

The Effect of Beta-Hydroxy-Beta-Methyl Butyrate in Response to Exercise and High-Fat Diet on Body Weight, Body Composition and Characteristics of Metabolic Syndrome in Male Wistar Rats

Original Research

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Abstract

Introduction: The favorable effect of Beta-hydroxy-beta-methyl butyrate (HMB) on body weight, body composition, and food intake is reported. However, its effect in response to a Western diet and exercise was unclear.

Methods: Wistar rats (n=36) received a regular diet for 4 weeks. Thereafter, they were allocated to three groups and received a high-fat diet for 8 weeks. Group one received HMB (320mg/kg BW /d) and an exercise regimen; group two received HMB with no exercise and group three received a placebo with no exercise. Bodyweight (BW), body composition, systolic (SBP) and diastolic (DBP) blood pressure, pulse rate, fasting blood glucose (FBG), and blood glucose (BG) response to a glucose load were measured.

Results: BW and food intake were not affected by either HMB supplement or exercise. Body fat was lower in HMB and HMB + exercise compared with the control group at week 12 (P<0.05). The glucose response to glucose preload was lower in HMB and HMB + exercise groups compared with control at week 12 (P<0.05).

Conclusion: The results of this study support the beneficial effects of HMB supplements on body composition and glucose metabolism. However, the HMB supplement did not have any additional effect when combined with an exercise regimen.

Keywords: food intake, glucose, blood pressure

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Introduction:

Beta-hydroxy-beta-methyl butyrate (HMB) is a metabolite of leucine, one of three branched-chain amino acids, that has been used extensively by athletes as a dietary supplement. Approximately 5% of leucine is being converted to HMB.^{1,2} It is suggested that the beneficial effects of leucine or alpha-ketoisocaproate (KIC) supplementation on performance, protein synthesis, and muscle hypertrophy can be partially attributed to HMB in both humans and rats.^{3,4} HMB is a multifunctional metabolite involved in protein metabolism, muscle hypertrophy, and insulin activity. There is a wide range of metabolic and physiological properties attributed to HMB including

promoting higher fat oxidation by activation of gamma co-activator 1-alpha α (PGC-1 α).⁵ HMB may also improve muscle glycogen synthesis by enhancing the insulin effect and amplifying phosphorylation.¹ In addition, HMB may enhance muscle protein synthesis³ by upregulation of the mammalian target of rapamycin (mTOR).⁶ Other mechanisms have also been suggested to explain the ergogenic effects of HMB. Downregulation of protein degradation through the ubiquitin pathway, development of sarcolemma integrity, decreased cell apoptosis, stimulation of the testosterone and growth hormone, and insulin-like growth factor-1 (GH/IGF-1) axis are some of the well-known mechanisms employed by HMB. Although the beneficial effects of HMB on various aspects of metabolism have been shown, these effects may be influenced by other factors including age, gender, exercise regimen (type and duration of exercise), dietary factors (e.g. total calorie intake, macronutrient composition, and other dietary bioactive components) and HMB regimen (dose, timing, and frequency).⁶

The effects of HMB on body weight and body composition are still unclear. The positive effects of HMB on body composition and/or performance during resistance exercise have been shown in previous studies in humans and in rats.⁷⁻¹⁰ Wilson et.al. found that HMB-free acid (HMB-FA) ingestion for 12 weeks enhanced resistance training-induced increases in total body mass and fat-free mass and fat mass loss in humans.¹¹ HMB resulted in increased muscle mass, muscle strength and anaerobic properties, fat-free mass, and peak and mean anaerobic power but had no effect on fatigue index and aerobic fitness or anabolic and catabolic and inflammatory mediators in elite adolescent volleyball players in humans.¹² However, in another study, while HMB increased total body mass, it did not affect fat-free mass after resistance exercise in men.^{13, 14}

Anti-catabolic effect of the HMB was found in studies on cancer patients.¹⁵ HMB supplementation reduced the downregulation of protein synthesis in skeletal muscle in response to cachectic stimuli.¹⁶ Moreover, HMB increased survival time and induced important metabolic changes in cancer-bearing rats.¹⁷

While the beneficial effects of HMB on body weight, body composition, and fat metabolism have received considerable studies, only a few studies examined its effect in response to a high fat, high carbohydrate diet as a typical western diet. In one study, HMB reduced body weight and improved insulin resistance in mice fed a high-fat diet (HFD).¹⁸ Authors suggested that this effect is mediated at least partially by gut microbiota. Moreover, as previously mentioned, the exercise regimen determines metabolic response to HMB supplementation. However, to the best of our knowledge, no study examined the interactive effects of HMB, high fat, high carbohydrate diet, and exercise in one single study. Therefore, this study aimed to examine the interactive effects of HMB, diet, and exercise on body weight, body composition, glucose metabolism, and blood pressure in Wistar rats. We hypothesized that HMB supplementation improves body composition, blood pressure and glucose metabolism in rats fed a high-fat diet. Exercise enhances the effect of HMB on body weight, body composition, and glucose metabolism.

Methods

Animals and Diets

Male Wistar Rats (BW: 194.33 \pm 4.44) were housed individually in ventilated plastic cages with bedding at 22 \pm 1 $^{\circ}$ C and a 12-h light-dark cycle (lights off at 0900 to 2100 h). The diets were provided ad libitum in glass jars. All rats had free access to water throughout the experiment. The regular (AIN-93G) and high-fat (60% FDC) diets were purchased from Dyets (Dyets Inc. Bethlehem, Pa, USA). The composition (per kilogram diet) of the diets is illustrated in Table 1. The protocol was approved by the University of North Florida Institutional Animal Care and Use Committee.

HMB Supplement

Powdered Ca- β -hydroxy- β -methylbutyrate was supplied by Metabolic Technologies, Inc. (Ames, IA, USA). Dosage and delivery methods were determined based on previous studies.¹⁹⁻²⁴ A dose of 320mg/kg BW/d was administered to rats via combination with a food pellet. The calcium salt form of HMB was selected to be used in this study based on previous studies.⁶ The dosage of HMB supplementation for each rat was recalculated on weekly basis (as described earlier, the dosage was based on the body weight). The food pellets containing the HMB were separated into plastic bags labeled with the corresponding rat's number to ensure the proper dosage was administered. The weekly dosages were stored in the refrigerator.

Table 1. Composition of the Normal and High-fat Diets

	Regular Diet (AIN-93 G Diet)	High Fat Diet (Diet # 112252)
Cal	3597	3963
	g/kg	
Casein	200	200
Sucrose	92.7	68.8
Soybean oil	40	25
Lard	0	245
t-Butyhydroquinone	0	0.005
Cornstarch	426.79	0
Dyetrose	140	125
Cellulose	50	50
Mineral Mix	35	10
Calcium Carbonate	0	5.5
Potassium Citrate	0	16.5
Vitamin Mix	10	10
Choline Bitartrate	2.5	2
L-Cystine	3	3

Experimental design

Newly weaned Wistar rats (n=36) received a regular diet for 4 weeks to become more mature. Thereafter, rats were allocated to three groups (n = 12/group). All groups received a high-fat diet for 8 weeks. Group one received HMB (320mg/kg BW /d) and an exercise regimen; group two received HMB with no exercise and group three received a placebo with no exercise (control group) for 8 weeks. To ensure all the supplements are consumed, the HMB was given by mixing the daily dose with a pellet of food before introducing the rest of the food. Food and water were ad libitum. Bodyweight (BW) was measured weekly for 12 weeks. Systolic (SBP) and diastolic (DBP) blood pressure, pulse rate, fasting blood glucose (FBG), and glucose tolerance tests were measured at weeks 4, 8, and 12. Fat pad mass was measured at killing at week 12. Plasma glucose and insulin were measured at baseline and week 12.

Exercise Protocol

A 5-line rat's treadmill (Columbus Instrument, 950 North Hague Ave, Columbus, Ohio. Model: EXER-4 Treadmill) was used. The adaptation process to the procedure was implemented for two weeks. Repeated bouts of running sessions (5 minutes/day) with a lower speed (15 m/min) were used when introducing rats to a treadmill for the first time, then the speed was slightly increased throughout training to reach the final speed and time (20 m/min for 30 min). An electrical stimulus was applied to ensure continual exercise (the amp was less than 0.5 amps and was limited to a max of 15 voltage). However, no individual rat received no more than two electrical stimuli in total since they were all good runners. During the intervention, the room temperature was kept around 21 degrees Celsius. The exercise regimen was 30 minutes/day, 5 days per week. The total exercise time (30 minutes) was split into 2 sets of active running for 15 minutes with a 30-minute interval time for rest.

Long-Term Food Intake

Long-term food intake was measured on a weekly basis by weighing the food at the beginning and at the end of each week. Spillage was collected, measured, and subtracted to obtain the actual intake.²⁵

Glucose tolerance test

After 12 h fasting overnight, blood samples were drawn from the tail vein at fasting and 15, 30, and 60 min after a glucose administration (0.375 g glucose per mL, 5 g glucose per kg BW).²⁵

Blood pressure

Systolic (SBP) and diastolic blood pressure (DBP) were measured by a non-invasive tail-cuff method (optical plethysmography) using a tail manometer tachometer system (BP-2000, Visitech system; Apex, NC, USA). After an adaptation process (5-day adaptation period), rats were restrained in holders on a constant warm platform (30 °C). On the day of measurement, five mock measurements preceded a series of ten measurements that were used to calculate the average as reported previously.²⁵

Blood glucose

Tail vein glucose concentration was assessed using test strips using a handheld commercial glucometer (Contour ® Next Blood Glucose Meter, Bayer Healthcare LLC, Mishawaka, IN, USA). The accuracy and variance of the glucometer and test strips were examined by applying control solutions (levels 1 and 2) provided by the manufacturer (Bayer, Bayer Healthcare LLC, Mishawaka, IN, USA).²⁵

Body composition

As previously described²⁵, fat mass and lean mass (fat-free mass) were measured right after killing at the end of the study. Fat mass was measured by dissection of extracted abdominal, epididymal, and perirenal fat. Lean mass was calculated by subtracting body weight from the fat-mass weight.

Statistical analyses

The effect of the HMB supplement and exercise and their interactions on BW, glucose response, SBP, and DBP were analyzed by two-way analysis of variance. When repeated measures were made over time on BW, food intake, SBP, DBP, pulse, BG response, and FBG, the PROC MIXED procedure was used with HMB supplement, exercise, and time as the main factors. When interactions were statistically significant, a one-way analysis of variance followed by a post hoc Tukey's test was conducted to evaluate treatment effects. The effects of HMB supplementation on plasma measures were compared using a Student's unpaired t-test. BG response was calculated as the total incremental area under the curve (tAUC) of the BG concentration over 1 h after receiving glucose administered for the glucose tolerance test. The homeostasis model assessment of insulin resistance index was calculated as fasting glucose multiplied by fasting insulin divided by 22. Data are expressed as means with standard errors. Statistical significance was defined at $P < 0.05$. All analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA).

Results

BW was not affected by either HMB supplement or exercise. However, there was an interactive effect of the treatment and time ($p < 0.05$) (**Fig 1**).

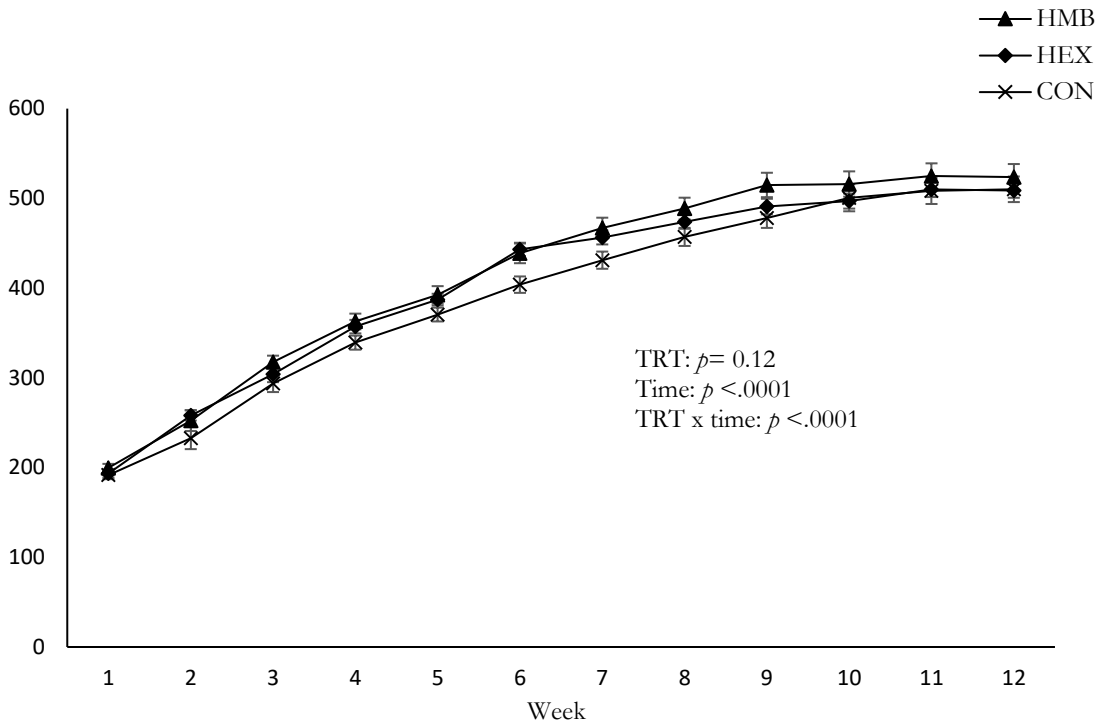


Fig 1. Effect of HMB supplement and exercise on body weight (n = 12/group). Data are means \pm SEM; BW was analyzed by one-way ANOVA. **HMB:** HMB supplement; **HEX:** HMB + Exercise; **CON:** Control group; **TRT:** Treatment

Similarly, food intake was not affected by either HMB supplement or exercise. However, there was an interactive effect of the treatment and time ($p < 0.05$) (**Fig 2**).

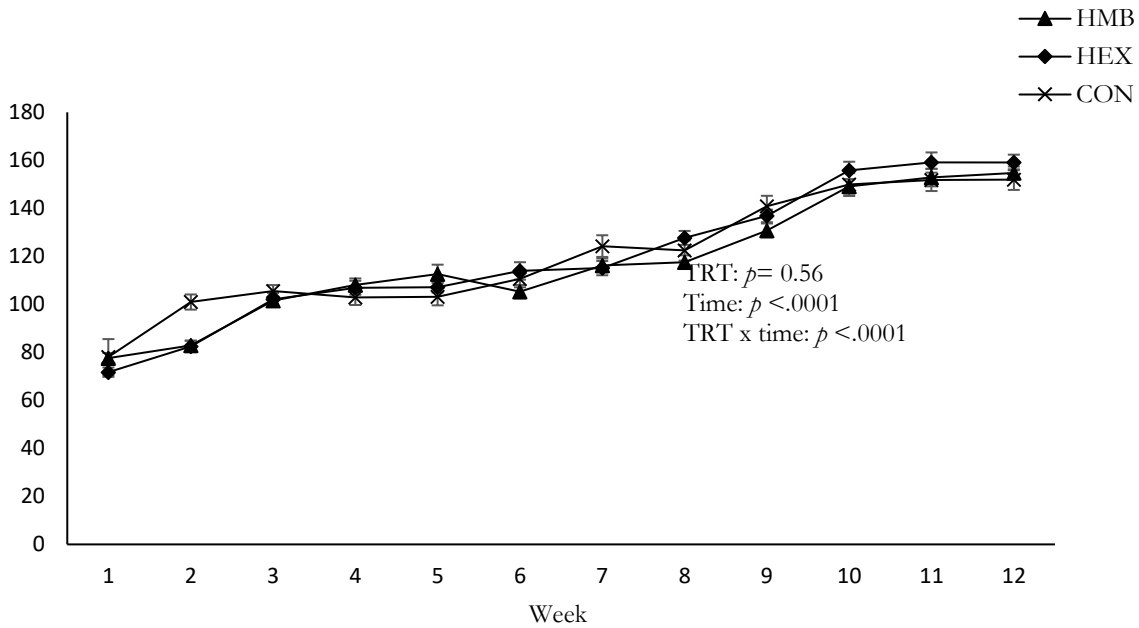


Fig 2. Effect of HMB supplement and exercise on Food Intake (n = 12/group). Data are means \pm SEM; BW was analyzed by one-way ANOVA. **HMB:** HMB supplement; **HEX:** HMB + Exercise; **CON:** Control group; **TRT:** Treatment

Body composition was altered by HMB and exercise: Body fat (as total and as a percentage) was lower in HMB and HMB + exercise compared with the control group at week 12 ($P < 0.05$) (**Table 2**)

Table 2. Effect of HMB and Exercise on body composition at week 12.

	HMB	HMB + Exercise	Control
Body Wt (g)	523.45 ± 15.98	511.22 ± 10.21	515.26 ± 14.92
Fat (g)	19.99 ± 1.73 ^b	18.90 ± 2.34 ^b	25.91 ± 1.89 ^a
Fat/Body Wt (%)	3.78 ± 0.26 ^b	3.71 ± 0.45 ^b	5.02 ± 0.27 ^a

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different ($p < 0.05$). The effect of HMB and exercise was analyzed by two-way ANOVA.

While fasting plasma glucose was not affected by either HMB or HMB + exercise, the glucose response to glucose preload was lower in HMB and HMB + exercise groups compared with control at week 12 ($P < 0.05$) (**Table 3**). The glucose response to administered insulin was not affected by either HMB or exercise. No effect of either HMB or exercise on systolic and diastolic blood pressure and pulse was observed (**Table 4**).

Table 3. Effect of HMB and Exercise on fasting blood glucose, glucose response to glucose preload, and glucose response to insulin administration at week 12.

	HMB	HMB + Exercise	Control
Glucose, mM	5.65 ± 0.16	5.93 ± 0.19	6.04 ± 0.18
OGTT	468.75 ± 17.99 ^b	484.75 ± 15.40 ^b	564.00 ± 82.38 ^a
ITT	213.40 ± 13.07	230.42 ± 5.98	220.83 ± 7.50

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different ($p < 0.05$). The effect of HMB and exercise was analyzed by two-way ANOVA. **OGTT**: Oral glucose tolerance test; **ITT**: Insulin tolerance test.

Table 4. Effect of HMB and Exercise on systolic and diastolic blood pressure and pulse rate at week 12.

	HMB	HMB + Exercise	Control
Systolic BP	120.5 ± 6.56	137.60 ± 5.86	127.38 ± 7.61
Diastolic BP	73.16 ± 15.58	92.1 ± 6.69	70.65 ± 7.57
Pulse	470.16 ± 17.86	466.8 ± 6.56	444.19 ± 16.02

Data are means ± SEM; (n = 12/group). Different letters in a row are significantly different ($p < 0.05$). The effect of HMB and exercise was analyzed by two-way ANOVA. **BP**: Blood pressure.

Discussion

Body weight and body composition:

The results of this study support our hypothesis that HMB supplementation improves body composition as illustrated by enhanced lean mass observed in this study. Moreover, HMB reduced fat mass (as an absolute content and as a percentage of body weight) in this study. The results are consistent with previous studies: In athletes, HMB supplements resulted in an increased lean body mass, muscle mass, and muscle strength.^{26, 27} In elderly men, supplementation of HMB combined with a 12-week resistance training program resulted in a decrease in abdominal adiposity.²⁸ HMB supplementation was also associated with the preservation of muscle mass during a 10-day bed rest period in elderly patients in a randomized control trial.²⁹ In cancer and AIDS-related wasting syndromes, HMB improved body composition by increasing fat-free mass through slowing protein breakdown and improving protein synthesis. However, these studies included the supplementation of arginine and glutamine along with HMB.^{30, 31} Finally, a meta-analysis, concluded that HMB supplementation contributed to the preservation of muscle mass and may be beneficial in the prevention of muscle atrophy related to bed rest.³² In contrast, a meta-analysis found no beneficial effects of HMB as a supplement on body composition in athletes.³³ This discrepancy can be explained by the differences in study designs implemented in these studies including the length of the study, type of population, exercise regimen, and the dose of the HMB applied in these studies. The majority of studies on HMB were in clinical settings examining its effect on body composition in patients with waste syndromes which is based on the fact that HMB downregulates glucocorticoid-induced muscle loss.³⁴ Moreover, a great number of studies tested its effect on muscle synthesis in athletes.

Interactive effects of HMB and exercise on body weight and body composition:

Only a few studies cross-examined the interaction between HMB and exercise. Results from 10 randomized controlled trials (RCTs) examining the interactive effect of HMB supplementation and physical activity in elderly adults showed that there is no additional effect of HMB supplementation over physical exercise on body composition, muscle strength, or physical performance compared to exercise alone.³⁵

HMB had minimal effect on total body mass gain when combined with an exercise regimen in the elderly, and this effect was not reflected in greater increases in fat-free mass, strength, or decreases in fat mass.³⁶ Authors suggested the HMB has no beneficial effect on the improvement of body composition or strength when added to the resistance training exercise regimen. These results are consistent with our observation indicating that HMB and exercise had no interactive effect on body composition: Exercise did not have any robust effect on body composition in addition to the effect of the HMB supplementation and vice versa. However, in another clinical study, HMB but not exercise enhanced protein synthesis in older adults only in the first two weeks of the study.³⁷

Food intake:

The effect of HMB on food intake is studied. HMB supplementation for 10 days significantly decreased food intake and body weight in mice.³⁸ The authors suggested that these results can be attributed to an increase in β -hydroxybutyrate since it suppresses food intake and reduces skeletal muscle catabolism.³⁸ The results of this study did not support any main effect of either HMB supplement or exercise on food intake in rats. However, there was an interactive effect of HMB supplement and time. In another word, the effect of HMB has become more significant over time. It can be suggested the length of this study was not enough long to determine the effect of HMB on food intake.

Glucose metabolism:

HMB altered glucose metabolism in this study. While no effect on plasma fasting glucose was observed, the glucose total AUC was lower in the HMB and HMB + exercise groups compared with the control. However, the results from previous studies are not consistent which can be explained by various study designs: In a clinical study, in younger subjects, coadministration of HMB and insulin resulted in a smaller insulin AUC compared with insulin alone. It did not affect glucose total AUC.³⁹ In mice, the HMB supplement increased fasting blood glucose and insulin resistance compared with the non-supplemented trained group.⁴⁰

Blood pressure:

Only a few studies examined the role of HMB in the prevention of cardiovascular diseases in general and blood pressure in particular. HMB supplementation reduced total cholesterol and systolic blood pressure but did not affect diastolic blood pressure in hypertensive subjects.⁴¹ The results of this study did not support any effect of HMB on either systolic, diastolic blood pressure, or pulse. A longer study with a higher dosage of HMB supplement might be a better approach for future studies.

HMB and high-fat diet:

It is notable that the significant effect of the HMB on body composition and glucose response to glucose preload observed in this study was in rats fed a high-fat diet throughout the study. Similarly, HMB reduced body weight and improved insulin resistance in mice fed a high-fat diet (HFD).²⁰

Dose of HMB supplement:

It has been shown that HMB affects body composition in a dose-dependent manner.¹⁶ The dosage of the HMB supplementation implemented in this study (320mg/kg BW /d) and the implementation was based on previous studies.¹⁹⁻²⁴ The recommended dose of HMB in humans is around 3 g per day.^{6, 34, 42-43} However, the metabolic rate in rodents is at least 6 times higher than in humans.⁶ Therefore, the suggested dose in rodents is up to 500 mg/kg BW. However, even 125 mg/kg BW caused a significant reduction in BW loss in mice.¹⁵

Conclusion:

These results support our hypothesis that HMB has a favorable effect on body composition and glucose metabolism when rats were on a high-fat diet. However, no effect of either HMB, exercise, or their combination on body weight and blood pressure was observed. Although there is no direct implementation of these results in humans, it may help to have a better understanding of the interactive effects of HMB, exercise, and diet on food intake and characteristics of Metabolic syndrome. A clinical study with a similar study design can be considered as a further step.

As a future direction, studies examining the effect of sex (male vs. female) may help to understand the sex-specific parameters related to the effect of HMB on body weight and body composition in response to a high-fat diet. Moreover, comparing normal-weight subjects vs. obese subjects can help to understand the interactive effect of HMB and obesity on metabolic and physiologic responses to exercise.

The main potential limitation was the duration of the study. A longer study may help to have a better understanding of the physiological properties of HMB in the long term.

This study was reviewed and approved by the University of North Florida Institutional Animal Care and Use Committee (IA 16-007).

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