

## **Improving Post-Operative Outcomes in Aged and Diabetic Obese Mice**

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Due to their small size, high metabolic rate, and large surface to volume ratio, mice are a challenge to work with surgically and peri-operatively. Working with mice that are more susceptible to anesthetic agents, aged, or obese (e.g., diabetic mice), provides even more challenges. In two separate studies, we found simple that supportive care measures during and after surgery improved post-operative outcomes.

### **Introduction**

The vast majority of literature on rodent anesthesia and analgesia, and what little has been published on peri- and post-operative care,<sup>7,11</sup> addresses young and healthy animals. In reality, an increasing number of studies rely on rodents that are neither young nor healthy. These models are more challenging to work with and warrant extra care to avoid loss due to potentially avoidable complications.<sup>6,9</sup> Consistent with the 3Rs, decreasing mortality would reduce the number of animals required to achieve study significance. Publishing modifications that reduce adverse events, as encouraged by the ARRIVE Guidelines<sup>4</sup>, allows IACUCs, veterinary and husbandry staff, and researchers to provide improved animal welfare, and fulfills another 3R, refinement.

The two studies explored here include a bone healing study involving aged mice and a skin wound healing study involving diabetic obese mice. Few studies have completed major survival

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This is the author's manuscript of the article published in final edited form as:

Keely L. Szilagyi, Michael A. Zieger, Jiliang Li, and Melissa A. Kacena. Improving Postoperative Outcomes in Aged and Diabetic Obese Mice. *Laboratory Animal Science Professional (LAS Pro)*. Sept 2018; p. 61-63.

surgery in mice that are 2+ years of age (old) and reported complications including survival rates. In one study, at 10 days post-operative a 30% mortality rate was documented for 18-month-old male mice undergoing fracture surgery as compared to a 10% mortality rate for 6-month-old male mice.<sup>5</sup> Mortality rates for diabetic mice in wound healing studies were reported to be as high as 75% within 10 days post-wounding when mice were subjected to repeated anesthesia for wound analyses.<sup>1</sup> Here we present several simple supportive care measures that improved survival post-operatively including: providing additional fluids, housing mice under static conditions, placing cages on low heat circulating water blankets, providing mice with wet floor feed, switching anesthesia method, and/or modifying analgesic dosing.

## **Materials and Methods**

Mice were housed in microisolator cages, corncob bedding, and received enrichment of either a tissue paper and/or Enviropak. Mouse housing rooms were maintained at 22°C±2°C with 30-70% relative humidity on a 12:12hr light:dark cycle. All mice were part of IACUC-approved studies and were housed in an AAALAC accredited institution.

Forty-two male 24-26-month-old C57BL/6JN mice underwent a segmental bone healing surgery. C57BL/6 is a common background strain for genetically modified mice and using this strain provides a baseline in preparation for future studies assessing genetic impact on bone healing. Male mice were used as their femur is larger than females, and the larger bone marrow cavity allows for better stabilization with the needle and better technique by the surgeons on the small bone (personal observation, MAK). Mice were anesthetized with isoflurane and 0.5ml buprenorphine HCl (0.05mg/kg, diluted in 0.9% NaCl USP) was administered SC pre-operatively. Mice were maintained on a circulating water blanket (T/Pump Classic, Gaymar,

38°C). The surgical site was prepared aseptically, and the fracture and repair surgery performed as previously described.<sup>2,8,10</sup> In brief, the mouse femur was exposed through a lateral incision of the skin of the thigh and the muscle was bluntly separated to expose the full length of the bone. An osteotomy producing a 2-mm-long segmental defect was made at the femoral mid-diaphysis. A 2mm synthetic, biocompatible, biodegradable scaffold was implanted to bridge the gap, and was then secured in place with an intramedullary pin (27G needle). A type I collagen membrane was placed around the femoral diaphysis and fixed into place with absorbable suture. Muscle tissue and skin were then closed with absorbable suture. Additional supportive care measures: An additional 0.5ml of warmed saline (0.9% NaCl USP) was administered SC post-operatively. 0.5ml buprenorphine HCl (0.05mg/kg, diluted in warm 0.9% NaCl USP) was administered SC every 12 hours for 48hrs post-operatively (increased volume from 0.1ml to 0.5ml). Mice were housed in static microisolator cages, half-on circulating water blankets, with wet feed (2018SX soaked in water from facility water bottles) provided on the floor of the cage daily, for 5 days post-operatively.

A preliminary wound healing study was performed using 15 (9-12-week-old, male) diabetic obese (BKS.Cg-*Dock7<sup>m</sup>* *+/+* *Lepr<sup>db</sup>/J*) mice. Diabetic mice are frequently used to study chronic wound healing. The mice were anesthetized with isoflurane and 0.9 mg/kg (original protocol) or 0.6-0.7 mg/kg (modified treatment) buprenorphine SR-LAB was administered SC pre-operatively. Mice were maintained on a circulating water blanket. The surgical site was prepared aseptically and the skin wound surgery performed as described by Wang et al.<sup>12</sup> Briefly, two circular full-thickness cutaneous wounds were created about the midline of the back using an 8-mm biopsy punch and a donut-shaped silicone splint (14-mm outer diameter, 10-mm internal diameter; Grace Bio-Labs) was affixed to the skin surrounding each wound using tissue adhesive

(Vetbond, 3M) and 6 interrupted sutures (4-0 silk, Ethicon). Wounds were covered with a single sheet of transparent dressing (Tegaderm, 3M) and with an elastic wrap. Post-operatively, the original protocol group were placed on the ventilated caging racks and received no further supportive care, except 2 mice were placed half-on circulating water blankets 3 days after surgery (Table 1). The modified treatment group, post-operatively, received room temperature 1.0ml saline (0.9% NaCl USP) SC and were housed in static microisolator cages, 1 per cage, half-on circulating water blankets for the duration of the experiment (Figure 1, Table 1). Mice underwent subsequent isoflurane anesthetic events for the study (Table 1) and received 1.0ml saline (0.9%NaCl USP) and wet feed on the floor of the cage after each event.

## **Results**

For the bone healing study, 83% (88% if not including 2 mice that were euthanized due to age related neoplasia; Table 2) of the mice survived to the study endpoint 28 days post-surgery. The majority of the mice that were lost (n = 5, not counting the 2 mice that were euthanized due to age related neoplasia) were found dead within 2 days post-surgery (n = 3, without notable symptoms pre-death). Of the remaining 2 losses, 1 mouse died during/after anesthesia at the 14 day study related radiograph and 1 mouse was found dead 20 days post-surgery (without notable symptoms pre-death). In the immediate 3 day post-operative period, there was 92.5% survival with the peri- and post-operative care provided. This improved survival rate is consistent with our survival rate in young male mice ~95% (MAK personal observation), the survival rate reported by others in 6-month-old male mice (~90 %), and is better than the survival rate observed by others when operating on 18-month-old male mice (~70% survival).<sup>5</sup>

For the wound healing study, 33% of the mice survived in the original treatment group and 100% mice survived in the modified treatment group to the respective study endpoint, 8 or 14 days post-surgery (Table 3). The mice that were lost in the original treatment group died within 3 days post-surgery (n = 4) and exhibited signs of lethargy pre-death. There was a 100% survival with the modified peri- and post-operative care provided.

## **Conclusion**

In providing extra supportive care for the diabetic obese mice peri- and post-operatively, we were able to see substantially improved post-operative survival rates (33% to 100%). In the aged mice, there is no original treatment group for comparison, but there was a 92.5% survival rate in the immediate 3-day post-surgery period. Supportive care measures included: providing warm or room temperature saline injections, removing the cages from ventilated racks to create static cages (likely reducing chilling due to air flow in the cage)<sup>3</sup>, and placing the cages partially on low heat circulating water blankets. An additional modification of lowering the dose of buprenorphine SR-LAB (to accommodate for the lean body mass) also may have contributed to improving the post-operative survival rate in the diabetic obese mice. Likewise, providing additional fluids through saline injection or wet feed, may have also contributed to improved survival rates. These simple modifications may improve post-operative outcomes and animal welfare and at the same time reduce the number of mice required for sensitive models.

***Acknowledgments:** This work was supported in part by an Indiana University Collaborative Research Grant (MAK, JL), NIH-NIA R01 AG046246 (MAK), and by a grant from NextFlex (no. 16088525, subaward no. 4104-76414 to MZ). This material is also the result of work supported*

*with resources and the use of facilities at the Richard L. Roudebush VA Medical Center, Indianapolis, IN: VA Merit #BX003751 (MAK).*

***Disclaimer:*** *The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.*

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**Tables & Figures:**

**Table 1.** Skin wound healing study timeline by cohort.

Cohort	Post-Surgery Group	Surgery Date	n	Post-surgery Treatment		Subsequent Anesthetic Events*	Death/Euthanasia
				Saline (ml)	Circulating Warm Water Blanket	Date(s)	Date(s)
A-1	Original	2/23/18	4	0	None	None	2/25, 2/26/18
A-2	Original	2/23/18	2	0	Starting 2/26, continuous	3/2/18	3/9/18
B-1	Modified	3/5/18	3	1	Continuous	8x/12 days	3/19/18
B-2	Modified	3/5/18	2	1	Continuous	3/12/18	3/19/18
B-3	Modified	3/22/18	3	1	Continuous	5x/7 days	3/30/18
B-4	Modified	3/22/18	1	1	Continuous	3/26/18	3/30/18

\* 1ml saline administered SC after each anesthetic event.  
n = number of mice in each cohort.

**Table 2.** Post-operative survival outcomes, bone healing study.

1 Day	2 Days	3 Days	14 Days	20 Days	28 Days
38/40	37/40	37/40	36/40	35/40	35/40

Numerator = surviving mice, denominator = total mice in cohort. Days = days post-surgery, day 0 = day of surgery. 2 mice of the total 42 have been omitted from the total cohort number, due to missing data for date of euthanasia, these mice were euthanized due to age related neoplasia. The day 14 death coincided with an anesthetic event for a study related radiograph.

**Table 3.** Post-operative survival outcomes, skin wound healing study.

Cohort	1 Day	2 Days	3 Days	8 Days	14 Days
A-1	4/4	2/4	0/4	0/4	0/4
A-2	2/2	2/2	2/2	2/2	2/2
B-1,2	5/5	5/5	5/5	5/5	5/5
B-3,4	4/4	4/4	4/4	4/4	NA

Numerator = surviving mice, denominator = total mice in cohort. Days = days post-surgery, day 0 = day of surgery. Cohort: A-1,2 = original protocol, B-1,2 = modified treatment, 14 day endpoint, B-3,4 = modified treatment, 8 day endpoint.



**Figure 1.** Typical setup of the static microisolator cages, half-on circulating water blankets, maintained in the standard animal housing room.