/affective state in insulin-resistant (defined by low insulin sensitivity) women. We will evaluate women after placebo and metformin treatment in a randomized cross-over study design, with baseline comparison to a sample of healthy control women. We will approach this investigation using metabolic assessment of insulin and endocrine function, neuropsychological assessment of affective and mood state, fMRI, and PET imaging with a selective μ-opioid receptor radiotracer to quantify 1) μ-opioid receptor availability *in vivo* (binding potential, BP_{ND}) at baseline, and 2) endogenous opioid release in response to a standardized stress challenge known to induce the release of endogenous opioids. We hypothesize that metabolic and mood symptoms in women with non-diabetic metabolic dysfunction, reflected by insulin resistance, will be associated with reduced μ-opioid receptor-mediated neurotransmission and alterations in fMRI activation patterns during emotional processing.

Aim 1: establish the relationships between insulin resistance, affective state, and μ-opioid system function.

Aim 2: examine the effects of insulin regulation on μ-opioid system function and affective state after 16 weeks of metformin treatment in insulin-resistant women.

Aim 3: determine whether identifiable differences can be observed in emotional processing between insulin resistant women compared to normal controls using validated functional MRI tasks.

Aim 4: explore relationship between insulin metabolism and cognitive function using neuropsychological assessments and functional MRI tasks of visual and verbal cognitive domains.

The expected results of this study would show that the endogenous opioid system has a central role in mediating the relationship between metabolic function and emotional processes in neural circuits involved in motivated behavior and

mood and stress regulation. This work aims to increase our understanding of the factors that influence the development of depression, and to identify potential targets for optimal treatments, in a population at higher risk due to metabolic dysfunction. As the rate of metabolic dysfunction in the United States increases, a more complete understanding of the relationship between mood and metabolism has direct clinical translation to the treatment and prevention of mood disorders in a growing at-risk population.

3. Background

As many as a third of American adults are affected by insulin resistance[1], and over 70% of reproductive-age women meet the criteria for at least one component of the metabolic syndrome, a group of risk factors that include obesity, hyperglycemia, hypertension, elevated triglycerides, low HDL, and pro-inflammatory state[2]. The metabolic syndrome and insulin resistance in particular are associated with increased risk of significant medical complications, including obesity, type II diabetes mellitus, cardiovascular disease, stroke, and in women, altered reproductive and hormonal function[3-6]. The public health impact is exceedingly significant considering the rapidly rising prevalence of insulin resistance, the lack of routine screening, and the increasing number of insulin resistant individuals, many of whom are not aware of their metabolic status[7].
In addition to the medical complications associated with insulin resistance, metabolic dysfunction is also associated with mood disturbances[8-22]. Bidirectional predictive relationships have been described for mood and metabolic disorders, and the presence of metabolic dysfunction doubles the risk for mood disorder[9,12,17,18,21-27]. Importantly, regulation of insulin function with the thiazolidinedione class of insulin sensitizing drugs has been shown to alleviate depressive symptoms in insulin resistant and diabetic patients, suggesting that dysregulated insulin metabolism may contribute to negative mood symptoms in this population[28-30].

Insulin has more diverse functions in the brain than peripherally, where its primary action is to regulate glucose metabolism[31]. Central and peripheral insulin receptors are distinct in composition and binding kinetics, and high insulin concentrations do not cause central insulin receptors to down-regulate, as in the periphery[32,33,34]. Despite these distinctions, central insulin levels are influenced by peripheral concentrations through transport across the blood brain barrier, and there is evidence of increased transport under some metabolic conditions, including diabetes[35].
Functionally, neurons rely comparatively less on insulin for glucose uptake than peripheral cells[32,33], and central insulin primarily facilitates neuronal signaling, with neurotransmitter, metabolic, neurotrophic, neuromodulatory, as well as neuroendocrine roles[34,36,37]. Insulin interacts with several neurotransmitter systems, including monoamine and peptide neurotransmitters[36,38], and has been shown to regulate the cell-surface expression of excitatory and inhibitory receptors[39]. Insulin receptors are selectively localized throughout the brain, including in the amygdala and limbic-hypothalamic system[32,40]. Almost 90% of insulin receptors in the hypothalamus/pituitary co-localize with β-endorphin synthesizing cells[41].

The endogenous opioid system has been separately linked to both depression and insulin metabolism, and may represent a critical interface between metabolic dysfunction and mood disorders. Opioid transmission is implicated in the pathogenesis of depression[42-44], and the system is responsive to physically and emotionally stressful stimuli[45-51]. PET imaging studies have shown μ-opioid system responses to sadness and emotional stress within neural circuitry responsible for mood regulation, the so-called motivational circuitry, but also for neuroendocrine function, including the anterior cingulate, nucleus accumbens, ventral pallidum, thalamus, hypothalamus, insular cortex, and amygdala[49,50,52,53]. Heightened opioid response to stressors has been demonstrated in patients diagnosed with Major Depression and Borderline Personality Disorder, examples of mood and affective dysregulation respectively. In the latter group, both increases in baseline μ-opioid receptor availability and increased μ-opioid neurotransmission during an affective challenge were observed[54,54].

Peripheral interactions between insulin and circulating β-endorphin contribute to the regulation of metabolic homeostasis, but the relationship between central opioid and insulin signaling appears to be mediated through different mechanisms[55-57]. Peripheral β-endorphin concentrations are elevated in diabetic patients, and opioid antagonist administration has been shown to improve insulin function and metabolic homeostasis, particularly in women[57,58]. These findings have led to the hypothesis that elevated peripheral β-endorphin may worsen metabolic dysfunction via decreased insulin secretion[57]. While dysregulated insulin metabolism has also been linked to behavioral changes, including appetite, cognition, motivation, and mood[10,59-66], less is known about central opioid transmission in metabolic disorders. **Evidence suggests that in the brain, metabolic dysregulation may be associated with reductions in baseline opioid tone, especially in limbic regions associated with mood regulation, hedonic state, and reward**. This evidence

includes decreased opioid concentrations in the cerebrospinal fluid of diabetic rats[67] and increased central μ-opioid receptor binding and expression – both consistent with upregulatory receptor expression in response to chronic opioid reductions – in rats susceptible to diet-induced obesity[68,69]. These findings are consistent with our preliminary results of increased central resting μ-opioid receptor availability (binding potential, BP_{ND}) in women with insulin resistance, particularly in the nucleus accumbens and amygdala (regions with significant resting state opioid tone[70,71]). These are regions where μ-opioid receptor-mediated neurotransmission is implicated in mood regulation, as well as food reward and appetite for high fat and high sugar foods, and specifically in the attribution of hedonic value to those foods[50,59,60,72-74]. If the preliminary relationships between measures of insulin resistance and μ-opioid receptor function are replicated and enhanced in the proposed studies, they would represent an important advance in the understanding of the relationships between obesity, insulin resistance, and mood/affective state.

The relationships between insulin metabolism and mood and affective regulation may be more pronounced in women than men, as reproductive and hormone status can influence susceptibility to both mood and cognitive disturbances, as well as metabolic disorders, and each has higher prevalence in women than men[21,75-77]. Several population-based studies have shown that depression is a risk factor for the metabolic syndrome in women but not in men, and women with bipolar disorder are at increased risk of metabolic dysfunction, effects that are further enhanced by some of the medications used to treat theses illnesses[11,14,16,19]. Reproductive hormone status and circulating hormones further interface with the insulin and opioid systems in women: opioid receptor antagonists improve metabolic function in insulin resistant menopausal women, and in women with polycystic ovarian syndrome, additionally improve ovarian function[57,78-81]. This evidence suggests that the mechanisms mediating the relationship between mood and metabolic dysfunction may differ between the sexes, and this study will focus on defining these relationships in women.

***Innovation*:** We propose to investigate the association between central endogenous opioid neurotransmission, metabolic function, and emotional processing in healthy and insulin-resistant non-diabetic women. Our approach includes assessment of metabolic and endocrine function, neuropsychological assessment of emotion and mood state, fMRI during emotional processing, and PET imaging with a selective μ-opioid receptor radiotracer to visualize and quantify 1) μ-opioid receptor non-displaceable binding potential (BP_{ND}), a measure of available receptors, and 2) endogenous opioid release in response to a standardized stress challenge, measured by the reduction in BP_{ND} from baseline to stress state, in limbic emotional regulation regions. **The expected results of this investigation would demonstrate that μ-opioid neurotransmission has a role in mediating the relationship between metabolic function and emotional processes, and will provide tools to identify patients at risk of developing metabolic and mood comorbidities**. This research may lead to novel therapy to prevent the development of severe mood disorders or to adjuvant therapy for treatment of mood symptoms, and lessen the risk of metabolic comorbidities in patients being treated for mood disorders.

Appropriate treatment of insulin dysfunction with insulin regulating agents may have positive effects on mood and emotional processing, potentially via regulation of the endogenous opioid system. This is exemplified by evidence that the thiazolidinedione class of anti-diabetic drugs have positive effects on depressive symptoms and related cognitive complaints in diabetic and insulin-resistant patients[28,30,82]. Despite this documented ability of the thiazolidinediones to alleviate mood symptoms associated with metabolic dysfunction, we are not aware of similar studies of metformin. Metformin has a favorable side-effect profile for use in non-diabetic individuals, and lacks the appetite-inducing actions on leptin, an effect see with use of the thiazolidinediones[83,84]. Further, metformin will not induce hypoglycemia, even in non-diabetic individuals[85], and has been shown to increase plasma β-endorphin levels[86].

To our knowledge, the proposed studies would be the first to examine the effects of any anti-diabetic agent on mood/affective state in insulin resistant individuals without a diagnosis of diabetes or a depressive disorder, with comparison to placebo and metabolically normal controls. This would make our proposed studies the first to examine the relationship between metabolic dysfunction and mood disorders at the very earliest stages of each disorder. This will also be the first study to provide a mechanistic explanation for those relationships by examining central opioid system involvement in human metabolic dysregulation, and to investigate the endogenous opioid system as a potential mediator of the relationship between metabolic homeostasis and mood and affective regulation.

4. Study Team Expertise

Alison Berent-Spillson, Ph.D., Principal Investigator,: Dr. Berent-Spillson is a Research Investigator in the Psychiatry Department at the University of Michigan. Dr. Berent-Spillson will be responsible for directing the research project, with assistance from study co-investigators. She will conduct all aspects of the research project, including IRB applications, subject recruitment, screening, & scheduling, data collection & analysis, and research manuscript preparation and

submission. She will coordinate drug dispensing under the guidance of the University of Michigan Investigational Drug Service, with prescription and oversight by Dr. Yolanda Smith, M.D. Dr. Berent-Spillson has extensive training and experience in basic and clinical/translational neuroscience research, with an emphasis on endocrine and metabolic influences on brain function in women. She has over six years of experience performing neuroimaging research in women.

Jon-Kar Zubieta, M.D., Ph.D., Co-Investigator: Dr. Zubieta is Professor and Chair in the Department of Psychiatry at the University of Utah, and adjunct Professor of Psychiatry at the University of Michigan. . Dr. Zubieta has over 10 years of experience in the use of MR data and acquisition protocols, PET and SPECT for the quantification of metabolism, blood flow, and neuroreceptor sites in human subjects, including cholinergic, aminergic, and opioid markers. Dr. Zubieta will provide guidance on all aspects of PET assessment of the endogenous opioid system, including experimental design, data analysis, manuscript preparation, and dissemination of results.

Charles Burant, M.D., Ph.D., Co-Investigator: Dr. Burant is a Professor in the Department of Internal Medicine, Metabolism, Endocrinology, & Diabetes division, and Professor in the Department of Molecular and Integrative Physiology at the University of Michigan. Dr. Burant is a clinical endocrinologist, and is the Director of the Michigan Metabolomics and Obesity Center, Director of University of Michigan Nutrition Obesity Research Center, which provides training and infrastructure for basic, clinical and translational research in obesity and related metabolic diseases, and Director of the University of Michigan Postdoctoral Translational Scholars Program. Dr. Burant has research expertise in obesity and development of insulin resistance and related complications.

Yolanda R. Smith, M.S., M.D., Co-Investigator: Dr. Smith is a Professor in the Department of Obstetrics and Gynecology, and Research Professor in the Reproductive Sciences Program at the University of Michigan. Dr. Smith is the Associate Chair for Research for the Department of Obstetrics and Gynecology, the research director for the Women's Reproductive Health Research program, and Executive Committee member for the Reproductive Sciences program. Dr. Smith is a reproductive endocrinologist with expertise in the evaluation of gonadal steroid states in women and neuroendocrinology research, including the application of neuroimaging techniques to the study of endocrinological effects on brain function. Dr. Smith will oversee drug treatment (metformin) prescribing and monitoring.

Carol Persad, Ph.D., Co-Investigator: Dr. Persad is an Associate Professor in the Department of Psychiatry, and Clinical Director of the Neuropsychology Section, at the University of Michigan. Dr. Persad is an expert in the neuropsychological assessment of cognitive and emotional function, and application of neuropsychological assessment results to PET and fMRI neuroimaging research studies. Dr. Persad will provide guidance in the design and interpretation of neuropsychological emotional processing assessment.

Robert Koeppe, M.S., Ph.D., (Co-Investigator): Dr. Koeppe is a Professor in the Department of Radiology, and the Director of the PET Physics Section, Division of Nuclear Medicine. His research interests center around the quantitative aspects of positron emission tomography (PET). Specific research areas include the development and implementation of tracer kinetic models for new and existing positron labeled radiotracers, development of optimal techniques for estimation of physiological quantities, and development and implementation of automated image analysis routines for use with PET. Another specific area of research is the use of PET cerebral blood flow activation studies to examine various cognitive and neurological functions of the brain. Other research interests include the correlation and comparison of information obtained from PET studies to that obtained from corresponding anatomic imaging procedures such as magnetic resonance imaging.

Kirk Frey, M.D., Ph.D. (Co-Investigator): is a Professor in the Departments of Radiology Neurology and MBNI. He has over 20 years of experience in radioisotope research and is 102R Authorized User.

5. Methodology

a. Inclusion/Exclusion Criteria

Inclusion criteria: women aged 18-40, defined as metabolically healthy (n = 18) or insulin resistant (n = 18) based on insulin sensitivity (Si) values ("insulin resistance" defined as Si < 1.61×10^{-4}; healthy as Si > 1.89×10^{-4} ($min^{-1} \times \mu U^{-1} \times mL^{-1}$; calculated by minimal model assessment of glucose tolerance test, based on the median of the non-diabetic population[87,88]), and body mass index (BMI = weight (kg) / height^2 (m^2)) between 18 kg/m^2 and 35 kg/m^2. Women with mild or moderate depressive symptoms not meeting the criteria for Major Depressive Disorder will be included.

Exclusion criteria: men, left handed, acute medical illness, uncorrected thyroid disease, diabetes (fasting glucose ≥126 mg/dL), neurological disease, major depression, substance abuse, claustrophobia or MRI contraindications (pacemakers, pumps, or metallic agents or devices), severe calorie restriction, intense physical exercise ≥1 hour/day, smoking within 6 months, hormonal, insulin sensitizing, or centrally acting medications within 2 months, pregnancy within 6 months, lactation, cardiac or pulmonary insufficiency, liver or renal insufficiency (>2.5 x normal transaminases levels, plasma creatinine ≥1.4 mg/dL), history of lactic acidosis, BMI ≥35 kg/m^2, and opioid allergy.

b. Recruitment Plan and Study Design

i. Number of Subjects

We plan to enroll 36 women aged 18-40, defined as metabolically healthy (n = 18) or insulin resistant (n = 18). We expect to perform initial clinical screening assessments on 60 women to achieve our target enrollment. We will replace drop outs with new subjects to complete studies on 36 subjects.

ii. Method of Contact

Women will be recruited through news boards, websites, UMClinicalStudies Registry, the Reproductive Endocrinology Clinics, and from the Investigational Weight Management study, a study of phenotypic influences on weight loss and metabolic function in normal and obese individuals, conducted by Dr. Burant at the Michigan Nutrition and Obesity Research Center (MNORC) in the Michigan Metabolomics and Obesity Center (MMOC). Participants enrolled in this study may choose to participate in the proposed project, and have been evaluated for general health, mood, physical fitness, body composition, and metabolic, metabolomic, and hormonal phenotyping.

iii. Method of Consent

After an initial phone screen, a comprehensive written IRB approved consent form is mailed to the volunteer for her review. An appointment is made for further screening and review of study entry criteria. The investigators or research personnel will explain the nature of the study and answer the questions of the potential participant. If the subject qualifies and agrees to participate, she will sign a consent form.

iv. Method of Interaction/Procedure/Intervention

Insulin resistant women will be scheduled for up to 8 visits, and controls for 3 visits. Controls will not receive metformin and placebo treatments, and will not have repeat studies. Visits will be scheduled during the follicular phase of the menstrual cycle. Prior to all imaging procedures (PET and MRI), a urine pregnancy test will be administered. A description and summary of each visit is provided in the table below, followed by further experimental details:

Phone screening to determine initial eligibility to participate; schedule clinical screening & send review materials	
Details for each participant visit:	
1	*Visit 1 will occur at the Michigan Clinical Research Unit (MCRU), and will last approximately 4-5 hours.* Obtain informed consent and medical, neurological, and psychiatric histories. Perform structured diagnostic interview to rule out current clinical Major Depression or other psychiatric diagnoses[89,90]. Clinical assessment: collect initial clinical measures and confirm eligibility for continued participation. Assay samples collected after overnight fast. *Clinical measures*: thyroid stimulating hormone (TSH), comprehensive chemistry panel (CCP), blood counts, and a glucose tolerance test. *Samples will also be obtained for hormonal and metabolic measures, pending eligibility*. Additional samples will be collected and stored for future investigation of potential biomarkers for comorbid metabolic and mood dysfunction. Anthropomorphic measurements: measure height, weight, and sizes for BMI and waist-to-hip ratio and circumference calculations. Eligibility for continued enrollment in study will be determined from these assessments. If enrolled, glucose tolerance results will also be used to calculate baseline glucose, insulin, and insulin resistance measures. Assessment of affective and mood state: state measures include the Positive and Negative Affect Schedule (PANAS)[91], Profile of Mood States (POMS)[92], State portion of the Spielberg Anxiety Inventory (STAI)[93], and Snaith-Hamilton Pleasure Scale measure of anhedonia[94]. Trait measures include the Trait portion of the STAI, Beck Depression Index (BDI)[95], Patient Health Questionnaire-9 (PHQ-9)[96] and depression and anxiety subscales of the NEO Personality Inventory (NEO-PI-R)[97]. Cognitive assessment: measures of cognitive function may include California Verbal learning Test – Second Edition (CVLT-II; verbal memory), Controlled Oral Word Association (COWA; verbal fluency), and Brief Visual Memory Test – Revised (BVMT-R; visual memory).
2	*Visit 2 will occur at the University of Michigan Functional MRI Laboratory, and will last approximately 2 hours.* Anatomical and functional MRI: to collect brain structural and anatomical T1-weighted images for PET image data processing and normalization, and to assess responses to words or images with an affective valence in comparison to neutral words or images. Scanning session may also include tasks of verbal and visual cognitive function. Prior to scanning, PANAS, POMS, and STAI will be administered to assess mood and affective state at time of scanning.
3	*Visit 3 will occur at the Nuclear Medicine Imaging Facilities in the University Hospital, and will last approximately 4 hours.*

	PET scan: to quantify endogenous μ-opioid receptors using the specific μ-opioid receptor radiotracer [^{11}C]carfentanil. The PET scan will consist of a baseline scan to assess baseline μ-opioid BP_{ND}, and a stress challenge scan, consisting of a 40 minute pain-free segment and a 20 minute pain stress challenge segment, to induce endogenous opioid release and receptor occupancy by the endogenous ligand(s). The challenge scan will allow assessment of presynaptic endogenous opioid function at μ-opioid receptors. Affective state will be assessed with PANAS at the beginning and end of each scanning segment. For cycling women, PET scans will be scheduled during the follicular phase of menstrual cycle.. Pain profile assessment: We will utilize a validated[49,98] physical and emotional stressor, moderate levels of sustained deep muscular pain. An infusion profile will be developed in each subject that will maintain pain at a target 40 VAS units in a 0-100 scale, making it possible to draw comparisons between subjects and conditions for the same experiential stimulus.
End of study for metabolically healthy participants	
Begin 1st treatment arm: insulin resistant participants randomized to 16 weeks metformin or placebo treatment. Dosing: 500 mg metformin tablets or identically compounded placebo tablets orally after meals. Dosing schedule: *Week 1*: 1 tablet after breakfast (500 mg daily dose) *Week 2*: 1 tablet after breakfast and 1 tablet after lunch (1000 mg daily dose) *Week 3*: 1 tablet after breakfast, after lunch, and after dinner (1500 mg daily dose) Dr. Smith will prescribe medication and supervise monitoring during treatment. If subjects have minor side effects, the dose escalation may be slower. Women will be maintained in the study if they can at least reach a dose of 1000 mg/day. Treatment will be monitored by phone calls to the subjects at 2 week intervals throughout each treatment arm. *Treatment will be coordinated through the Investigational Dug Services (IDS) in the University of Michigan Department of Pharmacy. IDS will manage treatment-related details including randomization and blinding, drug and placebo acquisition and compounding, medication storage and dispensing, treatment compliance, accountability records, and adherence to regulatory requirements.*	
4	Clinical (except histories, TSH, CCP, and blood count), anthropomorphic, and affect and mood assessments, similar to Visit 1.
5	PET scan, similar to Visit 3, but consisting of a single baseline PET scan without the stress challenge scan.
6	MRI scan, similar to Visit 2, may be performed depending on funding availability.
Four week medication-free wash-out period between treatment arms	
Begin 2nd treatment arm: participants begin the 2nd arm of 16 weeks metformin or placebo treatment. Dosing and medication details identical to those described for 1st treatment arm; *treatment will be coordinated through the IDS*	
7	Clinical, anthropomorphic, and affect and mood assessments, similar to Visits 1 and 4.
8	PET scan, similar to Visits 5.
9	MRI scan, similar to Visit 2, may be performed depending on funding availability.

Clinical measures: clinical assessments of metabolic and endocrine environment to determine overall status at each of the time points and to assess response to treatment. Metabolic measures include a 2 hour 75g oral glucose tolerance test, with samples collected at -30, -15, 0, 30, 60, 90, and 120 minutes. Anthropomorphic measures will also be collected to control for changes in affective state that may be attributed to change in body composition. Sample collection and clinical assays will be performed at the MCRU and associated core laboratories.

PET imaging of μ-opioid receptors: PET scanning, acquisition, and reconstruction will be performed as previously described[48-50]. Subjects will be positioned in the PET scanner gantry, and an intravenous (antecubital) line will be placed. Positioning will use the orbito-meatal line as reference line, and markings will be placed on the subject's scalp using the gantry laser lights. A light forehead restraint will be used to eliminate intrascan movement. The intravenous line is used for the administration of the radiotracer [^{11}C]carfentanil. The length of the visit is approximately 4 hours for the baseline double PET scan, and approximately 2 hours for post-treatment arm PET scans. Four 90 minute PET scans,one with 20 minute stress challenge to induce opioid release during one scan, will be performed to quantify μ-opioid system activity, using tracer doses of the specific μ-opioid radiotracer [^{11}C]carfentanil. [^{11}C]carfentanil (CFN), a selective μ-receptor radioligand is synthesized at high specific activity by the reaction of ^{11}C methyliodide and non-methyl precursor, with minor modifications to improve its synthetic yield. 15 mCi (maximum) are administered to each subject in each of the three scans proposed with a maximum mass injected 0.05 μg/kg per study. This ensures that the compound is administered in true tracer quantities, therefore eliminating significant receptor occupancy and physiological effects. Fifty percent of [^{11}C]CFN dose will be administered as a bolus, and the remainder as a continuous infusion, using a computer-controlled automated pump. During the stress challenge scan, the initial 40 minute pain-free segment will include infusion of isotonic saline into the masseter muscle. The 20 minute pain challenge segment will achieve a steady state of sustained pain through infusion of 5% hypertonic saline into the masseter muscle[99], which has been shown to activate endogenous opioid neurotransmission in a number of previous studies[49,50,52,53]. After an initial 0.15 ml bolus, pain is maintained at subject-defined ~ 40/100 intensity rating. PET image data will be transformed on a voxel-by-voxel

basis into tracer transport (K_1 ratio) and receptor-related BP_{ND} parametric maps, co-registered to each other. PET images will be co-registered to the T1-weighted MR structural, anatomical image, affine transformed and warped to standard MNI stereotactic space using mutual information algorithms[100]. Inclusion of the pain challenge in a separate scan will allow comparison of pain and baseline conditions at the same timepoint in relation to tracer administration. The reduction in BP_{ND} from baseline to stress challenge provides a measure of endogenous opioid release in response to the stressor (related to the occupancy and endogenous ligand-receptor interactions)[48,49]. The stress challenge scan will occur after the baseline scan to avoid carry-over effects from active to baseline states, as previously described[50,101].

MRI: structural, anatomical, and functional images will be acquired using a 3T whole-body magnetic resonance imaging scanner with a head coil. Images will be acquired axially with a three-dimensional, volumetric acquisition. Functional images will be collected during a presentation of words or images with an affective valence (positive or negative) or affectively neutral. Images may also be collected during validated tasks of verbal processing (women must decide whether presented words are written in upper or lower case letters, or if they have abstract or concrete word meaning) and visual memory (women must determine which of two test patterns matches the stimulus pattern).

Drug treatment: the University of Michigan Investigational Drug Service (IDS) will manage and coordinate metformin and placebo treatment. Metformin is the most widely perscribed drug for type 2 diabetes, and while generally not recognized as a true insulin sensitizing agent, it has been shown to improve insulin resistance and reduce fasting and postprandial insulin concentration without hypoglycemia. Dr. Smith will provide medical oversight and prescribe treatment medication. After baseline assessments, IDS will randomize participants to the order in which they receive metformin or placebo using a randomly permuted block design. Investigators and participants will be blinded to treatment order randomizations. Placebo pills will look identical to metformin pills but contain no active ingredients, and will be taken on an identical schedule as the active drug.

v. Survey Instruments

1. What are they?

1) Positive and Negative Affective Scale (PANAS)
2) Profile of Mood States (POMS)
3) Spielberg State and Trait Anxiety Inventory (STAI)
4) Snaith-Hamilton Pleasure Scale
5) Beck Depression Index (BDI)
6) NEO Personality Inventory
7) Patient Health Questionnaire -9 (PHQ-9)

Affective state will be assessed using PANAS (positive and negative affect score), POMS (total mood disturbance score), and the State portion of the STAI. Trait measures include the Trait portion of the STAI and the NEO Personality Inventory (Neuroticism domain and depression and anxiety subscales). The Beck Depression Index and PHQ-9 will assess the severity of depressive symptoms even in the absence of frank Major Depression (exclusion criterion).

2. Under what conditions will they be administered

Self-administered. Questionnaires will be completed online using Research Electronic Data Capture (REDCap) or paper and pencil as necessary.

3. How many questions and how long will each instrument take?

1) Positive and Negative Affective Scale (PANAS) – 5 minutes
2) Profile of Mood States (POMS) – 5 minutes
3) Spielberg State and Trait Anxiety Inventory (STAI) – 10 minutes
4) Snaith-Hamilton Pleasure Scale – 5 minutes
5) Beck Depression Index (BDI) – 10 minutes
6) NEO Personality Inventory –30-45minutes
7) Patient Health Questionnaire -9 (PHQ-9) – 5 minutes

i. Compensation

1. Are subjects being paid? How much? (justification for offering these payments)

2. If they do not complete the entire study, will they receive partial payment and how is that determined?
3. Will they be compensated for parking?

Subjects will be paid for each study visit, to compensate them for their time spent. Payments will be made after each study visit, whether or not the entire study is completed. All subjects will be compensated $50 for labs visits, $150 for the PET visit, and $50 for the MRI visit, for $250 total study compensation. Insulin resistant subjects will be further compensated $50 at each of 2 post-treatment arm labs visits, $150 at each of 2 post-treatment arm PET visits, and $50 at each of the 2 possible post-treatment arm fMRI visits, for $750 total study compensation. Free parking will be provided at all visits, in visitor parking lots or by valet.

b. Subject Withdrawal

i. Under what conditions will a subject be withdrawn prior to completion

Potential subjects will be informed that becoming a participant in this study is entirely by their own free choice. Subjects may drop out of the project by their own free will, after having agreed to become a subject. They may refuse to enroll in this project, or drop out of this project without any penalty. By doing so, participants will not lose any benefits that they are entitled to. Subjects will be asked to discontinue their participation if they no longer meet criteria, did not comply or the investigator no longer thought it was safe for the subject to be involved with the study.

ii. If a subject withdraws prior to completion, what is the plan for the use of their data

Subjects who have withdrawn prior to the completion of the study and have provided usable data in terms of the study's criteria will be included in the final analysis. Incomplete data sets which will not add any meaningful material for the research goals will not become part of the analysis.

8) Identifiable Data

a. Indicate how subjects are identified in research records

Subjects will be indirectly identified in research records: personal identification (e.g. name, phone number, SSN, medical record number) will be replaced with coded identifiers in study databases. Coding will be kept secured and stored separately from study data.

- Explain the necessity for collection or maintaining data linked to subjects' identities.

Contact information for each will be collected and maintained in order to inform the subject of any necessary or pertinent information about the study prior to or following their participation. Also contact information such as mailing addresses are needed in order to mail the subject a packet of information about the study prior to their involvement. Name or medical record numbers may be used to confirm medical histories with subject permission.

- How long will the identifiers be retained?

Identifiers will be retained for 2 years following the final publications associated with the study, or when at least 2 years have elapsed since the formal discontinuation of the study.

9) Data Retention and/or Data Destruction Plan

i. How long will you keep subject data?
ii. If you plan to destroy the data, how will you destroy it?

Data collected will be retained for study recordkeeping purposes and for future research use. Data will be destroyed after 15 years by deleting the electronic files and shredding the paper documents.

10) <u>Risks & Benefits</u>

Potential risks to the subjects participating in the study include those associated with confidentiality, clinical and laboratory assessments, MRI and PET imaging, and side effects of metformin and placebo treatments. In the case of adverse events or physical injury resulting from participation in these studies, subjects are provided with a 24 hour emergency access number to obtain immediate medical care.

Breach of confidentiality: to minimize the breach of confidentiality risk, subjects will not be identified in any reports on this study. All personal identification data will be stored securely and separately from the study databases, and replaced with coded identifiers. Records will be kept confidential to the extent provided by Federal, State, and local law. Nevertheless, subjects will be informed that the sponsor and the Institutional Review Board for the use of human subjects in research may inspect the records of this investigation.

Clinical and lab assessments: blood drawing is mildly painful from the insertion of the needle, and can cause bruising and very rarely, fainting, blood clots, or an infection at the needle stick site. Blood drawing will be done by experienced personnel in the MCRU, and all care will be taken to minimize any discomfort.

MRI imaging: MRI imaging will occur in the facilities of the University of Michigan, and will include limited anatomic MRI acquisition. Anatomic MRI images will be used for analysis, providing anatomical information for structure identification and non-linear warping of PET images to standardized stereotactic coordinates. MRI is used routinely in clinical practice, and it does not involve radiation exposure. Possible risks are minor, and include:

1. The MRI magnet may interfere with the functioning of any electric or mechanical devises implanted with the subject. Prior to inclusion in the study, the presence of potential MRI risks, such as pacemakers, surgical clips, metallic surgical devices, or medication patches will be excluded by medical and surgical history using a standard review form.
2. Functional MRI studies have the potential to cause "peripheral nerve stimulation" (PNS). PNS is a light touching sensation on the skin surface, lasting only for a few seconds. It may cause mild discomfort, but is not harmful. The MRI machine is operated within FDA guidelines so the potential for inducing PNS is low.
3. There is a minor risk of discomfort or anxiety from being in the confided space of the MRI scanner. We will provide pads and blankets to make the subject as comfortable as possible. In addition, the MRI scanner makes loud vibrating noises. The subject will wear foam earplugs to reduce the noises made the scanner. During scanning, communication with the subjects will be maintained through the use of a microphone and speaker in the scanner, and the subject will be provided with a "panic" squeeze ball that triggers an audible alarm in the control room. Subjects will be instructed to squeeze the ball if they become frightened or unable to tolerate the scanning procedures.
4. There is the potential that the magnetic resonance image may reveal an abnormality. Such a finding might require additional studies and treatment. Any significant abnormality will be reported to the subject and her primary care physician with written consent.

PET imaging: PET imaging involves low-level radiation exposure, which amounts to 2.4 mSv per scan at 15 mCi of carfentanil administration, 7.2 mSv for 3 scans, and 9.6 mSv for 4 scans. The radiation doses arising from these studies are within the range of commonly utilized diagnostic studies. There is no documented biologic effect of radiation exposure at these levels. The risks are minimized by administering the smallest radiotracer doses acceptable for acquiring the necessary PET data, and by strict adherence to accepted protocols for dispensing and administration of radiotracers. We will also require the subjects to drink several glasses of water (or non-caffeinated beverages), and encourage voiding after completion of the scanning session to reduce further radiation exposure to kidney, bowel, and bladder. To minimize psychological discomfort, the subjects will be familiarized with the PET scanner environment, and scanning will be initiated only after they are comfortable with and fully understand the experimental sequence.

Pain stressor challenge: the stressor challenge that will be used for this protocol consists of moderate levels of sustained deep muscular pain, achieved by infusing medication-grade 5% hypertonic saline into the relaxed masseter (jaw) muscle. Infusion is controlled by a computerized pump, through which participants maintain a pain intensity level of 40/100 via an electronic visual analog scale. Participants may experience physical or emotional discomfort during the challenge, however we will ensure that they are aware of the level of pain they will experience and that they understand the scale ratings of pain and internal states and can use them in a reliable manner.

Metformin/placebo side effects: metformin is in the biguanidine class of anti-diabetic drugs, and regulates hepatic gluconeogenesis, with secondary action enhancing glucose uptake at peripheral tissues through increased transport across the cell membrane. While metformin is not recognized as a true insulin sensitizing agent, it has been shown to improve insulin resistance and reduce fasting and postprandial insulin concentration, and has benefits over the thiazolidinedione class of drugs in terms of weight gain, improvements in lipid, vascular, and inflammaory profiles, and does not induce hypoglycemia even in non-diabetic individuals. Side effects of metformin include abnormal liver

changes, lactic acidosis and the more common side effects of gastric distress, nausea and vomiting. All subjects will be monitored for possible side effects such as nausea, vomiting, diarrhea, anorexia, and abdominal discomfort. These side effects tend to be mild, dose-related and improve with continued use of metformin. GI side effects will be minimized using a low dose of medication titrated slowly over a three-week period. Hypoglycemia is rare and tends to occur in the setting of alcohol abuse or prolonged starvation. Malabsorption of vitamin B_{12} and folate occurs with long-term treatment, although it usually does not lead to anemia. Non-hormonal contraception will be recommended.

B) Discuss they the risks to the subjects are reasonable in relation to the anticipated benefits

Experimental work using imaging is essential for the advancement of knowledge in the field. This lays a rationale foundation for understanding the relationship between metabolic and mood disorders, the role of the endogenous opioid system in mediating that relationship, and the potential benefits of insulin-regulating or μ-opioid system-targeting treatments. The knowledge to be gained through these studies will also provide important objective information on the biochemistry and function of neurochemical systems in the human not obtainable by other means, and central to the study of metabolic dysregulation influences on affect/mood and novel avenues for intervention. Since risks to participants are of quite low order, we believe the gain of knowledge about the mood regulation effects of insulin metabolism outweigh these risks.

C) What are the benefits?

i. To the individual

There is no direct benefit to the participants in this project, except that the volunteers will have the benefit of being screened for insulin resistance. Patients will be informed that the medical significance of these studies is presently unknown, and the results will not influence their subsequent medical care. The risks to subjects participating in the project are minimal, as outlined above.

ii. To society

Experimental work using imaging is essential for the advancement of knowledge in the field. This lays a rationale foundation for understanding the relationship between metabolic and mood disorders, the role of the endogenous opioid system in mediating that relationship, and the potential benefits of insulin-regulating or μ-opioid system-targeting treatments. The knowledge to be gained through these studies will also provide important objective information on the biochemistry and function of neurochemical systems in the human not obtainable by other means, and central to the study of metabolic dysregulation influences on affect/mood and novel avenues for intervention. Since risks to participants are of quite low order, we believe the gain of knowledge about the mood regulation effects of insulin metabolism outweigh these risks.

11) Data & Safety Monitoring

a. Will there be a board your study will report adverse events and other problems to?

As this study does not constitute significant risk to patients through participation, a data monitoring board should not be required. All personnel involved with patients or their data will have formal training in human subjects protection and confidentiality. Subjects will not be identified in any reports or presentations generated by the study. All data will be kept confidential to the extent provided by local, state, and federal law. No identifiers will be kept with the data. New identification numbers will be assigned to each woman as she is recruited. This identification number will be used for the neuropsychological testing data, fMRI and PET scans. Images will be coded with this number. Those performing image analysis will be blind to subject information. Data collected during the performance of the studies is stored in mirrored RAID arrays which are both firewall-protected and isolated from access outside the immediate local network. The RAID arrays are backed up daily with digital tape. Failure of up to two drives in the RAID array does not result in the loss of data. RAID array drives are hot-swappable, and back-up drives are available in the event that individual drives may fail. Electronic data files in Excel or Word containing information about the study participants will be stored on password-protected drives, and separate from subject identifiers.

b. Adverse Events

i. Method of Identifying, Recording, Monitoring and Reporting Adverse Events

Adverse events are unlikely. If an adverse event should occur during PET scans, fMRI, blood draws, OGGT or neuropsychological testing, it would be expected to occur while the participant is in the presence of one of the investigators or their staff and the P.I. would be notified. Prior to the studies, volunteers will undergo physical exams to rule out the presence of undetected medical or psychological/psychiatric problems. During the performance of the studies, the volunteers will be monitored at all times by research personnel associated with the project (research assistant/associate, radiology technologists) or the investigators themselves. All the volunteers will have direct access to the phone numbers and pagers of the study coordinator and the responsible physicians, as well as a 24-hour contact number (emergency room services). These numbers are additionally included in the consent form provided to the volunteers. They will be informed of all the possible adverse events that could be encountered during the studies which may include, in very rare cases, allergic reactions to the radiopharmaceuticals, fatigue, thromboembolism, phlebitis and limb pain due to indwelling catheters, headaches or nausea. They will be encouraged to contact the investigators if they notice any symptoms of side effects. The investigators have ample prior experience in the utilization of the study protocols. All adverse events are immediately communicated to the IRB and the committee for human use of radioisotopes (RDRC/SHUR).

If the subject is having side effects of the metformin treatment they will have a contact number to reach the P.I. The P.I. will be reviewing all adverse events and they will be reported to the IRBMED committee and Health Science IRB regardless of severity or study relatedness, in compliance with the IRBMED Standard AE Guidelines. IRB Medical School definitions of severities (mild, moderate, severe), attribution, and expectedness will be used.

12) Statistical Design

PET imaging analysis: PET image data of 1) μ-opioid receptor BP_{ND} and 2) opioid release in response to stress challenge, calculated as the difference in BP_{ND} between baseline and challenge conditions, will be analyzed using Statistical Parametric Mapping software (SPM8, Wellcome Department of Cognitive Neurology, London, UK) with 1) volume-of-interest (VOI) analyses and 2) whole-brain statistical parametric mapping. VOIs will be defined using Montreal Neurological Institute (MNI) coordinates in affect/mood regulating regions (amygdala, nucleus accumbens, and rostral anterior cingulate). BP_{ND} values for these regions will be extracted for outlier assessment and further analyses in SPSS (SPSS Inc, Chicago, IL) or SAS (SAS Institute Inc, Cary, NC). In a separate set of analyses, whole-brain statistical parametric maps will be created for comparisons which do not assume selective involvement of specific regions, but will only include regions with specific μ-opioid receptor binding.

A global model based on the general linear model (GLM) will also be used to compare relationships betweem metabolic measures and mood/affective state, and to determine potential interactions and control for variables (e.g., age, BMI, estradiol and testosterone plasma levels) that could potentially confound the relationship between insulin resistance and mood state or opioid system activity.

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