United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1033877

Project Title

Evaluating the impact of increasing dietary intake of vitamin B12 on ischemic stroke outcomes

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2025
Grant No. 2025-67018-44947	**Cumulative Award Amt.** $300,000.00
Proposal No. 2024-10290	**Multistate No.** (N/A)
Project Start Date Aug 15, 2025	**Project End Date** Aug 14, 2027
Program Code [A1344] Diet, Nutrition and the Prevention of Chronic Disease	

Project Director

Jadavji, N.

Recipient Organization

BOARD OF TRUSTEES OF SOUTHERN ILLINOIS UNIVERSITY

900 S NORMAL AVE

Carbondale, ILLINOIS 629014302

Performing Department

Biomedical Sciences

Non Technical Summary

Ischemic stroke occurs when there is a lack of blood flow to the brain as a result of a blockage in a blood vessel. There is a significant cost related to this disease. For example, stroke-related costs in the US between 2019 and 2020 were estimated to be $56.2 billion. There are several factors that increase risk of developing a stroke, some include age and nutrition. The risk of stroke increases each year after the age of 55. Reduced levels of vitamin B12 increased risk of developing stroke and lead to worse outcomes post-stroke. Most older adults in the US are vitamin B12 deficient due to changes in metabolism associated with aging. Therefore, older adults in the US that are vitamin B12 deficient are at a higher risk for developing stroke and have worse outcomes if they do indeed have a stroke. This is a significant problem.Our funded work will investigate whether increasing dietary intake of vitamin B12 after stroke improves outcomes. In our research, we will use both female and male mice, and all animals will be aged to model what is seen in the human clinical stroke population. The proposed work will generate a foundational set of data. We

will provide insights on multiple levels of function, including what is happening at the genetic level all the way to behavioral changes with increased levels of dietary vitamin B12 intake after stroke. A major source of vitamin B12 are animal products, the potential of this project is to increase intake of these animal products generated by the U.S. agriculture and food systems.Therapies for stroke patients are limited and the only approved drug that is used currently for individual affected by stroke is tissue plasminogen activator (tPA), an anti- thrombolytic agent which is only effective when administered within 4.5 hours after the onset of a stroke. Patients treated with tPA are often left with significant functional impairments. It is therefore imperative to understand potential mechanisms to promote repair, by investigating potential therapies that could rewire the brain and promote recovery following stroke.

Animal Health Component

Animal Health Component: 10%

Research Effort Categories

Basic	100%
Applied	0%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
724	3320	1010	100%

Knowledge Area

[724] Healthy Lifestyle

Subject Of Investigation

[3320] Meat, beef cattle

Field Of Science

[1010] Nutrition and metabolism

Keywords

mice, vitamin B12, female and male, ischemic stroke, stroke outcome

Goals / Objectives

Clinical research has demonstrated that reduced levels of vitamin B12 increase risk of ischemic stroke and lead to worse outcomes post-stroke . Our previous research has demonstrated that reduced intake of dietary vitamin B12 result in worse stroke outcome in preclinical models through increased apoptosis and changes in mitochondrial metabolism. The goal of the proposed project is to understand the impact of increasing dietary levels of vitamin B12 on ischemic stroke outcome in a preclinical model. We plan to accomplish this goal through 2 objectives:The first is to determine whether increased dietary intake of vitamin B12 improves stroke outcome. Briefly, aged female and male mice (~17 months) will be acclimatized for a 1 week and then be put on a control diet for a period of 4 weeks.

United States Department of Agriculture
Project Initiation

Ischemic stroke will be induced in all animals using photothrombosis. Prior to and after inducing ischemic stroke in vivo imaging using laser speckle imaging will be conducted. Additionally, after ischemic stroke a subset of animals will be placed on a vitamin B12 supplemented diet, other animals will remain on a control diet. Four weeks after ischemic stroke motor function in mice will be measured using behavioral tests. After the completion of testing, animals will be euthanized, and tissues will be collected for further analysis.The second is to measure whether an increased dietary intake of vitamin B12 can improve stroke outcome and vitamin B12 dietary deficiency pre-stroke. Briefly, aged female and male mice (~17 months) will be put on a vitamin B12 deficient diet for a period of 4 weeks. After which animals will undergo an ischemic stroke using the photothrombosis model, a subset of animals will be placed on a vitamin B12 supplemented diet, and another group will be maintained on a vitamin B12 deficient diet. In vivo imaging to measure blood flow and behavioral testing will be conducted after which animals euthanized and tissue will be collected.The proposed research will provide vital information on whether increasing dietary intake of vitamin B12 can help improve stroke outcomes. The data generated will be important for the development of health policy and how early lifestyle choices could have prophylactic consequences on health outcome.

Project Methods

EffortsChanging Vitamin B12 Dietary IntakesOur research will investigate the impact of reduced and supplemented dietary intake of vitamin B12 in experimental animals. The reduced vitamin B12 diet will have reduced levels of vitamin B12, and the control diet will be sufficient to meet all dietary requirements. A supplemented vitamin B12 diet will be used to complete both aims 1 and 2These diets were formulated according to the Recommended Dietary Allowance (RDA) for rodents set by the American Institute of Nutrition. We and others have used this approach previously to ensure vitamin B12 levels are depleted in the test groups prior to conception. As indicated in previous published work by our team and others, all diets also contain 1% succinylsulfathiazole to prevent vitamin synthesis by intestinal flora. Dr. Jadavji (PI) has extensive experience using Envigo's products in previous animal studies. All experimental animals will have access to food and water ad libitum throughout the duration of the study.PhotothrombosisDr. Jadavji (PI) has developed and established a working protocol to induce ischemic damage to the mouse brain using the photothrombosis (PT) model in her laboratory and published using the model. The model relies upon an intraperitoneal injection of a photoactive dye (e.g., Rose Bengal), after which the intact skull is irradiated using a light beam at 532 nm, resulting in sensorimotor cortical damage. The damage is a result of singlet oxygen and superoxide formation, which results in endothelial injury, platelet activation, and aggregation. This reproducible and reliable method has been standardized over the past decade in mice. The major advantage of this model of ischemic stroke is that it results in highly reproducible damage within a localized region of the brain and there is also minimal surgical intervention involved. This stroke model shows brain characteristics reminiscent of the clinical circumstance and is being adopted by several laboratories around the world.Laser Speckle imaging systemUsing laser speckle imaging, we can non-invasively measure cerebral blood flow and peripheral blood flow, including the left ventricle and limbs. To reduce the noise from the heart we will use laser speckle contrast imaging. Measurements will take place at 7 days prior to induction of ischemic stroke, 7 and 30 days after, we will evaluate cerebral and peripheral blood flow. Behavioral testingThe PT model we will be using will target the sensorimotor cortex, so we will measure motor function in mice only. We previously demonstrated motor impairment in the animals after damage. Four weeks after ischemic stroke we will measure motor function using the ladder beam walking, accelerating rotarod, and forepaw placement tasks set-up in the laboratory. The 4-week post-stroke time point has previously been used in our studies as it provides enough time for stroke to develop and dietary manipulation to take effect. After the completion of behavioral testing and in vivo imaging, we will euthanize animals and collect tissue.Brain Tissue Measurements Lesion VolumeBrain tissue was sectioned using a cryostat and sections were slide mounted on microscope slides in serial order. One series of brain tissue was stained with cresyl violet (Sigma). Lesion volume was quantified using ImageJ (NIH) software. The lesion area was traced, and the area was multiplied by the section thickness and number of sections.Central Immune System ActivationInflammation in the central and peripheral nervous system is a main hallmark of ischemic stroke. We will measure microglial activation by counting the number of cells when Iba1 is co-localized with CD68 using the open-source software package NIH Image J.Global DNA methylationOne-carbon metabolism generates methyl groups, some of which are used for methylation through S-adenosylmethionine. Once

brain tissue is collected from both aims, global methylation measurements will be completed by Azenta Life Science and the data analysis will be completed in Dr. Jadavji's (PI) laboratory.One-carbon metabolite measurements in blood samplesBlood tissue will be collected measured to confirm decreased or increased dietary intake of vitamin B12 using chromatography tandem mass spectrometry (LC-MS/MS). These measurements will be done in collaboration with Dr. Teodoro Bottiglieri (Consultant) at Baylor University. Dr. Bottiglieri will be measuring levels of total homocysteine, methionine, and other 1C metabolites.Plans to communicate results; We plan to write up the results from this proposed research for publication in peer review journals, such as Nutrients and Neurobiology of Disease. We will also publish our work using open access option, so that other scientists and the public access can readily access our findings. Additionally, Dr. Jadavji (PI) and graduate student (Hassanat Etti-Balogun) will attend American Society for Nutrition and American Heart Association International Stroke meeting in year 2 of the grant to share the data generated from the proposed study with peers. Additionally, Dr. Jadavji (PI) will organize a public talk through the Office of Student Engagement at SIUC and share a lay person blog post on her laboratory website. These activities combined will ensure that the results generated from this proposed research are shared with scientific peers, stakeholders, and the public.EvaluationData collection:Expected results: We anticipate that increasing dietary intake of vitamin B12 will provide some protection against ischemic damage in female and male mice. This will include better ischemic outcome and less changes in vasculature, such as reduced blood flow within the same region. We have previously demonstrated that dietary supplementation of 5-methyltetrahydrofolate, vitamin B12, folic acid, choline, ribofalvin results in less motor impairments and reduced damage volume. In supplemented brain tissue samples, we expect to see less damage and apoptosis within the ischemic tissue. In blood tissue we expect to see increased levels of 1C metabolites. These studies will provide insight into the mechanisms on how increasing dietary intake of vitamin B12 impacts ischemic stroke outcome.How data will be analyzed or interpreted; Laser Speckle imaging, behavioral, and lesion volume, immunofluorescence data analysis will be conducted independently by two individuals that are blinded to experimental groups. If there is a discrepancy with data analysis, a third individual will perform the analysis. Statistical analysis will be conducted using SPSS and GraphPad Prism 8 Software. We will use mixed design ANOVA (factors: sex (Male or Female), and diet (factors: Control, Vitamin B12 deficient or supplemented diets) for vasculature and histological measurements, as well as motor function assessments. We will employ several measures to ensure that our methods and data analysis are rigorous and reproducible as summarized. Dr. Jadavji (PI) will mentor students involved in the research project on reproducibility techniques through her work with Reproducibility for Everyone (R4E). Additionally, the methods proposed in this study have been established in our laboratories.For our outreach event, we will provide feedback forms to TEDXSIUC attendees. These forms will ask questions about the effectiveness and understandability of the presentation, as well as other topics that can be covered within the area of nutrition and ischemic stroke.