**SPAN STANDARD OPERATING PROCEDURE**

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| **#1** | **INFORMATION** |

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| **Procedure Title** | | | | **Obesity Induced Hyperglycemic Model** |
| **Originators** | | | | **SPAN Coordinating Center** |
| **Creation/Revision Date** | | | | **3/18/21** |
| |  |  | | --- | --- | | **SOP: 52**  **Version No: 1.0**  **Effective Date: TBD** | **Supersedes**  **Document:**  **Effective Date:** | | | | | |
| **#2** | **POLICY** | | | |
| SPAN seeks to reduce variation among study sites. All sites will use the same procedures to induce comorbidities in research models. | | | | |
| **#3** | **SCOPE** | | | |
| This procedure applies to all study sites. | | | | |
| **#4** | | **ROLES AND RESPONSIBILITIES** | | |
| Coordinating Center: draft all SOPs  Study Site Principal Investigator: read and distribute all SOPs to relevant study team members and assure compliance. | | | | |
| **#5** | **APPLICABLE REGULATIONS AND GUIDELINES** | | | |
| <https://olaw.nih.gov/sites/default/files/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf> | | | | |
| **#6** | **REFERENCES TO OTHER APPLICABLE SOPS** | | | |
| SPAN SOP 2 Animal Acquisition | | | | |
| **#7** | **ATTACHMENTS AND REFERENCES** | | | |
| Togashi, Yu. Shirakawa J, Okuyama T. Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. Nature Scientific Reports. 2016 May 6; 25464 doi: [10.1038/srep25465](https://dx.doi.org/10.1038%2Fsrep25465) PMID: [27151424](https://www.ncbi.nlm.nih.gov/pubmed/27151424); PMCID: PMC4858715  Liu Z, Patil IY, Jiang T, Sancheti H, Walsh JP, Stiles BL, Yin F, Cadenas E. High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. PLoS One. 2015 May 29;10(5):e0128274. doi: 10.1371/journal.pone.0128274. PMID: 26023930; PMCID: PMC4449222.  Deji N, Kume S, Araki S, Soumura M, Sugimoto T, Isshiki K, Chin-Kanasaki M, Sakaguchi M, Koya D, Haneda M, Kashiwagi A, Uzu T. Structural and functional changes in the kidneys of high-fat diet-induced obese mice. Am J Physiol Renal Physiol. 2009 Jan;296(1):F118-26. doi: 10.1152/ajprenal.00110.2008. Epub 2008 Oct 29. PMID: 18971213.  Griffin LE, Essenmacher L, Racine KC, Iglesias-Carres L, Tessem JS, Smith SM, Neilson AP. Diet-induced obesity in genetically diverse collaborative cross mouse founder strains reveals diverse phenotype response and amelioration by quercetin treatment in 129S1/SvImJ, PWK/EiJ, CAST/PhJ, and WSB/EiJ mice. J Nutr Biochem. 2021 Jan;87:108521. doi: 10.1016/j.jnutbio.2020.108521. Epub 2020 Oct 8. PMID: 33039581. | | | | |
| **#8** | **TERMS AND ABBREVIATIONS** | | | |
| SOP: standard operating procedure  NINDS: National Institutes for Neurological Disorders and Stroke  SPAN: Stroke Preclinical Assessment Network | | | | |
| **#9** | **TRAINING REQUIREMENTS** | | | |
| **General Training:**  Site animal handling training   |  |  | | --- | --- | | **Location Where Records Maintained:** | Site | | | | | |
| **#10** | | | **SPECIFIC PROCEDURES** | |
| |  |  | | --- | --- | | **Glucose Testing** | **Notes** | | 1. Measure a morning blood glucose 24 hours before surgery using one of the recommended glucometers on the first 10 animals (5M/5F). | Notes: Recommended glucometer  Glutest neo alfa®  Freestyle Freedom Lite®  ONETOUCH Ultra Vue® | | 1. Swab the animal’s tail with alcohol. Place Vasoline on the tail surface before making a small nick. A bubble of blood will appear. Apply blood to the glucose reading strip. |  | | **Description** | **Notes** | | 1. On Surgery day animals will be **15-17 weeks** of age. | **See Experimental Protocol** for Stage specific details  See **SPAN SOP 6** Mouse Middle Cerebral Artery Occlusion | | 1. Induction with inhaled anesthetic should proceed more cautiously than usual. | Note: Beware prolonged and un-predictable fat-stored isoflurane during recovery. |  |  |  | | --- | --- | | **Acquisition** | **Notes** | | 1. Sites must plan anticipated date of surgery and Order animals from the closest vendor site with timepoints outlined in Steps 2-3. | **See SPAN SOP 2 Animal Acquisition** | | 1. Use strain/species specified in Experimental Protocol | **See Experimental protocol** for Stage specific details for vendor site/strain/species. | | 1. Animals will be fed (**Teklad TD.06414**) beginning around 4 weeks of age no later than 6 weeks of age. Diet must be fed for at least **12 weeks** and no more than 13 weeks prior to surgery date. Diet will continue through survival period. |  | | **Model Specific Guidelines** | **Notes** | | 1. Each site’s animal facility should provide guidance on any special considerations (eg. bunker, petri dish, etc.) |  | | 1. Fresh diet should be replaced weekly at minimum. Before use food should be stored at 4°C |  | | 1. Animals should be housed in groups. Group density might need to be adjusted as animals get larger. | **Note:** CC suggests organizing cages according to week of surgery | | 1. Animals may need help grooming as the food accumulates on their paws. This may also include more frequent cage changes. |  | | 1. Obese Hyperglycemic animals should be gently handled to reduce stress. |  |  |  |  | | --- | --- | | **Post-Operative Care** | **Notes** | | 1. Administer subcutaneous injections of 0.9% sodium chloride or LRS (1 ml into two sites) twice a day for 7 days |  | | 1. Remove penile plugs with 70% ethanol- soaked cotton wipes. This helps to avoid any distress associated with bladder emptying. | Note: Penile plugs are very common in obese mice post MCAo. | | 1. Provide animals with a petri dish of the high fat-diet on the floor of the cage. |  | | 1. Record Body weight for the first 2 days in REDCap database. | Note: Sites should be aware of prolonged and un-predictable fat-stored isoflurane during recovery. |  |  |  | | --- | --- | | **Animal Handling Guidelines** | **Notes** | | 1. Change the cage before surgery day and 3-4 days post MCAo. This will minimize stress due to cage change. |  | | 1. Limit the surgery duration <10 minutes. This will limit over-exposure to anesthesia. | Note: This implies awakening animal during occlusion and briefly de-anesthetizing for de-occlusion | | 1. Place the animal in the recovery cage on heating pads. Arrange the heating pad in the cage so that the heating pad is on one half of the cage. This will allow the animal to move to the cold side of the cage if overheated. |  | | 1. Perform Neuro Deficit Score for the first 2 days after MCAo to minimize overhandling of the animal. | Note: They look fragile and sick for almost a week. Most of the mortality is observed within first 4-7 days post-surgery | | 1. Place shacks or little house in the cages so that the animal feels secure | Note: Sites to consult with onsite vet regarding enrichment. | | **Euthanasia Criterion** | **Notes** | | Euthanize Animal in Case of:  ☐ Body Score: BCS-2 or lower  ☐ Severe deficit such that animal cannot eat, drink or groom.  Complete the **End of Study form** in the REDCap database. | Note: weight cannot be solely used as indicator of decline in obese animals. They can lose a significant amount of weight before attaining BCS-2. Consult with your onsite vet for more guidance. | | | | | |

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| **#11** | **REVIEWED AND APPROVED BY** |
| *Patrick Lyden, Principal Investigator*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *Jessica Lamb, SPAN Manager*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)*  *\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_*  *(Printed Name/Title) (Signature)* | |