2001

The rat model for metabolic studies during spaceflight

Mary Eulalia Miller
San Jose State University

Follow this and additional works at: https://scholarworks.sjsu.edu/etd_theses

Recommended Citation

Miller, Mary Eulalia, "The rat model for metabolic studies during spaceflight" (2001). *Master's Theses*. 2190.

DOI: <https://doi.org/10.31979/etd.j6me-wn6u>
https://scholarworks.sjsu.edu/etd_theses/2190

This Thesis is brought to you for free and open access by the Master's Theses and Graduate Research at SJSU ScholarWorks. It has been accepted for inclusion in Master's Theses by an authorized administrator of SJSU ScholarWorks. For more information, please contact scholarworks@sjsu.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI[®]

THE RAT MODEL FOR METABOLIC STUDIES DURING SPACEFLIGHT

**A Thesis Presented to
The Faculty of the Department of Biological Sciences
San Jose State University**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Sciences**

**by
Mary Eulalia Miller**

August 2001

UMI Number: 1405512

UMI[®]

UMI Microform 1405512

Copyright 2001 by Bell & Howell Information and Learning Company.

All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

© 2001

Mary Eulalia Miller

ALL RIGHTS RESERVED

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Daniel C. Holley (Graduate Advisor)

Dr. Adrian Rodriguez

Dr. Charles E. Wade (Senior Scientist, NASA Ames Research Center)

APPROVED FOR THE UNIVERSITY

ABSTRACT

THE RAT MODEL FOR METABOLIC STUDIES DURING SPACEFLIGHT

by Mary Eulalia Miller

Negative energy balance is consistently noted in astronauts returning from spaceflight. Efforts to resolve the mechanisms leading to this imbalance and determine the effects of microgravity on human metabolism during spaceflight are complicated by factors such as decreased caloric consumption and unknown energy requirements during spaceflight. Due to its adaptability to microgravity the rat may serve as an appropriate model in future spaceflight metabolic studies. To determine the feasibility of using a rat model, archived data from fifteen spaceflights were evaluated. This included evaluation of the Animal Enclosure Module as a calorimeter.

Rats appear to adapt more favorably to spaceflight than humans. Analyses of rat body mass, growth rate, and food and water consumption indicate that rats maintain a balanced energy state during 4.1 to 17.7 days of spaceflight. This balanced energy state makes the rat an appropriate model for studies on metabolic changes caused by microgravity.

ACKNOWLEDGEMENTS

I would like to acknowledge the NASA Ames personnel who contributed to this project, Rudy Aquilina, Lisa Baer, Mike Eodice, Cyra Fung, Dr. Richard Grindeland, Megan Moran, Dr. Marianne Steele, and Tom Wang. I greatly appreciate their efforts and assistance. I would like to thank my thesis committee, Dr. Daniel Holley (San Jose State University), Dr. Adrian Rodriguez (San Jose State University), and Dr. Charles Wade (NASA Ames Research Center) for their contributions of time, support, and insight to my thesis project. Dr. Wade's continual guidance and direction throughout this effort have been invaluable and are specially noted.

This project was completed using the facilities in Dr. Wade's Lab at NASA Ames Research Center. The work was supported by NASA grants 121-10-50 and 121-40-10.

TABLE OF CONTENTS

	Page
Introduction	1
Materials and Methods	11
Results	21
Discussion	28
Bibliography	42

LIST OF TABLES

	Page
Table 1. Mission plans for fifteen spaceflight missions considered	A-1
Table 2. Rat details for fifteen spaceflight missions considered.....	A-3
Table 3. Body mass measurements taken when the rats were initially placed in the AEMs.....	A-5
Table 4. Body mass measurements taken when the rats were recovered following flight.....	A-7
Table 5. Average body mass and body mass gain measurements for rat groups.....	A-9
Table 6. Food consumption.....	A-11
Table 7. Water consumption	A-13
Table 8. Details of ATR-4 data recordings.....	A-14
Table 9. ATR-4 temperature data.....	A-15
Table 10. Total body mass of all rats within an AEM and the average delta habitat temperature.....	A-16
Table 11. Comparison of male Sprague-Dawley rats from AEM flight and control groups to male Sprague-Dawley laboratory rats housed and fed under standardized conditions.....	A-17

LIST OF FIGURES

	Page
Figure 1. Starting body mass of AEM rats	A-18
Figure 2. Final body mass of AEM rats	A-19
Figure 3. Average body mass of AEM rats	A-20
Figure 4. Daily mass gain of AEM rats	A-21
Figure 5. Taconic growth chart and AEM rats	A-22
Figure 6. Daily food consumption of AEM rats	A-23
Figure 7. Daily food consumption of AEM rats adjusted for body mass	A-24
Figure 8. Daily caloric consumption AEM vs. control rats	A-25
Figure 9. Daily water consumption from water bladders of AEM rats.....	A-26
Figure 10. Daily water consumption from water bladders and moisture in food bars.....	A-27
Figure 11. Total body mass vs. delta temperature.....	A-28

INTRODUCTION

Physical challenges facing astronauts in spaceflight:

Physiological changes during and following spaceflight have been well documented. In a microgravity environment body fluid compartment shifts occur and astronauts face proprioceptive challenges. During spaceflight the musculo-skeletal system is remodeled and bone and muscle are lost. Other changes include electrolyte imbalances, cardiovascular deconditioning, loss of red blood cell mass, and changes in immunocompetence (4). Additionally, astronauts consistently lose body mass during flight. A decrease in body mass is indicative of a negative energy balance (52,54,63,71).

The negative energy balance state:

The implications of a chronic negative energy balance state are serious. Long term exposure to a state of negative energy balance results in decreased physical performance and decreased immunocompetence (63). Such complications pose a threat to astronauts' well being. If astronauts were in a poor state of health, mission success would be jeopardized and long-term habitation in space would be infeasible.

The potential causes of the negative energy balance seen in astronauts include changes in caloric consumption, energy expenditure requirements, or increase in metabolism. Efforts continue to determine the effects of microgravity on metabolism. Such findings will be helpful in future efforts to maintain energy balance in astronauts. Human, monkey, and rat models have been used in previous spaceflight studies to give

insight related to changes in metabolism during spaceflight. However, interpretation of data collected from humans and monkeys has been confounded in the past, as will be discussed below. The rat appears to be a less complicated model for metabolic studies during spaceflight.

Metabolic studies in spaceflight:

The human model:

Several confounding variables make the human a poor model for metabolic studies during spaceflight. These confounding variables include decreased caloric consumption, unknown energy requirements during spaceflight, and work / sleep schedule variations between missions.

Decreased food consumption: Factors contributing to decreased caloric intake by astronauts include space motion sickness, decreased appetite, poor food palatability, and astronaut compliance (63). Motion sickness, a problem for some astronauts during spaceflight, generally resolves itself within the first day or two in flight. Stein notes a 20% decrease in voluntary dietary intake by astronauts considered in his report (63) and he argues that there is nothing unique about the nutritional requirements in spaceflight, rather, the problem is getting the astronauts to eat (63). Astronauts, particularly in the earlier missions, noted that foods were unappetizing and difficult to prepare and eat without creating crumbs, which could potentially float into instrument panels and cause malfunctions (http://lsda.jsc.nasa.gov/kids/L&W/eat_drink.htm, May 28, 2001, 8:00 am).

Unknown energy requirements: It was originally thought that astronauts would

expend less energy in space as a result of not having to overcome the force of gravity (7). Accordingly, it was assumed that a reduction in food consumption would follow without serious threat to energy balance in the astronauts. However, the energy expenditure requirements of astronauts in space have yet to be fully determined and are mission and individual specific. A better understanding of the metabolic responses to demanding physical extravehicular activities is also needed, for it is now thought that the metabolic cost of movement in microgravity has been underestimated. Changes in body composition, increased metabolic rates for physically demanding activities, and increases in energy expenditure (energy metabolism) during long missions have been noted, and considered by some to reflect a decrease in metabolic efficiency (4,8). Contributing to this higher than anticipated metabolic demand may be the lack of force from gravity-induced friction present on Earth. Altman proposes that during spaceflight astronauts must exert extra muscular effort for such a reactive force (4).

It is important to consider the potential shifts in energy expenditure during spaceflight caused by novel use of muscles and motor patterns. The noted deconditioning of the muscles, most evident in the antigravity slow-twitch muscles, is part of an adaptive process to a microgravity environment where astronauts use novel motor patterns. During spaceflight astronauts rely heavily on the upper body (forelimbs) for movement and the lower limbs are used in perching-type activities (12). These changes could alter energy requirements during spaceflight.

Lane and Schoeller considered energy requirements during spaceflight and stated that the energy requirements for spaceflight are not dramatically different for those on

Earth. Lane, however, noted that this prediction on the energy requirements during spaceflight did not account for the potential changes in body size during a long mission and noted that predictions were not exact for individuals (31).

Mission variation: Missions vary in workload demand and duration. Missions requiring astronauts to perform extensive extravehicular activity are more physically demanding. Also, in shorter flights astronauts have less time to rest and are allotted a limited amount of time for eating (personal communication – Charles Wade, 2001). However, during longer missions, astronauts are given vacation time. Significant variation in the energy requirements from mission to mission complicate the interpretation of data. Comparisons of flight data collected from different missions are not possible when there is significant variation in flight duration and workload demands.

The human is a confounded model: The aforementioned variables make the human a confounded model for metabolic studies during spaceflight. Several investigators have noted this in their attempts to study metabolism in the human during spaceflight. For example, body composition studies performed on astronauts returning from spaceflight have been complicated by the astronauts' states of negative energy balance (32,65,72). It has been proposed that providing "caloric balance" during spaceflight will enable scientists to better understand the mechanisms involved in the body composition changes caused by exposure to a microgravity environment (28), ultimately indicating how metabolism is altered by microgravity environments. However, data from previous flights was not collected while maintaining a "caloric balance" and there is still uncertainty about future efforts to maintain "caloric balance,"

especially in long-term missions.

The monkey model:

In a continuing effort to better understand microgravity's effect on metabolism, experiments have been performed on primates flown aboard satellites. Studies of energy expenditure in spaceflight primates have been done using chair-adapted rhesus monkeys and a doubly labeled water technique. Chair-restraint limits the monkey's level of activity making it easier to account for energy expenditure. Two monkeys were flown aboard the COSMOS 2044 and two aboard COSMOS 2229 producing results that indicated a significant reduction in energy expenditure during flight (64). A later study involving two monkeys flown aboard the Bion 11 mission also indicated a decrease in energy expenditure relative to control monkeys and, additionally, to the monkeys flown previously on COSMOS 2044 and 2229 (24,64). Hoban-Higgans *et al.* (24) suggest that the Bion 11 results were complicated by the influence of frequent handling of the monkeys and its subsequent effect on control energy expenditure. While past studies involving primates provide some information on energy expenditure in spaceflight, due to the limited data, small population size, and confounding interactions, they offer little insight into the details of potential metabolic shifts in spaceflight.

The rat model:

The rat has been a popular subject in past spaceflight studies of metabolism.

Three methods of studying metabolic shifts in the rat model include analyses of the metabolic pathways, body composition, and energy expenditure. Several metabolic pathways in spaceflight rats have been investigated, with special focus on changes in enzymatic activity. Efforts have been made to determine changes in body composition caused by exposure to microgravity, but have been greatly confounded by operational constraints. Examples of such operational constraints will be discussed in depth below and include delayed recovery of the rats following landing (COSMOS 1129) and deviation from feeding protocol (COSMOS 1887).

Male rats flown aboard the Soviet biosatellite COSMOS 936 were used to examine the effects of weightlessness on liver metabolism (2). The changes in activities of carbohydrate and lipid enzymes were analyzed. A centrifuge onboard the biosatellite was used to eliminate the confounding gravity differences between control and flight animals. Decreases in activity were reported in the following enzymes for rats sacrificed 4.5 hours post-recovery: glycogen phosphorylase, 6-phosphogluconate dehydrogenase, aconitate hydratase, alpha-glycerolphosphate acyltransferase, and diglyceride acyltransferase, while the activity levels of palmitoyl-CoA desaturase and lactate dehydrogenase were elevated. There were no significant changes in these enzymes for the rats sacrificed after 25 days of recovery at 1 G. Glycogen synthetase activity was measured in all cases and showed no significant difference between groups. The liver showed an altered ability to complex long-chain fatty esters and an impaired ability to form triglycerides and phospholipids due to decreases in enzymatic activity. Increases in the listed enzymes caused altered lipid composition with an increase in the fraction of

unsaturated fatty acids. Increased liver glycogen levels were also noted in the rats sacrificed at 4.5 hours post-recovery (2).

Livers from male rats flown for fourteen days aboard COSMOS 2044 were analyzed for protein, carbohydrate (glycogen), and lipids. In addition, the activities of key enzymes involved in metabolism of these compounds and xenobiotics were analyzed (34). Rats were sacrificed 8 to 11 hours post-landing. Parameters found to be significantly altered for the flight rats were 1) increased glycogen, tyrosine aminotransferase, and tryptophan oxygenase levels, and 2) decreased hepatic cholesterol, sphingolipids, and hepatic delta-aminolevulinic acid synthase levels. The causes and mechanisms behind the observed increase in glycogen are not clear. Of particular interest was the decrease in delta-aminolevulinic acid synthase activity levels, as this enzyme functions as the rate-controlling enzyme of heme synthesis and is repressed by the increased supply of glucose or glucogenic intermediates to rat livers. The hypothesis put forth by this group was that the earliest biochemical changes in the liver (due to microgravity or the recovery from microgravity) are alterations in glucose homeostasis. These alterations likely result in other metabolic changes such as the noted decrease in porphyrin and heme synthesis rates and the decrease in heme-dependent systems represented by cytochrome P-450. However, great uncertainty surrounds the contrasting data in this area and the data cannot clearly be attributed to the effect of varying gravitational loads. Investigators have proposed that additional flight and ground based experiments be performed (36).

At present centrifuge studies provide the most reliable results for studying

gravitational effects on metabolism. Ideally metabolic shifts associated with exposure to varying gravitational loads are studied by comparisons of findings in hypergravity, normal gravity (1G at Earth), and microgravity (0 G in space) environments, when possible. Hypergravity environments of varying G-loads are created by centrifugation or radial acceleration. The effects of acute exposure to hypergravity on liver gluconeogenesis has been a popular area of study. The most common metabolic changes seen in these studies are increased gluconeogenesis which is promoted by the release of stress hormones (corticosterone and epinephrine) and results in increased liver glycogenesis and thus increased in glycogen deposition (14,15,41,43). Chronic hypergravity studies consistently show decreases in body fat depots (42,44,46,49,50). Decreases in fat-free muscle and bone and in liver and kidney lipids in chronically centrifuged rats have also been noted (44). In a one-year centrifuge study rat liver weights were increased in the hypergravity rats (42). Evans using a chicken model also observed increased liver masses in a chronic hypergravity study. Analyses of this finding and others indicate that the bird model adapted to chronic hypergravity exposure through changes in free fatty acid metabolism and changes in lipogenic enzymes (19).

Hypothesis:

The rat is the most feasible model to study the physiology of metabolic changes induced by exposure to microgravity in future spaceflight studies. The main goal of this report is to evaluate and validate the use of the rat model for metabolic studies during spaceflight. It is hypothesized that the rat is a less confounded model than the human and

monkey models. As a less confounded model, the rat is expected to maintain energy balance, as would be indicated by similar body mass, body mass gain, and caloric consumption when compared to ground control. To test this hypothesis rat data from fifteen spaceflight missions were analyzed.

Additional considerations:

Comparisons to rats maintained under standardized conditions

Mission flight and ground control groups were compared to control ("normal") rats which were maintained under standardized laboratory conditions. It is expected that the mission rats will have similar caloric consumption as "normal" rats with similar body masses. Likewise, in a comparison of body mass over time between our subjects and "normal" rats, there should be no difference.

Measurement of energy expenditure:

The potential for measuring the energy expenditure of the rat during spaceflight from archived temperature data is also investigated in this report. It is proposed that the animal habitat used during these spaceflight missions (the Animal Enclosure Module, AEM) can function as a direct calorimeter. The heat produced by the rats is measured based on the assumption that the animal habitat functions as a direct calorimeter. The determined heat production values are expected to correlate with the energy expenditure (and thus caloric consumption values) of the rats housed in the animal habitats. It is

impossible to predict energy expenditure of the rats without body composition information. Therefore, comparisons will be made between the calculated heat values and the caloric consumption of the rats.

MATERIALS AND METHODS

Data were obtained from fifteen Space Transportation System (STS) missions carrying rats housed in specialized rodent habitats, Animal Enclosure Modules (AEMs). Analyses of food and water consumption, body mass, growth rate, and temperature changes within the AEM were used to compare flight and ground control groups and formulate predictions of energy expenditure.

MISSIONS:

Data sets were compiled from fifteen spaceflight missions (STS 40, 41, 48, 52, 54, 56, 57, 60, 62, 63, 66, 70, 77, 78, and 80) and sixteen groups of rats. In this report, the rats in the STS 80 mission are divided into two groups based on their treatment protocol which included either a high calcium or low calcium diet. Most of the missions considered in this report, (with the exceptions of STS 48 and 54, which use one flight and one ground control AEM each) used four AEMs over the study duration. Two AEM were housed onboard the shuttle and other two were used as ground control AEMs. There are three types of ground controls used in these missions, the synchronous ground control, the asynchronous ground control, and the vivarium ground control. Most comparisons in this report are made between flight rats and synchronous ground control rats. Synchronous ground control rats are housed in the AEMs, maintained in a simulated spacecraft environment, and follow a procedural time course that is identical to the flight

rats. Asynchronous ground control rats are the equivalent of delayed synchronous controls. They follow the same procedures as the synchronous controls, but these procedures begin several hours after the flight. Flight duration ranged from approximately 4.1 to 17.7 days.

ANIMALS:

The total population of flight rats (N=174) analyzed in this report included Sprague-Dawley male (n=122), Sprague-Dawley female (n=28), Fisher male (n=12), and Fisher female (n=12). The rats were housed in AEMs (5-8 rats/AEM) for the duration of the flight plus approximately one day. The rats were loaded into the AEMs approximately one day before launch. Control rats were housed in ground AEMs under the same lighting, feeding, watering, and community grouping conditions. The starting weight of the animals at the time of launch ranged from 47 to 321 grams. STSs 66 and 70 housed pregnant Sprague-Dawley rats, while STS 80, the longest shuttle flight considered in this report, housed Sprague-Dawley rats with genetically induced hypertension and were fed either a high calcium or low calcium diet.

HOUSING:

Animal Enclosure Module (Diagram 1):

The total volume of an AEM is 17 X 20 X 9.62 inches, however the actual rodent cage in which the rats were housed occupied approximately a third (or 1100 inches³) of the total AEM volume (62). Drinking water and feeding systems were mounted within

AEM Top View
(drawing not to scale)

Diagram 1. Top view of an Animal Enclosure Module (AEM). Temperature probe locations are identified on the diagram. Diagram created by Mike Eodice of Lockheed and Martin and reproduced with permission.

the animal cage area and reduced the habitable area (61,62). The total animal floor space with the water box installed was 645 cm² (61). Four food bars (1.8 X 1 X 8 inches per food bar) were attached to four slide-in food bar plates inside the animal cage area and had a combined volume of approximately 58 inches³. The AEM with rats, food, and water weighed approximately 55-60 pounds. AEMs are placed in standard Shuttle middeck lockers while in flight. In flight AEMs can be partially removed from the locker so that the interior can be viewed and photographed through a Lexan cover on the top of the unit. Rats housed in the rodent cage are provided with ventilation, lighting, food, and water.

Ventilation:

A specialized air flow system created by four fans outside the cage area filtered the cabin air before it entered (rate = 0.0083 m³/second) the cage area, reduced the heat accumulation inside the cage area, and filtered the air prior to exhausting it into the cabin. The air entered the AEM through openings at the right and left sides of the front panel and eventually exited the AEM through the center of the front panel. Treated charcoal within the filters helped contain animal odor and neutralize urine within the AEM. It is a single pass, non-recirculating system.

Filtering System:

Waste products and odor are controlled through a filtering system.

Lighting and electrical system:

Rats are exposed to 12 hour light : 12 hour dark cycles. There are four lamps within the cage area, two produce light, while the other two are used as backups. The two

lamps produced an average of 14 lux of illumination (NASA TM#101077). Four fans, two lamps, and a timer operate at 28 VDC and require 28 watts.

Temperature:

Ambient Temperature Recorders (ATR-4) are small (23 x 41 x 86 mm and 135 grams), self-contained, battery charged temperature detectors capable of recording four channels of temperature data (60). They are capable of recording temperature ranging from -50 to +50 °C within 1° of accuracy. They are housed within the AEM and record the temperature at four different locations. For the purposes of this report, the temperature recordings from two locations were considered, the temperature of the air entering the cage area (T in) and the temperature of the air exiting the cage area (T out). The increase in temperature of the air flowing out of the cage area is attributed to the heat produced by the lamps and the rats. Temperature recordings were taken every 3.75 or 7.5 minutes depending on the mission. Approximately 90,000 temperature recordings, ranging from 22 to 35 °C, were considered in this report. Temperatures within the AEMs are reportedly elevated 3 to 8 °C above on-orbit ambient temperature.

Food and Water Consumption:

Rats had access to food and water *ad libitum*. Rats were fed hydrated Teklad food bars (Teklad, Inc. Madison, WI). When completely dry, the food bars have a caloric value of 3.45 kcal/gram. However, relatively new food bars contain 25% moisture and have a caloric value of 2.59 kcal/gram. For the purposes of this report, food bars are assumed to be relatively new and the caloric value of 2.59 kcal/gram will be used for all calculations. Food bars were attached to plates within the animal cage area via expoxy

glue. Adequate food was available for the duration of the flight. Food mass measurements were made before and after flight. Food consumption was determined based on the difference in these two measurements. Water was provided via the Rodent Drinking Water System and was refilled in flight as necessary via the AEM Water Refill Box stored in a middeck locker. Refill capabilities are necessary in longer flights (over five days). It has been noted that when the water supply is refilled on-orbit, it is difficult to determine the amount of water used in flight because the amount of water added cannot be accurately measured (60). Attempts to monitor water consumption involved observation of water levels through a Lexan window on the top of the water box (61). Final water consumption values were derived from a similar delta calculation using initial and final values and accounting for the water additions supplied during flight.

DATA COLLECTION AND STATISTICAL ANALYSES:

Data were compiled from an official AEM Database Report prepared by Dr. Marianne Steele at NASA Ames Research Center and from official reports housed in the document centers at NASA Ames Research Center. Official reports were housed in Building 240 Annex in Room 174. Reports were located using the NASA server, selecting the Life Sciences (Life Science) File, then accessing the Engineering Release Center (ERC) databases and searching for documents using key words to determine the appropriate protocol numbers (PCNs). Once the appropriate PCNs were determined, reports filed in Room 174 were located. Sharon Redfern, Engineering Release Center Administrator, oversaw these operations. Data were also compiled from NASA Life

Science Data Archive (<http://lsda.jsc.nasa.gov>), Life into Space, Space Life Sciences Experiments, Volumes 1 and 2 (61,60), extensive literature searches cited throughout this report, and personal communication with principal investigators involved in the STS missions. Additional information was obtained from sources at Harlan Teklad in Madison, WI, Lockheed Martin engineers at NASA Ames Research Center, the 2001 Taconic product guide publication, and set of control data obtained from a rat study at Davis, CA.

Excel and Statview were used to compile data, plot bivariate linear regressions, and perform statistical analyses with paired t-tests. Significant differences are noted at $p \leq 0.05$.

FORMULAS and CALCULATIONS:

Energy expenditure of the rats in term of kcal/rat/day and kcal/kg body mass/day was calculated using two independent methods (11):

Equation 1: $EE = E_{in} - E_{wg}$ EE proportional to $E_{in} - wg$	Equation 2: $Q = m \times c \times \Delta T$
Where: EE = energy expenditure in terms of food consumption and weight gain	Where: Q = heat produced by rats = cal/day
E_{in} = energy of food intake = total food use (grams) * 2.59 kcal/gram	m = mass of air flowing through the animal cage = 846 kg/day

E_{wg} = energy of weight gain	c = specific heat of air = 0.24cal/g/degree C
Wg = weight gain	ΔT = temperature difference (air in vs. air out) = change in air temperature caused by rat body heat

Equation 1: EE proportional to E_{in} – wg.

This equation is based on the first law of thermodynamics or conservation of energy. As simply stated by Carlson and Hsieh when E_{in} (or caloric intake by the animal) = E_{out} (or the heat produced or work done by the animal) body mass remains constant (11). However, when there is an imbalance between E_{in} and E_{out} , body mass changes. In Equation 1 energy expenditure (or EE, which for the purposes of this report represents Carlson and Hsieh's E_{out}) is shown to be proportional to caloric intake (E_{in}) and weight gain. This equation has been set up as a proportionality because body composition studies were not performed and only predictions could be made regarding the weight gain distribution and the consistency of this distribution. As a result it is impossible to predict E_{wg} . The EE values derived based on the assumption the weight gain distribution was constant ($EE = E_{in} - E_{wg}$) were compared to the Q values derived from the equation discussed below. Additionally, because EE was proportional to $E_{in} - wg$, comparisons were made between E_{in} values and the Q values.

Equation 2: $Q = m \times c \times \Delta T$

In this method the AEM is used as a calorimeter. We propose that the AEM, although not specifically designed for such use, could be used as a direct calorimeter due

to the peculiarities of the microgravity environment. The animal and the internal animal cage environment are in temperature equilibrium in the AEM and it was assumed that no heat was lost to or added from the external environment. In a normal calorimeter you must account for the transfer of heat through, conduction, convection, and radiation. However, in a microgravity environment, there is no convection (34) and heat loss through conduction and radiation was assumed to be minimal. Potential evaporative heat loss due to urine and feces production was not considered in these calculations. While it is possible that there was some evaporative heat loss to urine and feces production, it was not considered significant for the purpose of this report. It has been noted that the heat loss accounted for in a calorimeter does not include the heat given up by the body to bring ingested food and drinking water to body temperature, however in a standard calorimeter heat lost by the excreta cooling to calorimeter temperature is accounted for by the system (9). The primary goal of this research was to determine if there were trends and correlations that would support the hypothesis that the AEM could function as a calorimeter in future studies in which special efforts are made to account for variations in heat flux.

ADDITIONAL COMPARISONS:

Body mass gain and caloric consumption comparisons were also made between AEM rats and reference rats. Male Sprague-Dawleys, the largest subpopulation of AEM rats considered in this report, were selected from STSs 40, 52, 54, 60, 63, 77, 78 and were compared in terms of age and body weight on a Taconic reference growth chart for rats of

the same strain and gender. The averaged caloric intake of all AEM rats was compared to an independent group of control rats that were maintained under standardized conditions during earlier studies in the laboratory. These control rats were similar in body weight and age, however, they consumed a standardized laboratory diet and were housed under standardized conditions.

RESULTS

Mission details:

The details for each mission regarding flight name, date, time in flight, and time housing rats in AEMs are reported in Table 1. It should be noted that the rats spent approximately one day in the AEM before launch. Approximate time in AEM (spaceflight duration + 1 day) and actual time in flight are listed separately. Specific details on the rats housed within the AEMs during these missions are found in Table 2. Number of rats housed aboard each AEM, gender, and strain were important considerations in final comparisons used in this report. Calculations of daily body mass gain and food and water consumption are made based on the time spent in the AEM, whereas calculations made using ATR-4 temperature data are based on the actual time spent in flight. The specific details for each mission were needed to account for individual rat averages in body mass, body mass gain, food and water consumption, and in efforts to determine the average heat production of individual rats. Male Sprague-Dawleys were the most commonly used subject in these fifteen missions, however four missions used female rats, two of these missions studied pregnant rats (STS 66 and 70), and two missions (one with males, STS 57, and one with females, STS 62,) used a Fisher strain of rats. Starting ages of the rats ranged from 26 days old (STS 48) to 58 days old (STS 40). All flight data were compiled from spaceflight recordings collected from different missions flown between October 1990 and October 1996. The shortest flight

lasted just over four days (STS 41), whereas the longest time spent in flight was nearly eighteen days (STS 80).

Body Mass:

The body mass data presented in Table 3 represents the body mass determinations made at the time the rats were placed in the AEMs. The body masses were measured approximately one day before launch. Body mass data are reported as total group start body mass and individual mean rat start body mass. Total body mass data represent the combined body mass of all rats housed in a single AEM. The average individual rat mass is reported as rat start body mass. The standard deviations and ranges are listed for the individual start body mass.

Body mass data collected following landing and recovery are listed in Table 4. Total end mass represents the sum of all rats in a particular AEM and the average body mass of the individual rats have been reported as rat end body mass. The standard deviations and ranges are listed for the individual end body mass.

Total body mass averages for each AEM were calculated based the average of the start and end masses (Table 5). Totals account for the body mass of the rats based on the amount of time spent in the AEM, not time spent in flight.

There were no significant differences between the AEM flight and control group totals for start, end, or average body mass. The individual body mass averages of the AEM flight and control groups were not significantly different in start, end, or average body mass and are plotted as bivariate linear regressions for individual rat start (Figure

1), end (Figure 2), and average body mass (Figures 3). Regression analyses show a high correlation between flight and control groups in all three measurements (average slope (or m value) = 0.91 and average correlation coefficient (or r value) = 0.98). Flight rats started with slightly elevated body masses (y-intercept = 19.57) relative to control rats. This elevated body mass was consistently noted throughout flight as is indicated by the 17.12 gram increase in the average body mass measurements, and the 15.40 gram increase in the end body mass measurements.

Body Mass Gain:

The body mass gain reported in Table 5 is the total body mass gain for all rats housed in the AEM for the duration of the study. The body mass gain per rat per day is reported in Table 6. There is no significant difference in the daily individual body mass gain between the AEM flight and control rats and regression analyses demonstrate a correlation between groups (Figure 4).

Additional comparisons of body mass and age are made between individual rats housed in the AEMs and a reference control group housed and fed under standardized conditions (Figure 5). The inner boundaries of the Taconic Growth Chart in Figure 5 capture the average body mass $\pm$ one standard deviation from the mean and the outer boundaries capture the average body mass $\pm$ two standard deviations from the mean for Taconic male Sprague-Dawley rats. Taconic rats used here as a reference population were housed and fed in control laboratories over time. The male Sprague-Dawley rats from seven AEM missions (five of which came from Taconic Farms) were similar in

body masses at various ages to the Taconic reference rats used to develop this standardized growth curve for male Sprague-Dawley rats. The male Sprague-Dawley group (STS 41, 40, 52, 54, 56, 60, 63, 77, and 78) selected for these comparisons had a body mass average of 249 grams (range of 146 to 336 grams) and the average age was 7.7 weeks (range 5.7 to 9.7 weeks). Standard errors were calculated from the original data sets listed in Table 4 and are shown on the AEM groups represented in Figure 5.

Food Consumption:

Food consumption comparisons made between AEM flight and control groups based on individual daily consumption (Figure 6) and individual daily consumption adjusted for body mass (Figure 7) showed no significant differences between the groups.

Male Sprague-Dawley rats from STS 40, 52, 54, 60, 63, 77 (control group only), and 78 ($n = 25$ AEMs) were selected based on gender and strain. Data from these six spaceflights were compared in terms of body mass and caloric consumption to a group of rats housed and fed under standardized conditions (Figure 8). There were no significant differences in the body masses or daily caloric consumption of the selected AEM flight, selected AEM control, and standard control rats. The average body mass of the AEM control rats was 246 ± 34 grams ($n = 12$ AEMs), AEM flight rats 236 ± 35 grams ($n = 13$ AEMs), and standard control rats 255 ± 7 grams ($n = 16$ rats). Caloric consumption adjusted for body mass and reported as kcal/kg body mass/day was 291 ± 44 for AEM control rats, 302 ± 40 for AEM flight rats, and 287 ± 13 for standard control rats. The significant differences in the coefficients of variation for the AEM group averages

(control AEM CV = 14 and flight AEM CV = 15) relative to the standard control (standard control CV = 3) is due to the populations considered. AEM control and flight groups represent a single averaged value from AEM populations rather than individual rats (n = 12 AEMs in control AEM group and n = 13 AEMs in flight AEM group), whereas values reported for the standard control group are averages for individual rats from a single study. Additional calculations showed that the average coefficients of variation for AEM data within a single mission were closer to the coefficient of variation values of the standard control groups. The average coefficient of variation within individual STSs for flight and control AEMs was 2.1 for caloric consumption adjusted for body mass and 4.4 for body mass.

Water Consumption:

There was no significant difference in water use between the AEM flight and control groups for individual daily water consumption reported in ml/rat (Figure 9) nor in individual daily water consumption adjusted for body mass reported in ml/kg body mass.

In addition, comparisons to rats maintained under standardized laboratory conditions (Table 9), daily water consumption adjusted for body mass was significantly different between AEM groups and standardized control groups ($p < 0.05$). Such differences are most likely the result of measuring errors and environmental differences, such as a broad temperature range of 22 to 35 °C in the animal cage areas. Reported values are likely invalid. Difficulties in determining water consumption will be discussed later below.

Temperature Data and Heat Calculations:

Temperature data were collected from flight AEMs aboard STS 54, 56, 57, 60, 63, 77, 78, and 80. These missions were selected based on temperature data availability, temperature data were not available for all missions considered in this report. Temperature data from STS 54, 56, 57, 60, 63, 77, 78, and 80 were stored electronically and available for analyses. Two temperature readings recorded by the ATR-4 at either 3.75 or 7.5 minute time intervals were used to calculate the temperature difference between the air flow entering and exiting the animal cage area. Approximately 90,000 temperature recordings were considered in these analyses and final delta temperatures are reported in Table 8. Air entering the animal cage area (T_{in}) was heated by animals and two lamps and eventually exhausted from the area (T_{out}). The mass flow rate of the air through the AEM was the average of values found in ground based studies. For the purposes of this report and in order to perform the necessary calculations, the airflow through the system was assumed to be constant. Based on the results from ground based testing the constant mass airflow rate used in the calculations was appropriate (personal communication – Mike Eodice, 2001). Heat values were derived using the mass of the air flowing through the system, the specific heat of air, and the delta value of the air temperature entering and exiting the animal cage area. The heat contributions of the rats alone were determined by deducting the heat contribution of the lamps.

It was hypothesized that if the AEM unit functioned as a calorimeter, the derived values for heat produced by the rats would correlate to the values derived using the body

mass gain and caloric intake equation. Results, however, showed no such correlation. Further investigation into the matter is needed, and it may be necessary to determine the potential for conductive heat loss to the flight middeck area and evaporative heat loss to urine and feces. However, there were several consistent findings in the analyses of these fifteen AEMs. All delta temperature values were positive and the delta temperature average for the fifteen AEMs considered was 2.2 ± 0.6 °C. The in flowing air had an average temperature of 27.7 ± 1.1 °C and the out flowing air had an average temperature of 29.9 ± 0.8 . The coefficients of variation for in flowing and out flowing air were relatively low, 4.0 and 2.7 respectively (Table 9). Also an important trend was noted in regression analyses of total body mass of rats in an AEM versus the average delta temperature value for the AEM (Figure 11). As the total body mass of a group of rats housed in an AEM increased, there was an increase in the change in temperature of air flowing in and out of the AEM ($r = 0.54$ when all AEMs with ATR-4 data recordings were considered, $n = 15$ and $r = 0.70$ when STS 78 AEM # 2, an outlier, was excluded).

DISCUSSION

Metabolic Spaceflight Models:

The Human Model:

Scientists are trying to determine microgravity's effects on metabolism. Understanding metabolic changes resulting from exposure to microgravity may provide useful information that will guide scientists in their efforts to maintain the energy balance of astronauts. Currently astronauts are a poor model for such studies because they generally return from space having lost weight and in a negative state of energy balance (53,63,66,71). It is very difficult to distinguish between the possible impact of microgravity versus those of confounding variables and operational constraints on this negative state of energy balance. Such factors make the human a complicated model for studying microgravity's effects on metabolism. It seems that a more practical approach to studying the metabolic changes in spaceflight may be the use of a less confounded and more stable animal model.

The Monkey Model:

Use of the monkey model has provided limited data due to the small population size and limited number of times primates have flown in space. Additionally, frequent handling of the monkeys and its subsequent effect on control energy expenditure may have confounded data collected from studies of energy expenditure in spaceflight primates (24). Because of these experimental problems and limitations, past studies using

the primate model offer little insight into the details of potential metabolic shifts in spaceflight.

The Rat Model:

The rat is the most popular laboratory subject in space and a well studied subject in biology. A large database on the effects of microgravity on physiological systems has been established for rats. The rat has served as a useful model in several spaceflight studies involving bone metabolism (38,73), skeletal muscle (10,56), hormonal changes (37,40), and hepatic enzymes (1,2,23,25,35). Initial concerns over the responsiveness of the rat to weightlessness due to its small size and the direct relationship between size and sensitivity to gravitational loading (59) have been dismissed by the observed physiological responses of rats to spaceflight in several studies (38,47,73). Accordingly it seems that the rat would be sensitive to metabolic changes caused by microgravity and, therefore, serve as appropriate experimental model.

Furthermore, using the rat as a model has several advantages when compared to using humans or primates. The small size of the rat allows for a large population relative to those attainable using humans or monkeys. Metabolism in rats can be studied through energy expenditure measurements, body composition analyses, and examination of the extensive metabolic pathways mentioned above. Different techniques can also be utilized with rats, such as direct calorimetry and telemetry. Also advantageous is the ability to eliminate several of the variables that complicate human studies. Rats are not exposed to varying workloads that have confounded our understanding of human energy

requirements in spaceflight. Additionally, measuring caloric intake involves fewer variables with rats. Rats given free access to water and a uniformed diet in spaceflight can be compared more appropriately to controls than can astronauts, who are allotted a limited amount of time for eating in short missions. There is also greater variation in astronauts' diets while in flight depending on mission plan and personal dietary preferences compared to rats receiving a common diet. And finally, subject compliance is not an issue with rats, as it has been with astronauts in certain missions (63).

To better establish the rat's suitability as a model for future spaceflight studies on metabolism, it is important to consider factors that would indicate how well the rat adapts to spaceflight. Rats exposed to stress generally decrease food intake and lose weight, placing them in a negative energy state. This has been well established in studies inducing stress through restraint (6,33,58,74), housing and environmental changes (22,26,39), administration of electrical shocks, (16,17,27,55), and exposure to noise (3, 30). It has also been shown that a change in feeding container or location alone can cause a temporary decrease food intake by rats (13). Acceleration or centrifugation has been used to create a hypergravity environment in numerous animal studies and has also been noted to cause a stress response in rats. Centrifuged rats show decreases in body mass depending on gravitational load and exposure duration (42,48,50,57,68,69). Centrifuged rats show a dose-related decrease in food and water intake at the onset of centrifugation as well as a dose related increase in urine corticosterone levels dependent on gravitational load (40,43). As the rats adapt (5 to 7 days) food and water intake levels return to those of baseline and test rats have a mass gain rate similar to control rats (69). Corticosterone

levels elevated at the onset of centrifugation return to baseline levels after 4 days (40) and indicate that animals have adapted to the new gravitational load of centrifugation.

Reports indicating that rat body mass is unaffected or increased following spaceflight, such as those from Space Lab 3, support the hypothesis that rats adapt well to spaceflight and do not exhibit the typical stress response. Twelve large and twelve small rats were flown on Space Lab 3 to assess the husbandry capabilities of the Research Animal Holding Facility during spaceflight. Additionally, all animals consumed a Teklad 356 diet (20). Evaluations of the rats based on physical appearance, weight gain, and food and water consumption indicated that the rats were healthy, well nourished, and experienced minimal stress during spaceflight. Flight and control rats were housed individually and had the same weight gain and food and water consumption with food and water available *ad libitum*.

Flight and synchronous control rats from COSMOS 1129 and 1887 reportedly consumed similar amounts of food and water. However, it should be noted that in both cases the food was not provided *ad libitum* (51,21) . This confounds analyses if the rats could have consumed more and were limited by the amount provided. Also the finding that flight and control rats in COSMOS 1129 had similar growth rates (51,20) supports the hypothesis that rats may adapt better to spaceflight than humans. Unlike the human model, these rats appear to have an adequate appetite and no complications with food palatability.

Data from fifteen missions have been analyzed in the present study to evaluate the metabolic response of the rat to spaceflight. Analyses show no difference in food and

water consumption, body masses, and gain rates between the two AEM groups, flight and synchronous control. The high correlation between body mass gain rates of the flight and control rats considered here indicate that the flight and control groups share a common and apparently balanced energy state. However, it is necessary to determine whether these rats differ in these areas from rats housed and fed under standardized laboratory conditions. Additional comparisons were made to control groups housed and fed under standardized laboratory conditions to better assess the normality of these rats and evaluate their state of energy balance relative to “normal” rats. These analyses were necessary to determine if the space foodbar diet or the unique housing system affected the AEM population in a way that might complicate attempts to study metabolism as affected by microgravity per se.

Caloric intake comparisons were made between the AEM population and rats of similar age and weight to demonstrate that although they were on a different diet and in a different housing system, their caloric intake was comparable to control laboratory rats on a standardized diet. In addition, the largest subpopulation of the AEM population, the Sprague-Dawley male rats, was plotted on a standardized reference growth chart to show that AEM rats exhibit growth rates falling within the reported range for control laboratory rats. These findings indicate that AEM rats (flight and control) appear to be relatively “normal” rats based on caloric consumption and growth rate.

The data and analyses presented from the fifteen missions considered in this report indicate that the rat is an appropriate model for future metabolic studies on microgravity. The findings reported indicate that rats adapt favorably to spaceflight.

They do not exhibit signs of stress most often noted by significant changes in body weight. Even in the face of uncontrolled variables and inevitable complications in these missions, the results from the rats flown in AEMs support their potential use as models in future spaceflight studies on metabolism. The reported results, which show no significant differences between control and flight groups in body mass, growth rate, or food and water use, support adaptation and indicate that AEM rats are in a healthy state. Additional outside comparisons showing no difference between caloric intake and growth rate in AEM rat and control rats maintained under standardized conditions offer additional support for the use of the rat model future spaceflight studies on metabolism.

Based on the findings noted above, the rat appears to be an appropriate model for studying metabolic changes resulting from exposure to microgravity. Metabolic studies on rats can be performed using different methods and combinations of these methods. Three separate approaches are considered in this report. Studies on different metabolic pathways and key enzymatic shifts have been performed and were discussed in the introduction. Body composition studies have been performed on spaceflight rats, however due to study complications, the findings have been inconclusive and further research is needed in this area (45,51,67). The potential for future energy expenditure studies using rats is promising and this report considers the potential for use of direct calorimetry in future studies on energy expenditure in spaceflight.

Body Composition:

COSMOS 1129 caged five rats individually onboard the 18.5 day mission. One objective of the study was to determine the effects of weightlessness on body composition. Comparisons were made among the flight group, a synchronous control group with the same housing situation and feeding regimen (10 grams every 6 hours), and a vivarium control group where rats were housed 3 rats per cage and fed a different diet and had a different feeding regimen (120 grams/cage/day). It was difficult to make comparisons between body masses due to age differences among groups. Relative body composition analyses were made, but results were likely confounded by an unanticipated delay in animal recovery following flight and a 25 ± 1 hour fast prior to death. Additionally, the flight and synchronous control rats consumed a paste mixture diet composed of 60% water. Reports indicate significantly increased body fat and decreased fat-free body mass in flight animals versus synchronous control and in synchronous control versus vivarium control. Synchronous control rats showed a 79% increase in body fat and a 5% decrease in fat-free body mass when compared to the vivarium controls, both statistically significant (51). In an additional study, the effect of the unique paste diet used in spaceflight on body composition was assessed. Results show that rats fed 40 grams/day of the paste diet used in COSMOS 1129 over 18.5 days have experienced a 52% increase in body fat and a 16% decrease in fat-free body mass compared to rats consuming a standard laboratory diet (45). Thus assessing microgravity's role in body composition changes in spaceflight rats for these studies

requires separating the effects of this unique diet from those thought to be caused by microgravity, making analyses complicated.

Ten rats group housed onboard the COSMOS 1887 biosatellite were fed 14 grams of a Soviet paste diet of 70% water every 6 hours. In this study there were complications in recovering the rats, the animals were fasted for 42 hours after landing, and exposed to lower temperatures than those during flight. Although the flight rats consumed approximately the same amount of food and water as the synchronous controls, they gained only 16% of the amount gained by the synchronous controls. It was hypothesized that flight rats in this study expended more energy and/or absorbed and metabolized the diet less efficiently. Findings in this study such as increased liver glycogen levels of flight rats support the theory of metabolic shifts (21).

While the aforementioned studies using the rat model have provided some information on potential metabolic changes in spaceflight, they are limited by variation between studies and confounded by several variables. Comparisons made between flights are confounded by flight duration, diet and housing conditions, rat age and strain, complications in recovery of rats, and variation in treatment and exposure time to 1G before death. The conflicting data over growth rates in these rats have prompted further investigation into the matter. Using literature from hypergravity and spaceflight studies Wade *et al.* developed two models to explore potential shifts in rat body mass during spaceflight (70). Centrifuged rats experience a dose related weight loss dependent on the gravitational load during centrifugation. When rats return to a 1G environment, they gain weight indicating that body mass is greatly influenced by gravitational deviations from

the 1G environment. Using data from eighteen publications on centrifugation Wade *et al.* produced a model demonstrating the inverse relationship between gravitational load and body mass, and predicted a 27% increase in body mass during exposure to microgravity. Twenty-three publications were used to create a model representative of the change in body mass following return from spaceflight. The sooner the flight animals were weighed after landing, the greater the positive deviation from the control animals masses. In the three days post-landing the flight animals lose body mass, following which they show an increase in mass gain and body masses six to seven days after landing are comparable to the control body masses. (70)

Delay in recovery and weighing of the rats in previous spaceflights have complicate interpretations of the past body weight data. Rats in COSMOS 1129 were sacrificed 36 ± 2 hours after reentry (25 ± 2 hours after last feeding) and rats from COSMOS1887 were weighed and sacrificed 53-56 hours after reentry (with a 42 hour fast during recovery). These delays in obtaining body weight data following landing, considered in light of Wade *et al.*'s microgravity model, indicate that the reported body masses for these flight rats are most likely not representative of a spaceflight model. Changes reported from COSMOS 1129 on body composition are probably not the result of exposure to a microgravity environment *per se*. Extended periods of fasting in these studies complicate matters further because it has been shown that the relationship between feeding and weight is not always clear. In a study on the effects of fasting in rats, rats fasted for 24 to 96 hours were not always able to return to a nondeprived control level (5). Additional studies with fewer complications are needed to resolve these issues.

Energy Expenditure:

It was hypothesized that the AEMs could function as direct calorimeters and used to determine heat production by the rats. In his book, Control of Energy Exchange, Carlson and Hsieh introduces a direct calorimetry system that relies on a temperature difference between the inlet and outlet temperatures and a constant and known airflow. These are the same parameters that were measured in the AEM. However, in Carlson and Hsieh's model, the heat loss, while it is minimal because the outer room is maintained at calorimeter temperature, is accounted for through disks mounted on the walls of the system (11). Assumptions were made regarding heat loss from the AEM to the cabin. Heat will not be lost through convection, which is a streaming of heating into the air as a result of the air's temperature dependent density gradient, because in a microgravity environment such a gradient does not exist. The potential for heat escape through conduction, the transfer of heat between molecules of different substances, was assumed to be minimal and was not accounted for in these AEM calorimetry calculations. As noted earlier, temperatures within the AEMs are elevated 3 to 8 °C above on-orbit ambient temperature. Studies on the AEM's thermal performance in the space shuttle middeck revealed that accurate predictions of AEM internal temperature cannot be determined based on shuttle middeck ambient temperature because of the numerous variables present in the middeck environment (18). Both the elevated ambient air temperature of the middeck and the inability to predict AEM internal temperature based on the ambient middeck air temperature complicate the calorimetry equations in this

report. Based on a calorimetry system devised by Carlson and Hsieh the proposal to use the AEM as a calorimetry systems seems very feasible, especially when the unique benefits a microgravity environment are considered.

Additional data collected from the fifteen flights analyzed in this report were used to consider new methods for studying metabolism in spaceflight rats. Analyses of the body mass, growth rate, and food and water use data collected from AEM rats show no significant differences between flight and control groups. Assuming weight gain distribution is uniform for the flight and control rats a high correlation in energy expenditure between the groups was expected. Energy expenditure was determined based on Equation 1, $EE = E_{in} - E_{wg}$. The values determined for EE were then compared to animal heat production (Q) values derived based on the assumption that the AEM was functioning as a calorimeter. When these values did not show a correlation, comparisons were made between caloric intake (E_{in}) and Q because E_{in} is assumed to be proportional to EE and this would remove the weight gain element of the equation. Again there was no correlation. There was not a correlation between the EE and Q values derived in this report, nor the caloric intake (E_{in}) and Q values.

While there was no correlation between the energy expenditure values derived using the two separate methods, there were many positive findings in the data analyses and the idea of using calorimeters in future spaceflight studies with rats should not be dismissed. The decision to consider the AEM's potential for use as a calorimeter was made after the missions had been completed. Special efforts to control for complicating variables were not made. As noted earlier, differences in AEM internal temperatures and

ambient middeck temperatures could have confounded our attempts to use the AEM as a calorimeter. Also, while likely an insignificant factor, it was impossible to account for evaporative heat loss to urine and feces. Flight variations, as well as in others unaccounted for variables may have influenced temperature readings. Perhaps the ATR-4 with four temperature probes capable of taking four independent temperature readings could be used in future studies to account for potential heat loss to the middeck environment if placed strategically within the AEM and outside the AEM.

However, even with the confounding variables involved in these missions, consistent findings in the data analyses indicate the AEM's potential for use as a calorimetry system in future spaceflights. The delta temperatures reported for the AEMs considered in this report had a temperature range of 1.3 to 2.8 °C. All delta temperatures were positive. This indicates that the animals and lamps had heated the air leaving the animal caging area of the AEM. Additionally, the trend in increasing body mass and increasing delta temperature values (Figure 11) mimics correlations noted in metabolic studies on mammals by Kleiber. Kleiber's findings show that metabolic rate (or heat production in kcal/day) is to correlates positively to body weight (29). The derived delta temperature values from the AEM animal cage areas should be an indication of the amount of heat produced by the animals (or an indication of metabolic rate). Analyses in this report show a correlation between these delta temperature values and the body masses of the rats, as would be predicted by Kleiber's findings. Furthermore, Kleiber has shown that metabolic rate is proportional to the three-fourth power of body weight. The body mass totals of the AEM rats were taken to the three-fourths power and regressional

analyses performed against the delta temperature values. However, because of the narrow body mass range of the AEM subjects in this report relative to the wide body mass range used in Kleiber's studies, the correlation values from this analysis were the same as those found in the delta temperature versus total body mass analysis (Figure 11).

Future:

Although extensive research has been done on certain areas of metabolism in rats exposed to hypergravity and microgravity, it is difficult to understand the true metabolic changes induced by microgravity. Additional research is needed in this area and particularly research on the effects of chronic exposure to microgravity. Results from future research will be more convincing if the confounding variables that complicated data interpretations in the past are resolved before future testing.

The rat's sensitivity to confounding experimental variables has made past data interpretation difficult, however the rat is a valuable model in that it has demonstrated noted physiological responses to microgravity in past studies. The rat's sensitivity to microgravity exposure has been useful in past spaceflight studies on microgravity's effects on biology and will be helpful in the future studies where investigators hope to have better control over experimental variables and can more definitively attribute metabolic changes noted during spaceflight to microgravity.

The primary objective of this report is to offer further support for the use of the rat model in future spaceflight studies of metabolism. The findings reported here show that AEM flight rats appear to be metabolically stable. AEM flight and control rats have similar body masses, mass gain rates, and consume the same amount of food. In

additional comparisons to control laboratory rats housed and fed under standardized conditions AEM rats did not differ significantly from “normal” rats in terms of caloric intake and body mass gain rate. AEM flight rats appear to maintain energy balance and in such a balanced state could provide insight to metabolic shifts via body composition analyses or energy expenditure studies in the future.

Overall the AEM rats appear to adapt favorably to their unique housing environment and diet. Rats in these fifteen missions housed in AEMs appear to be healthy and well adapted. AEM flight and control rats with similar caloric intakes and growth rates, and maintained under identical housing and feeding conditions are a promising model for metabolic studies. Future research using the rat in balanced energy states for body composition, energy expenditure, and metabolic pathway analyses will be key to understanding metabolic changes in flight rats. Such insight into microgravity’s effect on metabolism is needed to resolve problems facing astronauts chronically exposed to microgravity in long-term missions and for habitation of environments with gravity levels other than 1G.

BIBLIOGRAPHY

1. Abraham S, Lin CY, Klein, Volkmann C, Tigranyan RA, Vetrova EG. Studies of specific hepatic enzymes involved in the conversion of carbohydrates to lipids in rats exposed to prolonged spaceflight aboard COSMOS-1129. *Physiologist* 1980; 23:S55-8.
2. Abraham S, Lin CY, Volkman, and Klein HP. Biochemical changes in rat liver after 18.5 days of spaceflight. *Proc Soc Exptl Biol Med* 1983; 172:334-9.
3. Alario P, Gamallo A, Beato MJ, Trancho G. Body weight gain, food intake and adrenal development in chronic noise stressed rats. *Physiol Behav* 1987; 40:29-32.
4. Altman PL and Talbot JM. Nutrition and metabolism in spaceflight. *J Nutr* 1987; 117:421-7.
5. Armstong S, Coleman G, Singer G. Food and water deprivation: changes in rat feeding, drinking, activity and body weight. *Neurosci Biobehav Rev* 1980; 4:377-402.
6. Bell EM, Bhatnagar S, Akana SF, Choi S, Dallman MF. Disruption of arcuate/paraventricular nucleus connections changes body energy balance and response to acute stress. *J Neurosci* 2000; 20(17):6707-13.
7. Berry CA. Summary of medical experience in the Apollo 7 through 11 manned spaceflights. *Aerospace Med* 1970; 41(5):500-19
8. Blanc S, Normand S, Ritz P, Pachiaudi C, Vico L, Gharib C, Gauquelin-Koch G. Energy and water metabolism, body composition, and hormonal changes induced by 42 days of enforced inactivity and simulated weightlessness. *J Clin Endocrinol Metab* 1998; 83:4289-97.
9. Blaxter, K. Energy Metabolism in Animals and Man. 1989. Cambridge University Press, New York. p. 41.
10. Bonting, SL. Advances in Space Biology and Medicine. Vol. 7.1999. JAI Press Inc., Connecticut. p. 2-22
11. Carlson LD and Hsieh AC. Control of Energy Exchange. 1970. The MacMillan Company, New York. p.122-123.

12. Committee on Space Biology and Medicine Space Studies Board. A Strategy for Research in Space Biology and Medicine in the New Century. 1998. National Academy Press, Washington, D.C. p. 100-101.
13. Corey DT. The determinants of exploration and neophobia, *Neurosci Biobehav Rev* 1978; 2:235-53.
14. Daligcon BC and Oyama J. Increased gluconeogenesis in hyper-G stressed rats. *Physiologist* 1982; 25 (6): S87-8.
15. Daligcon BC, Oyama J, Hannak K. Increased gluconeogenesis in rats exposed to hyper-G stress. *Life Sciences* 1985; 37: 235-241.
16. Dess NK, Minor TR, Brewer J. Suppression of feeding and body weight by inescapable shock: modulation by quinine adulteration, stress reinstatement, and controllability. *Physiol Behav*. 1989; 45:975-83.
17. Dess NK, Raizer J, Chapman CD, Garcia J. Stressors in the learned helplessness paradigm: effects on body weight and conditioned taste aversion in rats. *Physiol Behav* 1988; 44:483-90.
18. Eodice MT. Modeling Animal Enclosure Module (AEM) thermal performance in the Space Shuttle Middeck. SAE Paper 961362.1996. NASA Ames Research Center, Moffett Field, CA. p.1-4.
19. Evans JW, Smith AH, Boda JM. Fat metabolism and chronic acceleration. *Am J Physiol* 1969; 216(6):1468-71.
20. Fast T, Grindeland R, Kraft, L, Ruder M, Vasques M, Lundgren P, Scibetta S, Tremor J, Buckendahl P, Keil L, Chee O, Reilly T, Dalton B, Callahan p.. Rat maintenance in the Research Animal Holding Facility during the flight of Space Lab 3. *Physiologist* 1985; 28(6Suppl):S187-8.
21. Grindeland RE, Popova IA, Vasques M, Arnaud SB. Cosmos 1887 mission overview: effects of microgravity on rat body and adrenal weights and plasma constituents. *FASEB J* 1990; 4:105-9.
22. Hall FS, Humby T, Wilkinson LS, Robbins TW. The effects of isolation-rearing of rats on behavioral responses to food and environmental novelty. *Physiol Behav* 1997; 62:281-90.
23. Hargrove JL and Jones DP. Hepatic enzyme adaptation in rats after spaceflight. *Physiologist*; 1985; 28(6):S230.

24. Hoban-Higgins TA, Stein TP, Dotsenko MA, Korolkov VI, Fuller CA. Energy metabolism of *Macaca mulatta* during spaceflight. *J Grav Physiol* 2000; 7(1):S145-8.
25. Hollander J, Gore M, Fiebig R, Mazzeo R, Ohishi S, Ji LL. Spaceflight downregulates antioxidant defense systems in the rat liver. *Free Radical Bio and Med* 1998; 24(2):385-90.
26. Honey RC, Pye C, Lightbown Y, Rey V, Hall G. Contextual factors in neophobia and its habituation: the role of absolute and relative novelty. *Q J Exp Psychol B* 1992; 45:327-47.
27. Job RFS and Barnes BW. Stress and consumption: inescapable shock, neophobia, and quinine finickiness in rats. *Behav Neurosci* 1995; 109:106-26.
28. Johnson PC, Rambaut PC, and Leach CS. Apollo16 bio energetic considerations. *Nutr Metabol* 1974; 16:119-126.
29. Kleiber, M. The Fire of Life, an introduction to animal energetics. 1975. John Wiley & Sons, Inc. New York.
30. Krebs H, Macht M, Weyers P, Weijers H, Janke W. Effects of stressful noise on eating and non-eating behavior in rats. *Appetite* 1996; 26:193-202.
31. Lane HW and Schoeller DA. Nutrition in Spaceflight and Weightlessness Models. 2000. CRC Press, New York. p.113-4.
32. Leonard JJ, Leach CS, Rambaut PC. Quantitation of tissue loss during prolonged space flight. *Amer J Clin Nutr* 1983; 38:667-79.
33. Marti O, Marti J, Armario A. Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol Behav* 1994; 55:747-53.
34. McCally, M. Hypodynamics and Hypogravics. The Physiology of Inactivity and Weightlessness. 1968. Academic Press. New York. p 114.
35. Merrill AH, Wang E, Hargrove JL. Hepatic enzymes of sphingolipid and glycerolipid biosynthesis in rats from Space Lab 3. *Physiologist* 1985; 28(6):S229.
36. Merrill AH, Wang E, LaRocque R, Mullins RE, Morgan ET, Hargrove JL, Bonkovsky HL, Popova IA. Differences in glycogen, lipids, and enzymes in livers from rats flown on COSMOS 2044. *J App Physiol* 1992; 73(2):142S-147S.

37. Merrill AH, Wang E, Mullins RE, Grindeland RE, Popova, IA. Analyses of plasma for metabolic and hormonal changes in rats flown aboard COSMOS 2044. *J Appl Physiol* 1992; 73(2):132S-135S.
38. Morey-Holton ER, Halloran BP, Garetto LP, Doty SB. Animal housing influences the response of bone to spaceflight in juvenile rats. *J Appl Physiol* 2000; 88(4):1303-1309.
39. O'Connor R and Eikelboom R. The effects of changes in housing on feeding and wheel running. *Physiol Behav* 2000; 68:631-71.
40. Ortiz RM, Wang TJ, Wade CE. Influence of centrifugation and hindlimb suspension on testosterone and corticosterone excretion in rats. *Aviat Space Environ Med* 1999; 70(5):499-504.
41. Oyama J and Daligcon BC. Liver Glycogenesis, Glycogen Synthetase and Adrenal Responsiveness of Rats Exposed to Acceleration Stress. *Endocrinology* 1967; 80:707-13.
42. Oyama J and Platt WT. Effects of prolonged centrifugation on growth and organ development of rats. *Am J Physiol* 1965; 209(3):611-5.
43. Oyama J and Platt WT. Metabolic Alterations in Rats Exposed to Acute Acceleration Stress. *Endocrinology*. 1965; 76: 203-9.
44. Oyama J and Zeitman B. Tissue composition of rats exposed to chronic centrifugation. *Am J Physiol* 1967; 213(5):1305-10.
45. Pace N, Rahlmann DF, Smith AH, Pitts GC. Effects of the Cosmos-1129 Soviet paste diet on body composition in the growing rat. NASA-CR-164725. National Aeronautics and Space Administration, Washinton D.C. 1981.
46. Pace N, Smith AH, Rahlmann DF. Skeletal mass change as a function of gravitational loading. *Physiologist* 1985; 28(6):S17-S20.
47. Pitts GC. Body size and chronic acceleration. *Life Sci Space Res* 1977; 15:251-6.
48. Pitts GC. Effects of chronic acceleration on body composition. *Physiologist* 1982; 25(6):S13-S16.
49. Pitts GC, Bull LS, Oyama J. Effect of chronic centrifugation on body composition in the rat. *Am J Physiol* 1972; 223(5):1044-8.

50. Pitts GC, Bull LS, Oyama J. Regulation of body mass in rats exposed to chronic acceleration. *Am J Physiol* 1975; 228:714-7.
51. Pitts GC, Ushakov AS, Pace N, Rahlmann DF, Smirnova TA. Effects of weightlessness on body composition in the rat. *Am J Physiol* 1983; 244:R332-7.
52. Rambaut PC, Heidelbaugh ND, Reid JM, Smith MS. Caloric balance during simulated and actual space flight. *Aerospace Med* 1973; 44(11):1264-9.
53. Rambaut PC, Leach CS, Leonard JJ. Observations in energy balance on man during spaceflight. *Am J Physiol* 1977; 2(3):R208-R212.
54. Rambaut PC, Smith MC, Leach CS, Whedon GD, Reid J. Nutrition and responses to zero gravity. *Fed Proc* 1977; 36(5):1678-82.
55. Rickards SL, Job RFS, Boakes RA. Effects of acute shock on body weight are mediated by changes in food intake. *Anim Learn Behav.* 1997; 25:437-45.
56. Riley DA, Ellis S, Slocum GR, Sedlak FR, Bain JLW, Krippendorf BB, Lehman CT, Macias MY, Thompson JL, Vijayan K, De Bruin JA. In-flight and postflight changes in skeletal muscles of SLS-1 and SLS-2 spaceflown rats. *J Appl Physiol* 1996; 81(1):133-144
57. Roy RR, Roy ME, Talmadge RJ, Mendoza R, Grindeland RE, Vasques M.. Size and myosin heavy chain profiles of rat hindlimb extensor muscle fibers after two weeks at 2 G. *Aviat Space Environ Med* 1996; 67(9): 854-8.
58. Rybkin II, Zhou Y, Volaufova J, Smagin GN, Ryan DH, Harris RBS. Effect of restraint stress in food intake and body weight is determined by the time of day. *Am J Physiol* 1997; 273: R1612-22.
59. Smith, AH and Burton, RR. Chronic acceleration of animals. *Gravity and the Organism*, edited by SA Gordon and MJ Cohen. Chicago: Univ. Chicago Press, 1971, p. 371-88.
60. Souza K, Etheridge G, Callahan PX. Life into Space, Space Life Sciences Experiments NASA Ames Research Center 1991-1998. Volume 2. NASA/SP – 2000-534. National Aeronautics and Space Administration, Ames Research Center. p. 464,468.
61. Souza K, Hogan R, Ballard R. Life into Space, Space Life Sciences Experiments NASA Ames Research Center 1965-1990. Volume 1. NASA Reference Publication – 1372. National Aeronautics and Space Administration, Ames Research Center. p. 460.

62. Stehle S. Animal Enclosure Module (AEM) Crew Training Familiarization Manual, Lockheed Martin Engineering & Sciences, NASA Ames Research Center 1996; CT-080, Rev. D. p. 1,11.
63. Stein TP. Nutrition in the space station era. J Nutr (in press) p.1,29.
64. Stein TP, Dotsenko MA, Korolkov VI, Griffin DW, Fuller CA. Energy expenditure in rhesus monkeys (*Macaca mulatta*) during space flight using doubly labeled water ($^2\text{H}_2^{18}\text{O}$). J Appl Physiol 1996; 81(1):201-7.
65. Stein TP and Gaprindashvili T. Spaceflight and protein metabolism, with special reference to humans. Am J Clin Nutr 1994; 60:806S-19S.
66. Stein TP, Leskiw MJ, Schluter MD, Hoyt RW, Lane HW, Gretebeck RE, LeBlanc AD. Energy expenditure and balance during spaceflight on the space shuttle. Am J Physiol 1999; 276:R1739-R1748.
67. Ushakov AS, Smirnova TA, Pitts GC, Pace N, Smith AH, Rahlmann DF. Body composition of rats flown aboard COSMOS-1129. Physiologist 1980; 23:S41-4.
68. Vico L, Barou O, Laroche N, Alexandre C, Lafage-Proust MH. Effects of centrifuging at 2 g on rat long bone metaphyses. Eur J Appl Physiol Occup Physiol. 1999; 80(4):360-6.
69. Wade CE, Harper JS, Daunton NG, Corcoran ML, Morey-Holton E. Body mass change during altered gravity: spaceflight, centrifugation, and return to 1 G. J Grav Physiol 1997; 4:43-8.
70. Wade CE, Ortiz RM, Baer LA. Increases in body mass of rats during spaceflight: models and measurements. Aviat Space Environ Med 2000; 71:1-5.
71. Webb P. Weight loss in men in space. Science 1967; 155:558-9.
72. Whittle MW. Caloric and exercise requirements of space flight: biostereometric results from Skylab. Aviat Space Environ Med 1979; 50(2):163-7.
73. Wronski TJ, Li M, Shen Y, Miller SC, Bowman BM, Kostenuik P, Halloran BP. Lack of effect of spaceflight on bone mass and bone formation in group-housed rats. J of Appl Physiol 1998; 85(1):279-285.
74. Zylan KD and Brown SD. Effect of stress and food variety on food intake in male and female rats. Physiol Behav 1996; 59:165-9.

APPENDIX

Table 1. Mission plans for fifteen spaceflight missions considered.

STS* Animal Group	PAYLOAD	AEM** Serial Number	DATE	AEM TIME*** (APPROX = flight + 1 day)	FLIGHT TIME **** (days)
41-F	PSE.01	1	Oct-90	5.0	4.1
41-F	PSE.01	2		5.0	4.1
41-AC	PSE.01	1	Dec-90	5.0	
41-AC	PSE.01	2		5.0	
40-GC	SLS-1	1	May-91	10.0	
40-F	SLS-1	2		10.0	9.1
40-F	SLS-1	3		10.0	9.1
40-GC	SLS-1	4		10.0	
48-F	PARE.01	1	Sep-91	6.4	5.4
48-AC	PARE.01	1	Oct-91	6.4	
52-F	PSE.02	1	Oct-92	11.0	10.0
52-F	PSE.02	2		11.0	10.0
52-SC	PSE.02	1	Oct-92	11.0	
52-SC	PSE.02	2		11.0	
54-F	PARE.02	1	Jan-93	7.0	6.0
54-SC	PARE.02	1	Jan-93	7.0	
56-F	PARE.03	1	Apr-93	10.0	9.3
56-F	PARE.03	2		10.0	9.3
56-SC	PARE.03	1	Apr-93	10.0	
56-SC	PARE.03	2		10.0	
57-F	PSE.03	1	Jun-93	12.0	10.0
57-F	PSE.03	2		12.0	10.0
57-SC	PSE.03	1	Jun-93	12.0	
57-SC	PSE.03	2		12.0	
60-F	IMMUNE.1	1	Feb-94	9.0	8.3
60-F	IMMUNE.1	2		9.0	8.3
60-SC	IMMUNE.1	1	Feb-94	9.0	
60-SC	IMMUNE.1	2		9.0	

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AEM** = Animal Enclosure Module

AEM time*** = Animals were placed in the AEMs approximately one day before launch.

Flight time****= reference:[http:// alsda.arc.nasa.gov/office/pao/History/pocketstats/sect%20B/P&E.pd](http://alsda.arc.nasa.gov/office/pao/History/pocketstats/sect%20B/P&E.pd)

Table 1 (continued). Mission plans for fifteen spaceflight missions considered.

STS* Animal Group	PAYLOAD	AEM** Serial Number	DATE	AEM TIME*** (APPROX = flight + 1 day)	FLIGHT TIME **** (days)
62-F	PSE.04	1	Mar-94	15.0	14.0
62-F	PSE.04	2		15.0	14.0
62-SC	PSE.04	1	Mar-94	15.0	
62-SC	PSE.04	2		15.0	
66-F	NIH.R1	1	Nov-94	11.0	na
66-F	NIH.R1	2		11.0	
66-SC	NIH.R1	3	Nov-94	11.0	
66-SC	NIH.R1	4		11.0	
63-F	IMMUNE.2	1	Feb-95	9.0	8.2
63-F	IMMUNE.2	2		9.0	8.2
63-SC	IMMUNE.2	1	Feb-95	9.0	
63-SC	IMMUNE.2	2		9.0	
63-SC	IMMUNE.2	3		9.0	
70-F	NIH.R2	1	Jul-95	10.0	8.9
70-F	NIH.R2	2		10.0	8.9
70-SC	NIH.R2	3	Jul-95	10.0	
70-SC	NIH.R2	4		10.0	
77-F	IMMUNE.3	7	May-96	11.0	10.0
77-F	IMMUNE.3	8		11.0	10.0
77-VC	IMMUNE.3			11.0	
77-VC	IMMUNE.3			11.0	
78-F	LMS	3	Jun-96	18.0	16.9
78-F	LMS	4		18.0	16.9
78-GC	LMS	1		18.0	
78-GC	LMS	2		18.0	
80-F	NIH.R4	7	Oct-96	18.5	17.7
80-F	NIH.R4	8		18.5	17.7
80-VC	NIH.R4	1		18.5	
80-VC	NIH.R4	2		18.5	

na = not available

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AEM** = Animal Enclosure Module

AEM time*** = Animals were placed in the AEMs approximately one day before launch.

Flight time****= reference: <http://alsda.arc.nasa.gov/office/pao/History/pocketstats/sect%20B/P&E.pdf>

Table 2. Rat details for fifteen spaceflight missions considered.

STS* Animal Group	AEM#	# of RATS	GENDER	STRAIN	VENDOR
41-F	1	8	male	S-D	Charles River
41-F	2	8	male	S-D	Charles River
41-AC	1	8	male	S-D	Charles River
41-AC	2	8	male	S-D	Charles River
40-GC	1	5	male	S-D	Taconic
40-F	2	5	male	S-D	Taconic
40-F	3	5	male	S-D	Taconic
40-GC	4	5	male	S-D	Taconic
48-F	1	8	female	S-D	Taconic
48-AC	1	8	female	S-D	Taconic
52-F	1	6	male	S-D	Taconic
52-F	2	6	male	S-D	Taconic
52-SC	1	6	male	S-D	Taconic
52-SC	2	6	male	S-D	Taconic
54-F	1	6	male	S-D	Taconic
54-SC	1	6	male	S-D	Taconic
56-F	1	8	male	S-D	Taconic
56-F	2	8	male	S-D	Taconic
56-SC	1	8	male	S-D	Taconic
56-SC	2	8	male	S-D	Taconic
57-F	1	6	male	Fisher	Taconic
57-F	2	6	male	Fisher	Taconic
57-SC	1	6	male	Fisher	Taconic
57-SC	2	6	male	Fisher	Taconic
60-F	1	6	male	S-D	Charles River
60-F	2	6	male	S-D	Charles River
60-SC	1	6	male	S-D	Charles River
60-SC	2	6	male	S-D	Charles River

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

S-D = Sprague-Dawley

Table 2 (continued). Rat details for fifteen spaceflight missions considered.

STS* Animal Group	AEM#	# of RATS	GENDER	STRAIN	VENDOR
62-F	1	6	female	Fisher	Taconic
62-F	2	6	female	Fisher	Taconic
62-SC	1	6	female	Fisher	Taconic
62-SC	2	6	female	Fisher	Taconic
66-F	1	5	female-P	S-D	Taconic
66-F	2	5	female-P	S-D	Taconic
66-SC	3	5	female-P	S-D	Taconic
66-SC	4	5	female-P	S-D	Taconic
63-F	1	6	male	S-D	Taconic
63-F	2	6	male	S-D	Taconic
63-SC	1	6	male	S-D	Taconic
63-SC	2	6	male	S-D	Taconic
63-SC	3	6	male	S-D	Taconic
70-F	1	5	female-P	S-D	Taconic
70-F	2	5	female-P	S-D	Taconic
70-SC	3	5	female-P	S-D	Taconic
70-SC	4	5	female-P	S-D	Taconic
77-F	7	6	male	S-D	Taconic
77-F	8	6	male	S-D	Taconic
77-VC		8	male	S-D	Taconic
77-VC		8	male	S-D	Taconic
78-F	3	6	male	S-D	Taconic
78-F	4	6	male	S-D	Taconic
78-GC	1	6	male	S-D	Taconic
78-GC	2	6	male	S-D	Taconic
80-F	7	7	male (SHR-L)	S-D	Taconic
80-F	8	7	male (SHR-H)	S-D	Taconic
80-VC	1	7	male (SHR-L)	S-D	Taconic
80-VC	2	7	male (SHR-H)	S-D	Taconic

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

P = pregnant

SHR-H = Spontaneously Hypertensive Rats fed a high calcium diet

SHR-L = Spontaneously Hypertensive Rats fed a low calcium diet

S-D = Sprague-Dawley

Table 3. Body mass data collected when the rats were placed in the AEMs approximately one day before launch. Total start body mass represents the combined body mass of all rats housed in a single AEM. Rat start body mass represents the average individual body mass of rats. Individual start body mass standard deviation and range are listed.

STS* Animal Group	AEM#	# of RATS	TOTAL START BM (grams)	RAT START AVE BM (grams)	RAT START AVE BM SD	RAT START AVE BM RANGE (grams)
41-F	1	8	912	114	5.4	103-120
41-F	2	8	1024	128	5.4	120-138
41-AC	1	8	984	123	8.5	111-138
41-AC	2	8	992	124	7.2	114-137
40-GC	1	5	1390	278	9.8	264-291
40-F	2	5	1430	286	25.0	249-321
40-F	3	5	1435	287	10.0	275-299
40-GC	4	5	1440	288	14.6	277-308
48-F	1	8	472	59	1.8	57-61
48-AC	1	8	416	52	3.0	47-56
52-F	1	6	1078	180	9.4	169-192
52-F	2	6	1068	178	2.4	175-181
52-SC	1	6	1112	185	8.1	171-194
52-SC	2	6	1102	184	7.7	174-194
54-F	1	6	1050	175	3.1	170-178
54-SC	1	6	1103	184	3.9	178-188
56-F	1	8	1116	140	5.3	133-148
56-F	2	8	1096	137	5.7	125-143
56-SC	1	8	1109	139	8.7	129-153
56-SC	2	8	1119	140	4.9	135-151
57-F	1	6	1275	213	5.9	205-220
57-F	2	6	1206	201	16.7	179-214
57-SC	1	6	1312	219	6.1	211-225
57-SC	2	6	1279	213	11.7	200-227
60-F	1	6	1148	191	3.4	187-195
60-F	2	6	1129	188	3.0	184-192
60-SC	1	6	1144	181	3.5	185-193
60-SC	2	6	1147	181	2.8	187-196

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

SD = Standard Deviation

Table 3 (continued). Body mass data collected when the rats were placed in the AEMs approximately one day before launch. Total start body mass represents the combined body mass of all rats housed in a single AEM. Rat start body mass represents the average individual body mass of rats. Individual start body mass standard deviation and range are listed.

STS* Animal Group	AEM#	# of RATS	TOTAL START BM (grams)	RAT START AVE BM (grams)	RAT START AVE BM SD	RAT START AVE BM RANGE (grams)
62-F	1	6	1026	171	8.7	162-183
62-F	2	6	1023	171	5.2	165-177
62-SC	1	6	1025	171	12.4	156-184
62-SC	2	6	1032	172	5.1	168-179
66-F	1	5	1090	218	5.6	211-225
66-F	2	5	1076	215	11.9	197-230
66-SC	3	5	1098	220	20.3	194-238
66-SC	4	5	1132	226	17.1	198-240
63-F	1	6	1219	203	2.2	200-206
63-F	2	6	1211	202	2.1	198-204
63-SC	1	6	1396	233	3.6	228-237
63-SC	2	6	1419	237	2.1	233-239
63-SC	3	6	1370	228	6.5	219-236
70-F	1	5	1112	222	12.2	203-236
70-F	2	5	1119	224	12.6	205-240
70-SC	3	5	1123	225	14.5	207-241
70-SC	4	5	1113	223	9.7	213-233
77-F	7	6	1046	174	7.2	166-179
77-F	8	6	1037	173	7.7	162-187
77-VC		8	1376	172	5.2	167-179
77-VC		8	1367	171	5.8	162-180
78-F	3	6	1006	168	8.2	156-181
78-F	4	6	981	164	3.5	130-168
78-GC	1	6	1020	170	6.1	161-180
78-GC	2	6	1006	168	3.2	164-172
80-F	7	7	976	135	7.9	126-146
80-F	8	7	921	132	7.9	122-142
80-VC	1	7	776	111	9.6	100-125
80-VC	2	7	811	116	8.8	105-130

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

SD = Standard Deviation

Table 4. Body mass data collected when the rats were recovered following landing.
Total end body mass represents the combined body mass of all rats housed in a single AEM. Rat end body mass represents the average individual body mass of rats. Individual end body mass standard deviation and range are listed.

STS* Animal Group	AEM#	# of RATS	TOTAL END BM (grams)	RAT END AVE BM (grams)	RAT END AVE BM SD	RAT END AVE BM RANGE (grams)
41-F	1	8	1114	139	5.9	127-146
41-F	2	8	1216	152	7.5	144-165
41-AC	1	8	1348	168	9.8	150-181
41-AC	2	8	1331	160	10.9	151-180
40-GC	1	5	1615	323	15.1	305-345
40-F	2	5	1665	333	28.5	310-383
40-F	3	5	1695	339	7.4	330-350
40-GC	4	5	1690	338	19.2	320-370
48-F	1	8	753	94	4.2	87-101
48-AC	1	8	617	77	3.5	71-83
52-F	1	6	1571	262	9.6	253-278
52-F	2	6	1568	261	6.2	253-271
52-SC	1	6	1583	264	8.3	248-270
52-SC	2	6	1539	257	9.4	247-269
54-F	1	6	1297	216	4.7	210-223
54-SC	1	6	1398	233	8.6	220-224
56-F	1	8	1609	201	9.4	185-214
56-F	2	8	1609	201	8.7	189-214
56-SC	1	8	1624	203	10.6	185-209
56-SC	2	8	1608	201	8.8	187-213
57-F	1	6	1505	251	6.4	245-261
57-F	2	6	1440	240	8.8	225-249
57-SC	1	6	1477	246	10.9	231-259
57-SC	2	6	1461	244	6.7	238-253
60-F	1	6	1508	251	11.0	236-263
60-F	2	6	1557	260	6.7	253-272
60-SC	1	6	1639	273	3.8	267-279
60-SC	2	6	1588	265	7.4	251-273

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

SD = Standard Deviation

Table 4 (continued). Body mass data collected when the rats were recovered following landing. Total end body mass represents the combined body mass of all rats housed in a single AEM. Rat end body mass represents the average individual body mass of rats. Individual end body mass standard deviation and range are listed.

STS* Animal Group	AEM#	# of RATS	TOTAL END BM (grams)	RAT END AVE BM (grams)	RAT END AVE BM SD	RAT END AVE BM RANGE (grams)
62-F	1	6	1205	201	14.0	182-219
62-F	2	6	1187	198	5.2	192-206
62-SC	1	6	1158	193	9.3	181-204
62-SC	2	6	1170	195	4.2	190-201
66-F	1	5	1584	317	13.4	302-338
66-F	2	5	1581	316	21.8	292-342
66-SC	3	5	1600	320	20.5	291-339
66-SC	4	5	1576	315	16.9	288-332
63-F	1	6	1596	266	8.7	257-275
63-F	2	6	1602	267	5.2	260-274
63-SC	1	6	1769	295	16.6	268-312
63-SC	2	6	1766	294	13.1	278-310
63-SC	3	6	1755	293	8.3	280-303
70-F	1	5	1477	295	19.0	274-326
70-F	2	5	1454	291	13.9	267-303
70-SC	3	5	1562	312	14.5	293-332
70-SC	4	5	1568	314	10.1	307-329
77-F	7	6	1522	254	20.5	242-277
77-F	8	6	1524	255	16.8	227-293
77-VC		8	1756	219	12.9	200-241
77-VC		8	1772	222	15.0	197-250
78-F	3	6	1768	295	26.8	243-317
78-F	4	6	1774	296	16.9	272-321
78-GC	1	6	1635	273	17.4	246-296
78-GC	2	6	1623	271	13.5	255-289
80-F	7	7	1404	201	7.7	190-212
80-F	8	7	1543	220	12.2	198-233
80-VC	1	7	1387	198	14.7	183-221
80-VC	2	7	1530	219	13.6	203-241

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

SD = Standard Deviation

Table 5. Average body mass and body mass gain data for rats in groups and individually.
Group totals were collected for all rats housed in a single AEM and reported as a single total.

STS* Animal Group	AEM#	TOTAL BM (grams)	TOTAL BM GAIN (grams)	RAT AVE BM (kg)	RAT AVE BM GAIN (grams/day)
41-F	1	1013	202	0.127	5.1
41-F	2	1120	192	0.140	4.8
41-AC	1	1166	364	0.146	9.1
41-AC	2	1162	339	0.145	8.4
40-GC	1	1503	225	0.301	4.5
40-F	2	1548	235	0.310	4.7
40-F	3	1565	260	0.313	5.2
40-GC	4	1565	250	0.313	5.0
48-F	1	613	281	0.077	5.5
48-AC	1	517	201	0.065	3.9
52-F	1	1325	493	0.221	7.5
52-F	2	1318	500	0.220	7.6
52-SC	1	1348	471	0.225	7.1
52-SC	2	1321	437	0.220	6.6
54-F	1	1174	247	0.196	5.9
54-SC	1	1251	295	0.208	7.0
56-F	1	1363	493	0.170	6.2
56-F	2	1353	513	0.169	6.4
56-SC	1	1367	515	0.171	6.4
56-SC	2	1364	489	0.170	6.1
57-F	1	1390	230	0.232	3.2
57-F	2	1323	234	0.221	3.3
57-SC	1	1395	165	0.232	2.3
57-SC	2	1370	182	0.228	2.5
60-F	1	1328	360	0.221	6.7
60-F	2	1343	428	0.224	7.9
60-SC	1	1392	495	0.232	9.2
60-SC	2	1368	441	0.228	8.2

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

Table 5 (continued). Average body mass and body mass gain data for rats in groups and individually. Group totals were collected for all rats housed in a single AEM and reported as a single total.

STS* Animal Group	AEM#	TOTAL BM (grams)	TOTAL BM GAIN (grams)	RAT AVE BM (kg)	RAT AVE BM GAIN (grams/day)
62-F	1	1116	179	0.186	2.0
62-F	2	1105	164	0.184	1.8
62-SC	1	1092	133	0.182	1.5
62-SC	2	1101	138	0.184	1.5
66-F	1	1337	494	0.267	9.0
66-F	2	1328	504	0.266	9.2
66-SC	3	1349	502	0.270	9.1
66-SC	4	1354	444	0.271	8.1
63-F	1	1408	377	0.235	7.0
63-F	2	1407	391	0.234	7.2
63-SC	1	1583	373	0.264	6.9
63-SC	2	1593	347	0.265	6.4
63-SC	3	1563	385	0.260	7.1
70-F	1	1295	365	0.259	7.3
70-F	2	1287	335	0.257	6.7
70-SC	3	1343	439	0.269	8.8
70-SC	4	1341	455	0.268	9.1
77-F	7	1284	476	0.214	7.2
77-F	8	1281	487	0.213	7.4
77-VC		1566	380	0.196	4.3
77-VC		1570	405	0.196	4.6
78-F	3	1387	762	0.231	7.1
78-F	4	1378	793	0.230	7.3
78-GC	1	1328	615	0.221	5.7
78-GC	2	1315	617	0.219	5.7
80-F	7	1190	428	0.170	3.3
80-F	8	1232	622	0.176	4.8
80-VC	1	1082	611	0.155	4.7
80-VC	2	1171	719	0.167	5.6

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

Table 6. Food consumption data. Total food represents food consumed by the group of rats in the AEM over the duration of time spent in the AEM. Rat food use represents the average daily consumption of individual rats. Rat E_{in} represents the daily caloric intake of the individual rats adjusted for body mass based on a consumption of a 3.45 kcal/gram food bar diet containing 25% moisture.

STS* Animal Group	AEM#	TOTAL FOOD USE (grams)	RAT AVE FOOD USE (grams/day)	RAT AVE E_{in} adjusted for BM (kcal/kg BM/day)
41-F	1	748	18.7	382
41-F	2	808	20.2	374
41-AC	1	936	23.4	416
41-AC	2	944	23.6	421
40-GC	1	1407	28.1	243
40-F	2	1418	28.4	237
40-F	3	1444	28.9	239
40-GC	4	1402	28.0	232
48-F	1	787	15.4	520
48-AC	1	590	11.5	463
52-F	1	1694	25.7	301
52-F	2	1722	26.1	308
52-SC	1	1620	24.5	283
52-SC	2	1585	24.0	283
54-F	1	1128	26.9	356
54-SC	1	1162	27.7	344
56-F	1	1631	20.4	310
56-F	2	1578	19.7	302
56-SC	1	1502	18.8	285
56-SC	2	1510	18.9	287
57-F	1	1468	20.4	228
57-F	2	1443	20.0	235
57-SC	1	1392	19.3	215
57-SC	2	1421	19.7	224
60-F	1	1559	28.9	338
60-F	2	1648	30.5	353
60-SC	1	1744	32.3	361
60-SC	2	1730	32.0	364

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AVE = Average

BM = Body Mass

na = not available (data not reported)

Table 6 (continued). Food consumption data. Total food represents food consumed by the group of rats in the AEM over the duration of time spent in the AEM. Rat food use represents the average daily consumption of individual rats. Rat E_{in} represents the daily caloric intake of the individual rats adjusted for body mass based on a consumption of a 3.45 kcal/gram food bar diet containing 25% moisture.

STS* Animal Group	AEM#	TOTAL FOOD USE (grams)	RAT AVE FOOD USE (grams/day)	RAT AVE E_{in} adjusted for BM (kcal/kg BM/day)
62-F	1	1718	19.1	266
62-F	2	1651	18.3	258
62-SC	1	1509	16.8	239
62-SC	2	1448	16.1	227
66-F	1	1713	31.1	302
66-F	2	1733	31.5	307
66-SC	3	1803	32.8	315
66-SC	4	1761	32.0	306
63-F	1	1523	28.2	311
63-F	2	1573	29.1	322
63-SC	1	1634	30.3	297
63-SC	2	1587	29.4	287
63-SC	3	1592	29.5	293
70-F	1	1465	29.3	293
70-F	2	1415	28.3	285
70-SC	3	1565	31.3	302
70-SC	4	1605	32.1	310
77-F	7	1742	26.4	319
77-F	8	1742	26.4	320
77-VC		na	na	na
77-VC		na	na	na
78-F	3	2495	23.1	259
78-F	4	2603	24.1	272
78-GC	1	2387	22.1	259
78-GC	2	2333	21.6	256
80-F	7	2396	18.5	na
80-F	8	2836	21.9	na
80-VC	1	2202	17.0	282
80-VC	2	2409	18.6	322

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AVE = Average

BM = Body Mass

na = not available (data not reported)

Table 7. Water consumption data. Total water use represents the amount of water consumed from the water bladders by the group of rats while housed in the AEM. Rat water use represents the average daily consumption of water from the water bladders for individual rats. Rat combined water use represent individual daily water consumed from bladders and food bar diet. Rat combined water use adjusted for body mass represents daily individual water consumption from water bladders and food bars and adjusted for rat body mass.

STS* Animal Group	AEM #	TOTAL Water Use from bladder (ml)	RAT AVE Water Use from bladder (ml)	RAT COMBINED Water Use (=25% bar + bladder) (ml/day)	RAT COMBINED Water Use adjusted for BM (ml water/kg bm/day)
41-F	1	1100	27.5	32.2	254
41-F	2	1084	27.1	32.2	230
41-AC	1	1004	25.1	31.0	212
41-AC	2	na	na	na	na
40-GC	1	2567	51.3	58.4	194
40-F	2	2279	45.6	52.7	170
40-F	3	1974	39.5	46.7	149
40-GC	4	2105	42.1	49.1	157
48-F	1	624	12.2	16.0	209
48-AC	1	590	11.5	14.4	223
52-F	1	2231	33.8	40.2	182
52-F	2	2466	37.4	43.9	200
52-SC	1	2701	40.9	47.1	210
52-SC	2	2571	39.0	45.0	204
54-F	1	1156	27.5	34.2	175
54-SC	1	1604	38.2	45.1	216
56-F	1	2338	29.2	34.3	202
56-F	2	2036	25.5	30.4	180
56-SC	1	2265	28.3	33.0	193
56-SC	2	2612	32.7	37.4	219
57-F	1	na	na	na	na
57-F	2	na	na	na	na
57-SC	1	1554	21.6	26.4	114
57-SC	2	1421	19.7	24.7	108
60-F	1	2126	39.4	46.6	210
60-F	2	1974	36.6	44.2	197
60-SC	1	2246	41.6	49.7	214
60-SC	2	1311	24.3	32.3	142

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AVE = Average

BM = Body Mass

na = not available (data not reported)

Table 8. Details of Ambient Temperature Recorder (ATR-4) data recordings.

STS* Animal Group	AEM #	FLIGHT TIME (days)	Time interval between recordings (minutes)	# of times temps were recorded in flight	# of temp recordings analyzed
54-F	1	6.0	3.75	2296	4593
56-F	1	9.3	3.75	3556	7112
56-F	2	9.3	3.75	3556	7112
57-F	1	10.0	3.75	3836	7672
57-F	2	10.0	3.75	3836	7672
60-F	1	8.3	7.5	1594	3187
60-F	2	8.3	7.5	1594	3187
63-F	1	8.2	3.75	3145	6290
63-F	2	8.2	3.75	3145	6290
77-F	7	10.0	7.5	1926	3852
77-F	8	10.0	7.5	1926	3852
78-F	3	16.9	7.5	3247	6493
78-F	4	16.9	7.5	3247	6493
80-F	7	17.7	7.5	3391	6781
80-F	8	17.7	7.5	3391	6781

STS* = Space Transportation System
Animal Groups : F, GC, AC, SC, and VC
F = Flight
GC = Ground Control
AC = Asynchronous Ground Control
SC = Synchronous Ground Control
VC = Vivarium Control

Total number of temperature recordings considered: approximately 87,368

Table 9. ATR-4 temperature data. Temp in represents the temperature of the air flowing into the animal cage area, temp out represents the temperature of the air flowing out of the animal cage area, and the delta temperatures are calculated based on these differences.

STS* Animal Group	AEM #	Temp In °C	Temp Out °C	Delta Temp Calculated (T out - T in)
54-F	1	29.3	30.7	1.4
56-F	1	28.5	30.4	1.9
56-F	2	27.6	30.3	2.7
57-F	1	27.0	29.9	2.9
57-F	2	27.6	29.7	2.1
60-F	1	26.0	28.6	2.6
60-F	2	25.3	27.9	2.6
63-F	1	27.5	30.3	2.8
63-F	2	26.9	28.9	2.0
77-F	7	27.5	30.2	2.7
77-F	8	28.8	30.5	1.7
78-F	3	27.7	30.2	2.5
78-F	4	28.2	29.5	1.3
80-F	7	29.1	30.8	1.7
80-F	8	28.6	30.0	1.4
AVE		27.7	29.9	2.2
SD		1.1	0.8	0.6
Range		25.3-29.3	27.9-30.8	1.3-2.9

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

AVE = Average

SD = Standard Deviation

Table 10. Total body mass of all rats within an AEM and the average delta temperature (temperature difference of in-flowing and out-flowing air) of the AEM. Values are plotted in Figure 11.

STS* Animal Group	AEM#	TOTAL BM (grams)	Delta Temp (°C)
54-F	1	1174	1.4
56-F	1	1363	1.9
56-F	2	1353	2.7
57-F	1	1390	2.9
57-F	2	1323	2.1
60-F	1	1328	2.6
60-F	2	1343	2.6
63-F	1	1408	2.8
63-F	2	1407	2.0
77-F	7	1284	2.7
77-F	8	1281	1.7
78-F	3	1387	2.5
78-F	4	1378	1.3
80-F	7	1190	1.7
80-F	8	1232	1.4
AVE		1323	2.2
SD		76	0.6
Range		1174-1408	1.3-2.9

STS* = Space Transportation System

Animal Groups : F, GC, AC, SC, and VC

F = Flight

GC = Ground Control

AC = Asynchronous Ground Control

SC = Synchronous Ground Control

VC = Vivarium Control

BM = Body Mass

AVE = Average

SD = Standard Deviation

Table II. Comparison of male Sprague-Dawley rats from AEM flight and control groups to male Sprague-Dawley laboratory rats housed and fed under standardized conditions. (AEM rats consumed a diet with a caloric value of 2.59 kcal/gram.)

	Standard Control	Control AEM	Flight AEM
Body Mass (g)	255	246	236
Population	n = 16 rats	n = 12 AEMs	n = 13 AEMs
SD	7	34	35
CV	3	14	15
SE	2	10	10
ADJ Caloric Consumption			
(kcal/kg bm/day)	287	291	302
Population	n = 16 rats	n = 12 AEMs	n = 13 AEMs
SD	13	44	40
CV	5	15	13
SE	3	13	11

SD = Standard Deviation
CV = Coefficient of Variation
SE = Standard Error
ADJ = Adjusted for Body Mass

Starting Body Mass of AEM Rats

Figure 1. Body mass comparisons between AEM control and flight group averages at the beginning of each mission. (AEM flight rat starting body mass = $0.88 \times$ (AEM control rat starting body mass) + 19.57, $r = 0.98$, $n = 16$.)

Final Body Mass of AEM Rats

Figure 2. Body mass comparisons between AEM control and flight group averages at the completion of each mission. (AEM flight rat final body mass = $0.93 \times (\text{AEM control rat final body mass}) + 15.40$, $r = 0.97$, $n = 16$.)

Average Body Mass of AEM Rats

Figure 3. Body mass comparisons between AEM control and flight group averages during each mission. (AEM flight rat average body mass = $0.91 \times (\text{AEM control rat average body mass}) + 17.12$, $r = 0.98$, $n = 16$.)

Daily Mass Gain of AEM Rats

Figure 4. Daily mass gain comparisons between AEM control and flight group averages during each mission. (AEM flight rat average daily mass gain = $0.60 \times$ (AEM control rat average daily mass gain) + 2.24, $r = 0.71$, $n = 16$.)

Taconic Growth Chart and AEM Rats

Figure 5. Standardized growth chart for male Sprague-Dawley rats (STS 40, 41, 52, 54, 56, 60, 63, 77, and 78) adapted from Taconic Product Guide (1/2001) with AEM flight and control groups ($n = 18$) plotted as points within the curve and error bars representing plus or minus one standard deviation (SD). The average coefficient of variation for final body mass of all AEM groups considered here was approximately 4.55. Inner boundaries (dashed lines) capture the average body mass plus or minus one standard deviation from the mean and outer boundaries (solid lines) capture average body mass plus or minus two standard deviations from the mean.

Daily Food Consumption of AEM Rats

Figure 6. Daily food consumption comparisons between AEM control and flight group averages during each mission. Data were obtained from fifteen missions. (AEM flight rat average daily food consumption = $0.73 \times$ (AEM control rat average daily food consumption) + 6.65, $r = 0.96$, $n = 15$.)

Daily Food Consumption Adjusted for Body Mass of AEM Rats

Figure 7. Daily food use adjusted for body mass of flight and control AEM rats. (Flight Food Use (g food/kg bm/day) = $0.98 \times$ (Control Food Use (g food/kg bm/day)) + 6.12, $r = 0.94$, $n = 15$.)

Daily Caloric Consumption AEM vs. Standard Control Rats

Figure 8. Average daily caloric consumption of AEM flight (body mass average = 236 ± 35 grams, $n = 13$ AEMs), AEM control (body mass average = 246 ± 34 grams, $n = 12$ AEMs), and standard control (body mass average = 255 ± 7 grams, $n = 16$ rats) groups. A subpopulation of male Sprague-Dawley rats similar in body mass to the reference group, standard control group, were selected from the entire AEM population for comparisons. The standard control rats were maintained under standardized housing and feeding conditions. Calculations for food consumption by the AEM rats accounted for the 25% hydration in the spacebars. Standard error bars are shown. Statistical analyses show no difference in daily caloric consumption between these three groups.

Daily Water Consumption from Water Bladders of AEM Rats

Figure 9. Daily water consumption (from water bladders) comparisons between AEM control and flight group averages during each mission. (AEM flight rat average daily water consumption = $0.77 \times (\text{AEM control rat average daily water consumption}) + 5.30$, $r = 0.89$, $n = 7$)

Daily Water Consumption from Water Bladders and Moisture in the Food Bar Diet of AEM Rats

Figure 10. Daily combined water consumption (from water bladders and moisture in food bars) comparisons between AEM control and flight group averages during each mission. (AEM flight rat average daily combined water consumption = $0.58 \times$ (AEM control rat average daily combined water consumption) + 79.38, $r = 0.40$, $n = 7$)

Total Body Mass vs. Delta Temperature

Figure 11. Total body mass of all rats housed in an AEM versus the average delta temperature (temperature difference of the in-flow and out-flow air) of the AEM while in flight. ($n = 15$ AEMs, $r = 0.54$).