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Oxygen Consumption and Carbon Dioxide Production in Prairie Deer mice (Peromyscus maniculatus bairdii) Kept in Various Group Densities and Reproductive Conditions

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OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION IN PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDII*) KEPT IN VARIOUS GROUP DENSITIES AND REPRODUCTIVE CONDITIONS

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APPROVAL SHEET

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the requirement for the degree of
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DEDICATION

To my parents who thankfully will never be more than a phone call away.

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Abstract

The induction of reproductive inhibition in laboratory populations of Peromyscus maniculatus appears to be dependent on the integration of physiological and behavioral events. The physiological components may include corticosterone hypersecretion from the adrenal and depressed circulating levels of thyroxine in the reproductively inhibited population animals. Reduction in the amount of food consumed per day and the formation of huddles are consistently observed behaviors in populations of P. maniculatus. Others have proposed that the elevated levels of carbon dioxide within the huddle may cause a suppression of the metabolic rate.

This study examined the metabolic rate (VO_2) of animals exposed to two significantly different carbon dioxide concentrations. Metabolic measurements were also made on animals at three densities to determine whether such changes in density affect metabolic rate. The metabolic rate of reproductively proven, reproductively inhibited animals, and reproductively recovered population animals was also evaluated. Gravimetric analyses of testes and seminal vesicles were conducted to confirm the reproductive condition of these animals.

Carbon dioxide concentrations ranging from 0.2% to 0.7% did not have a significant effect on VO_2. This concentration range was chosen because the instrumentation used in this study required a carbon dioxide concentration of at least 0.2% for accurate measurement. Therefore, any differences in the metabolic rate were not due to changes in carbon dioxide concentration within the chamber. Densities as high as six animals did not affect metabolic rate compared with values for individuals and pairs. This finding does not rule out the possibility that higher densities and/or a longer grouping time before measurement may have an effect on reducing metabolic rate.

The Active Metabolic Rate (AMR) for reproductively proven and inhibited animals was not significantly different. However, the Resting Metabolic Rate (RMR) was significantly lower in reproductively inhibited animals compared with proven animals. Previously inhibited animals paired with females showed a RMR that was significantly greater than that of proven animals. The Respiratory Exchange Ratio (RER) for proven animals was not significantly different from that of reproductively recovered animals. The RER for inhibited animals was significantly higher than that of proven and recovered animals.

OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION IN PRAIRIE DEERMICE (*PEROMYSCUS MANICULATUS BAIRDII*) KEPT IN VARIOUS GROUP DENSITIES AND REPRODUCTIVE CONDITIONS

INTRODUCTION

It is well known that in laboratory populations of Peromyscus maniculatus few animals within the population become reproductive while as many as 90% remain in a juvenile, non-reproductive condition with significantly smaller reproductive organ weights (Terman 1969, 1973a, 1974; Sung et al. 1977; Bradley and Terman 1981a, 1981c; Peebles et al. 1984). In contrast to Peromyscus, some other rodents do not exhibit depressed reproductive organ weights in crowded conditions. Gamallo et al. (1985) studied the effects of crowding in rats and found an increase in testes weight in crowd-reared rats in comparison with control rats. They attributed the larger testes weight in the crowd-reared animals to adrenal hypersecretion of corticosterone. Their conclusion was substantiated by the increased adrenal weights of the crowd-reared animals. Earlier work on rats and mice (Calhoun, 1962; Christian, 1950, 1956, 1971) has shown adrenal hypertrophy and hypersecretion is clearly associated with increased density in these species.

Investigations on the adrenals of Peromyscus have revealed as much as a five-fold elevation in corticosterone levels in reproductively inhibited animals from laboratory populations in comparison with controls, but the adrenal

weights in both sexes are not different or smaller than that of reproductively proven animals (Sung et al., 1977; Bradley and Terman, 1981a; Ransone and Bradley, in press). Andrews (1979) proposed that elevated levels of ACTH and stress led to a delay in sexual maturity in P. maniculatus; however, Coppes and Bradley (1984) found no difference in serum ACTH levels between population and reproductively capable animals. This suggests that while ACTH may be a factor, it is clearly not the sole cause of reproductive inhibition.

Terman (1974) reported that as populations reach a level at which they cease to grow, two behaviors are observed: a reduction in the number of food pellets consumed and an increase in huddling or aggregation behavior. More recently, Cronin and Bradley (1988) have shown that population animals consume less food per capita per day than the reproductively proven animals. They postulated that the energy requirements of population animals may be reduced through huddling by lessening the thermoregulatory demands on the animal. Huddling is a behavior commonly observed in many rodents such as Peromyscus (Hayward, 1965; Vogt and Lynch, 1982), Mus, and Meriones (Martin et al., 1980; Conteras, 1984). Energy consumption in huddled animals has been observed to be lower than that of solitary animals (Conteras, 1984; Martin et al., 1980). Vogt and Lynch (1982) found that huddled animals had an energy expenditure of 16 to 33% less than that of animals housed alone. The

energetic benefits that result from reducing the amount of heat lost to the environment may account for the reduced dietary intake in laboratory populations under ad lib conditions. Other authors have postulated that a behavioral component, such as contact with or the presence of other animals, is an important contributor to the suppression of metabolism (Martin et al., 1980; Vogt and Lynch; 1982).

Schlenker et al. (1981) proposed that the "group effect" may be directly caused by the elevated levels of carbon dioxide that exist within the microenvironment of the huddle. The possibility that high concentrations of carbon dioxide produced by dense aggregations of animals may actually cause a reduction in metabolic rate is strengthened by the fact that carbon dioxide does function as a parahormone in other systems, such as control of respiration.

Other reasons for a reduction in the energy requirement and food intake of population animals have been suggested. The thyroid plays a critical role in the regulation of metabolism. It has been reported that circulating levels of thyroid hormone are depressed in population animals in comparison with controls (Peebles et al., 1984; Pitman and Bradley, 1984). Depressed levels of thyroid hormone could lead to reduction of food intake and suppression of metabolism, and thereby affect gonadal development. Hogg et al. (1992) reported that supplementing triiodothyronine to

population animals produced a partial recovery from reproductive inhibition as indicated by an increase in gonad weights. These findings implicate the thyroid as a component in reproductive failure.

Reproductive inhibition is a reversible phenomenon. If animals are removed from the population context and paired with an animal of the opposite sex, recovery from inhibition occurs more than 75% of the time (Terman 1973b, 1987). These findings contrast with those of Andrews (1979) who reported that the high oxygen consumption rates induced in stressed population animals could not be reversed by removal from the crowded condition.

The purpose of this study was threefold. Experiment I was designed to test if the carbon dioxide levels necessarily produced during the measurement period had an effect on oxygen consumption (metabolic rate). In Experiment II, the effect of animal density on oxygen consumption was observed to determine if behavioral modification due to the presence of conspecifics in conjunction with relatively high carbon dioxide levels would have an effect on metabolic rate. In Experiment III, metabolic rate and quality of metabolism (Respiratory Exchange Ratio, RER) of inhibited population animals, proven animals, and reproductively recovered population animals were compared. Gravimetric analyses of testes and seminal vesicles were performed to determine the reproductive status

of the individuals in Experiment III. All of the experiments were conducted with males to ensure that ovarian cyclic variations in metabolic rate were avoided. Peromyscus maniculatus bairdii was chosen due to the plethora of literature available on this species and because reproductive inhibition is profound and well documented.

MATERIALS AND METHODS

Animal Maintenance

Peromyscus maniculatus bairdii, prairie deermice, were used for this study. Males were obtained from an outbred production colony maintained at the Laboratory of Endocrinology and Population Ecology. Reproductively proven and colony animals were kept in two-compartment, opaque plastic cages (12.8 X 27.8 X 14.5) covered with a wire top. Animals from the population were maintained in two metal population enclosures (1.5 m in diameter). Prior to testing, population animals were removed from the enclosures and placed in the two-compartment plastic cages mentioned previously. A layer of pine shavings on the bottom of the cage provided bedding. Food (Agway Prolab 3000) and water were provided *ad libitum*. The animals were maintained at a temperature of **23 ± 5 °C** and a photoperiod of 14:10 LD with a light period from 0600 to 2000. Light intensity in the cage averaged 129 lux.

Experimental Specifications

The Oxymax System (Columbus Instruments) was utilized for the measurement of oxygen consumption and carbon dioxide production in accordance with manufacturer's operating procedures. Oxygen consumption and carbon dioxide production were calculated and recorded in ml/kg/hr.

Measurements were taken at regular intervals over a 23 hour period commencing at approximately 1300 h. The remaining hour was used for calibration, changing the drying agent (indicating Drierite), cleaning the cage and weighing the animal. Three sampling modes were used in the experiment as necessary: 8 minute intervals (60 s measurement time, 180 s settle time), 10 minute intervals (30 s measurement time, 270 s settle time), 12 minute intervals (60 s measurement time, 300 s settle time). The mode used was dependent on the type of Drierite column used. When the smaller fast-running Drierite columns were used, measurements were recorded at 8 minute intervals because a shorter settle time could flush the lower volume column. The standard response column was used when the capacity of the fast running column was insufficient to last the 23 hour sample period. The increased volume of the standard column necessitated an increase in settle time, and consequently, total time.

At the beginning of each 23 hour sampling period animals were put into a clean cage with adequate amounts of food and water. A specially constructed 30 L acrylic, air-tight chamber within an incubator (Precision Low Temperature, Model #815) contained the cage and animal. The volume of the chamber was reduced to 26 L with a wood block that was placed in the unused cage compartment. A 22 W fan within the chamber circulated the air at a velocity of 2.5 m/s. A thermocouple inserted through a sealed grommet in

the top of the chamber provided a continuous monitor of the temperature. The incubator setting was adjusted to achieve a temperature of 24 $\pm$ 2 °C. The light intensity within the cage (0600-2000 h) was 161 lux. The Oxymax was calibrated for use daily with a gas standard of 20.50% oxygen and 0.500% carbon dioxide (Linde) and dry nitrogen (Linde and Airco).

Experiment I

Eight reproductively unproven males (aged 144-148 days) were selected from six litters to be tested individually for 2 consecutive days. The animals were kept in 2 groups of 5 except while being tested. Each animal was measured under two carbon dioxide concentrations that were created by adjusting the air flow through the chamber. On the first day of measurement for each animal, the air flow was adjusted between 0.54 L/min to 0.80 L/min, depending on the weight of the animal, to give initial differences of 0.20 to 0.25 between the percent CO_2 in the reference air relative to the returning expired air (delta CO_2) and on the second day of measurement, the flow was adjusted between 0.30 to 0.35 L/min to give initial delta CO_2 values of 0.50 to 0.55. Flow adjustments were made during the first hour in order to achieve the desired delta CO_2 values differences. The animals were returned to their respective groups upon the completion of testing.

Experiment II

Six additional reproductively unproven and unrelated males were obtained from the colony. The age range at the beginning of the experiment was from 85 to 93 days. For the first part of the experiment, each animal was housed separately and tested for one day. Values for all parameters were computed and printed at 8 minute intervals during the continuous 23 hour sampling period. The flow was set to 0.50 L/min to achieve delta CO_2 values between 0.20 and 0.50 during the sampling period.

After two animals had been tested individually, they were paired for 2 days, and then tested together for 2 consecutive days. The Drierite in the fast running column was exhausted before the completion of the sampling and had to be changed at least once during the sampling period. A flow of 1.00 L/min was used in order to achieve delta CO_2 values comparable with those obtained with individuals.

Upon the completion of pair testing, the 3 pairs (6 animals) were caged together for 2 days and then tested together for 6 consecutive days. A flow of 2.50 L/min was determined to produce values comparable with those of individuals and pairs. The standard Drierite column was used because of the increased water production with six animals. However, the water absorptive capacity of the standard column was exceeded and the Drierite was changed once during the sampling period.

Experiment III- Part I

Twelve reproductively inhibited males (aged 72-180 days) were obtained from two laboratory populations, six animals from each, of 38 (Population 1) and 44 animals (Population 2). Animals were selected if they were between 60 to 200 days of age and observed to continuously exhibit non-scrotal testes in semimonthly population checks. Animals were kept as two groups of six. The animals were grouped for one day, and were then tested as a group the following day. Each group of six was tested for 6 consecutive days. The flow of air to the chamber was set to achieve delta CO_2 values within the range established in Experiment I. The 12 minute sampling mode and standard response Drierite column were used.

Three days after the completion of measurement, the animals from Population 1 were separated into individual cage compartments to allow for a recovery from their reproductively inhibited state. They were kept as individuals for 10 days and then paired with nulliparous females for 24 additional days before being tested again. Each animal was chosen at random and tested for 2 non-consecutive days.

Part II

Six reproductively proven males, aged 264 to 285 days, were kept as pairs with females for a minimum of 13 days

before being individually tested for two non-consecutive days. Following the first day of measurement, the animal was returned to the cage with the female until the second measurement was taken.

Upon the completion of testing, the animals from Populations 1 and 2 and proven animals were sacrificed and placed into 10% buffered formalin. After at least 72 hours in the formalin, the testes and seminal vesicles were removed, cleaned of fat, and weighed to the nearest 0.1 mg.

Statistical Analysis

Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) values were recomputed to the nearest 0.1 g of body weight using Lotus 1-2-3. Lotus was also used to calculate means and standard deviations for oxygen consumption, delta CO_2, and the Respiratory Exchange Ratio (RER). Means and standard deviations were calculated for each parameter for four blocks: 1500-1100 hours (20 hour daily mean); 2030-0530 (the dark period); one hour on either side of the highest VO_2 value (Active Metabolic Rate or AMR); and, one hour on either side of the lowest VO_2 value (Resting Metabolic Rate or RMR). The values collected during any Drierite change were eliminated from statistical calculations. Statistical comparisons of the means were made by a one-way analysis of variance using the SPSS-X statistical package. Tukey's test was used if sample sizes were equal and there was

homogeneity of variance. Scheffe's test was used with unequal sample sizes and the non-parametric Kruskal-Wallis test was used if variances were significantly heterogenous.

Body weight, testes weight, and seminal vesicle weight for proven, population, and recovered animals were compared by a one-way analysis of variance. Spearman's correlation coefficients were used to compare testes weight and seminal vesicle weight with oxygen consumption. A probability of less than 0.05 was considered statistically significant for all comparisons.

RESULTS AND DISCUSSION

Experiment I

An earlier report by Schlenker et al. (1981) suggested that in mice there was a "group effect" (a reduction in metabolic rate) caused by an increase in the local carbon dioxide concentration from 0.04% to 0.20%. The instrumentation used in the present study required a carbon dioxide differential of at least 0.2% for accurate measurement. Therefore, I conducted an experiment to determine if a carbon dioxide concentrations above 0.2% would produce differences in oxygen consumption (metabolic rate).

Each animal was exposed to two carbon dioxide environments created by changing the rate of air flow to the chamber. The two air flow rates used produced significantly different delta CO_2 values (one approximately double the other) for all periods evaluated. The results of changing the carbon dioxide concentration within these limits (0.2% to 0.7%) did not affect the corresponding oxygen consumption (VO_2) for any period evaluated (Table 1).

The results of this experiment do not disprove the findings of Schlenker et al. (1981) because the carbon dioxide levels used in the two experiments do not overlap. However, some doubt is cast upon their findings because the oxygen consumption values in the present experiment are

Table 1. Experiment I. Oxygen consumption [VO_2] (ml/kg/hr) and change in carbon dioxide concentration [delta CO_2] (percent) under high and low air flows that produce low and high ambient carbon dioxide concentrations. Values are for eight males aged 144 to 149 days with each animal exposed to high and low flows. Mean values ± SEM.

Treatment Measurement	20 hour daily mean	Dark period 2030-0530 mean	Active Metabolic Rate	Resting Metabolic Rate
High flow				
VO_2	5892 ±765	7279 ±1042	8240 ±1091	3710 ±462
delta CO_2	0.317 ±0.0395	0.391 ±0.0544	0.343 ±0.0536	0.201 ±0.0355
Low flow				
VO_2	5633 ±703	6815 ±923	7823 ±1090	3848 ±462
delta CO_2	0.586* ±0.0376	0.678* ±0.0494	0.743* ±0.0471	0.428* ±0.0261

* $P<0.05$ with respect to the corresponding value for high flow delta CO_2.

similar to those found in other experiments with comparable conditions and carbon dioxide levels approximating ambient levels or under pure oxygen conditions (Hayward, 1965). Perhaps the differences found by Schlenker et al. (1981) were due to their using carbon dioxide production (VCO_2) as a measure of metabolic rate which is not as reflective of metabolism as is oxygen consumption. This is because variations in CO_2 produced to O_2 consumed are caused by the quality of food and state of the animal. Regardless, the data on VO_2 in the present study are not compromised by the changes in CO_2 concentration in the apparatus.

Experiment II

When animals were measured under relatively constant CO_2 conditions as either individuals, pairs, or in a group of six, they did not exhibit significantly different oxygen consumption in any of the periods evaluated (Table 2). The delta CO_2 values produced in this experiment were not significantly different from those values established in Experiment I (cf. Tables 1 and 2), so differences in delta CO_2 are inconsequential.

These data suggest that the pairing of individuals with other animals of the same sex in densities as high as six does not produce a change in their metabolism. Whether higher densities and/or a longer time period of assembly would have an effect is unknown. This suggests that if

Table 2. Experiment II. Oxygen consumption [VO_2] (ml/kg/h) and change in carbon dioxide concentration [delta CO_2] (percent) for six males aged 85 to 93 days and measured as individuals, pairs, and in a group of six. All dated based upon six daily measurement. Mean values ± SEM.

Treatment Measurement	20 hour daily mean	Dark period (2030-0530) mean	Active Metabolic Rate	Resting Metabolic Rate
Individual				
VO_2	5373 ±299.1	6287 ±474.9	7424 ±546.4	3858 ±182.2
delta CO_2	0.316 ±0.0174	0.375 ±0.0336	0.438 ±0.0353	0.239* ±0.0122
Pair				
VO_2	5083 ±463.4	6431 ±616.7	7190 ±625.2	3029 ±182.2
delta CO_2	0.285 ±0.0220	0.369 ±0.0330	0.400 ±0.0413	0.186 ±0.0084
Grouped				
VO_2	4580 ±121.5	6318 ±160.1	7049 ±192.2	2673 ±96.3
delta CO_2	0.329 ±0.0026	0.455 ±0.0043	0.494 ±0.0082	0.197 ±0.0028

* $P<0.05$ with respect to corresponding pair and group values.

differences in VO_2 exist in animals from laboratory populations compared with controls, these differences may not be due to density per se.

These findings in Peromyscus contrast with reports in Mus where it has been shown that three animals separated by barriers and housed in a single cage have suppressed oxygen consumption (Martin et al., 1980). This suggests that the mere presence of other animals may be sufficient to cause a change in metabolism. This may reflect another important difference between genera and indicates the danger of generalizing from a few well studied species to all rodents.

Experiment III

Body weight, testes weight, and seminal vesicle weight for proven animals were significantly different from Population 2 animals (Table 3). Body weight and testes weight of proven animals were significantly different from recovered Population 1 animals; however, seminal vesicle weight was not significantly different (Table 3, Figure 1). Body weight, testes weight, and seminal vesicle weight for Population 2 and the recovered animals from Population 1 were significantly different (Table 3, Figure 1).

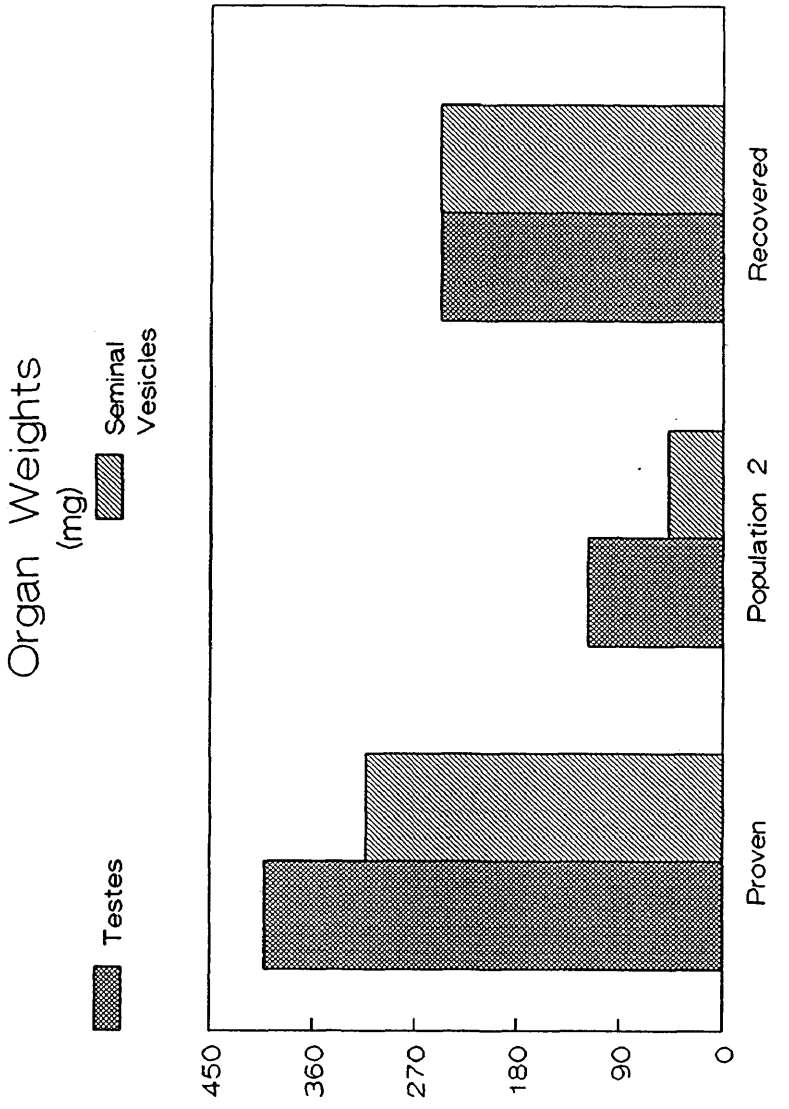
These data are consistent with several previous reports involving reproductively proven and inhibited animals of this species (Terman, 1969, 1974; Bradley and Terman,

Table 3. Body weight (grams), testes weight (mg), and seminal vesicles weight (mg) for proven, population 2, and recovered population 1 males. Mean values ± SEM.

Animals	Body weight (grams)	Testes Weight (mg)	Seminal Vesicles (mg)
Proven	23.1^{a} ±1.05	402.5^{a} ±10.91	313.8^{a} ±35.45
Population 2	14.2^{ab} ±0.29	117.5^{ab} ±28.53	48.5^{ab} ±14.48
Recovered Population 1	18.8^{ab} ±0.89	247.5^{ab} ±48.08	247.6^{b} ±18.67

Values with the same letter are significantly ($P<0.05$) different.
All groups contain 6 males.

Figure 1. Testis and seminal vesicle weight for reproductively proven, population, and reproductively recovered males.



1981a). As in these earlier studies, the reproductively inhibited animals from populations had drastically reduced reproductive organs when compared with reproductively capable animals. Testes and seminal vesicle weight has been reported as being positively correlated with testosterone concentration in P. leucopus (Feist et al., 1988). Bradley and Terman (1981b) have also shown a significant correlation between testosterone concentration and seminal vesicle weight in P. maniculatus, and that reproductively inhibited males from laboratory populations have significantly reduced serum testosterone concentration. Therefore, I am confident that the population animals used in this study were reproductively inhibited. These data also indicate a recovery from reproductive inhibition in the Population 1 animals housed as pairs with females. Circulating levels of testosterone are presumed to be higher in these animals due to the increase in seminal vesicle weight (Bradley and Terman, 1981b), and because four of the six recovered animals sired at least one litter.

Populations 1 and 2 were separated for all statistical analyses because of differences in oxygen consumption for all periods except the RMR. Variability between populations of animals is expected. Terman (1974) has reported population variation in the number of animals at asymptote and the time to reach controlled growth.

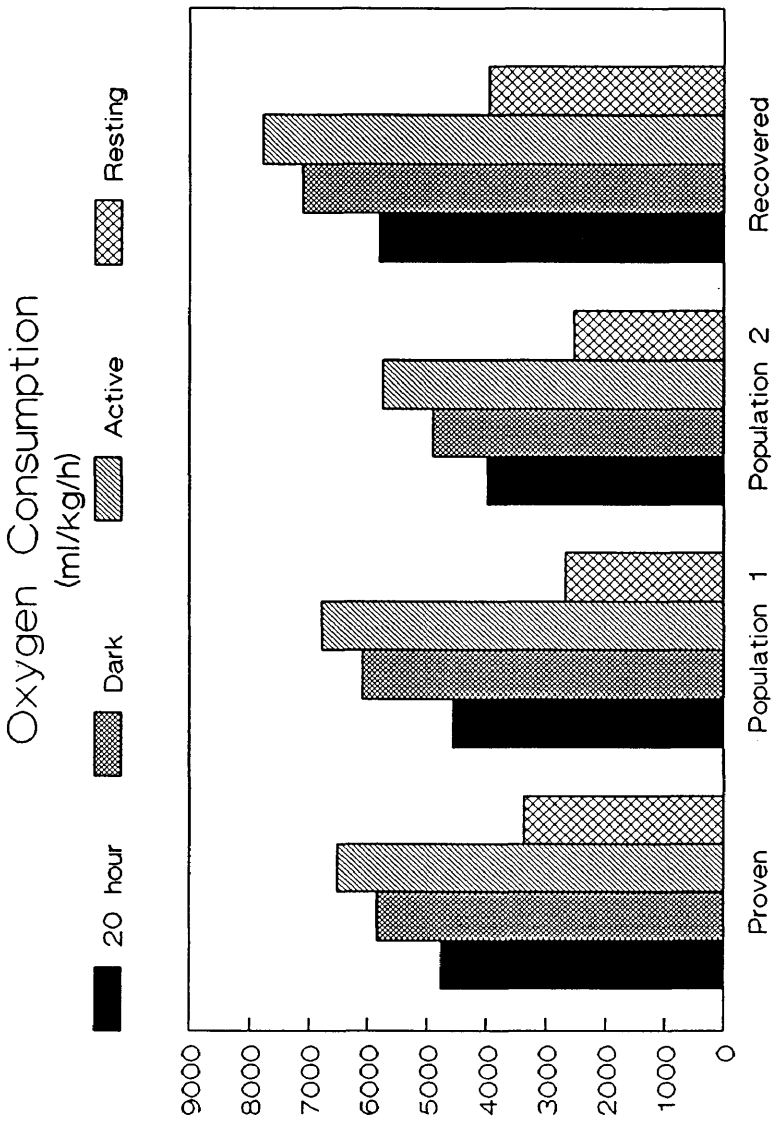
Differences between the proven animals and the two

Table 4. Oxygen consumption [VO_2] (ml/kg/h) for reproductively proven, population, and reproductively recovered population 1 males. Mean values ± SEM.

Animal Measurement	20 hour daily mean	Dark period (2030-0530) mean	Active Metabolic Rate	Resting Metabolic Rate
Proven				
VO_2	4760^{a} ±160.0	5838^{a} ±264.6	6522^{a} ±335.5	3370^{ab} ±126.7
Population 1				
VO_2	4571^{bc} ±137.3	6095^{c} ±131.4	6780^{c} ±178.2	2677^{a} ±73.3
Population 2				
VO_2	3992^{ac} ±213.9	4898^{bc} ±87.6	5750^{bc} ±173.8	2538^{b} ±102.6
Recovered				
VO_2	5823^{ab} ±144.8	7108^{ab} ±298.3	7779^{ab} ±361.5	3972^{ab} ±98.3

Values with the same letter are significantly ($P<0.05$) different from each other. All groups contain 6 animals.

Figure 2. Oxygen consumption for reproductively proven, population and reproductively recovered males.



populations for the 20 h daily mean are not consistent probably because this block of time contains merged values for both the AMR and the RMR (Table 4). Reproductively proven animals had a significantly higher RMR compared with reproductively inhibited animals from both Populations 1 and 2 (Table 4, Figure 2). However, the mean AMR values for proven and population animals were not significantly different. This finding is supported by the observation of Hayes (1989b) who also found no differences in the maximal VO_2 values between reproductive and non-reproductive Peromyscus. Reproductively inhibited animals from populations did not exhibit the chronically high metabolic rates predicted by Andrews (1979).

When animals from Population 1 were allowed to recover following pairing with a female, the oxygen consumption for all periods measured was significantly elevated compared with reproductively proven animals (Table 4, Figure 2). Oxygen consumption for the 20 hour daily mean and the RMR for the recovered Population 1 animals was also significantly different from that obtained when the same animals were tested immediately following removal from the population (Table 4, Figure 2). Population 2 animals were significantly different from recovered animals for all four periods (Table 4, Figure 2). These findings conflict with an earlier report of Andrews (1979) who found a high oxygen consumption in population animals that could not be reversed

by removal from the population. Not only did the population animals have lower oxygen consumption than the proven animals, but when removed from the population to allow for the maturation of the reproductive organs, the animals had a higher oxygen consumption. These recovered Population 1 animals clearly underwent a drastic and significant change from their previous condition. The elevation in oxygen consumption in the recovered animals over that of proven animals could be reflective of the initiation of sexual maturity after a significant delay, a condition resembling a pubertal transition.

Animal age and body composition should be considered in metabolic rate comparisons. A decline in metabolic rate with age has been repeatedly documented (Cook and Hannon, 1954; Kleiber, 1961; Gebczynski, 1971), and should be considered as a basis for the RMR differences observed in this study. However, in this case it is not a reasonable explanation since the younger population animals actually have a lower metabolic rate than the older proven animals. Body composition also has an influence on metabolic rate. Hayward (1965) stated that the basal metabolic rate (BMR) is affected by the percentage of body fat. Cronin and Bradley (1988) reported the body fat composition of population males to be 15.5% in comparison with 23.4% for proven males. While their data were only significant at the $P < 0.10$ level, the difference may be enough to influence the

metabolic rate.

Oxygen consumption values obtained in this experiment are comparable to those obtained in Peromyscus under similar conditions by Andrews and Belknap (1985) and Tomasi (1985). AMR and RMR values were usually obtained during the dark period and light period, respectively. This is also in agreement with previous work done on Peromyscus (Cook and Hannon, 1954) and Sorex (Gebczynski, 1971).

There were no correlations between testes weight or seminal vesicle weight with oxygen consumption. This suggests that reproductive inhibition in males ie., reduced testosterone levels, is not the direct cause of the metabolic rate reduction. The interplay of all the factors such as reduced testosterone levels, depressed thyroid activity, adrenal hypertrophy, and behavioral modification is probably important in inducing reproductive inhibition.

The Respiratory Exchange Ratio (RER) for P. maniculatus suggests that in a fed state on a lab diet consisting of 5% crude fat and 22% crude protein these animals have a metabolism based almost exclusively on carbohydrates in that they consistently have RERs in the 0.9 range. In order to confirm that these high RER values were not due to instrumentation or other error, Sprague-Dawley rats were measured and characteristic RER values of 0.8 were obtained. Also, a P. maniculatus male was tested while restricted to a Crisco diet and found to produce the

expected RER (0.7) for a pure lipid diet.

Further support for the normally high RER of P. maniculatus in the fed state was provided by Nagy and Pistole (1988) who reported that another cricetid, Microtus pennsylavanicus, has a metabolism based predominantly on carbohydrates. Carbohydrate appeared to be the primary fuel in both fed and fasted states. The animals in their study had a very low tolerance to fasting perhaps due to the inability to utilize fat stores. While it is possible that Peromyscus may also have a deficiency in their ability to use fat stores, it is unlikely because the RER does drop with time over a 24 h fasting period and usually results in a 5% to 22% weight loss (Bradley, personal communication). The significant weight loss and drop in RER suggest that P. maniculatus are capable of utilizing their fat stores when starved.

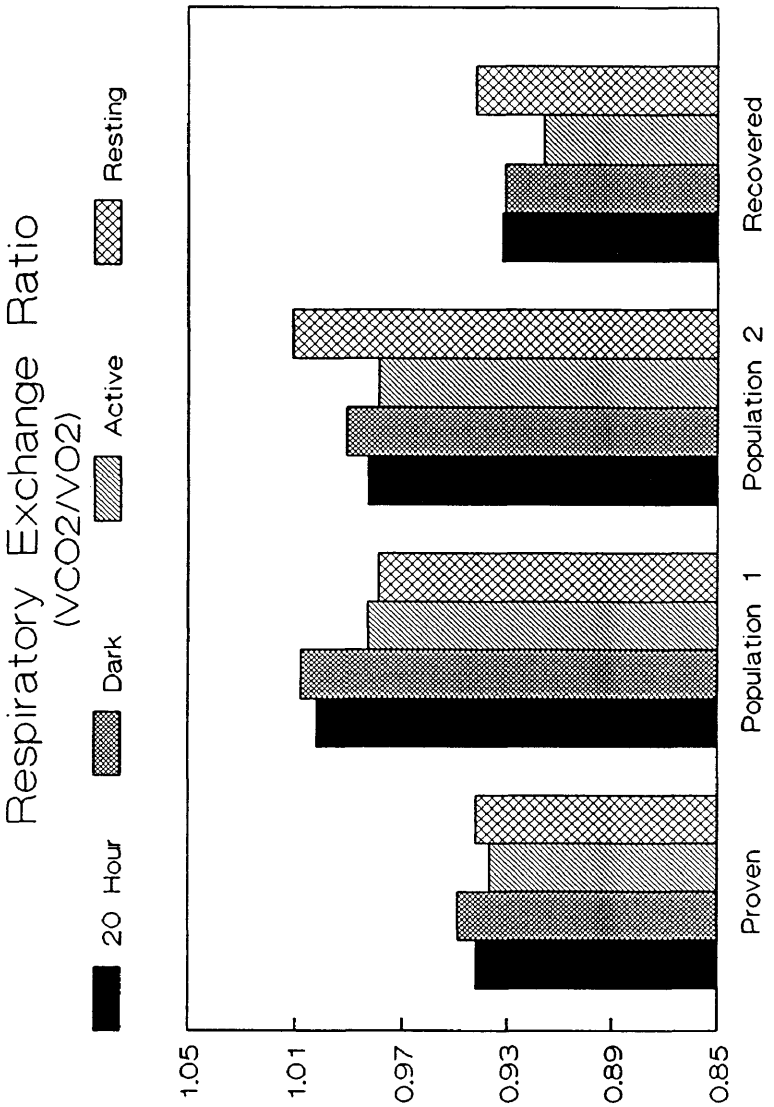
The RER for reproductively proven animals was not significantly different from recovered animals for any period evaluated (Table 5, Figure 3). Population 1 had significantly higher RER values when compared with themselves as reproductively recovered animals for all four periods, and the RER values for Population 2 animals were also significantly higher than the recovered animals for the 20 hour daily mean, the dark period, and the AMR (Table 5, Figure 3). The significantly higher RER values in the population inhibited may suggest that they were not

Table 5. Respiratory exchange ratio [RER] (VCO_2/VO_2) for reproductively proven, population, and reproductively recovered population 1 males. Mean values ± SEM.

Animal Measurement	20 hour daily mean	Dark period (2030-0530) mean	Active Metabolic Rate	Resting Metabolic Rate
Proven				
RER	0.942^{x} ±0.0101	0.949^{wx} ±0.0084	0.937^{wy} ±0.0101	0.942^{x} ±0.0178
Population 1				
RER	1.002^{z} ±0.0166	1.008^{wy} ±0.0103	0.983^{wx} ±0.0049	0.979^{xy} ±0.0443
Population 2				
RER	0.983^{xy} ±0.0087	0.991^{xz} ±0.0055	0.979^{yz} ±0.0084	1.011 ±0.0102
Recovered				
RER	0.932^{yz} ±0.0090	0.931^{yz} ±0.0129	0.916^{xz} ±0.0159	0.942^{y} ±0.0093

Values with the same letter are significantly ($P<0.05$) different from each other. All groups contain 6 animals.

Figure 3. Respiratory Exchange Ratio for reproductively proven, population, and reproductively recovered males.



experiencing an ACTH-induced lipolysis or that they had insufficient lipid to mobilize. Earlier work by Coppes and Bradley (1984) also reported no significant elevation of serum ACTH of reproductively inhibited animals. Both population inhibited and reproductively proven animals appear to utilize carbohydrate as the primary energy fuel.

The fact that some cricetids can utilize carbohydrate as their sole energy source casts some doubt on other studies that measure VCO_2 only and then calculate VO_2 by assuming RERs in the 0.8 range (Tomasi, 1985; Hayes, 1989a). Kleiber (1961) cautioned that RERs collected over short periods of time may be confounded by a "wash-out" of extra carbon dioxide from a carbonate pool; therefore, only RERs calculated over a sustained period of time would be reliable.

Many previous metabolic studies have ignored the circadian variation in metabolic rate and the activity state of the animal (Hayes, 1989a; Conteras, 1984). Haim et al. (1988) attributed the circadian variation in VO_2 in Saccostomus to an increase or decrease in the thermoregulatory demands on the animal. Seasonal variation in the maximal metabolic rate (VO_2) in Peromyscus is well documented (Hayes and Chappell, 1986; Andrews and Belknap, 1985).

Another common oversight in metabolic studies is that measurements are often taken before the animal has

acclimated to the study temperature and/or its test surroundings. In addition to the absence of acclimation time, the animals are further stressed by being tested in a food and water deprived or starved state (Cook and Hannon, 1954; Hayes, 1989b; Tomasi, 1985; Hayes and Chappell, 1986). Kleiber (1961) justified the practice of starving the animal prior to taking metabolic measurements by claiming that it standardized the activity state of the animal and made the measurements more reliable. The common practice of testing fasted animals in the thermoneutral zone ostensibly allows Basal Metabolic Rate (BMR) measurements to be attained. The RMR has been demonstrated to be significantly higher than the BMR in P. m. bairdii from populations (BMR= 2083 $\pm$ 87.8 vs RMR= 3231 $\pm$ 163 ml/kg/h) (Bradley, personal communication). However, the RMR is within approximately 30% to 40% of the actual BMR and the confounding varibles of starvation stress are removed by using the RMR.

In this experiment, measurements were taken continuously over a 23 hour period on fed animals. Taking measurements for this length of time allowed the observation of the entire range of activity without having to starve the animal and probably eliminated confounding variables related to choosing an arbitrary time block for resting or activity states.

The paucity of literature dealing with the metabolic differences between reproductively proven and reproductively

inhibited animals clearly indicates that more work needs to be done in this area. I found no papers concerning the RERs of fed Peromyscus. Studies focusing on the functionality of carbohydrate metabolism should also be performed in order to understand how and why these animals continually metabolize carbohydrates.

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