The effects of food restriction on wheel running in rats

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Abstract

The impact of food restriction and refeeding on wheel running was examined in 3 groups of rats: 55 day old male rats in Experiment 1; 140 day old male rats in Experiment 2; and 180 day old female rats in Experiment 3. After a baseline period of 24 or 40 days wheel experience with ad lib food access half of the animals were food restricted for 16 days by being given a limited amount of food daily to reduce (and maintain) their body weight at 85% of their baseline weight. After food restriction the rats were returned to ad lib food access and running was observed for another 16 days. Running performed by the food-restricted rats was compared to the running performed by continuously ad lib fed control rats. There were pronounced differences in the baseline running between the 3 groups of rats. The 55 day old males and 180 day old females showed high baseline levels of running while the 140 day old males showed low levels of running. Food restriction increased total wheel running by 70% from the high baseline levels in the 55 day old male group and by 112% from the low baseline levels in the 140 day old male group, but did not increase running in the female group. The food restriction induced increase in male running occurred only when the rats were decreasing in body weight; running returned to baseline levels for animals maintained at 85% baseline body weight. Baseline levels of running did not predict the increase in running for any of the rats. When ad lib food access was reinstated the high baseline running food-restricted 55 day old males showed a decrease in wheel running to below their baseline and control group levels. This decrease in running was negatively correlated with baseline levels of running, suggesting that all runners were approaching a floor level of running. The low baseline running food-restricted 140 day old male rats did not show a decrease in wheel running at refeeding; instead their running gradually declined to baseline levels over about 3 days. After ad lib food access was reinstated there were no long-term consequences of food restriction on wheel running. These results indicate that food restriction induced changes in wheel running are not influenced by age (or some covariate of age, such as body weight) in male rats, but are affected by gender, by baseline levels of running, and are controlled by a separate mechanism than that which causes the individual differences in baseline wheel running.

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The Effects of Food Restriction on Wheel Running in Rats

When given access to a running wheel rats will voluntarily run (Richter, 1927), and will even work for the opportunity to run (Iversen, 1993). Further, when they are placed on restricted food access they will, as a consequence, increase their wheel running (Richter, 1927). Food restriction has been argued to increase the amount of wheel running rats will perform by making it more rewarding (Pierce, Epling & Boer, 1986). Moreover, this combination of wheel access and food restriction resulting in arguably excessive amounts of wheel running has been used as an animal model for the human disorder anorexia nervosa (Epling, Pierce & Stefan, 1983). However, despite 80 years of investigation into the interactions between food restriction and wheel running, a number of questions remain to be explored. First, the effects of age and gender on wheel running induced by food restriction are not clear. Second, the ways in which the large individual differences in wheel running interact with the effects of food deprivation need to be determined. And finally, the long-term consequences that a period of food restriction has on wheel running are not well documented.

In the literature on wheel running induced by food restriction a number of different parameters have been explored. Researchers have used different food restriction methodologies with differing degrees of deprivation; they have deprived rats at wheel introduction or after extensive wheel experience, and they have used rats of different ages, strains, and genders, sometimes interchangeably. Many of these factors influence pre-restriction baseline levels of wheel running, and so it is questionable if these studies are directly comparable. Moreover, there are conflicting data on what happens to wheel running after food restriction is discontinued and the rat is returned to unlimited, ad lib food access. Since the food restriction paradigm for wheel running has been suggested as a model of human eating disorders (Epling et al., 1983), it would seem important to document similarities and inconsistencies in the literature, as they may influence the interpretation of any study using this paradigm.

It is my goal in this introduction to review past studies in this area to analyse how pre-restriction baseline running and methodological differences influence the impact of food restriction on wheel running. This will lead to the present experiments which study the effects of food restriction on wheel running in 55 day old adult male, 140 day old adult male and 180 day old adult female rats, focusing particularly on baseline pre-restriction differences in running and on how this running is changed by food restriction. It was expected that the 55 day old male and 180 day old female rats would show similar levels of baseline wheel running, thus making it easy to compare the effects of food restriction across genders given similar baseline levels of running. The 140 day old male rats were expected to run at low baseline levels, thus allowing for comparisons between high and low baseline running male rats. I will also examine the after-effects of food restriction on wheel running when ad lib food access is reinstated.

Ad Lib Food Access and Wheel Running

When young, ad lib fed male rats are first given access to a running wheel they will run about 1,000 wheel turns per 24 hours, and increase their daily running over 2 to 3 weeks (Looy & Eikelboom, 1989; Reid & Finger, 1955). This increased running is followed by a stable plateau, which in our lab is usually between 5,000-6,000 wheel turns per 24 hours, run mostly at night (Eikelboom & Mills, 1988; Looy & Eikelboom, 1989; Mueller, Loft & Eikelboom, 1997). Other researchers have found the same general pattern of wheel running, although the absolute amount of running tends to vary between labs (Richter, 1927; Peng, Jian, & Hsu, 1980; Mondon, Dolkas, Sims & Reaven, 1985).

The high intensity running found in young, ad lib fed, adult male rats changes with age. Peng et al. (1980) found voluntary wheel running peaked at around 10 to 11 weeks of age, after which there was a gradual decline in running.

The stabilised level of wheel running seems to be related to the age of the rat when the running wheel is first made available (Jakubczak, 1973; Looy & Eikelboom, 1989; unpublished observations). Jakubczak (1973) found that ad lib fed rats that are older (293 and 746 days old) at the time of wheel introduction show less frequent and slower wheel running than younger rats (67 days old), and the running is maintained for shorter periods of time. Our lab has found that ad lib fed rats given wheel access at 55 days of age show a rapid increase in wheel running, reaching a plateau after about 2-3 weeks at relatively high levels. Conversely, rats that are 140 days old at wheel introduction will continue to run at the initial low level, not showing any increase in running. This level of running is less than that of 140 day old male rats that have had wheel access since they were 55 days of age. The older the rats are when the wheel is introduced, the slower and less pronounced is their increase in wheel running (90 day old male rats showed a small increase in wheel running, between that of the 55 and 140 day old rats). It is important to note that older male rats also weigh more than younger male rats, making it difficult to distinguish between the effects of age and weight on wheel running. The weight difference, or some other age related difference, may be responsible for the differences in running. However, heavy, weight matched rats that have been introduced to the running wheel at an early age run at higher levels than rats introduced when both heavy and older.

While most research is done using young adult male rats, male and female rats behave differently when given access to a running wheel. Female rats are known to run more than males (Tokuyama, Saito & Okuda, 1982). As well, the female rat has a 4-day estrus cycle, and her running follows this cycle, peaking on the night of estrus (see early review by Shirley, 1929). In our laboratory young adult female rats will typically plateau at about 10,000 wheel turns in 24 hours (Eikelboom & Mills, 1988). Our lab has found that older female rats still increase their wheel running when introduced to the running wheel. Females introduced to the wheel at 180 days old rapidly increase their running, plateauing at about 6,000 wheel turns after 3 weeks (unpublished observations). Thus, female rats not only run more, but they also seem resistant to the dramatic decline in plateau levels of running associated with age of wheel introduction in male rats. It should be noted that, unlike males, female rats do not gain much weight as they age, suggesting that the weight of the rats at wheel introduction influences running levels.

While rates of wheel running are relatively consistent from day to day for an individual rat, groups of rats often show a large variance in running (Reed, 1947). Some labs report a 40-fold difference in running levels between animals (Riss, Burstein, Johnson, & Lutz, 1959), and our lab has seen groups of rats display a stable average running of 5,000 wheel turns a day, with some rats running over 12,000 wheel turns and others running only 1,000 (Eikelboom, 2001). Afonso and Eikelboom (2002) found that the only reliable predictor (among food and water consumption, body weight, weight gain, or wheel running) of how much wheel running rats will perform after 32 days of running is the amount of running they performed in the first 3 days of wheel access, but this only accounted for 23 % of the variance in running. This correlation demonstrates the consistency in running found for individual animals, as high wheel runners at wheel introduction will remain high wheel runners 32 days later.

Surprisingly there is a drop in the rat's food consumption when the running wheel is first introduced, which, over about 10 days, returns to, and later surpasses, baseline feeding levels (Looy & Eikelboom, 1989). Even if at wheel introduction access to the running wheel is restricted to two daytime hours, food intake decreases over the next 24 hours by an amount similar to that in rats with ad lib wheel access (Lattanzio & Eikelboom, 2001). This wheel induced feeding suppression is also evident if animals are placed on food restriction when wheels are introduced and may play a critical role in the death this activity anorexia induces (Epling & Pierce, 1984; Routtenberg & Kuznesof, 1967). A result of the feeding suppression is that running rats come to weigh less than homecage controls, a weight loss they maintain as long as they have wheel access (Collier, Hirsch, & Leshner, 1972; Looy & Eikelboom, 1989). The fact that the weight difference is maintained despite the recovery of food intake suggests to some that wheel running changes the animal's weight 'set point' (Collier et al., 1972).

Food Deprivation and Food Restriction

There are 3 methodologies commonly used to limit a rats' access to its food supply. First, one may completely deprive the animal of food for 24 hours (or multiples of 24 hours), not allowing it access to any food. Secondly, one may give the rat a fixed daily time period when food is made available ad lib, such as restricting it to 1 hour of food access, subsequently depriving it of food for 23 hours. This procedure enables the rats to be maintained on food restriction for several days or weeks. It should be noted that this method of food restriction typically entails giving the rats their 1 hour of food access at the same time each day. It is known that the rat will learn when food is made available, even if it is given during the light cycle when rats typically eat very little. Lastly, during the third method of restricted food access a rat may be given a set amount of food to last it for 24 hours. Each of these methodologies has unique characteristics. The amount of weight the animals will lose, the time it takes to recover from the food deprivation or restriction, and the ways food consumption changes to accommodate the recovery are different for each method. In this thesis food deprivation will refer to when an animal is deprived of all food, going without food access for a certain period of time. Conversely, food restriction occurs when the animals has a limited amount of access to food within 24 hours, be it measured by a set amount of time when food is made available, or a set amount of food made available for the 24 hours.

One measure of the effectiveness of food deprivation or food restriction is the amount of body weight the animal loses. Loss of body weight is typically reported as a percentage of weight lost during deprivation relative to the animal's baseline body weight prior to the manipulation. Two other ways of reporting weight loss are percentages relative to age-matched control animals not given restricted food access, and relative to the animal's own projected body weight. Because adult male rats tend to gain weight throughout their life, the second and third way of reporting weight loss would yield larger percentages of weight loss than the first. Unless otherwise stated, in this thesis studies using food deprivation or food restriction report loss of body weight relative to baseline measures.

Each of the 3 methods of food deprivation or food restriction results in different amounts of body weight lost. Armstrong, Coleman, and Singer (1980) deprived rats of food for 24, 48, 72, or 96 hours and measured their body weight. The longer the rat was on food deprivation the more weight it lost. Rats deprived for 24 hours lost about 7% body weight, while those deprived for 48, 72, and 96 hours lost about 10%, 17% and 19% respectively. The researchers calculated these percentages relative to the animals' expected body weight, so these values may be slightly inflated relative to measures using baseline weights. Larue-Achagiotis and Thouzeau (1996) deprived young male rats of food for 6 and 12 days, and found that 6 days of fasting resulted in a 19% decrease in body weight relative to baseline measures, while 12 days of deprivation resulted in a 35% body weight loss. These studies suggest that when a rat is deprived of food it will lose weight in a monotonic fashion.

When rats are placed on a food restriction schedule that gives them unlimited, ad lib food access for 2 hours each day, Hurwitz, Stewart, and Wasservogel (1958) found that, after 3 weeks, rats lost between 18% and 24% of their baseline body weight. They also found that the lighter rats at the start of the food restriction lost the highest percentage of weight. Hurwitz and Davis (1983) argued that 2 hours of food access each day could result in the long-term maintenance of a stable weight of about 80% baseline body weight. When Ehrenfreund (1959) gave rats 1 hour of food access each day, he found that they declined in body weight for the first 8 days of food restriction. This decline was followed by a steady increase in body weight, which reached baseline levels after about 55 days and continued to rise until 100 days of food restriction, when the study was terminated. The food-restricted rats came to weigh 30 g more than their baseline body weight by day 100. Ehrenfreund (1959) also found that the food-restricted rats increased their food intake during the 1 hour they had food access over about 15 days, increasing from 18 g of food to about 38 g after 20 days of food restriction. For the remainder of the study they maintained a food intake of about

38 g in the hour they were fed. Giving rats a set amount of time when food is made available ad lib will allow them to maintain their body weight for long periods of time.

When Bolles (1963) gave rats access to 10 g of food every 24 hours for 12 days he found that by the end of the food restriction period the rats had lost between 15% and 20% of their baseline body weight. He also noted that the rats usually ate the food within the first hour of receiving it, indicating that this method of food restriction may be quite similar to giving rats 1 hour of food access each day. Brownlow, Park, Schwartz and Woods (1993) found that when rats were given 70% or 50% of their ad lib food intake either at one time or split over two meals, the rats that were given 50% lost weight faster. The rats fed once each day lost weight at the same rate as rats fed twice each day. When the food restricted rats reached a criterion 75% of the body weight of ad lib fed control rats the food restriction was discontinued. The rats that received 50% ad lib food intake reached this criterion in about 24 days while the rats that received 70% ad lib food intake failed to reach the criterion after 60 days of the food restriction schedule. Their body weight plateaued at about 80% of control body weight by about day 40.

When rats were returned to ad lib food access after a period of food deprivation, Armstrong et al. (1980) found that those deprived for 24 hours were able to recover the weight they had lost after 15 days. They report that even 40 days after food deprivation those deprived for 48, 72, and 96 hours never came to weigh as much as was expected according to the growth curves obtained during pre-restriction baseline. However, the growth curves the rats were compared to were linear, and rats may gain weight in a non-linear way as they age, so age-matched control rats would have been a more appropriate comparison for body weight.

Lawrence and Mason (1955) restricted rats of food by allowing them 2 hours of ad lib food access for 61 days, after which they were returned to ad lib food access for 14 days. They found that the rats recovered to baseline body weight levels by the third day of ad lib food access and continued to increase their body weight over the 14 days. After Brownlow et al. (1993) restricted rats to 50% or 70% of their ad lib food intake, under which the 50% restricted rats declined in body weight to 75% of their pre-restriction body weight where as the 70% restricted rats declined to 80% of their baseline body weight, the researchers returned the rats to ad lib feeding for 120 days. They found that both food restriction groups rapidly increased their body weight over the first 20 days of ad lib food access, plateauing at about 90% of continuously ad lib fed control animals, where they remained for the rest of the study. During the refeeding period there were no differences in percentage body weight between those rats that had been given 50% or 70% of their ad lib food consumption.

When animals are returned to ad lib feeding conditions after a period of deprivation there is a change in their food consumption pattern compared to baseline and control rats. Levitsky (1970) found that rats that were food deprived for 24 or 72 hours increased their food intake on the first day of recovery, with no difference between the 2 deprivation groups. After about 4 days food intake returned to normal, baseline levels for the 24-hour deprivation group, but remained above normal after 12 days for the 72-hour deprivation group. The total number of meals eaten per day did not change in either group, but the amount of food consumed in those meals increased similarly for both deprivation groups in the initial refeeding period. Levitsky (1970) concluded that, regardless of the length of food deprivation, the rats recovered by adjusting the size rather then the frequency of their meals. Baker (1955), who deprived rats for 12, 24, and 36 hours, and Larue-Achagiotis and Thouzeau (1996), who deprived rats for 6 and 12 days, reported similar results.

Rats given ad lib food access after 61 days of 2 hours of food access each day tend to 'overeat' the first 5 to 7 days of ad lib food access, initially consuming about 29 g of food, decreasing to about 23 g of food by day 6 (Lawrence & Mason, 1955). The rats further decreased their food consumption to about 18 g per 24 hours for the next 8 days. Rats given 70% of their ad lib food consumption in either 1 or 2 meals per day, as well as rats given 50% of their ad lib food in 1 meal per day, and then subsequently given ad lib food access will demonstrate increased food consumption compared to control rats (Brownlow et al., 1993). By the second week there were no group differences in the amount of food the rats consumed compared to continuously ad lib fed control rats.

Each of the 3 methods of food deprivation and food restriction have unique consequences in terms of the amount of body weight lost while under food deprivation or restriction, how long the rat can be maintained on the restricted food access schedule, and the ways in which the rat recovers when given ad lib food access. Since these methods vary in terms of their effects on the rat, the increased levels of wheel running induced by food deprivation or food restriction may also be different in each method.

Food Deprivation, Food Restriction and Wheel Running

Absolute food deprivation has been used by researchers exploring wheel running, with fairly consistent results (Finger, 1951; Finger & Reid, 1952). When Finger (1951) food deprived wheel-experienced rats for 24 or 72 hours, he found there was a marked increase in the rat's wheel running. During this 24 and 72 hours of deprivation, wheel running increased 121% and 194% respectively, relative to baseline running levels (absolute levels of running were not reported). Finger (1951) reported a corresponding decrease in the body weight of the rats during deprivation, at 92% baseline after 24 hours of deprivation, and 81% baseline after 72 hours of deprivation. Livesely, Egger, and Meyer (1972) found that rats increase their wheel running more when under both food and water deprivation than when deprived of just food or just water, and the decrease in body weight was also larger under the combined condition.

Sclafani and Rendel (1978) examined food deprivation induced wheel running in rats made obese or lean by altering the palatability of their diet, and compared them to normal diet control rats. At wheel introduction the obese rats weighed about 400 g, the lean rats weighed about 310 g, and the normal control rats weighed about 360 g. All rats had similar baseline running levels of about 200 wheel turns per 24 hours, despite the differences in initial body weight. They found the dietary lean rats increased their wheel running the fastest; peaking at 1,600 wheel turns, and died after about 3 days of food deprivation. The normal control rats increased their wheel running to about 1,200 wheel turns and died after about 6 days. The dietary obese rats increased their wheel running to about 1,450 wheel turns, and died after about 12 days. While the obese rats took longer to reach their peak running than the normal controls, which took longer than the dietary lean rats, all of the rats peaked at about the same number of wheel turns (approximately 600% of baseline wheel running). The obese rats were also slower to lose weight than the normal and the lean rats, but all of the groups weighed about the same at death (between 260 and 240 g, which was about 69% of baseline of the normal control rats).

Another way to restrict a rat of food is to allow ad lib access to food for a 1 hour period each day (Hall, Smith, Schnitzer, & Hanford, 1953). This very common form of food restriction can be maintained longer than acute deprivation, while still increasing wheel running in the rat. When Hall et al. (1953) gave male rats 1 hour of food access for 21 days they saw an increase in wheel running from about 250 to 1,400 wheel turns per 24 hours. Tsuda, Tanaka, Iimori, Ida and Nagasaki (1981) divided rats' access to food into 2, 30-minute meals or 1, 1-hour meal, and found that when the food access was divided, the rats consumed more food and lost less weight than when the rats were given food access only once per day. The rats that were given 2, 30-minute meals per day ran about 3,900 wheel turns, while the rats given only 1 hour of food access once per day ran more, approximately 4,200 wheel turns.

Lastly, rats can be food restricted by allowing them a set amount of food per 24 hours. This method of food restriction has been argued to be less stressful on the rat than both acute deprivation and a limited time of food access, as it allowed researchers to increase the amount of food made available to the rat, controlling the loss of body weight (Morse, Russell, Hunt, Wood, Epling, & Pierce, 1995). Limiting the amount of food made available is known to affect the subsequent amount of wheel running (Russell, Epling, Pierce, Amy & Boer, 1987). Young adult male rats (63 days old) given 15-18 g of food per day were found to increase their wheel running over about 20 days, reaching a plateau of about 8,000 wheel turns (Russell et al., 1987). Russell et al. (1987) were able to influence the amount of running performed by each rat by manipulating the amount of food given so that running was maintained between 6,000 and 10,000 wheel turns per 24 hours. The researchers noted that the body weight of the rats given 15 g of food per day rapidly decreased during the same time the wheel running of the rats increased. When weight was stabilised (by giving the rats 18 g of food per day) the wheel running also plateaued.

Few studies have compared the effects of the 3 methods of food restriction on wheel running. Moskowitz (1959) put rats on either a fixed amount of 40% of their baseline food consumption, fed the rats for a fixed time of 1 hour each day, or brought the rats weight down and maintained it at 80% of their baseline body weight. The rats were given 1 hour of wheel access just prior to being fed in their home cages. She found that only the rats in the fixed percentage weight group maintained a stable body weight over the 25 days of food restriction, and also showed a stable level of wheel running – the rats in the other groups continued to lose weight and increased their running throughout the study. The rats in the fixed percentage weight group decreased in body weight to 80%, with the fixed amount of food group decreased to 70%, and the fixed time rats decreased to 76% over the 25 days of deprivation. Moskowitz (1959) found that activity did not increase until body weight had decreased to a 'breaking point' of between 85-90% of normal, age-matched control animals' ad lib fed body weight. She argued that below this point activity levels were a direct function of weight loss. Thus, if body weight was not decreasing, as with the fixed percentage weight group, wheel running plateaus.

Morse et al. (1995) compared wheel running of rats given ad lib food access, restricted to 90 minutes of food access, or to 15 g of food per day. The rats given 90 minutes of food access each day lost body weight so rapidly they were removed from the study after 4 days, while those given 15 g of food each day were able to maintain their body weight after an initial decrease. Both food-restricted groups increased their wheel running compared to the ad lib fed group, with the 90-minute group increasing more than the 15 g group. The researchers concluded that the excessive wheel running seen in food restricted animals is dependent on the severity of the food restriction. They suggest that allowing a rat 90 minutes of ad lib food access per day is a more severe form of food restriction than giving the rat 15 g of food per day, as the 15 g group was able to maintain their body weight despite increased levels of running.

Characteristics of Food Restricted Wheel Running

When a rat is exposed to food restriction or food deprivation schedule several changes in the rats' wheel running have been reported. When under food restriction the rats will show anticipatory running, which is an increase in wheel running during the hour prior to the rats being fed (Reid & Finger, 1955). Food restriction has also been known to increase the amount of daytime running (Armstrong et al., 1980), and has been argued to change the reinforcing value of wheel access (Pierce et al., 1986). Furthermore, wheel access and food restriction applied simultaneously will result in activity anorexia (Epling & Pierce, 1984). The degree of weight loss the rats experience has been argued to play an important role in increased wheel running induced by food restriction (Kanarek & Collier, 1981).

Reid and Finger (1955) reported steadily increasing anticipatory running for rats given 1 hour of food access per day over 35 days of food restriction, starting at 0 wheel turns in 1 hour on the first day, and ending at 1,671 wheel turns in 1 hour by day 35 (the total daily running during the same time period increased from 493 to 6,664 wheel turns). This increase was evident even though the researchers fed the rats during the light part of their light: dark cycle, when wheel running is typically low. When the rats were removed from the food restriction schedule, and returned to ad lib food access, the rats quickly decreased their running in the hour preceding when they usually got fed to only 16 wheel turns on the first day of ad lib food access, and decreased to 0 wheel turns in 1 hour by day 7. The development of anticipatory running led the researchers to suggest that the rats were being rewarded for their increased wheel running by food access. Finger, Reid and Weasner (1957) subsequently examined rats given 1 hour of wheel access and 1 hour of food access each day. They had one group receiving food immediately after the wheel access, while another group was placed in a neutral delay chamber for their 1 hour after wheel access before being given 1 hour of food access. The researchers found that the rats given food access immediately ran more than those placed in the delay chamber after running. This suggests that when the rats are on 1 hour a day of food access anticipatory running is strongest just before food is made available.

It has been hypothesised that food restriction also changes the reinforcing value of wheel running. Pierce et al. (1986) had rats bar press for wheel access on a progressive ratio schedule after various degrees of food deprivation. They found that the animals would continue to bar press to higher ratios when food deprived than when food satiated. They argued that deprivation or satiation in one behaviour may change the rewarding effects of another behaviour, specifically, that food deprivation increases the reinforcing value of wheel access.

When a food restriction or deprivation schedule is imposed there have been reports of a shift in the light: dark cycle of the rat's wheel running. Armstrong et al. (1980) found that rats would increase their running in the light hours during 96 hours of food deprivation, until the rats ran as much during the light as during the dark. It seems wheel running during the light is particularly sensitive to the effects of food deprivation.

When rats are given only 1 hour of food access per day at the same time a running wheel is first made available the increase in running is even larger than that naturally seen in non-food restricted rats. This procedure is termed self-starvation (Routtenberg & Kuznesof, 1967) or activity anorexia (Epling & Pierce, 1984). This resulting increase in running is argued to be excessive (Epling & Pierce, 1984). The animals rapidly increase their wheel running and consume less food than food restricted control rats not given a running wheel, resulting in a marked weight loss, which eventually leads to their death. Kanarek and Collier (1981) argue that a restricted feeding schedule combined with the introduction of a running wheel compounds the weight loss effects of wheel introduction, resulting in a weight loss larger than that of either manipulation alone. The researchers suggest that this weight loss leads to increased running and failure to increase food consumption in the restricted feeding schedule, and the rats fail to recover food intake under food restriction. This immediate effect of food restriction on wheel running suggests profound interactions between food consumption and running.

Exploring the relationship between the increase in wheel running induced by food restriction and body weight, Routtonberg and Kuznesof (1967) report a negative correlation between weight and wheel running, suggesting that as the rats' body weight decreases in response to food restriction, their wheel running increases. Moreover, other labs have argued that the increase in wheel running under food restriction is due to the decrease in body weight, not the decrease in food consumption (Duda & Bolles, 1963).

Factors Influencing Food Restricted Wheel Running

There are many factors known to affect wheel running in general which may play a role in food restriction induced increases in wheel running. The age and gender of the rats, as well as individual differences in wheel running may all influence increased wheel running induced by food restriction.

Examining the age of the rats under food deprivation, Koubi, Robin, Dewasmes, Le Maho, Frutoso and Minaire (1991) exposed 63, 126, and 231 day old male rats to 6, 10, and 15 days of deprivation respectively. They found that, while all groups increased their wheel running during the food deprivation, the older the rats were, the slower was the increase in running, and the lower the final level of running. In this study the older male rats weighed more than the younger ones, so the influence of age may also be the influence of the weight of the animals. Boakes, Mills, and Single (1999) examined gave 52 day old male rats, 52 day old female rats, and 136 day old female rats ad lib wheel access and 90 minutes of food access, and examined the affects on rate of body weight loss. At the start of the study the older female rats weighed about the same as the young male rats, which both weighed more than the young female rats, thus the researchers were able to compare the influence of age and body weight. They found that, during food restriction, the young female rats lost body weight at the same rate as the young (but heavier) male rats, and the older (but weight matched) female rats lost weight slower than the young male rats. While this suggests that rats of the same age lose weight in a similar manner, and older rats lose weight slower, this comparison is across genders. Therefore, gender differences in weight loss may also be influencing these results. There are some studies suggest that age influences food restriction induced wheel running; however, body weight, or some other covariate of age (such as hormone levels or percentage of body fat), have not been ruled out as possible explanations for the differences in wheel running and rate of body weight loss during food restriction.

There are very few studies that examine differences between male and female rats' wheel running while under food deprivation or restriction. Boakes et al. (1999) compared 52 day old male and female rats given ad lib wheel access at the same time their food was restricted to 90 minutes of food access per day. They found that, while both groups of rats lost weight at the same rate on this food restriction schedule, the wheel running in the females was always higher than the males. In a second experiment the researchers decreased male and female rats' body weight until it reached 95%, 90%, 85%, and 80% of projected ad lib body weight, and then tested the 8 groups of rats with 1 hour of wheel access. Again, they found that the female rats were always higher runners than the males despite the male rats increasing their running in response to decreased body weight. While the male rats increased their wheel running as their weight decreased the females did not show significantly higher levels of wheel running at lower body weights. Their research suggests that with male rats increases in wheel running are closely related to decreases in body weight, but the same relationship may not exist in female rats.

While rats are known to show individual differences in wheel running levels under ad lib food access conditions (Reed, 1947), it may be that these individual differences play an important role in predicting wheel running while under food restriction. Tyler, Waag and Darnall (1974) divided rats into high and low wheel runners during ad lib food access. They found that, when given 2 hours of food access per 24 hours, the high running rats had quicker start latencies and faster running times to food reward in a straight alley runway than low running rats. This suggests that there may be qualitative differences between high and low wheel runners. However, there are no studies that explicitly examine food deprivation induced wheel running in individual rats, or that determine if baseline differences in running can predict subsequent increases under food restriction. However, if high runners respond to food restriction differently than low runners, it is hypothesised that they may increase their wheel running differently.

Factors such as method of food restriction, age (or some covariate of age, such as body weight), and gender of the rat, as well as individual differences seem to play important roles in wheel running induced by food restriction in the rat. The purpose of my thesis is to describe the increase in wheel running under food restriction in different rat populations that were expected to show differences in baseline levels of wheel running: 55 day old male rats, 140 day old male rats, and 180 day old female rats, all who have had extensive wheel experience. The food restriction method used was similar to Russell et al (1987), in that the rats were fed a set amount of food every 24 hours, ensuring that their body weight did not drop below 85% of their baseline body weight. Based on the work of Moskowitz (1959) a contributing factor in the food restriction induced increase in wheel running is the decrease in the rats' body weight during restriction. As such, the food restriction period was divided into two sections: 1) the time in which the food restricted rats were decreasing their weight to 85% of their baseline body weight, and 2) the time in which the rats were being maintained at this 85% baseline body weight. Differences were expected in the number of days it would take each rat to reach 85% of their baseline body weight. Thus, for period 1 when weight was declining analysis was done for the last days it took each rat to reach 85% baseline body weight, using the longest common number of days when all rats were still declining. Furthermore, the influence individual differences in baseline wheel running had on running while the rats were under food restriction were examined.

After Effects of Food Deprivation on Wheel Running

The increase in wheel running seen with food restriction and food deprivation suggests that wheel running becomes a more important behaviour under conditions of food scarcity, but is the importance of wheel running sustained after the period of food restriction? Using a progressive ratio operant procedure, Pierce et al. (1986) found that food restriction enhances the rewarding effects of wheel running, so it would seem important to know if this altered rewarding value maintains itself when ad lib food access is reinstated. If the running changes, in which direction does it go, and for how long are these changes evident? It is possible that, after a period of food restriction when the animal is returned to ad lib food access, the animal's motivation may also return to normal and running might return to pre-deprivation baseline levels.

Alternately, the period of elevated running might have permanently changed the animal, resulting in a maintained higher level of running.

There have been only a few studies that have followed the wheel running behaviour of rats after food restriction or deprivation, when ad lib food access is reinstated, and varied results have been reported. In the early literature, when rats were food restricted or food deprived and subsequently refed, some researchers have reported a 'satiety effect'. This term was coined by Finger (1951), and occurs when a rat's wheel running decreases below that of baseline or control levels when it is given ad lib food access after a period of restriction. This decrease in running was seen in rats that, at the start of the study were as young as 30 days (Finger, 1951) and as old as 115 days (Reid & Finger, 1955). The satiety effect has also been observed using both 24 hour or 72 hour acute food deprivation (Finger, 1951; Finger & Reid, 1952), as well as after 35 days of only 1 hour of food access per day (Reid & Finger, 1955). After 24 hours of food deprivation, when ad lib food access was reinstated, Finger (1951) reported wheel running to be 57% of pre-deprivation running on the first day, but the running returned to pre-deprivation levels by the second day of ad lib food access. Finger (1951) also reported a decrease to 17% of baseline running when ad lib food was reinstated after 72 hours of food deprivation. The running increased reaching 48% the second day, 68% the fifth day, 84% the twelfth day, and 88% of pre-deprivation baseline by the nineteenth day of ad lib food access. Unfortunately, the pre-deprivation baseline running was not specifically identified, as Finger did not differentiate between 4 groups of 30 and 115 day old male and female rats in his analysis of the satiety effect. (He did report that, at baseline, males ran less than females, and that the older rats ran less than the younger rats, reporting that the young female rats ran a maximum of 12,310 wheel turns, and the older males ran a minimum of 610 wheel turns per 24 hours.)

Finger and Reid (1952) reported a similar satiety pattern of running, but again their data was only presented as a percentage of baseline wheel running. Reid and Finger (1955) found a satiety effect when rats were returned to ad lib feeding after getting 1 hour of food access for 35 days. The baseline wheel running of 493 wheel turns per day in 140 day old male rats increased to over 6,000 wheel turns during food restriction. and subsequently decreased to 192 wheel turns when ad lib food access was reinstated, which was 39% of pre-restriction running. It would seem that the satiety effect has been observed across ages, levels of baseline running, and methods of food deprivation and food restriction.

Not all researchers have reported a satiety effect. In fact, while some researchers hint at a satiety effect (Hall et al., 1953), there are no labs outside of Finger's that report a significant decrease in running at refeeding. Instead, others report wheel running returns to baseline levels after food restriction or deprivation, either immediately or over 2-3 days (Hall et al., 1953; Koubi et al., 1991). This return from restriction induced elevation to pre-restriction baseline running levels occurs in ages ranging from 63 to 150 days, and with a variety of food restriction methods, including acute deprivation, 1 hour of food access per day, and a limited amount of food per day. After rats are given 1 hour of food access per day for 7 days, and are returned to ad lib food access, they immediately returned to baseline levels of running of approximately 250 wheel turns per day (Hall et al., 1953).

Similarly, when Stevenson and Rixon (1957) gave 150 day old rats one third of their daily food intake each day, and returned them to ad lib food access, they immediately returned to pre-restriction baseline levels of running of about 400 wheel turns. After fasting 63, 126, and 231 day old rats for 6, 10, and 15 days respectively, Koubi et al. (1991) reported a gradual decline in wheel running when the rats were given ad lib food access, returning to baseline levels over 3 days; however, the researchers report their data in terms of percentage of baseline running, not absolute values.

Many of the pre-restriction baseline levels of running are low, and the lack of a satiety effect may be the result of a floor effect on wheel running. However, Reid and Finger (1955) report a satiety effect (of 192 wheel turns) in rats that had a baseline level of running as low as 493 wheel turns per 24 hours. Both a satiety effect and an immediate return to pre-restriction baseline levels of running have been reported in low baseline running rats.

While Finger and associates have reported a satiety effect when ad lib food access is reinstated, others have not reported such a phenomenon. This difference does not seem to be a function of method of restriction, as the satiety effect has been reported in both acute deprivation conditions, as well as when the rats are given 1 hour of food access per 24 hours. It is possible that low levels of baseline running may hide the satiety effect, as a low running rat may not be able to run any less, but this does not seem to be the case. The satiety effect has been reported under both high and low baseline conditions, as well as in both young and older rats. Furthermore, others have argued that wheel running returns to baseline levels when the rats are returned to ad lib feeding, despite different food restriction methods, different aged rats, and different baseline running levels. Therefore, a second purpose of this thesis is to examine what happens to wheel running after food restriction is discontinued in rats showing varying levels of pre-restriction baseline running, clarifying this inconsistency in the literature.

Experiment 1

The immediate and long-term effects of food restriction on wheel running were examined in this study, using young adult male Sprague Dawley rats. The rats were followed for a baseline period of 24 days, during which time their running was expected to plateau. Then half of the rats remained on ad lib food access while the other half were placed on a food restriction schedule that was a modification of the procedure used by Russell et al. (1987). The food-restricted rats were given 15 g of food every 24 hours until they reached 85% (± 1 %) of their baseline body weight, after which their daily food was increased to maintain their body weight at this level. It was expected that the rat's wheel running would increase in response to the food restriction, plateauing at an elevated level at the time their weight reached 85% (± 1 %) of their baseline body weight and their food ration was increased. It was also predicted that the rats' wheel running would plateau at the increased level during the period of food restriction when the rats were being maintained at 85% (± 1 %) of their baseline body weight. It was further predicted that the rats would increase their wheel running in a manner proportional to their baseline running. Thus, individual differences in baseline wheel running should predict differences in the elevated wheel running while the rats were under food restriction. Low runners during baseline should increase their running but remain the low runners in the group during food restriction.

The second objective of this study was to examine wheel running after food restriction was discontinued. Some researchers have reported a satiety effect (Finger, 1951) while others did not (Hall et al., 1953). This experiment examined if a satiety effect was present in the high running young adult male rats when ad lib food was restored after 16 days of food restriction, and if individual differences during baseline or food restriction could predict wheel running levels when the rats were refed.

Method

Subjects. Sixteen male Sprague-Dawley rats from Charles River Canada weighing between 200-225 g (47-49 days old) at arrival were pair housed in 48 x 27 x 20 cm polycarbonate shoebox homecages. Each cage had a wire lid for holding food and water bottles, 1 cm of hardwood Beta Chip bedding, and an 8 cm diameter x 15 cm long ABS tube for environmental enrichment. The rats had ad lib access to tap water and to PMI Feeds Inc. Rat Diet pellets, except when on food restriction, as described below. The animals were exposed to a 12:12 light: dark cycle, with the lights on at 06:00 h, and were maintained at a temperature of approximately 21° Celsius. All manipulations and handling took place during the light phase. The rats were handled and weighed daily between 09:00 h and 10:00 h throughout the experiment, and the cages were changed bi-weekly at the time of weighing.

Apparatus. Nalgene wheels (diameter 30 cm, width 11 cm) were placed in shoebox cages the same size as the homecages. VitalView, a Mini Mitter Co. PC based data collection system, continuously recorded wheel revolutions in 5 s bins using a magnetic contact closure system.

Procedure. Upon their arrival at the laboratory the rats in this study were pair housed for a period of 6 days in the homecages. The rats were then placed individually in the Nalgene wheel cages and given ad lib access to the running wheel for 24 days (days 1 to 24). After this baseline period of wheel running, the rats were rank ordered based on their average wheel running during days 21 to 24, and each consecutive matched pair of rats were randomly assigned into one of two groups of 8 rats: an ADLIB group, and a RESTRICT group. The rats in the ADLIB group remained on ad lib food access throughout the entire experiment. The rats in the RESTRICT group were put on a food restriction schedule, a procedure similar to that used by Russell et al. (1987). These rats were given 15 g of food each day at 11:15 h, for 16 days (days 25 to 40). It was expected that this limited food access would cause the RESTRICT group rats to lose weight. The amount of food made available for each rat was adjusted to maintain the rats at or above 85% (± 1 %) of their initial body weight. When the rats reached 85% (± 1 %) body weight their daily ration of food was increased to 18 g each day. If this increase was not enough to maintain the rats body weight at 85% (± 1 %) baseline weight their food ration was further increased in 2 g intervals until weight was maintained.

On the fourteenth day of food restriction (day 38) the running wheels were disassembled and cleaned from 07:00h to 11:30h, so all of the rats were without running wheels during this time. This day is excluded from all analysis.

The RESTRICT group of rats were returned to ad lib feeding after 16 days of the food restriction schedule. Animals were followed under ad lib conditions for another 16 days (days 41 to 56). Wheel turns were recorded continuously throughout the entire experiment.

Analysis. The experiment was divided into 5 sections: baseline, days 21 to 24; 2 sections during food restriction based on the weight of the rats in the group RESTRICT; and 2 sections after refeeding, days 41 to 44 and the last 12 days of the experiment, days 45 to 56. A mixed measures days by group (ADLIB and RESTRICT) analysis of variance (ANOVA) was conducted on each section for each dependent variable. Appropriate simple main effects were analysed in the case of a significant Days x Group interaction. Significance is set at $p < .05$, but any repeated factor is only reported as significant if it is still significant after the Greenhouse-Geisser correction for possible sphericity violations, and when means are reported they are followed by the Standard Error of the Mean. In this and following experiments all significant results are reported. Results which approach significance ($.1 > p > .05$) and are meaningful are reported as a marginally significant finding.

The first section analysed was the last 4 days of baseline (days 21 to 24). This was to assess if there were any initial differences between the groups ADLIB and RESTRICT. The second section analysed was the period of food restriction when the weight of the rats in the RESTRICT group was decreasing to 85% (± 1 %) of their baseline body weight. In this experiment the first RESTRICT rat reached 85% (± 1 %) of it's baseline body weight on the third day of food restriction so the last 3 days of declining weight were analysed for all animals in the RESTRICT group. For each RESTRICT group rat the data for the same 3 days of the pair matched animal in the ADLIB group was used for the control measure. The third section of analysis was the 4 days at the end of the food restriction period (days 36 to 40, excluding day 38), a time when the rats in the RESTRICT group were maintained at 85% of their baseline body weight. The last 2 sections that were analysed were during the refeeding period. Since the satiety effect reported by Finger (1951) occurred only for one or two days, the first refeeding period was chosen to capture any possible satiety effect, analysing the first 4 days after ad lib feeding was reinstated (days 41 to 44). The final 12 days of refeeding (days 45 to 56) was analysed to examine the long-term consequences of refeeding.

Data analysis was conducted on a number of dependent measures, including the total number of wheel turns per 24 hours, the number of wheel turns performed in the hour prior to food access (10:15 h – 11:15 h), and the number of wheel turns done during the dark (18:00 h – 06:00 h) and during the light (06:00 h – 18:00 h) cycle.

Thus, wheel turns in the hour prior to food access during food restriction was included in the light cycle wheel turns.

To examine the influence individual differences may have on wheel running for the rats in the group RESTRICT correlations were determined between the total number of wheel turns performed on the last day of baseline running (day 24) and the change in wheel running on the day the rats reached 85% of their baseline body weight, the last day of food restriction (day 40) and the first day of refeeding (day 41). The change in wheel running was calculated as the difference between the baseline day and the day of interest, and was used to evaluate if all of the rats changed their level of running in proportion to baseline levels of running.

Results

Due to technical difficulties there were some days where a particular running wheel was disconnected from the data collection system (but the animals still had a free turning running wheel available ad lib). This occurred on day 43 for rat 12 (group ADLIB), day 45 for rat 3 (group RESTRICT), and day 47 for rat 7 (group ADLIB), all during the refeeding period. For this missing data the mean of the previous and subsequent days wheel turns for the animal were used to represent the day when the wheel was disconnected from the data collection system, and the degrees of freedom of the analysis was decreased accordingly.

In the food restriction period the rats in the RESTRICT group declined in weight at different rates and thus reached a point where they were fed more than the initial 15 g of food every 24 hours at different times, see Table 1. By the third day of food restriction, day 27, 1 rat was given an increased amount of food, and by day 29 the majority of the rats, 5 out of 8, were given more than 15 g of food every 24 hours. On day 36 all of the rats were given more than 15 g of food, and their food intake was adjusted for the rest of the food restriction period to ensure that the rats maintained 85% of their baseline body weight. It is interesting to note that by day 31 more than half of the rats in the RESTRICT group were receiving more than 23 g of food.

From Figure 1 it is clear that, on average, the rats in the RESTRICT group decreased in body weight over the first 6 days of food restriction. During the remaining 10 days of food restriction the rats in the RESTRICT group maintained a fairly stable average body weight of approximately 85% of their baseline body weight (which was approximately 79% of the ad lib fed control rats' body weight on the last day of food restriction). Table 2 shows the number of rats that reached 85% (± 1 %) of their baseline body weight on each day of food restriction. The analysis of wheel running during the first food restriction period when the rats in the group RESTRICT were decreasing in body weight consists of the days just before the rats reached 85% (± 1 %) baseline body weight. This period is limited to 3 days because on the third day of food restriction, day 27, the first rat reached the 85% (± 1 %) criterion. Wheel running analysis was conducted using the day when each rat reached the 85% (± 1 %) criterion and the 2 prior days, to total 3 days of food restriction leading up to the point when the rat reached 85% (± 1 %) of its baseline body weight. It is clear from Table 2 that by the tenth day of food restriction, day 34, all of the rats had reached the 85% (± 1 %) criterion. This suggests that using the last 4 days at the end of food restriction, days 36 to 40 (excluding day 38 as the wheels were being cleaned), adequately represents a time when all rats in the RESTRICT group were being maintained at the 85% (± 1 %) criterion.

At refeeding the rats in the RESTRICT group rapidly increased their body weight by an average of 38.56 (± 3.44) g on the first day, see Figure 1. This suggests that despite receiving over 23 g of food each day at the end of food restriction, this was clearly still food restriction, and was just enough food to maintain their body weight. When they were given ad lib food access their body weight increased rapidly. The rats in the RESTRICT group weighted as much as the rats in the ADLIB group by about day 48.

Total Wheel Running Data

Figure 2 shows the total number of wheel turns the rats in the groups ADLIB and RESTRICT ran from days 21 to 56. The mixed measures, 4 (days) x 2 (group) ANOVA revealed that during the last 4 days of baseline there was a significant days effect, $F(3, 42) = 3.78, p < .02$, with a significant linear trend, $F(1, 14) = 10.90, p < .01$. While both the ADLIB and RESTRICT groups of rats were declining in their wheel running linearly during the last 4 days of baseline the two groups did not differ.

The Figure 2 Insert shows the total number of wheel turns the rats in the 2 groups ran during the 3 days leading to when the rats in the group RESTRICT reached 85% (± 1 %) of their baseline body weight. A mixed measures, 3 (days) x 2 (group) ANOVA during this period revealed a significant Days x Group interaction, $F(2, 28) = 3.47, p < .05$. A repeated measures ANOVA on the 3 days during this period for each group revealed no days effect for the rats in the group ADLIB ($F < 1$), and a days effect that approached significance for rats in the group RESTRICT, $F(2, 14) = 3.16$, $p = .074$, with a significant linear trend, $F(1, 7) = 6.42$, $p < .05$. During this period the rats in the RESTRICT group were linearly increasing their wheel running from 10,260 (± 1,361) wheel turns on the first day to 13,214 (± 1,590) on the third day.

During the last 4 days of food restriction (days 36, 37, 39 and 40) there were no differences in wheel running between the rats in the group ADLIB and the rats in the group RESTRICT; the 4 (days) x 2 (group) ANOVA revealed no significant effects or interaction.

During refeeding Figure 2 shows that the rats in the group RESTRICT displayed a satiety effect; their running was less than that of the rats in the group ADLIB over days 41 to 44. The mixed measures, 4 (days) x 2 (group) ANOVA of these first 4 days of refeeding revealed a significant days, $F(3, 41) = 9.68$, $p < .001$, and a significant group effect, $F(1, 14) = 7.09$, $p < .02$ (a t-test between the ADLIB and RESTRICT groups on the first day of refeeding showed that the rats in the RESTRICT group ran less than the rats in the ADLIB group, $t(14) = 4.83$, $p < .001$). During the last 12 days of the refeeding period the 12 (days) x 2 (group) ANOVA revealed no significant effects. During the first part of refeeding the rats in the RESTRICT group ran less than those in the ADLIB group, but during the last 12 days of refeeding the running in both groups had stabilised and was no longer different.

Anticipatory Running Data

As shown in Figure 3 the rats in the RESTRICT group displayed anticipatory running during the period of food restriction. There were no group differences in running between 10:15 h and 11:15 h during the last 4 days of baseline (F is < 1) with both groups running a mean of 18 (± 8) wheel turns in the hour.

When the rats in the group RESTRICT were decreasing in body weight during food restriction the 3 (days) by 2 (group) ANOVA revealed a significant group effect, $F(1, 14) = 6.91, p < .02$, as well as a significant Days x Group interaction, $F(2, 28) = 3.92, p < .05$. A repeated measures ANOVA on the 3 days during this period for each group revealed no days effect for the rats in the group ADLIB, $F(2, 14) = 2.13, p = .156$, and a days effect that approached significance for rats in the group RESTRICT, $F(2, 14) = 2.9, p = .091$. The rats in the RESTRICT group were running more than the rats in the ADLIB group, and they tended to be increasing this running over the 3 days that they were decreasing in body weight, see Figure 3 Insert. During the last 4 days at the end of food restriction the rats in the group RESTRICT continued to run more than the rats in the ADLIB group, $F(1, 14) = 27.32, p < .001$. The rats in the group RESTRICT ran an average of 426 (± 75) wheel turns in the hour prior to being fed, while the rats in the ADLIB group ran an average of 28 (± 11) wheel turns in the same hour.

Once food restriction was discontinued the rats in the group RESTRICT ran the same as the rats in the ADLIB group for both the first 4 days of refeeding and the last 12 days of refeeding (both F-ratios for the group effects are < 1).

Dark and Light Cycle Data

Figure 4 represents the number of wheel turns the rats in the groups ADLIB and RESTRICT performed in the dark cycle (A) and in the light cycle (B) (note scale differences). As shown, the number of wheel turns the rats performed in the dark during the last 4 days of baseline was generally declining as there was a significant days effect, $F(3, 42) = 6.11, p < .01$, with a significant linear trend, $F(1, 14) = 14.01, p < .01$. In the light part of the cycle during the last 4 days of baseline there was only a significant days effect, $F(3, 42) = 3.24, p < .05$.

During the period of food restriction when the rats in the RESTRICT group were decreasing to 85% (± 1 %) of their baseline body weight a 3 (days) x 2 (group) mixed ANOVA on wheel running performed during the dark cycle revealed no significant effects. The wheel running performed during the dark cycle did not differ between the ADLIB and RESTRICT group, see Figure 4A Insert. In the light cycle the similar analysis during the same period revealed a significant days effect, $F(2, 28) = 4.46, p < .05$, and a group effect that approaches significance, $F(1, 14) = 4.07, p = .063$. As illustrated in Figure 4B Insert, the running in the light cycle was generally increasing, but the difference in running displayed by the rats in the 2 groups only approached significance. During the last 4 days at the end of food restriction the analysis on the dark cycle running showed no significant effects. The rats' wheel running had stabilised at 5,760 (± 1,175) wheel turns in the ADLIB group and 4,400 (± 769) wheel turns in the RESTRICT group, with no differences between the 2 groups. Conversely, light cycle running during the same period showed a significant group effect, $F(1, 14) = 36.34, p < .001$. As Figure 4B shows, the rats in the group RESTRICT were running more than the rats in the group ADLIB during the last 4 days at the end of food restriction.

During the first 4 days after the rats in the RESTRICT group were returned to ad lib food access, a 4 (days) x 2 (group) mixed ANOVA on the dark cycle running revealed significant days, $F(3, 41) = 5.89, p < .01$, and group effects, $F(1, 14) = 8.75$, $p < .01$. While running was generally increasing over the 4 days, the rats in the RESTRICT group were running less than the rats in the ADLIB group, see Figure 4A. Analysis on the light cycle during the same period revealed only a days effect that approached significance, $F(3, 41) = 2.60, p = .064$. The wheel running in both groups did not differ in the light cycle during the first 4 days of refeeding. The immediate decrease in running during the dark cycle and not the light cycle suggests that the rats expressed the decreased running observed in the satiety effect only in their dark cycle running. However, since light cycle running is low, a satiety effect may not be present during the light cycle because the rats may be limited by a floor level for wheel turns possible. During the remainder of the refeeding period there were no group differences in running during the dark or light cycle.

Individual Differences

Examining the last day of baseline running there was a large variance in total wheel running in both the ADLIB and RESTRICT groups. The rats in the ADLIB group ran a mean of 8,501 (± 1,425) wheel turns, with the highest rat running 13,983 wheel turns and the lowest rat running 2,368 wheel turns. A similar variance in wheel running was seen in the RESTRICT group, which ran mean of 7,771 (± 1,389) wheel turns on the last day of baseline, with the highest rat running 12,478 wheel turns and the lowest rat running 1,910 wheel turns (there are no differences in wheel running between the 2 groups, $t(14) = .37, p = .719$). When the rats in the RESTRICT group reached 85% (± 1 %) of their baseline body weight their wheel running had increased by an average of 5,443 (± 841) wheel turns. All of the rats in this group increased their wheel running, with the largest increase being 9,428 wheel turns and the smallest increase being 1,628 wheel turns. During this period the rats in the ADLIB group showed a mean difference score of 631 (± 602) wheel turns, ranging from 2,758 to –2,587 wheel turns. The rats in the RESTRICT group increased their running from baseline more than the rats in the ADLIB group, $t(14) = -4.65, p < .001$. The baseline wheel running for the RESTRICT group of rats was not correlated with the increase in wheel running on the day the rats reached 85% (± 1 %) baseline body weight, $r = -.046, p = .913$. This suggests there is no relationship between the baseline level of wheel running and the increase in wheel running seen under food restriction; however, since the rats did reach the 85% (± 1 %) criterion on different days, practice may be confounding this result.

On the last day of food restriction the rats in the RESTRICT group had a mean difference score of –63 (± 998) wheel turns, ranging from 4,446 to –4,545 wheel turns. The rats in the ADLIB group had a mean difference score of –2,876 (± 847) wheel turns, with a range from 787 to –6,223 wheel turns. The rats in the RESTRICT group decreased their running from baseline less than the rats in the ADLIB group,

$t(14) = -2.15, p = .05$. The change in running of the rats in the RESTRICT group during this period was not significantly correlated with baseline levels of running, $r = -.581, p = .131$. This indicates that there likely was no relationship between baseline levels of running and the increase in running on the last day of food restriction; however, with such a small sample size (n = 8), caution should be used in interpreting this finding.

On the first day of refeeding the rats in the RESTRICT group had a mean change from baseline of –6,308 (± 1,284) wheel turns, ranging from –598 to –10,252 wheel turns. All rats in this group decreased their running compared to baseline. Conversely, the rats in the ADLIB group had a significantly smaller ($t(14) = 2.58, p < .05$) mean change in running of –2,488 (± 847) wheel turns, ranging from 593 to –4,891 wheel turns. The baseline wheel running the rats in the RESTRICT group ran was significantly correlated with the change in wheel running the first day of refeeding, $r = -.84, p < .01$. This indicates that the high baseline runners in the RESTRICT group showed the largest decrease in wheel running, and the low baseline runners in the RESTRICT group showed the smallest decrease in wheel running at refeeding.

Discussion

The food restriction manipulation used in Experiment 1 was effective in reducing the body weight of the rats in the RESTRICT group to 85% (± 1 %) of their baseline body weight in as quickly as 3 days. It was also effective in producing an extensive period of time when the food-restricted rats were being maintained at this weight. The rats were maintained at 85% (± 1 %) of their baseline body weight at a time when age-matched control rats were steadily increasing in body weight, reducing the RESTRICT group of rats to 79% of the body weight of the ADLIB group. Furthermore, when the rats in the RESTRICT group were returned to ad lib food access they quickly increased their body weight and by the sixteenth day of refeeding they weighed about the same as the ad lib fed control group. Thus, 3 distinctive phases developed during Experiment 1: a decreasing phase, when the rats were decreasing in body weight; a maintenance phase, when they were food restricted but maintained at 85% (± 1 %) of their baseline body weight; and a recovery phase when they were gaining after being returned to ad lib food access.

Within the decreasing phase of Experiment 1 the food-restricted rats marginally increased in their total wheel running compared to the rats in the ADLIB group. However it should also be noted that the rats in the RESTRICT group all increased in their wheel running from baseline by the day they reached 85% (± 1 %) of their baseline body weight (as indicated from their change in wheel running during this period), and this increase was significantly higher than that of the rats in the ADLIB group. Moskowitz (1959) has speculated that the amount of wheel running rats will perform is related to the amount of weight the rats are losing. In the present study the young adult rats lost weight under food restriction quickly, with only 3 days of food restriction when all of the rats were decreasing in body weight. The lack of a dramatic increase in wheel running by the rats in the RESTRICT group may have been a result of the young rats losing weight too quickly, thereby not providing a long enough time window when they were decreasing in weight to allow for a large increase in running.

It is also possible that the lack of a dramatic increase in wheel running is the result of the high baseline wheel running, and the rats in the RESTRICT group may have reached a ceiling level of wheel turns possible in 24 hours. However, in this case the correlation between baseline and the increase during this period should have been negative. The high runners would have increased little to reach the ceiling and the low runners would have increased a large amount. As well, it is unlikely that the rats all proportionally increased their wheel running, with high runners and low runners increasing the same percentage. If this were the case a positive correlation would have been expected between the increase and baseline running, as all of the rats would then be increasing in proportion to their baseline running. The fact that all of the rats showed an increase in running during food restriction demonstrates that all rats were effected by food restriction and not just one subgroup of runners. The lack of correlation between the baseline levels of running and the increase suggests that different processes may be responsible for the increase relative to baseline levels of wheel running.

When the RESTRICT group of rats were decreasing in their body weight during food restriction they increased the running they performed in the hour prior to being fed. The running during the light cycle also tended to be higher in the RESTRICT group than in the ADLIB group, but there were no differences between the 2 groups in the running they performed during the dark cycle. The increase in light cycle running may be due to the increase in anticipatory running, which could have occurred in over a longer period than 1 hour.

When the rats in the RESTRICT group were being maintained at 85% (± 1 %) of their baseline body weight their wheel running did not differ from the rats in the ADLIB group. The marginally increased wheel running during the decreasing phase was not sustained. Anticipatory running continued to increase during this period of food restriction, as did the light cycle running. Moreover, while not significant, the wheel running the rats in the RESTRICT group ran during the dark cycle was slightly decreased compared to the ADLIB group. This indicates that the food restricted rats were allocating their wheel running differently than the rats in the ADLIB group: they are running more in the light cycle, while maintaining their total number of wheel turns, possibly by slightly decreasing dark cycle running.

The lack of increase in running for the rats in the RESTRICT group during the weight maintenance period of food restriction may be due to the increased amount of food the rats were receiving while in this phase. Most of the rats were receiving more than 23 g of food by the end of the food restriction period. However, our lab has found wheel experienced rats of the same strain, age, and supplier will typically eat about 35 g of food each day (Mueller et al., 1997), indicating that the rats in the RESTRICT group were still consuming less food than normal.

Russell et al. (1987) argue that prolonged increases in wheel running are possible using the procedure of food restriction that was implemented in the present study. However, they used 63 day old male rats and food restricted them after only 3 days of wheel access. Our lab has found that rats this age will increase in their wheel running over at least a 2 week period (unpublished observations). The increase in wheel running Russell et al. (1987) reported may have been confounded by the natural increase in wheel running observed in rats that age. Furthermore, Russell et al. (1987) did not have a control group of non-food-restricted rats given wheel access. While an increase in wheel running of about 12,000 wheel turns was observed over about 20 days when the rats were placed on their food restriction schedule, the 'prolonged increased' level of running described by the researchers was about 8,000 wheel turns after about 45 days of food restriction. This level of running is comparable to the wheel running seen in the present experiment in the ADLIB group of rats, as well as the RESTRICT group of rats when they were being maintained at 85% (± 1 %) of their baseline body weight during food restriction. It is possible that the food restriction used by Russell et al. (1987) was not effective in inducing prolonged increases in wheel running in high running rats.

After the rats in the RESTRICT group were returned to ad lib food access they decreased their total wheel running the first day, displaying a satiety effect. Running was significantly decreased in this group compared to the ADLIB group for the first 4 days of refeeding, and, while it remained less than the ADLIB group for the remainder of the study, there were no significant differences in running for the last 12 days of refeeding. The dark cycle running was decreased during the first 4 days of refeeding, and there were no differences between the 2 groups in light cycle running or anticipatory running. This suggests that the difference in total wheel running may be due to the rats running less during the dark cycle at the same time anticipatory and light cycle running immediately returned to control levels.

Interestingly, during the last 4 days of the food restriction period, there were no differences in total wheel running between the RESTRICT and ADLIB groups; however, the RESTRICT group of rats decreased their running and rapidly increased their body weight at refeeding. This indicates that, even though the groups did not differ in wheel running at the end of the food restriction period, the RESTRICT group rats were experiencing the food restriction in a way that led to the satiety effect at refeeding. This satiety effect, resulting in decreased levels of wheel running, lasted about 1-2 days, and there were no long-term consequences of food restriction on wheel running in Experiment 1.

The decrease in wheel running seen on the first day of refeeding was significantly correlated with baseline wheel running, indicating that high runners decreased the most, and low runners decreased the least. This suggests that all of the rats in the RESTRICT group decreased in their wheel running to a minimum level of wheel turns, showing a floor effect; that is, to reach the 2,000 wheel turns seen on the first day of refeeding, the high baseline runners had to decrease their wheel turns more than the low baseline runners. Perhaps low runners are less prone to a satiety effect because they are running at such low levels, and any decreases in wheel running would not be different from low running control rats.

Experiment 2

Experiment 2 explores the effects of food restriction in 140 day old male rats. It was expected that, unlike the 55 day old adult male rats used in Experiment 1, these rats would not show an increase in wheel running when they were introduced to the wheel, producing a considerably lower level of baseline running. By using the same food restriction manipulation it is possible to explore if the high baseline running levels in the young rats used in Experiment 1 was critical for the small effects of food restriction on wheel running. As well, these older rats would be heavier and thus take longer to reach 85% (± 1 %) of their baseline body weight under food restriction, allowing for more time to increase wheel running if weight loss is an important factor in the increased wheel running induced by food restriction. It was hypothesised that the food-restricted rats would increase their wheel running, and that in these low level runners baseline levels of wheel running would predict changes in wheel running under food restriction. It was also expected that, unlike in Experiment 1, these rats would not show a satiety effect when placed back on ad lib food access, as these already low running rats may not decrease their running significantly when refed. Furthermore, it was predicted that the individual differences seen in wheel running during baseline would predict levels of wheel running when the rats were refed.

Method

Subjects. Sixteen male Sprague-Dawley rats from Charles River Canada served as subjects. Upon arrival the rats weighed between 200-225 g (47-49 days old), and were maintained as in Experiment 1.

Apparatus. The Nalgene wheels and cages described in Experiment 1 were used as an apparatus.

Procedure. All of the rats in this study were pair housed upon their arrival at the laboratory. They were initially used as a control group in a 24-day study that examined the effects of moving rats from pair to individual housing. In that study the rats were pair housed for a period of 20 days and then given 90 minutes of contact with a novel rat each day for 4 days. On day 24 the rats were rehoused in pairs with a new partner until 90 days after their arrival.

After 90 days of being pair housed in the homecages the rats, which were about 140 days old, were individually housed in the Nalgene wheel cages, and given 40 days of continuous wheel access. This extended baseline period was to ensure that the rats were running at a stable plateau. After the baseline period the rats were randomly assigned to 2 groups of 8 rats based on their last 4 days of baseline wheel running, as in Experiment 1. The rats in the group ADLIB continued to have ad lib access to food. The rats in the group RESTRICT were put on a food restriction schedule for 16 days, days 41 to 56, similar to that described in Experiment 1. When it was observed that the rats in the group RESTRICT were not losing much weight on 15 g of food each day, the food administered was further reduced to as little as 10 g each day. The amount of food made available for each rat was then adjusted to maintain the rats at or above 85% (± 1 %) of their initial body weight. The RESTRICT group of rats were returned to ad lib feeding after 16 days of food restriction and followed for another 16 days, days 57 to 72. The rats were handled and body weight was measured every day between 08:30 h and 09:30 h, and, while on the food restriction schedule, the rats in the group RESTRICT were given food daily at 09:30 h. Cages were changed bi-weekly, and wheel turns were recorded continuously throughout the entire experiment. On day 38 and day 56 the running wheels were disassembled and cleaned, thus the rats did not have access to the running wheels for approximately 4 hours in the early morning. Therefore, these days were excluded from all analysis.

Analysis. This mixed measures design was analysed as in Experiment 1, across Days during baseline, food restriction, and refeeding. The analysis was divided into 4 sections: the last 4 days of baseline (days 36, 37, 39, and 40, excluding day 38 as the wheels were cleaned at that time), a period of food restriction when all of the rats were decreasing to 85% (± 1 %) of their baseline body weight, the first 4 days at the beginning of refeeding (days 57 to 60), and the last 12 days of refeeding (days 61 to 72). The rats in the RESTRICT group were decreasing in body weight throughout the food restriction period, thus there was not an extended period of time during food restriction when the rats were being maintained at 85% (± 1 %) of their baseline body weight (see Results). Therefore, a period when the rats had plateaued in body weight was not included in this analysis. The remaining 4 periods were chosen on a basis similar to that in Experiment 1. In this experiment the first rat to reach 85% (± 1 %) of it's baseline body weight did so after 6 days of food restriction; however, as a result of a computer crash during the food restriction period, the decreasing period consists of 5 days of analysis.

Data analysis was done on all 4 periods for similar dependent variables as analysed in Experiment 1. The number of wheel turns performed by each rat in the hour prior to being fed was calculated from 08:30 h to 09:30 h. All other variables were calculated as in Experiment 1.

Results

During the course of this experiment some wheels for individual animals became disconnected from the data collection system. While the rats still had access to a free turning running wheel, their wheel counts were not accumulated. This occurred on days 55 and 56 (during food restriction) and days 59, 60, 61, 62, and 63 (during refeeding) for rat #7, from the ADLIB group. Due to the number of consecutive days of lost data, analysis of the refeeding periods excluded this rat. The running wheel was also disconnected from the data collection system on day 71 for rat #5, from the ADLIB group, and this was corrected by using the average of the prior day's wheel turns with the next day's wheel turns, and decreasing the degrees of freedom in the analysis.

On Day 50 the data collection computer crashed resulting in the loss of the wheel running data from about 02:00 h to 09:00 h. While the rats still had free turning wheel access, the wheel counts during those hours were not collected, and thus day 50 is excluded from all wheel running analysis. Since this day was in the period when the rats in the RESTRICT group were decreasing in body weight for 7 of the 8 rats, 5 days will be used in the analysis of this period.

Table 3 shows the amount of food the rats were given throughout the food restriction period. During the first 5 days of food restriction all rats in the RESTRICT group were given about 15 g of food, which was then decreased for most of the rats until day 55. One rat's food was increased from day 49 until the end of the food restriction period, receiving more than 23 g of food each day starting at day 51.

Examining the amount of weight the rats lost during food restriction, Figure 5 shows that rats in the RESTRICT group decreased their body weight throughout the food restriction period. By the last day of food restriction the rats declined to an average of 85% (± 1 %) of their baseline body weight, which was 79% of the body weight of the ADLIB group (although, as shown in Figure 5, the rats in the RESTRICT group consistently weighed less than the rats in the ADLIB group, a 4 (days) by 2(group) ANOVA revealed no significant effects). Table 4 shows that all rats remained above 85% (± 1 %) of their baseline body weight until day 45. Only 1 rat had reached 85% by day 46, and 6 rats had reached 85% (± 1 %) by day 53. Only on days 55 and 56 were all animals at 85% (± 1 %) of their baseline body weight. As such, animals were all maintained at 85% (± 1 %) weight for only two days. Therefore, it was not possible to analyse running when these rats were being maintained at 85% (± 1 %). Thus, analysis of the food restriction period only included the time when all of the rats were decreasing in body weight, not a period of weight maintenance. Once returned to ad lib food access the rats in the RESTRICT group quickly gained weight, increasing 41.5 (± 4.03) g the first day. The body weight of the rats in the RESTRICT group remained below the ADLIB group of rats at the end of the experiment.

Total Wheel Running Data

Figure 6 shows the total number of daily wheel turns the rats in the ADLIB and RESTRICT groups performed from days 37 to 72. During the last 4 days of baseline a 4 (days) x 2 (group) mixed measures ANOVA revealed that the running in the 2 groups had stabilised and did not differ, as there were no significant effects or interactions.

During the period of food restriction when the rats were decreasing to 85% (± 1 %) of their baseline body weight, the 5 (days) x 2 (group) ANOVA revealed a significant days effect, $F(4, 56) = 4.33, p < .01$, a group effect that approached significance, $F(1, 14) = 3.45, p = .084$, and a significant Days x Group interaction, $F(4, 56) = 4.90, p < .01$, see Figure 6 Insert. A 5 (days) repeated measures ANOVA on the ADLIB group revealed no significant Days effect ($F < 1$), indicating that the wheel running in this group remained stable. Similar analysis on the RESTRICT group revealed a significant days effect, $F(4, 28) = 5.86, p < .001$, with a significant linear trend, $F(1, 7) = 10.73, p < .05$. The rats in the RESTRICT group significantly increased their wheel running linearly from 1,145 (± 356) to 1,580 (± 423) wheel turns when they were decreasing in body weight during food restriction.

During the first 4 days at the beginning of the refeeding period the 4 (days) x 2 (group) ANOVA showed a significant days effect, $F(3, 39) = 5.72, p < .01$, with a significant linear trend, $F(1, 13) = 13.86, p < .01$, indicating that, on average, the 2 groups were decreasing in their running linearly. On the last 12 days of refeeding there were no significant effects. Figure 6 shows that clearly there was no satiety effect, in fact, it appears that the rats in the RESTRICT group tended to run more than the rats in the ADLIB group for the entire refeeding period, but this was not statistically significant.

Anticipatory Running Data

The rats in the RESTRICT group displayed anticipatory running throughout the food restriction period, see Figure 7. During baseline there were no group differences in wheel running performed between 08:30 h and 09:30 h, (Group effect $F < 1$). During food restriction when the rats in the RESTRICT group were decreasing to 85% (± 1 %) body weight a 5 (days) x 2 (group) mixed ANOVA revealed significant days, $F(4, 56) = 9.56, p < .001$, and group effects, $F(1, 14) = 10.05, p < .01$, as well as a significant Days x Group interaction, $F(4, 56) = 6.29, p < .001$, see Figure 7 Insert. A 5 (days) repeated measures ANOVA on the ADLIB group showed no significant days effect $F(4, 28) = 1.05, p = .399$, while a similar analysis on the RESTRICT group showed a significant days effect, $F(4, 28) = 9.44, p < .001$ with a significant linear trend, $F(1, 7) = 18.50, p < .01$. As the Figure 7 Insert shows, during the period of food restriction when the rats in the RESTRICT group were decreasing in body weight they were linearly increasing their wheel running in the hour prior to being fed compared to the rats in the ADLIB group, who were running a stable number of wheel turns.

A 4 (days) x 2 (group) ANOVA on the first 4 days at the beginning of refeeding showed no significant effects. The rats in the RESTRICT group no longer displayed significant anticipatory running. Similar analysis on the last 12 days of refeeding also showed no significant effects. As Figure 7 shows, during the refeeding period both the RESTRICT and ADLIB groups of rats did not differ in the wheel turns they ran in the hour prior to when the RESTRICT rats used to be fed.

Dark and Light Cycle Data

Figure 8 shows that during the last 4 days of baseline the rats in the RESTRICT and ADLIB groups ran the same amount during the dark and light cycles (both group effect F-ratios < 1). During food restriction when the rats in the RESTRICT group were decreasing in body weight there was no difference between the groups in their dark cycle running ($F < 1$), see Figure 8A Insert. Analysis of the light cycle running over the same period revealed significant days, $F(4, 56) = 6.99$, $p < .001$, and group effects, $F(1, 14) = 9.66$, $p < .01$, and a significant Days x Group interaction, $F(4, 56) = 7.39$, $p < .001$. A 5 (days) repeated measures ANOVA on each group revealed no significant days effect ($F < 1$) for the ADLIB group, and a significant days effect, $F(4, 28) = 7.42$, $p < .001$, with a significant linear trend, $F(1, 7) = 14.89$, $p < .01$, for the RESTRICT group. As shown in Figure 8B Insert, during the light cycle, the rats in the RESTRICT group were running more than the rats in the ADLIB group when they were decreasing in body weight, and the food restricted rats were increasing their light cycle running linearly during this period. However, their dark cycle running remained the same, see Figure 8A Insert.

Analysis on the first 4 days of refeeding showed no significant differences between the RESTRICT and ADLIB groups in their dark cycle running ($F < 1$). As well, there were no differences in the dark cycle wheel running between the 2 groups on the last 12 days of the refeeding period, $F(1, 13) = 1.18$, $p = .296$. In the light cycle, during the first 4 days of refeeding there was a significant days effect, $F(3, 38) = 6.08$, $p < .01$, as both groups of rats were changing their light cycle wheel running over the 4 days. During the last 12 days of the refeeding period there were no significant effects on light cycle running. As Figures 8A and 8B show, there were no group differences in wheel running throughout the refeeding period in either the light or dark cycle.

Individual Differences

In this experiment there was variation in the last day of baseline wheel running for both the ADLIB and RESTRICT groups of rats. The rats in the ADLIB group ran a mean of 663 (± 163) wheel turns, with a maximum of 1,325 wheel turns and a minimum of 4 wheel turns on the last day of baseline (because of the lower levels of running the absolute range was smaller than that of the rats in Experiment 1). The rats in the RESTRICT group showed a similar pattern, running a mean of 799 (± 247) wheel turns, ranging from 1,909 to 21 wheel turns on the last day of baseline (there were no differences between the 2 groups, $t(14) = -.46$, $p = .66$).

When the rats in the RESTRICT group reached 85% (± 1 %) of their baseline body weight all of the rats had increased their wheel running by a mean of 781 (± 261) wheel turns, with the highest increase being 2,280 wheel turns while the lowest was 43 wheel turns. The rats in the ADLIB group decreased in their wheel running by 129 (± 64) wheel turns, ranging from an increase of 101 wheel turns to a decrease of 382 wheel turns from baseline. The increase seen in the RESTRICT group was significantly different from the decrease seen in the ADLIB group, $t(14) = -3.39, p < .01$. The increase the RESTRICT group of rats experienced was not correlated with their baseline level of wheel running, $r = .38, p = .35$. This indicates that there was no relationship between the number of wheel turns the rats in the RESTRICT group performed the last day of baseline and how much they increased their wheel running on the day they reached 85% of their baseline body weight.

On the first day of refeeding the rats in the RESTRICT group still showed an increase in wheel running from their baseline running, with a mean increase of 1,012 (± 469) wheel turns more than baseline, which ranged from 4,085 wheel turns, to 18 wheel turns. The rats in the ADLIB group showed a mean increase in wheel running during this time of 10 (± 154) wheel turns from baseline, ranging from an increase of 760 wheel turns to a decrease of 530 wheel turns. The difference in increase between the ADLIB and RESTRICT groups was marginally significant, $t(13) = -1.91, p = .078$. At this time the increase in wheel running displayed by the rats in the RESTRICT group was significantly correlated with their baseline levels of wheel running, $r = .72$, $p = .044$, indicating that the rats increased in their wheel running in a manner that was proportional to their baseline levels of running.

Discussion

In this experiment the 140 day old food-restricted male rats did not show an extended period during food restriction when they were being maintained at 85% (± 1 %) of their baseline body weight. Throughout the 16 days of food restriction some of the rats took as long as 14 days to reach 85% (± 1 %) of their baseline body weight despite receiving less than 15 g of food each day. This may be because their initial body weight was considerably higher than the weight of the rats used in Experiment 1, and so the absolute decrease needed to reach 85% was much larger. Alternatively, the lack of a maintenance period during food restriction may be because they were all low runners and not expending as much energy as the high runners used in the first study, thus not decreasing their weight as quickly. When ad lib food access was reinstated to the rats in the RESTRICT group they quickly gained weight. On the first day of refeeding they gained about 41 g, similar to the 38 g gained by the rats in the first study. Unlike in the first study which had 3 phases, the food restriction manipulation in the present study resulted in 2 phases: a decreasing phase, when the rats were decreasing in body weight while under food restriction; and a recovery phase, when the rats were increasing in body weight upon refeeding.

As anticipated, the rats in Experiment 2 showed lower baseline levels of wheel running (about 600 wheel turns) than the rats in Experiment 1 (who ran about 8000 wheel turns during baseline). The rats in the RESTRICT group in the present study showed a significant increase in wheel running the last 5 days they were all decreasing in body weight.

As in Experiment 1, the RESTRICT group of rats in the present study showed anticipatory running during food restriction. They also showed increased light cycle running, but food restriction had no effect on dark cycle running. This suggests that, as in Experiment 1, the rats may be reallocating their wheel running to run more in the light cycle, accounting for some of the anticipatory running, while their dark cycle running remains unchanged.

As mentioned above, the rats in the RESTRICT group took between 6 and 14 days to decrease their body weight. It is possible that practice effects may influence the results, as some rats were decreasing in body weight for a longer period of time than others. Furthermore, the rats in Experiment 1 were decreasing in body weight for between 3 and 9 days. This difference in the amount of time it took to decrease in weight may account for the differences in the relative strength of the increase in wheel running between the first and second experiment.

Interestingly, the increase in wheel running the rats in the RESTRICT group ran on the day they reached 85% (± 1 %) body weight was not significantly correlated with their baseline running. As in Experiment 1, this indicates that the level of baseline running was not a predictor of the amount of wheel turns the rats increased in response to the food restriction.

When the RESTRICT group of rats were returned to ad lib food access they did not differ in their total wheel running from the ADLIB groups. Unlike in Experiment 1, they did not show a satiety effect. In fact, they tended to run more than the ADLIB group rats throughout the refeeding period, while the food restricted rats in

Experiment 1 tended to run less than the ad lib fed rats when they were returned to ad lib food access. In the present study the anticipatory and light cycle running by the rats in the RESTRICT group tended to be higher than the ADLIB group (although not significantly different), while the dark cycle running clearly returned to control levels.

In the literature many of the studies that refed rats after a period of food restriction or deprivation involved low running rats that showed either an immediate or gradual decline to baseline and control levels of running (Hall et al., 1953; Stevenson & Rixon, 1957), or a satiety effect (Finger, 1951; Reid & Finger, 1955). Reid and Finger (1955) did report low baseline running rats (about 500 wheel turns after 20 days of wheel experience) that had increased their running to 6,000 wheel turns during food restriction, showed a satiety effect of 192 wheel turns on the first day of refeeding. While they reported this decrease as less than the control rats that were running 493 wheel turns, no statistics were reported to determine if this difference was statistically significant. It is conceivable that what Reid and Finger actually saw was an immediate decrease to baseline levels of wheel running.

The lack of a satiety effect in the present study may be because, compared to the food restricted rats in Experiment 1, RESTRICT group of rats in the present study were running at relatively low levels during food restriction. The satiety effect seen in Experiment 1 had the food restricted rats running about 1,800 wheel turns the first day of refeeding, which is similar to the 2,000 wheel turns the RESTRICT group of rats in Experiment 2 were performing the first day of refeeding. Perhaps rats that are returned to ad lib food access after a period of food restriction will reach some common floor level of wheel turns, regardless of how much they were running prior to refeeding.

The change in running on the first day of refeeding was significantly correlated with baseline wheel running, suggesting that, at refeeding, the rats had changed their wheel running proportionally to baseline. It is interesting that in Experiment 1 the RESTRICT group of rats had a negative correlation between baseline running and the decrease from baseline to the first day of refeeding. The high runners decreased their wheel running the most while low runners decreased the least. As previously mentioned, the present study used rats that showed lower baseline levels of running than the rats in the first study. Perhaps these rats would be considered low runners in terms of Experiment 1, and all showed a small change in running, and no satiety effect.

Experiment 3

In the first and second experiments high baseline running and low baseline running male rats were exposed to a food restriction schedule, which resulted in differing effects on wheel running. The high baseline running rats in Experiment 1 showed a slight increase in wheel running while they were decreasing to 85% (± 1 %) of their baseline body weight, and a satiety effect immediately at refeeding. Conversely, the low baseline running male rats in Experiment 2 showed a more sustained, but smaller absolute increase in wheel running in response to food restriction, and did not display a satiety effect. In these experiments the baseline running, age, and initial body weight of the animals differed. Old female rats are reported to show high levels of wheel running (Tokuyama, Saito, & Okuda, 1982), so in the present study the food restriction induced wheel running paradigm was expanded to include 180 day old female rats. It was expected that they would show baseline levels of wheel running similar to that of the young male rats used in Experiment 1. Thus, it was hypothesised that these older female rats would show an increase in wheel running when they were decreasing to 85% (± 1 %) of their baseline body weight, but return to running levels seen with ad lib feeding when they were being maintained at this reduced weight. As well, it was expected that these high running, food restricted female rats would show a satiety effect at refeeding, immediately decreasing their wheel running to below that of ad lib fed control rats.

Method

Subjects. Sixteen female Sprague-Dawley rats from Charles River Canada, weighing between 157-200 g (51-56 days old) at arrival, served as subjects. They were maintained as described in Experiment 1.

Apparatus. The Nalgene wheels and cages described in Experiment 1 were used in this study.

Procedure. In this study all of the rats were pair housed upon arrival at the laboratory. They served as a control group in a study that examined the effects of moving female rats between pair to individual housing. In that study the rats were pair-housed for 20 days and then re-housed in pairs with a new partner. They remained in those pair housing conditions until they were individually housed in the Nalgene wheel cages 129 days after their arrival.

In the Nalgene wheel cages the rats were given 24 hours of wheel access for 39 days. After this baseline period of wheel running the rats were randomly assigned to 2 groups based on their baseline wheel running. Six rats were assigned to the ADLIB group, which continued to have ad lib access to food. Ten rats were assigned to the RESTRICT group, which were put on the same food restriction schedule as described in Experiments 1 and 2. An unequal number of rats in the 2 groups was used, thus increasing the sample size of the correlations run between the baseline levels of wheel running of rats in the RESTRICT group and their change in wheel running during food restriction and refeeding. Rather than match animals with equivalent baseline running levels the animals for both groups were randomly chosen with the provision that the average running during the last 4 days of baseline was similar in both groups, and that both groups had high and low runners. The amount of food made available to the rats in the RESTRICT group was adjusted daily to maintain them at or above 85% (± 1 %) of their baseline body weight, and was decreased to no less than 10 g of food each day when the animals were not losing much body weight. After 16 days of food restriction (days 40 to 55) the rats in the RESTRICT group were returned to ad lib food access, and followed for another 16 days (days 56 to 71). The rats were handled and body weight was measured daily between 07:30 h and 08:30 h, and, while on food restriction, the rats in the RESTRICT group were fed daily at 10:00 h. Cages were changed bi-weekly and wheel turns were measured continuously throughout the experiment. On day 59 the running wheels were disassembled and cleaned, and the rats were without a running wheel for approximately 4 hours in the early morning. This day is excluded from all analysis.

Analysis. This experiment was analysed in a manner similar to Experiments 1 and 2, across Days during baseline, food restriction, and refeeding. Analysis was divided into 4 sections: the last 4 days of baseline (days 36 to 39), a period of food restriction when all of the rats were decreasing to 85% (± 1 %) of their baseline body weight, the first 4 days at the beginning of refeeding (days 56, 57, 58, and 60), and the last 11 days of refeeding (days 61 to 71). These 4 periods were chosen on a basis similar to that in Experiment 1. In this experiment the first rat to reach 85% (± 1 %) of it's baseline body weight did so after 5 days of food restriction, thus the decreasing period consists of 5 days of analysis. Three of the rats in the RESTRICT group did not reach 85% (± 1 %) of their baseline body weight during food restriction (see Results), with the highest of the 3 reaching only 89% baseline body weight. For these rats the last 5 days of food restriction were included in the period of analysis when they were decreasing in body weight. Therefore, as in Experiment 2, there was no period during food restriction when all of the rats were being maintained at 85% (± 1 %) of their baseline body weight. Thus a period when the rats had reached a plateau at 85% (± 1 %) body weight was not included in the analysis.

Results

Through the course of this experiment a rat from the ADLIB group developed a tumour on the top of her head, which became infected. This rat was removed from the study, leaving 5 rats in the ADLIB control condition.

All of the rats in the RESTRICT group were maintained on about 15 g of food each day until day 44, see Table 5. By day 51, 2 of the rats were maintained on less than 15 g of food, and 5 of the rats were given more than 15 g, with 2 receiving more than 23 g of food. At the end of food restriction 4 rats were being given 15 g of food or less, but the majority of the animals were maintained on more than 15 g of food.

Figure 9 shows that the rats in the RESTRICT group continued to lose weight throughout the food restriction period. By the last day of food restriction the RESTRICT group of rats weighed approximately 85% of their baseline body weight, which was 79% of the ADLIB group body weight on the same day. Table 6 shows the number of rats to reach 85% of their baseline body weight on each day of food restriction. The first rat to reach this criterion did so on day 44. By the last day of food restriction all but 3 rats had reached 85% (± 2 %). The 3 rats that never reached 85% were below 89%, and their last 5 days of food restriction are included in the analysis of the food restriction period. It is clear from Figure 9 that the rats in the RESTRICT group quickly increased in body weight once they were returned to ad lib food access, increasing 37 (± 3.17) g on the first day. The body weight of the rats in the RESTRICT group remained below the ADLIB group of rats at the end of the experiment.

Total Wheel Running Data

Figure 10 represents the total number of wheel turns the rats in the ADLIB and RESTRICT groups ran from days 36 to 71. During the last 4 days of baseline the rats in the 2 groups did not differ from each other on their running, as the 4 (days) x 2 (group) mixed ANOVA revealed no significant effects, see Figure 10. When the rats in the RESTRICT group were decreasing in body weight there were no differences in running between the 2 groups ($F < 1$), see Figure 10 Insert, indicating that the food restriction did not increase the wheel running of these female rats in the RESTRICT group.

On the first 4 days of refeeding Figure 10 shows that the rats in the RESTRICT group ran less than the rats in the ADLIB group; however this only approached significance, $F(1, 13) = 3.22$, $p = .096$. An 11 (days) x 2 (group) mixed measures ANOVA on the last 11 days of refeeding revealed only a marginally significant days effect, $F(10, 130) = 1.82$, $p = .063$. Running in both groups was not completely stable over the last 11 days of refeeding, as evident in Figure 10.

Anticipatory Running Data

There were no differences in the wheel running the rats in the ADLIB and RESTRICT groups performed between 09:00 h and 10:00 h during the last 4 days of baseline, $F(1, 13) = 1.26$, $p = .283$, see Figure 11. When the RESTRICT group of rats were decreasing in body weight a 5 (days) x 2 (group) ANOVA revealed a significant days effect, $F(4, 52) = 4.29$, $p < .01$, with a significant linear trend, $F(1, 13) = 6.29$, $p < .05$, see Figure 11 Insert. Both groups of rats were increasing in their wheel running in the hour prior to the RESTRICT group being fed.

Although the RESTRICT group of rats were not at a stable weight by the last 4 days of the food restriction period, it was decided to compare the anticipatory running over this period. If anticipatory running is a learned effect of the fixed food administration time, then it may be less weight sensitive. A 4 (days) x 2 (group) mixed measures ANOVA on the last 4 days of the food restriction period revealed only a significant group effect, $F(1, 10) = 12.00, p < .01$, with the rats in the RESTRICT group running more in the hour prior to being fed (displaying anticipatory running) than the rats in the ADLIB group, see Figure 11.

On the first 4 days of refeeding a 4 (days x 2 (group) ANOVA revealed a significant days effect, $F(3, 39) = 12.49, p < .001$, and a significant Days x Group interaction. $F(3, 39) = 2.85, p = .05$. A 4 (days) repeated measures ANOVA on each of the groups revealed a significant days effect, $F(3, 12) = 3.45, p < .05$, for the ADLIB group, and for the RESTRICT group, $F(3, 27) = 11.07, p < .001$. During the first 4 days of refeeding the rats in both the ADLIB group and RESTRICT groups were decreasing their wheel running they performed in the hour prior to when the RESTRICT group used to be fed. Over the last 11 days of refeeding analysis revealed a significant days effect, $F(10, 130) = 8.40$, $p < .001$, in the hour prior to when the RESTRICT group of rat were fed. Over days this hours running showed significant fluctuation, as evident in Figure 11.

Dark and Light Cycle Data

During the last 4 days of baseline there were no group difference in the wheel running the rats performed in the dark cycle, ($F < 1$), see Figure 12A. There was a marginal group difference in the running the rats performed in the light cycle, $F(1, 13) = 3.71, p = .076$, wit the rats in the RESTRICT group tending to run less than the rats in the ADLIB group, see Figure 12B. When the rats in the RESTRICT group were decreasing to 85% (± 1 %) of their baseline body weight there was no difference between groups in the dark cycle running ($F < 1$), see Figure 12A Insert, but a 5 (days) x 2 (group) ANOVA on the light cycle running revealed a significant days effect, $F(4, 52) = 3.04, p < .05$ with a significant linear trend, $F(1, 13) = 8.60, p < .05$. During this period both groups of rats were increasing their light cycle running, as shown in the Figure 12B Insert.

Since the food restriction schedule increases anticipatory running, which in turn alters the distribution of running over the light: dark cycle, it was decided to analyse the rats' dark cycle and light cycle running over the last 4 days of food restriction. A 4 (days) by 2 (group) ANOVA on the dark cycle running revealed a significant days effect, $F(3, 39) = 9.234, p < .001$, and a significant Days x Group interaction, $F(3, 39) = 3.168, p < .05$. A repeated measures ANOVA on the 4 days at the end of food restriction for each group revealed a significant days effect for the rats in the ADLIB group, $F(3, 12) = 4.24$, $p < .05$, and a significant days effect for the rats in the RESTRICT group, $F(3, 12) = 3.074$, $p < .05$. The rats in both groups were fluctuating in their dark cycle wheel running over the last 4 days of food restriction, see Figure 12A. The 4 (days) by 2 (group) ANOVA on the light cycle running revealed only a marginally significant group effect, $F(1, 13) = 3.87$, $p = .071$. The rats in the RESTRICT group tended to run more than the rats in the ADLIB group, see Figure 12B.

A 4 (days) x 2 (group) ANOVA on the dark cycle wheel running during the first 4 days of refeeding revealed no significant effects, as the running in the 2 groups did not differ, see Figure 12A. Similar analysis on the light cycle running also revealed no significant effects, with the RESTRICT group running as much as the ADLIB group during the first part of refeeding, see Figure 12B. An 11 (days) x 2 (group) ANOVA of the dark cycle running during the last 11 days of the refeeding revealed no significant effects. During the same period analysis of the light cycle running revealed a significant days effect, $F(10, 130) = 2.06, p < .05$. The number of wheel turns the rats ran in the dark cycle remained relatively steady, and the running in the light cycle tended to fluctuate, but there were no differences between the 2 groups during either dark or light periods.

Individual Differences

The rats in both the ADLIB and RESTRICT groups showed a large range in running on the last day of baseline. The ADLIB group had a mean total running of 6,347 (± 1,494) wheel turns, with the highest runner running 10,851 wheel turns, and the lowest runner running 1,850 wheel turns. A similar variance in running was seen in the RESTRICT group, which ran a mean of 5,295 (± 1,092) wheel turns, ranging from 9,144 to 53 wheel turns. The ADLIB and RESTRICT groups did not differ in their baseline wheel running ($t < 1$).

When the rats in the RESTRICT group reached 85% (± 1 %) of their baseline body weight they had increased their running by 3,023 (± 1,786) wheel turns from their baseline wheel running. The highest increase was 16,947 wheel turns, and the lowest decrease was 1,256 wheel turns. The ADLIB group increased their running by a mean of 1,967 (± 1,568) wheel turns, and the highest increase was 6,241 wheel turns while the lowest decrease was 1,381 wheel turns. There were no differences between the 2 groups on the change in wheel running ($t < 1$). The change in wheel running for the rats in the RESTRICT group on the day they reached 85% (± 1 %) body weight was not significantly correlated with their baseline wheel running, $r = -.29$, $p = .419$, indicating that there was no relationship between any change in wheel running while under food restriction and baseline levels of wheel running.

On the first day of refeeding the rats in the RESTRICT group decreased their wheel running from baseline by an average of 3,018 (± 1,121) wheel turns, ranging from an increase of 2,585 wheel turns to a decrease of 7,450 wheel turns. The ADLIB group decreased their wheel running by an average of 100 (± 1,511) wheel turns, ranging from an increase of 3,826 to a decrease of 4,756 wheel turns. The 2 groups did not differ in their change in wheel running from baseline, $t(13) = 1.52$, $p = .152$. The change in wheel running the rats in the RESTRICT group made on the first day of refeeding was significantly correlated with their baseline levels of wheel running, $r = -.96$, $p < .001$. This indicates that the high runners during baseline decreased their running the most, and the low runners were more likely to increase their wheel running.

Discussion

The food restriction manipulation did not induce a dramatic increase in total wheel running in the 180 day old female rats. The animals decreased their body weight throughout food restriction, and, like in Experiment 2, did not have a stable weight maintenance period during food restriction. There were some animals that did not even reach the 85% (± 1 %) body weight criterion. When the food-restricted rats were returned to ad lib food access they immediately increased in body weight, gaining about 37 g on the first day. This weight gain is similar to that experienced by the rats in Experiment 1 (38 g) and Experiment 2 (41 g), indicating that the food restriction in Experiment 3 had similar effects on body weight as in Experiments 1 and 2 despite different baseline weights. Like in Experiment 2, 2 phases of body weight change occurred in Experiment 3: a decreasing phase and a recovery phase.

When the food-restricted rats in the present study were decreasing in body weight their total wheel running did not increase. During the last 4 days of the food restriction period the rats in the RESTRICT group displayed anticipatory running. This increased anticipatory running indicates that, despite a lack of total increase in wheel running, the rats were sensitive to the food restriction manipulation.

When the food-restricted females were returned to ad lib food access they decreased their wheel running from about 6,100 to about 2,100 wheel turns. This lower level of running was not significantly different from the wheel running performed by the rats in the ADLIB group, perhaps because of the very large variance in running within the groups. Interestingly, baseline levels of wheel running were negatively correlated with the decrease in wheel running the rats in the RESTRICT group showed on the first day of refeeding. Similar to the young male rats in Experiment 1, the high running female rats in the group showed the biggest decrease in wheel running while the low running females in the group showed the smallest decrease.

Based on previous work by Finger (1951), it was predicted that male and female rats would express similar increases in wheel running in response to food restriction. Finger (1951) did not differentiate between males and females (or rats that were 30 and 115 days old), and reported that wheel running increased after 24 and 72 hours of food deprivation. The results of the present study suggest that there is a difference between male and female rats in the way they react to food restriction. Given the lack of an increase in wheel running observed in this present study, it is possible that the increase in wheel running reported by Finger (1951) was driven by a substantial increase in running performed by the male rats. The age and gender differences seen in Experiments 1, 2, and 3 suggest that Finger (1951) should have reported each of his 4 groups of rats separately.

Figure 10 of total wheel running illustrates that the rats were regularly fluctuating in their wheel running, possibly because of their estrus cycle. Female rats have long been known to change their wheel running in response to their 4-day estrus cycle (see early review by Shirley, 1929). The estrus cycle of female rats that are living together tend to become synchronized (McClintock, 1978). The rats in this third study had been living together in the same colony room, in pairs for 129 days prior to being placed in the running wheels. The regular fluctuations in wheel running suggests that the rats may have been cycling together, increasing their wheel running on similar nights in response to their estrus cycle. Future research should use vaginal smears to track the rats' cycle to account for this source of variance in wheel running.

General Discussion

The focus of this thesis was to determine if wheel experienced rats of different ages (and thus different baseline levels of wheel running) and genders given a specific restricted amount of food every day would show similar increases in wheel running, and, second, to see how differences in baseline running interact with the effects of food restriction. A final objective of this thesis was to clarify what happens to wheel running when ad lib food access is reinstated. These objectives were investigated in 55 and 140 day old male rats, and in 180 day old female rats with at least 24 days of wheel experience.

First, these studies indicate that, while age does not affect food restriction induced wheel running in male rats, there are gender differences in the effects of food restriction on wheel running. Both the younger, lighter male rats in Experiment 1 and the older, heavier male rats in Experiment 2 showed an increase in wheel running while they were decreasing in body weight. Conversely, in Experiment 3 the female rats' total amount of wheel running was not affected by the food restriction manipulation. These differences appear to be complex and may be the consequence of other factors related to gender, such as body weight and composition, and baseline levels of wheel running (Tokuyama et al., 1982; unpublished observations).

Secondly, the effects of individual differences in baseline running on food restriction induced increased wheel running were explored. In all 3 studies baseline levels of wheel running were not correlated with the change in running that occurred when the rats reached 85% (± 1 %) of their baseline body weight. This indicates that, regardless of age and gender, the change in running when under food restriction is not related to initial levels of baseline running. This constant finding is surprising given the wide differences in baseline levels of running, ranging from an average of over 7,000 wheel turns in Experiments 1 and 3 to a much lower average of around 750 wheel turns in Experiment 2. In all 3 cases there was considerable variation in both the baseline running and in the size of the change in running.

Lastly, wheel running was examined after the food-restricted rats were returned to ad lib food access. A satiety effect was present in the high running young male rats at refeeding, and was suggested in the high running female rats, but was not evident in the low running old male rats. In both the high running young males and the high running old females individual differences in baseline levels of running predicted the change in running from baseline to the first day of refeeding in a manner that is suggestive of a decrease to a floor level of running. All of the studies showed no long-term consequences of a period of food restriction after refeeding, as running quickly returned to ad lib fed control levels during refeeding in all 3 studies.

In Experiment 1 the young, high running male rats responded to food restriction by showing a 5,000 wheel turn increase in running as they were decreasing in body weight. This increase was from a baseline of about 7,000 wheel turns. While this marked increase in wheel running was only marginally significant, this may be because these high running rats showed considerable variance in their levels of wheel running; however the mean absolute increase in wheel running of the food restricted rats was large. Surprisingly, when these young male rats were being maintained at 85% (± 1 %) of their baseline body weight for a number of days their wheel running was similar to the ad lib fed control rats. When the food-restricted rats were returned to ad lib food access their wheel running dropped to below that of the ad lib fed control rats, showing a satiety effect. This lasted for about 3 days, after which running returned to ad lib control levels.

In Experiment 2 a different pattern of food restriction induced wheel running emerged. The older, low baseline running rats used in the second study significantly increased their wheel running in response to food restriction by about 800 wheel turns from a baseline of about 700 wheel turns. In this experiment there was no weight maintenance period of food restriction. When the food-restricted rats were returned to ad lib food access, in about 3 days their wheel running gradually returned to the same levels as the ad lib fed control group, but no satiety effect was evident. Again, there were no long-term consequences of the food restriction schedule on wheel running.

In Experiment 3 the female rats that were running at similar baseline levels as the young male rats, did not show any increase in total wheel running as a response to food restriction. Interestingly, during the last 4 days of food restriction the food-restricted female rats did show anticipatory running, suggesting that the food restriction schedule was changing their wheel running, even thought it did not increase their total wheel running. They did decrease their total wheel running on the day they were returned to ad lib food access, possibly showing a satiety effect; however, due to the large variance within the groups their running was not statistically different from ad lib fed control levels. There were no long-term consequences of the food restriction.

Both groups of male rats increased their absolute wheel running in response to food restriction, but the number of wheel turns they increased were very different. On the day they reached 85% (± 1 %) of their baseline body weight the young, high running males used in Experiment 1 increased 5,443 wheel turns from a baseline of about 7,000 wheel turns. On the same day the old, low running male rats used in Experiment 2 increased 781 wheel turns from a baseline of about 700 wheel turns. When the change in wheel running is expressed relative to baseline levels, the rats in Experiment 1 increased their running by about 77%, and the rats in Experiment 2 increased their running by 112%. In absolute terms, the high baseline running rats increased their wheel running substantially more than the low baseline running rats, but relative to baseline levels of running, both groups of rats showed similar percentages of increased running. This highlights the importance of reporting not only the changes in wheel running (be it in absolute number of wheel turns or as a percentage of baseline running) but also reporting baseline levels of running, because changes in running are relative to how much running the rats would normally perform.

In all 3 studies the food-restricted rats displayed anticipatory running, where they increased their wheel running in the hour prior to being fed, even though they were fed in the light hours, when rats typically run very little (Eikelboom & Mills, 1988). It is interesting to note that the anticipatory running was also present at the end of the food restriction period in Experiment 3 even though these rats had not increased their total wheel running. This suggests that anticipatory running is a learned effect of the food restriction manipulation, perhaps unrelated to any change in overall wheel running. It does make clear that the food restriction schedule was always effective in changing the rats' pattern of wheel running. Furthermore, in all 3 studies the rats increased their light cycle running, and, while not significant, tended to run less in the dark cycle. The food-restricted rats were allocating their running differently across the light: dark cycle. The anticipatory running did not account for the entire increase in light cycle running, suggesting that the rats were running more over several hours before feeding. Research has indicated that anticipatory running can occur as much as 3 hours prior to being fed (Dwyer & Boakes, 1997), therefore the increase in light cycle running may be the result of increased anticipatory running involving more than just the 1 hour prior to feeding.

The food restriction method was effective in decreasing the rats' body weight to 85% of their baseline body weight in all 3 studies. Because the ad lib fed rats continued to gain weight as they aged, when the food-restricted rats were decreased to

85% of their baseline body weight they weighed less than 80% of ad lib fed, age matched control rats in all 3 experiments. In the first experiment the young high running male rats quickly decreased in body weight, and thus experienced a period of weight maintenance during food restriction. The low running old male rats in Experiment 2, as well as the high running female rats in Experiment 3 decreased in body weight over a longer period of time, and some rats did not reach the 85% baseline body weight criterion in the 16 days of food restriction. Regardless of how long it took the rats to decrease to 85% body weight, and how much they were running, they all increased their body weight quite rapidly on the first day of refeeding. This indicates that the effect of food restriction on body weight was similar in all 3 studies. During food restriction both the young male and old female food-restricted rats lost about 50 g of their baseline body weight (and the old male rats lost about 95 g), but, by the end of the refeeding period, only the young male rats reached the body weight of the ad lib fed control rats. The old male and old female food-restricted rats in Experiments 2 and 3 remained lighter than the ad lib fed controls over the entire refeeding period. It is possible that the age of the young male rats used in Experiment 1 aided in their speedy weight recovery.

The results of this thesis indicate that food restriction induced increased wheel running is unaffected by the age, body weight, and baseline wheel running of male rats. While age (or body weight) appears to influence the baseline level of running in the rat (Jakubczak, 1973; Looy & Eikelboom, 1989; unpublished observations), Experiments 1 and 2 indicate that regardless of the age, weight, and baseline level of wheel running, male rats will increase their wheel running during food restriction. Boakes et al (1999) simultaneously gave young male rats, age-matched young female rats, and weight-matched old females rats ad lib wheel access and food restricted. They found that the rats of the same age decreased in body weight at the same rate, while the old female rats were slower to decrease in weight. This suggests that older, but not heavier, rats will lose weight slower than younger rats. While this may be true, and is supported by the findings of Koubi et al (1991), this does not necessarily indicate that wheel running is affected differently over different ages and body weights. In fact, both the young, light, high running rats in Experiment 1 and the old, heavy, low running rats in Experiment 2 increased their wheel running in response to food restriction, suggesting that male rats increase their wheel running during food restriction, regardless of age, weight, or baseline level of wheel running.

Decreasing body weight has been suggested as an important factor in food restriction induced increased wheel running (Moskowitz, 1959; Duda & Bolles, 1963; Routtonberg & Kuznesof, 1967). However, it does not appear to be the absolute percentage of body weight lost that is solely responsible for increases in wheel running, as all 3 experiments required that the food-restricted rats lose the same percentage of body weight. Also, in Experiment 1 the food-restricted rats had a period of weight maintenance during food restriction, and their wheel running decreased to the same levels as the ad lib fed control rats, despite the food-restricted rats being 20% lighter. This suggests that, for males, the decline in body weight may play an important role in increased levels of wheel running induced by food restriction. There may be other factors (such as gender) that also influence the increase in running. Unfortunately, a weight maintenance period only occurred in Experiment 1, so future research should examine the effects of a weight maintenance period in the 140 day old male rats to determine if the wheel running observed the food restricted rats in Experiment 2 would return to ad lib levels during such a maintenance period. This would help to further establish if decreasing body weight is a necessary if not sufficient condition for the food restriction induced wheel running.

Interestingly, for the male rats used in Experiments 1 and 2, the size of the increase in wheel running relative to baseline levels may be related to the amount of time the rats were declining in body weight. In Experiment 1 the high running food-restricted male rats were decreasing in body weight for between 3-9 days and increased their running by 77%. The low running food-restricted rats in Experiment 2 needed between 6-14 days to decrease in weight, resulting in a 112% increase in running. This difference in the number of days it took the rats to decrease to 85% of their baseline body weight may account for the difference in the relative strength of the increase in wheel running during food restriction. Perhaps the 6+ days of decreasing body weight in Experiment 2 gave the rats enough time to increase their wheel running in response to their decreasing weight. It remains unclear how much of an increase in wheel running would be seen in the high running food restricted rats in Experiment 1 if they had a period of decreasing body weight longer than 3+ days.

In all 3 studies of this thesis baseline levels of wheel running did not predict the change in wheel running evident when the rats reached 85% of their baseline body weight. If the rats had increased their wheel running in proportion to their baseline levels of wheel running a positive correlation would have been expected. Conversely, if the rats had reached a maximum possible number of wheel turns during food restriction a negative correlation would be expected, as the high runners would have had to increase very little and the low runners would have had to increase a large amount. This lack of correlation suggests that individual differences do not predict which rats will show the largest increase in wheel running during food restriction.

The lack of an increase in total wheel running observed in the female rats during food restriction suggests that there are gender differences in the ways that male and female rats react to the food restriction paradigm of wheel running. Finger (1951) indicated that there were no gender differences in food restriction induced increased wheel running when he reported male and females as one group. There are few other studies that have examined gender differences in the effects of food restriction on wheel running. In these studies some differences between male and females have been reported. Boakes et al. (1999) reported that male rats will increase their wheel running in response to decreased body weight, but female rats will maintain the same, albeit higher, level of wheel running across all weights. The researchers decreased rats to 95%, 90%, 85%, or 80% of their baseline body weight and tested them with an acute 1 hour of wheel access, which may not give the most accurate description of wheel running over time.

Gender differences also have been reported in studies that have employed the Activity Anorexia (AA) procedure, where wheel naïve rats are given wheel access and food restriction simultaneously. Doerries, Stanley, and Aravich (1991) reported that female rats are more resistant to the development of AA because, although the female rats ran more than the males, they also ate more and took longer to decrease their body weight by 25% or 30%. When the rats lost 25% or 30% of their baseline body weight the male rats ran 5,000 and 15,000 wheel turns respectively, and the female rats ran 12,000 and 20,000 wheel turns respectively. The different levels of running the female rats showed at the different body weights contrasts the findings of Boakes et al. (1999). The increase in running as weight declines suggests that females do increase their wheel running under food restriction, however the other characteristics of AA in these females (such as the amount of food consumed and the time to decrease in body weight) suggest that there are still pronounced gender differences. The present studies involved decreasing rats body weight by about 15%. Perhaps, while this level of weight loss was enough to increase wheel running in male rats, female rats need to decrease their body weight more to show similar levels of increased running as the males.

When the food-restricted rats were returned to ad lib food access a satiety effect was present in the first experiment. The high running female rats in Experiment 3 also tended to run less on the first day of refeeding compared to ad lib fed controls, although this comparison was not significant. The satiety effect was present in the first experiment despite the fact that, just prior to refeeding, the food restricted rats were running the same amount as the ad lib fed rats during food restriction. The decreased wheel running when the high running rats in Experiment 1 were returned to ad lib food access is similar to the effects of food access given every 48 hours (Goodrick, Ingram, Reynolds, Freeman, & Cider, 1983a, b; Weise & Eikelboom, 2000). When rats are given wheel access and then put on a food restriction schedule where ad lib food access is given every other day, they run the same as continuously ad lib fed rats on the days they do not have food access. On the days when food is made available their wheel running decreases to below ad lib fed levels (Goodrick, Ingram, Reynolds, Freeman, & Cider, 1983a, b; Weise & Eikelboom, 2000). This, as well as the satiety effect observed in Experiment 1 suggests that that the decrease in running is the result of the return to ad lib food access and not an after-effect of the period of increased running.

In both Experiments 1 and 3 baseline levels of wheel running were negatively correlated with the change in running on the first day of refeeding, indicating that the high runners decreased their running the most while the low runners decreased the least. This is consistent with the notion that the rats reached a floor level of wheel turns. It also suggests that high running rats may be more sensitive to the effects of refeeding, showing the greatest decrease in wheel turns, and the low running rats may be more resistant to such effects.

In all 3 experiments there were no long-term consequences of food restriction after ad lib food access was reinstated. Pierce et al. (1986) suggested that food restriction changes the rewarding value of wheel access. The present studies suggest that, if wheel running reward was changed during food restriction, this change was not permanent. Wheel running returned to ad lib fed control levels within about 3 days for all 3 studies, indicating that the rats rapidly adjusted to the change from food restriction to ad lib food access.

The present results have several implications. First, the results suggest that there is a gender difference in the influence of food restriction on wheel running. Female rats seem to be more resistant to the effects of food restriction, a result that is consistent with the findings of Boakes et al. (1999). Secondly, the increased wheel running under food restriction parallels increases in drug self-administration in response to food restriction (Carroll & Meisch, 1984). In fact, wheel running behaviour has several similarities to drug addiction and thus may be valuable as tool for examining addiction. Lastly, the results suggest that 3 separate mechanisms may be responsible for the level of wheel running seen in rats: one responsible for the initial low level of wheel running; a second responsible for increased running under normal conditions; a third responsible for increased running under food restriction.

The female rats used in this thesis did not show an increase in wheel running when exposed to food restriction. Increased levels of wheel running induced by food restriction have been suggested as an animal model for anorexia nervosa, specifically paralleling the increased levels of exercise and the associated decrease in food consumption seen in people who suffer from the disorder (Epling et al., 1983). While more females are diagnosed with anorexia nervosa than males (Patton, Selzer, Coffey, Carlin, & Wolfe, 1999), males who do have anorexia nervosa show a greater tendency to exercise excessively than females (Touyz, Kopec-Schrader, & Beumont, 1993). Thus, if this food restriction paradigm models anorexia nervosa, it may not be surprising that female rats do not show the same increase in wheel running when food restricted as male rats. However, given that one of the diagnostic criteria of anorexia nervosa is increased exercise, it is surprising that there was no increase in the total wheel running performed by the female rats in Experiment 3.

Just as restricted food access has been suggested to change wheel access reward (Pierce et al., 1986), it has also been suggested to elevate the reinforcing properties of drugs (de Vaca & Carr, 1998; Piazza & LeMoal, 1998). Food deprivation and food restriction have been demonstrated to increase the self-administration of a variety of abused drugs (see review by Carroll & Meisch, 1984). Food restriction will result in an upwards shift of the dose response curve for psychostimulant self administration, which seems to indicate a change in the rewarding value of the drug in the food restricted animal (Piazza & Le Moal, 1998).

Similar neural pathways for reward are activated in both drug self-administration and exercise. The mesolimbic dopamine system has been named as a site and neurochemical involved in the reward produced by drug self administration (Wise, 1981). Exercise has been found to activate the same mesolimbic dopamine system (Chaouloff, 1989), suggesting that there are neurochemical similarities between exercise reward and drug self administration reward.

Further linking drug self-administration and wheel running, Werme, Thoren, Olson, and Brene (1999) found that rats that have an increased tendency towards drug addiction also showed higher levels of wheel running. They compared Lewis and Fisher rats, suggesting that the genetic factors that predispose Lewis rats to high levels of drug self administration also predispose them to be higher runners, possibly the result of changes in the biochemistry involving receptor proteins in the dopamine system. Furthermore, Piazza, Deroche-Gammonent, Rouge-Ponte, and Le Moal (2000) identified 2 categories of drug self administrators: vulnerable rats and resistant rats. The researchers found that, over a range of doses of cocaine, vulnerable rats show a vertical shift in the dose response curve relative to resistant rats. These vulnerable rats have a higher rate of responding and consume more drug over a range of doses, and are also more willing to work for access to the drug. The researchers suggest that this indicates a difference in the rewarding value of the drug in these two groups of animals. They suggest that there is a drug prone phenotype, where some individuals may be more sensitive to the drug's rewarding effect.

If, as Werme et al (1998) suggests, there are links between high drug self administrators and wheel runners, perhaps the drug prone phenotype suggested by Piazza et al. (2000) are also high wheel runners. If so, wheel running may be a very effective, non-drug model of drug self administration, which may allow researchers to further explore addiction in the absence of a drug.

The present set of results also suggests that the level of wheel running may be determined by 3 separate mechanisms. A first mechanism is used to determine the initial, floor level of wheel running a rat will perform, which may be influenced by the age, weight, or gender of the rats. Young adult male rats first given access to a running wheel will start running about 1,000 wheel turns each day (Looy & Eikelboom, 1989), whereas older male rats will begin running at about 500 wheel turns (unpublished observations). A second mechanism is necessary, as it explains the natural increase in running seen in young male rats. This occurs over the 2 to 3 weeks of wheel access when the young male rats are increasing their wheel running to a stable plateau of about 7,000 wheel turns (Looy & Eikelboom, 1989). Old male rats seem to be resistant to this increase in wheel running, as they maintain a low stable level of running, which may be a result of their age or a secondary effect of their age, such as their increased body weight (unpublished observations). This suggests that the old males either lack the mechanism to increase their wheel running naturally or they experience such a small increase in running that it becomes undetectable. There is also a gender difference to be considered, as old female rats still show an increase in running.

A third mechanism may be responsible for the forced increase in wheel running seen under food restriction. The high baseline running 55 day old male rats in Experiment 1 increased their running under food restriction, however, the high running female rats in Experiment 3 did not change their total wheel running as a result of the food restriction. Thus, the two groups of rats showing similar natural increases in wheel running, the 55 day old males and 180 day old females, differed in their response to food restriction. Secondly, the 140 day old male rats did not increase their wheel running in response to food restriction to levels similar to the plateaued level of running observed in the 55 day old male rats. Finally, the increase in running under food restriction was not correlated with baseline levels of running, indicating that not all rats increase their running in similar ways. Therefore, this final mechanism is different from the mechanism responsible for the natural increase in wheel running, and necessary to explain the food restriction induced increase in wheel running.

When wheel experienced rats are exposed to a period of food restriction and then returned to ad lib food access several considerations must be made. First, there are clear gender differences in the effects of food restriction on wheel running, as females do not change their total wheel running in response to the same restricted food access that increses wheel running in males. Second, male rats of the different ages, body weights, and baseline levels of wheel running will increase their wheel running when exposed to food restriction. Third, the satiety effect at refeeding seems to be related to the rats' level of baseline wheel running. High running rats will show a satiety effect that is consistent with reaching a floor level of wheel turns, and low running rats decline in their wheel running to ad lib levels gradually, with no satiety effect. Lastly there appears to be no long-term consequences of the food restriction schedule after refeeding.

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Table 1

Number of Rats Given Differing Amounts of Food Across Food Restriction Period for Experiment 1

	Day of Study															
Amount of Food (g)	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
15.0-16.9	8	8	7	5	3	1	1	1	1	1	1				1	1
17.0-18.9			1	3	2	3						1		1		1
19.0-20.9					3	1							1		3	2
21.0-22.9						2	2	1	1	2	1		1	2		
>23							5	6	6	5	6	7	6	5	4	4

Table 2

Number of Rats and Percent of Body Weight Across Food Restriction Period for Experiment 1

Percent of Body Weight	Day of Study															
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
>86%	8	8	7	4	3	2	1	1	1							
85% (± 1%)			1	4	5	6	7	7	7	8	8	8	8	8	8	8

Table 3

Number of Rats Given Differing Amounts of Food Across Food Restriction Period for Experiment 2

Amount of Food (g)	Day of Study															
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
< 13									6	7	7	7	5	5	1	
13.0-14.9						1	7	7	1						1	
15.0-16.9	8	8	8	8	8	7	1	1					2	1	3	5
17.0-18.9									1					1	1	1
19.0-20.9										1					1	
21.0-22.9																1
> 23											1	1	1	1	1	1

Table 4

Number of Rats and Percent of Body Weight Across Food Restriction Period for Experiment 2

Percent of Body Weight	Day of Study															
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
>86%	8	8	8	8	8	7	7	7	7	7	6	4	2	1		
85% (± 1 %)						1	1	1	1	1	2	4	6	7	8	8

Table 5

Number of Rats Given Differing Amounts of Food Across Food Restriction Period for Experiment 3

	Day of Study															
Amount of Food (g)	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
< 13																
13.0-14.9								1	1	1	2	2	2	2	2	2
15.0-16.9	10	10	10	10	10	9	8	5	5	5	4	3	3	3	2	2
17.0-18.9						1	2	2	1	1	1	1	1	1	1	1
19.0-20.9								2	2	1	1	2	3	3	5	5
21.0-22.9									1	1	1		1	1		
> 23										1	1	2				

Table 6

Number of Rats and Percent of Body Weight Across Food Restriction Period for Experiment 3

Percent of Body Weight	Day of Study															
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
>86%	10	10	10	10	9	7	6	6	6	6	6	5	4	4	4	3
85% (± 1 %)					1	3	4	4	4	4	4	5	6	6	6	7

Figure Captions

Figure 1. Mean (± SEM) body weight of the 55 day old male rats in the groups ADLIB and RESTRICT during 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 1.

Figure 2. Mean (± SEM) number of wheel turns performed by the 55day old male rats in the groups ADLIB and RESTRICT in 24 hours, over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 1. The Insert shows the mean (± SEM) total number of wheel turns in the last 3 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 38 is excluded because the running wheels were cleaned on that day.

Figure 3. Mean (± SEM) number of wheel turns performed by the 55 day old male rats in the groups ADLIB and RESTRICT between 10:15 h and 11:15 h, over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 1. The Insert shows the mean (± SEM) number of wheel turns performed between 10:15 h and 11:15 h in the last 3 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 38 is excluded because the running wheels were cleaned on that day.

Figure 4. Mean (± SEM) number of wheel turns performed by the 55 day old male rats in the groups ADLIB and RESTRICT over 4 days of baseline, 16 days of food

restriction, and 16 days of refeeding in Experiment 1: **A** during the dark cycle, between the hours of 18:00 h and 06:00 h. **B** during the light cycles, between the hours of 06:00 h and 18:00 h. The Inserts show the mean (± SEM) number of wheel turns performed during the dark (A) and light (B) cycles in the last 3 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 38 is excluded because the running wheels were cleaned on that day.

Figure 5. Mean (± SEM) body weight of the 140 day old male rats in the groups ADLIB and RESTRICT during 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 2.

Figure 6. Mean (± SEM) number of wheel turns performed by the 140 day old male rats in the groups ADLIB and RESTRICT in 24 hours, over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 2. The Insert shows the mean (± SEM) total number of wheel turns performed in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 50 is excluded because the data collection computer crashed, so the wheel running data was unavailable. Day 56 is excluded because the running wheels were cleaned on that day.

Figure 7. Mean (± SEM) number of wheel turns performed by the 140 day old male rats in the groups ADLIB and RESTRICT between 08:30 h and 09:30 h, over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 2. The

Insert shows the mean (± SEM) number of wheel turns performed between 08:30 h and 09:30 h in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 50 is excluded because the data collection computer crashed, so the wheel running data was unavailable. Day 56 is excluded because the running wheels were cleaned on that day.

Figure 8. Mean (± SEM) number of wheel turns performed by the 140 day old male rats in the groups ADLIB and RESTRICT over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 2: **A** during the dark cycle, between the hours of 18:00 h and 06:00 h. **B** during the light cycles, between the hours of 06:00 h and 18:00 h. The Inserts show the mean (± SEM) number of wheel turns performed in the dark (A) and light (B) cycles in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 50 is excluded because the data collection computer crashed, so the wheel running data was unavailable. Day 56 is excluded because the running wheels were cleaned on that day.

Figure 9. Mean (± SEM) body weight of the 180 day old female rats in the groups ADLIB and RESTRICT during 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 3.

Figure 10. Mean (± SEM) number of wheel turns performed by the 180 day old female rats in the groups ADLIB and RESTRICT in 24 hours, over 4 days of baseline,

16 days of food restriction, and 16 days of refeeding in Experiment 3. The Insert shows the mean (± SEM) total number of wheel turns performed in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 59 is excluded because the running wheels were cleaned on that day.

Figure 11. Mean (± SEM) number of wheel turns performed by the 180 day old female rats in the groups ADLIB and RESTRICT between 09:00 h and 10:00 h, over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 3. The Insert shows the mean (± SEM) number of wheel turns performed between 09:00 h and 10:00 h in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 59 is excluded because the running wheels were cleaned on that day.

Figure 12. Mean (± SEM) number of wheel turns performed by the 180 day old female rats in the groups ADLIB and RESTRICT over 4 days of baseline, 16 days of food restriction, and 16 days of refeeding in Experiment 3: **A** during the dark cycle, between the hours of 18:00 h and 06:00 h. **B** during the light cycles, between the hours of 06:00 h and 18:00 h. The Inserts show the mean (± SEM) total number of wheel turns performed in the dark (A) and light (B) cycles in the last 5 days before the rats in the RESTRICT group reached 85% of their baseline body weight. Day 59 is excluded because the running wheels were cleaned on that day.

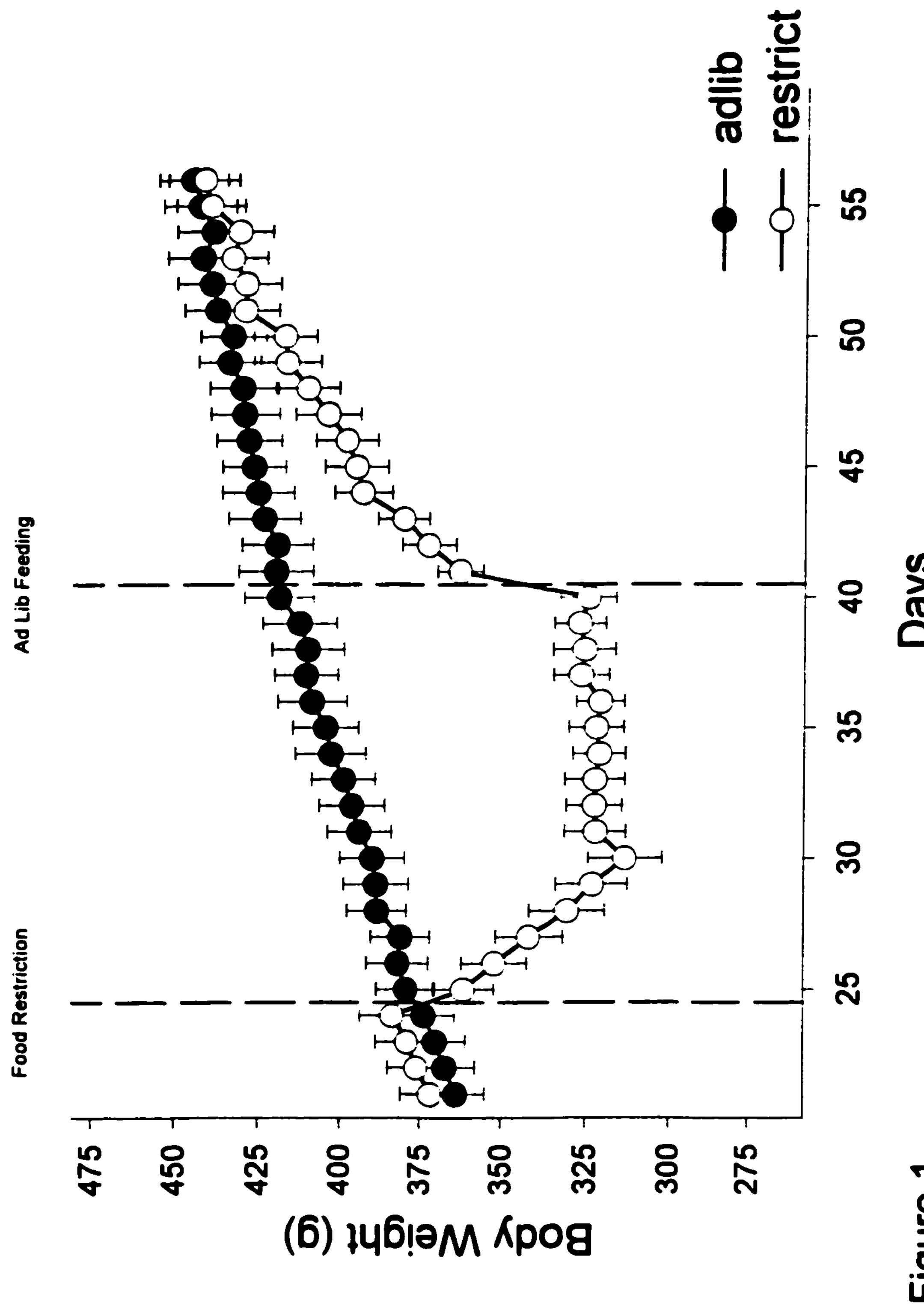
Food Restriction
Ad Lib Feeding
Body Weight (g)
475
450
425
400
375
350
325
300
275
25
30
35
40
45
50
55
Days
adlib
restrict

Figure 1

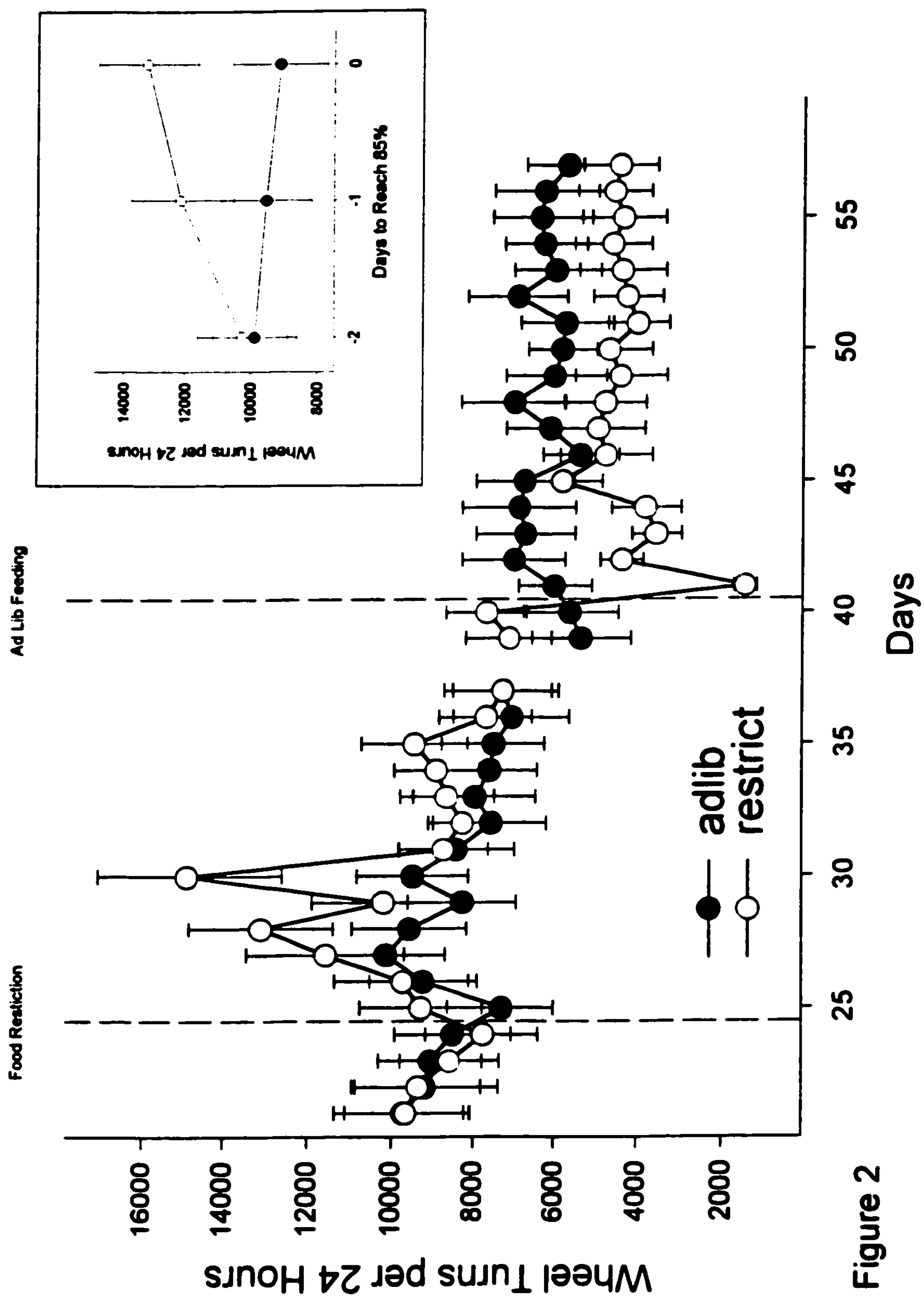
Food Restriction
Ad Lib Feeding
Wheel Turns per 24 Hours
16000
14000
12000
10000
8000
6000
4000
2000
25
30
35
40
45
50
55
Days
adlib
restrict
Wheel Turns per 24 Hours
14000
12000
10000
8000
-2
-1
0
Days to Reach 85%

Figure 2

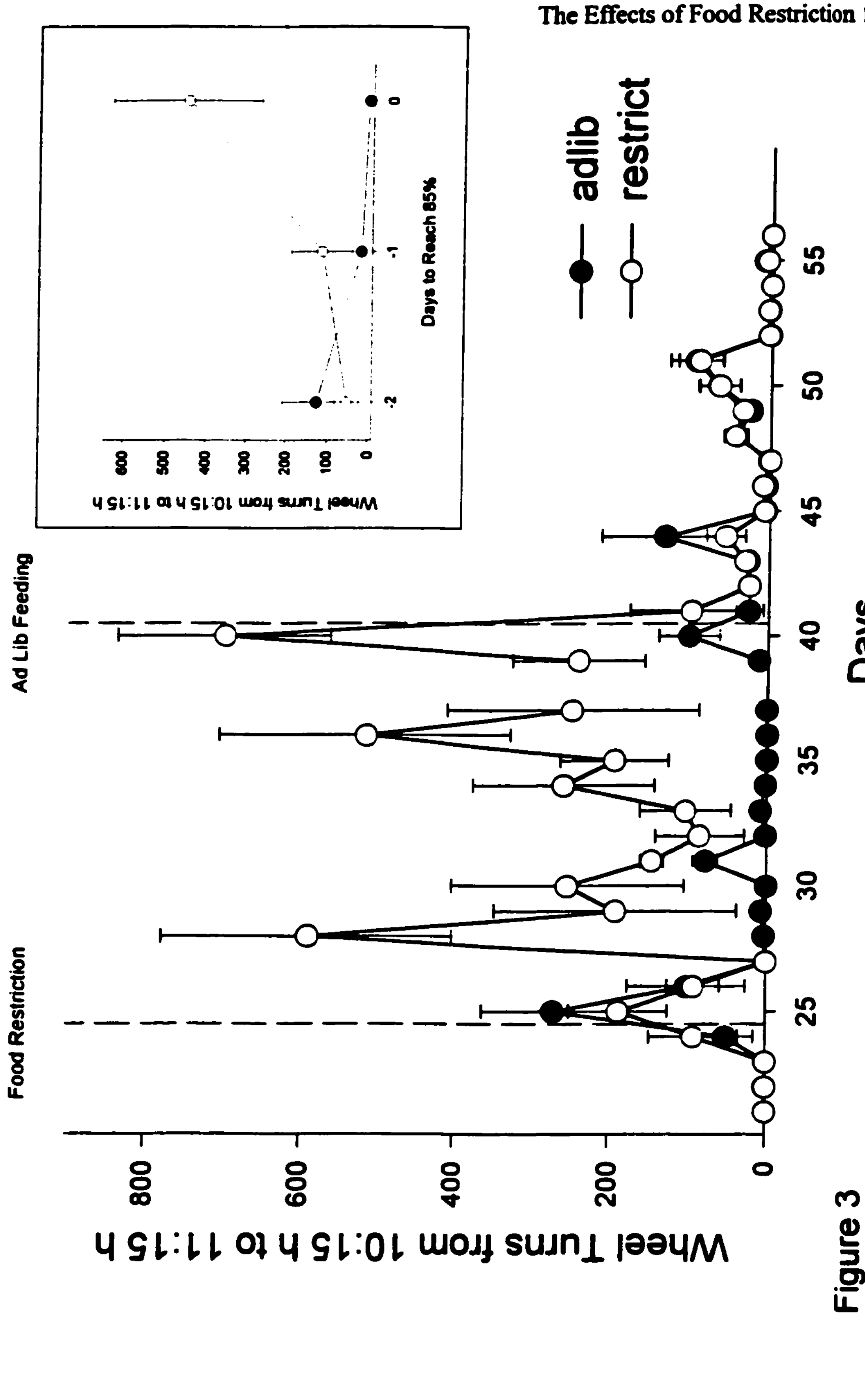
Food Restriction
Ad Lib Feeding
Wheel Turns from 10:15 h to 11:15 h
Days
adlib
restrict
Days to Reach 85%

Figure 3

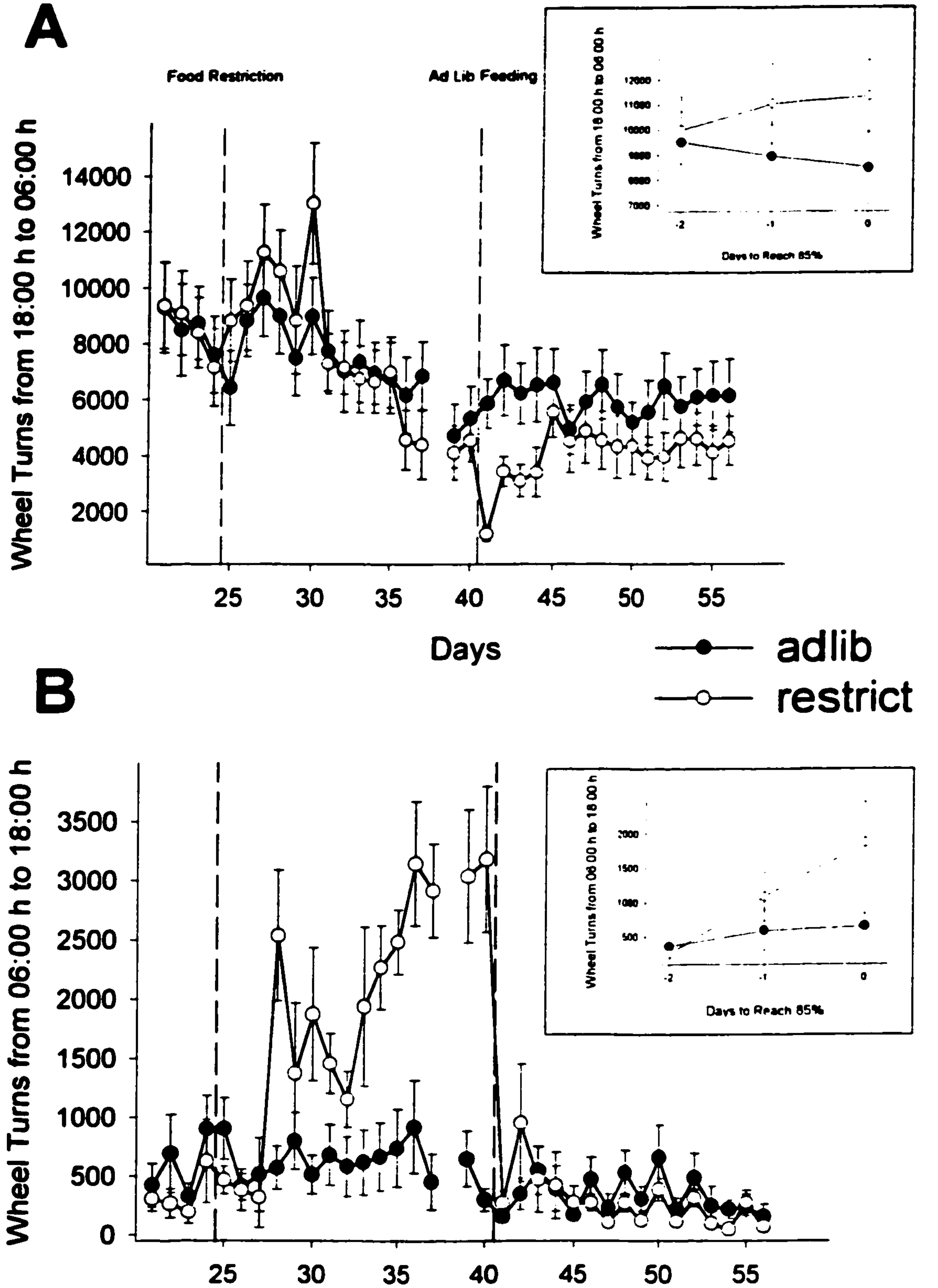
A
Food Restriction
Ad Lib Feeding
Wheel Turns from 18:00 h to 06:00 h
14000
12000
10000
8000
6000
4000
2000
25
30
35
40
45
50
55
Days
Days to Reach 85%
adlib
restrict
B
Wheel Turns from 06:00 h to 18:00 h
3500
3000
2500
2000
1500
1000
500
0
25
30
35
40
45
50
55
Days
Days to Reach 85%

Figure 4

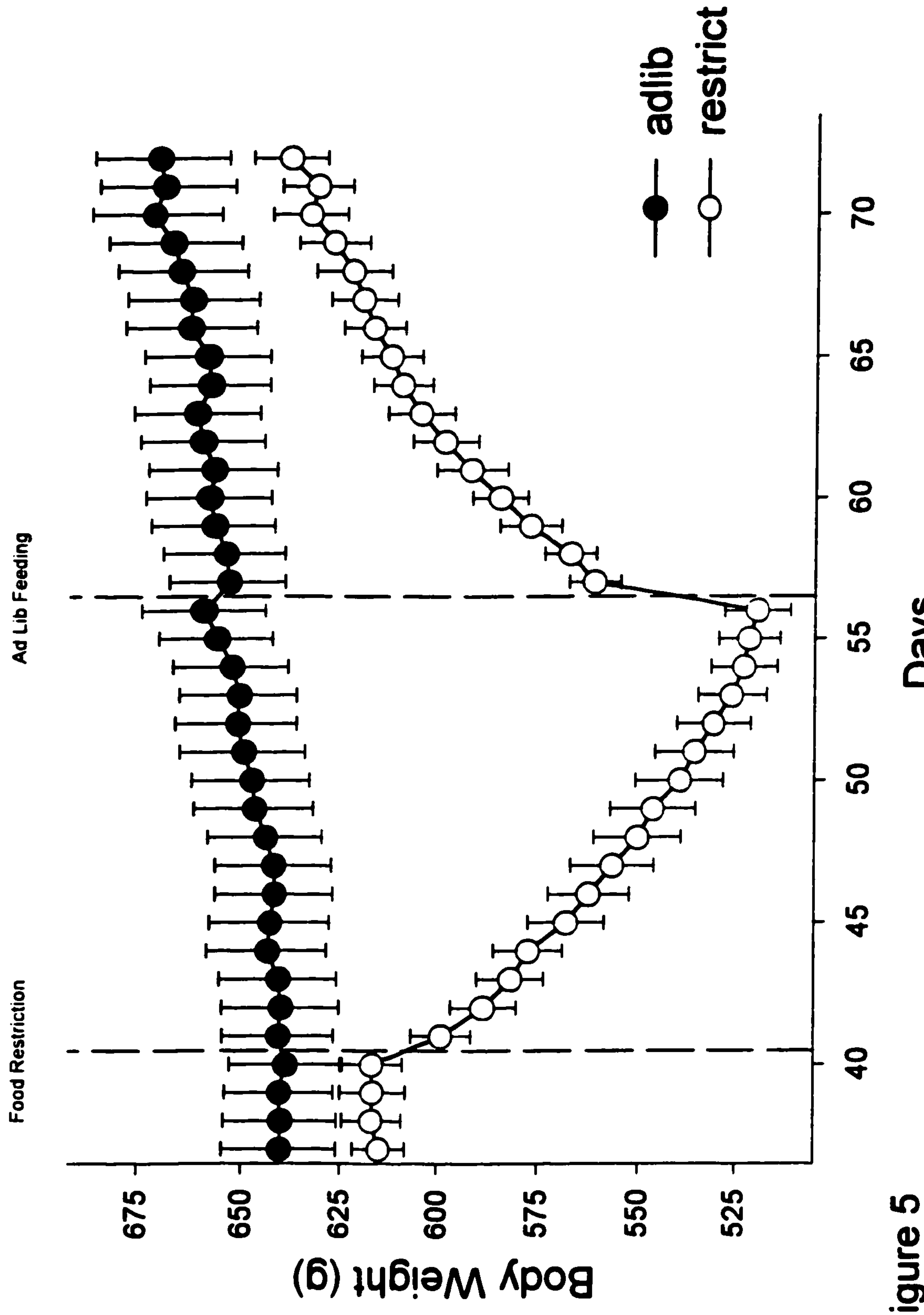
Food Restriction
Ad Lib Feeding
adlib
restrict
Body Weight (g)
675
650
625
600
575
550
525
Days
40
45
50
55
60
65
70

Figure 5

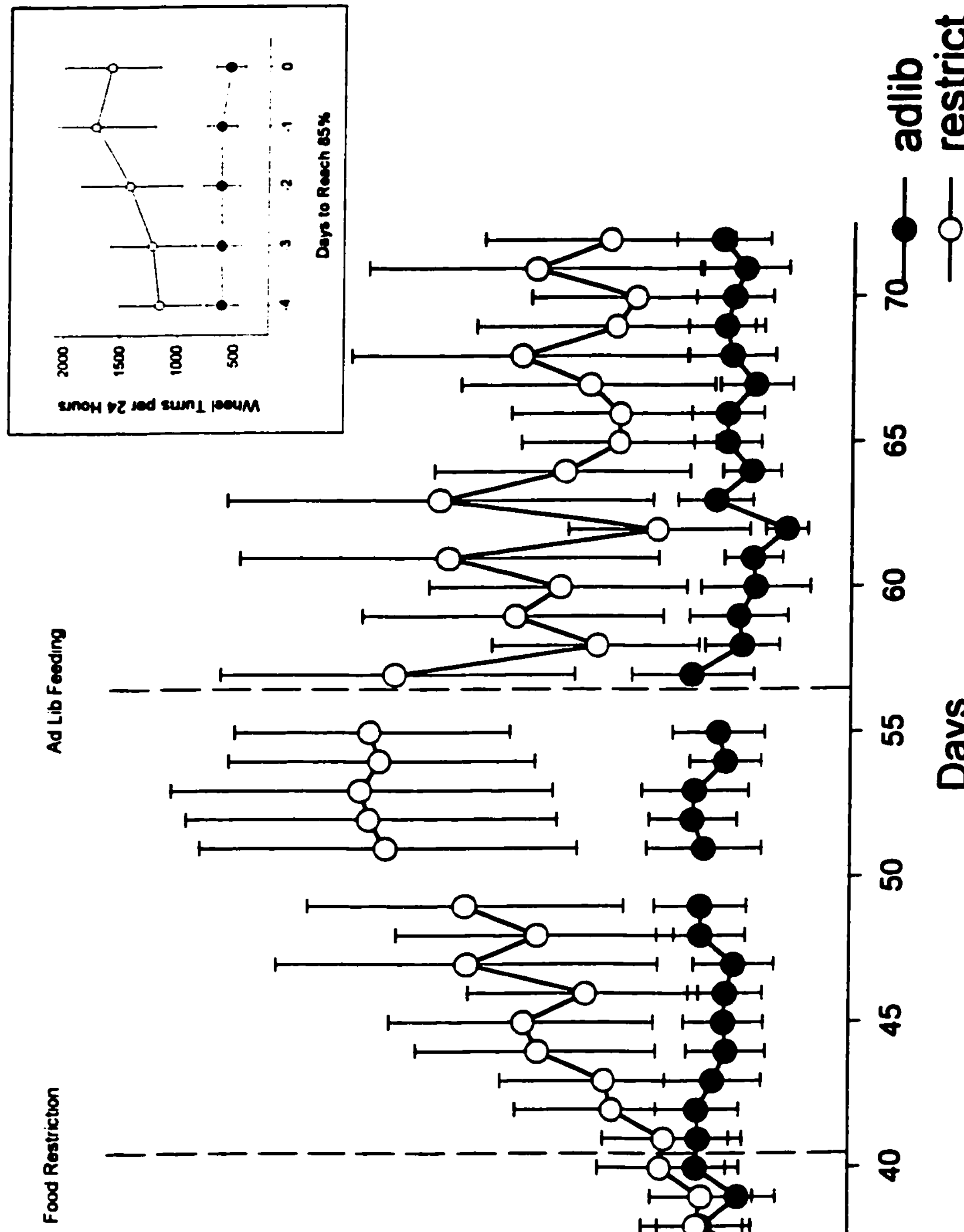
Food Restriction
Ad Lib Feeding
Wheel Turns per 24 Hours
2500
2000
1500
1000
500
40
45
50
55
60
65
70
Days
adlib
restrict
Days to Reach 85%
-4
-3
-2
-1
0

Figure 6

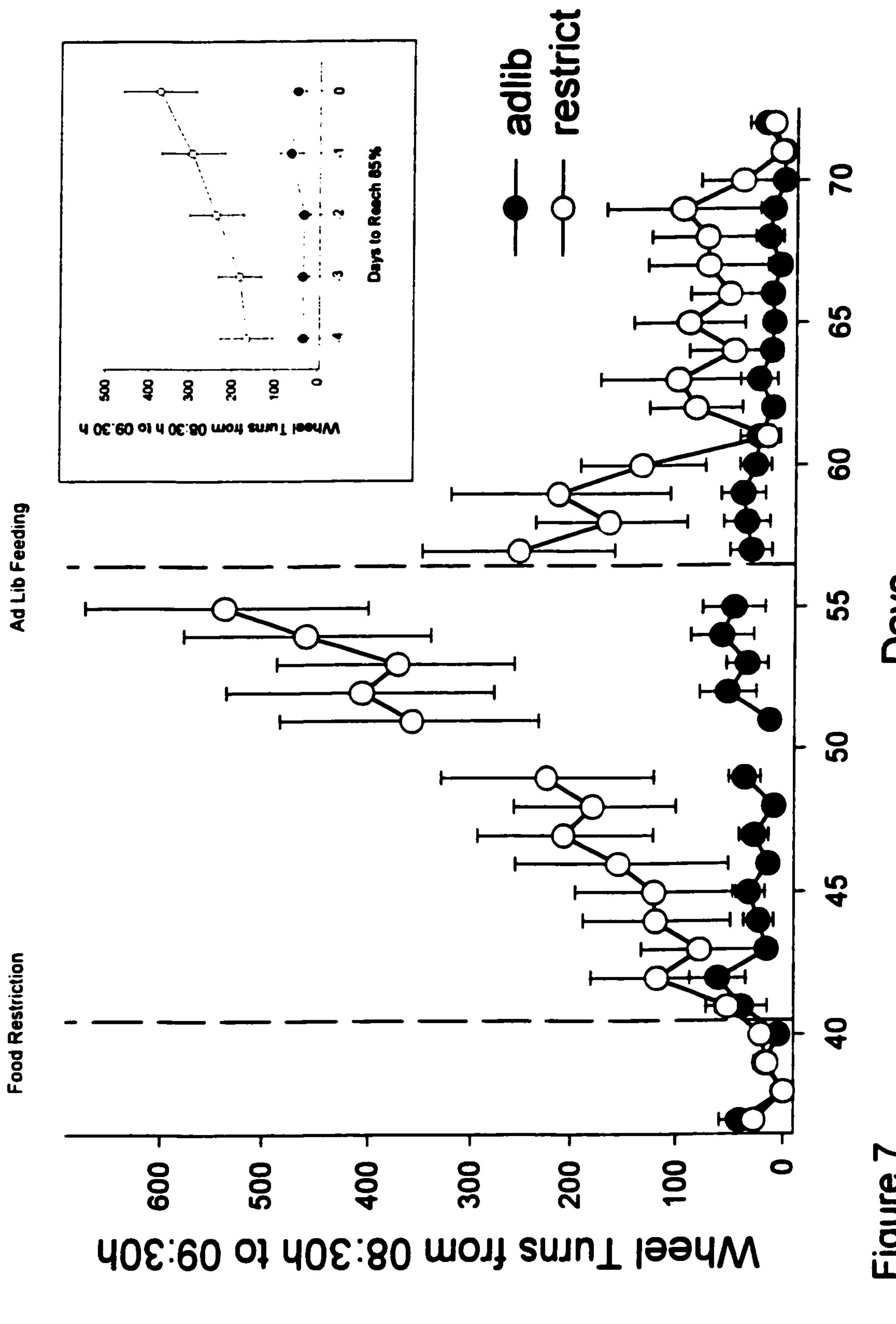
Food Restriction
Ad Lib Feeding
adlib
restrict
Wheel Turns from 08:30h to 09:30h
Days
Wheel Turns from 08:30 h to 09:30 h
Days to Reach 85%

Figure 7

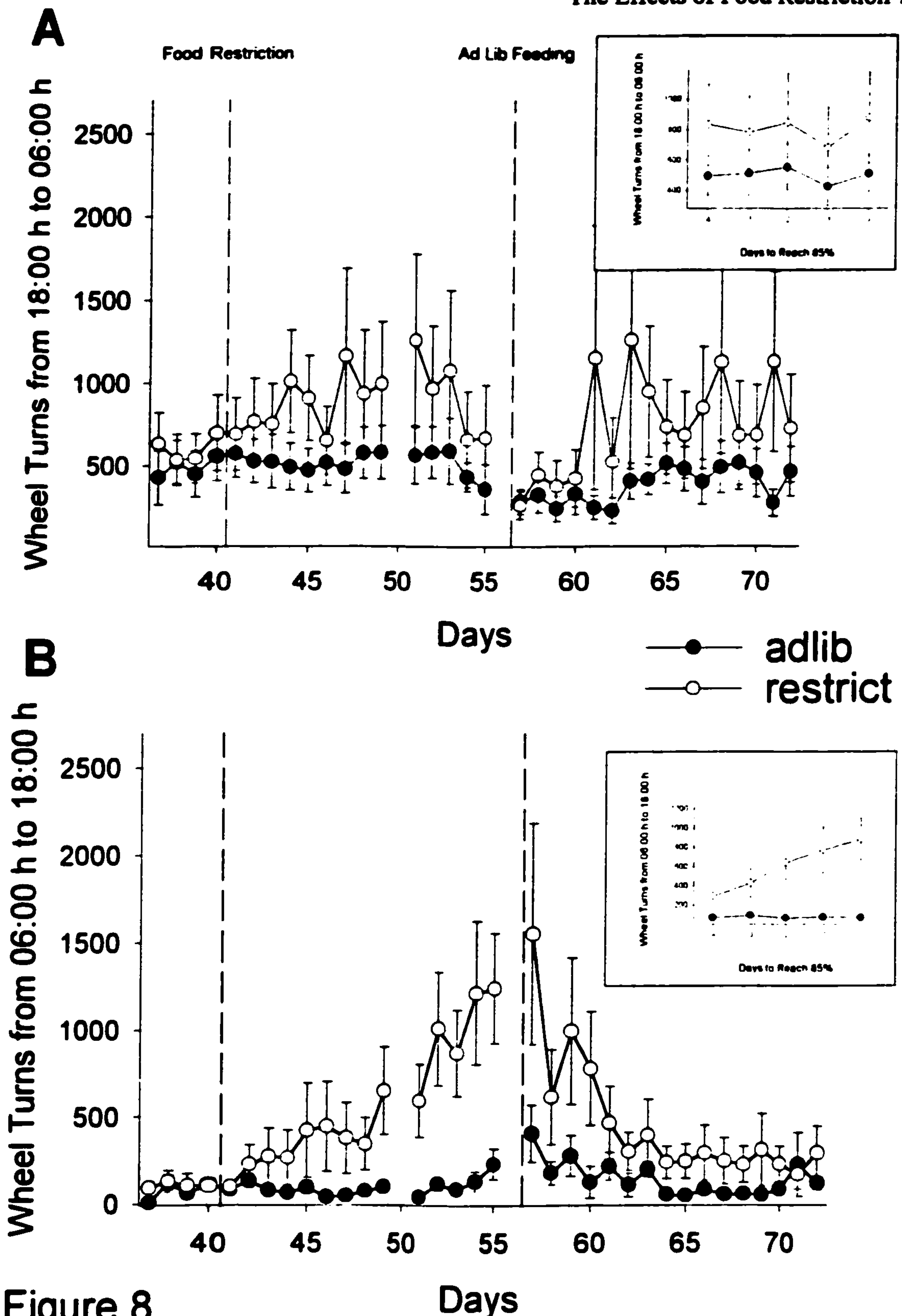
A
Food Restriction
Ad Lib Feeding
Wheel Turns from 18:00 h to 06:00 h
2500
2000
1500
1000
500
40
45
50
55
60
65
70
Days
B
adlib
restrict
Wheel Turns from 06:00 h to 18:00 h
2500
2000
1500
1000
500
0
40
45
50
55
60
65
70
Days

Figure 8

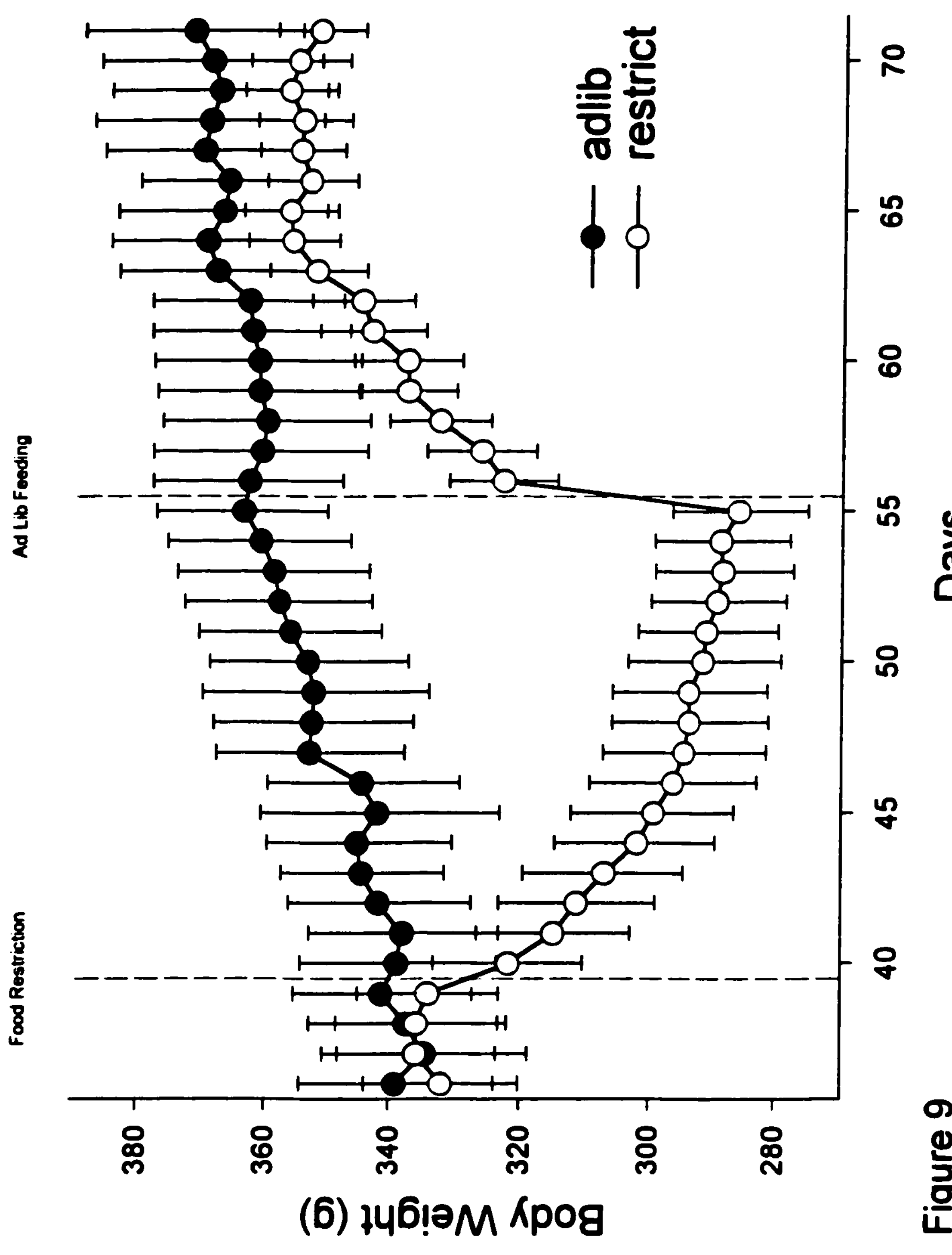
Food Restriction
Ad Lib Feeding
adlib
restrict
Body Weight (g)
380
360
340
320
300
280
40
45
50
55
60
65
70
Days

Figure 9

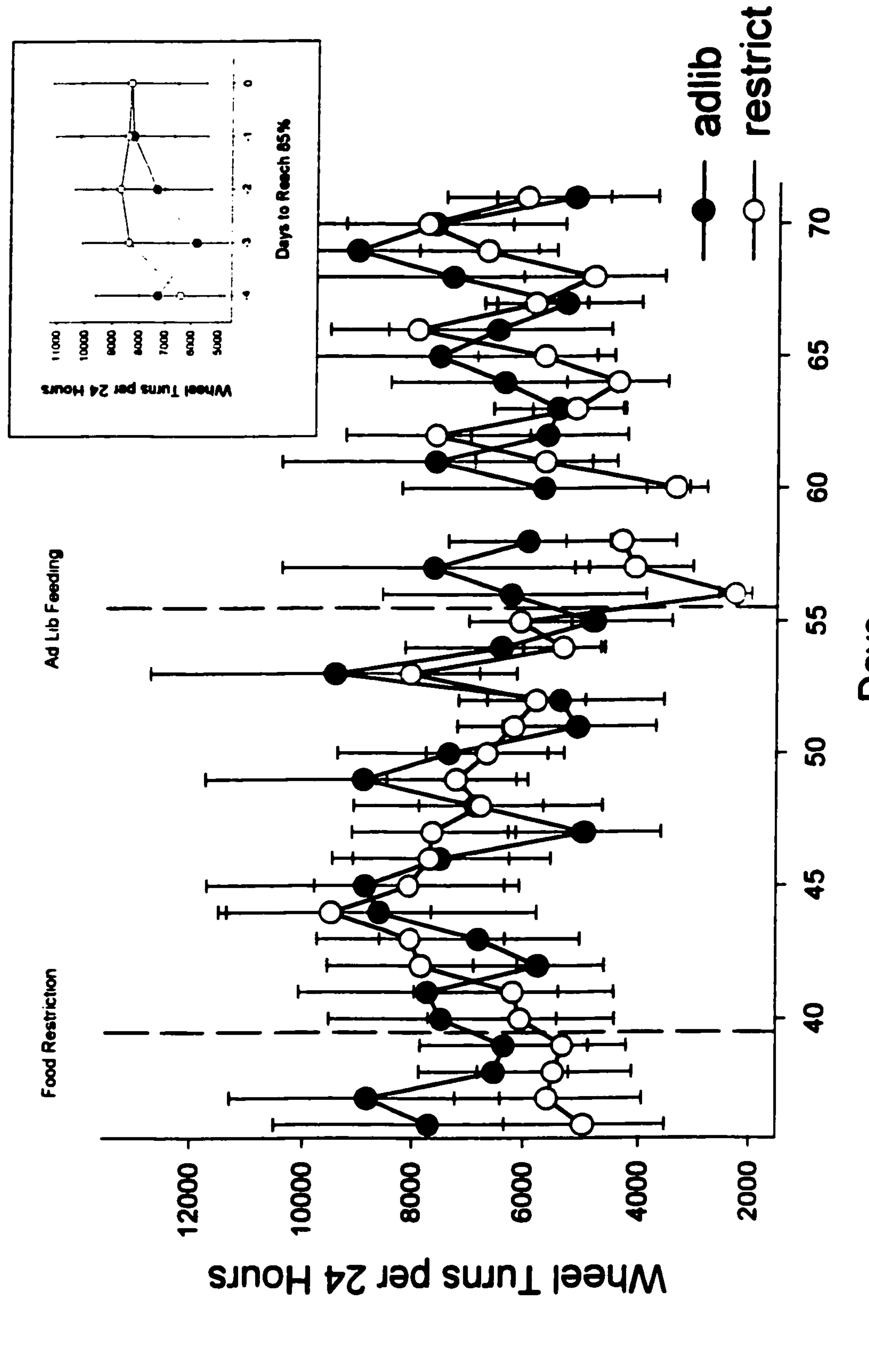
Food Restriction
Ad Lib Feeding
Wheel Turns per 24 Hours
12000
10000
8000
6000
4000
2000
40
45
50
55
60
65
70
Days
adlib
restrict
Days to Reach 85%

Figure 10

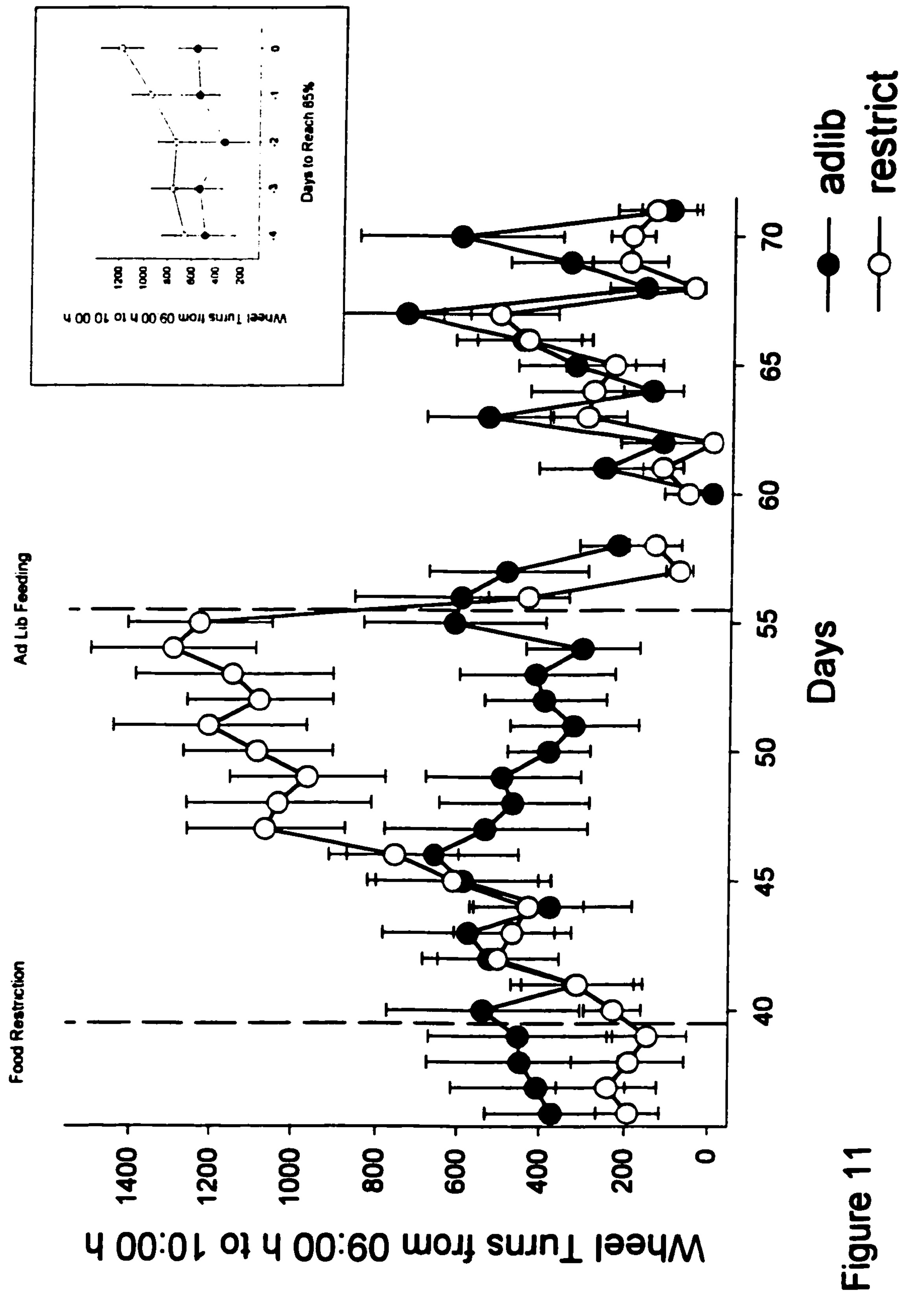
Wheel Turns from 09:00 h to 10:00 h
0
200
400
600
800
1000
1200
1400
Days
40
45
50
55
60
65
70
Food Restriction
Ad Lib Feeding
adlib
restrict
Days to Reach 85%
Wheel Turns from 09:00 h to 10:00 h

Figure 11

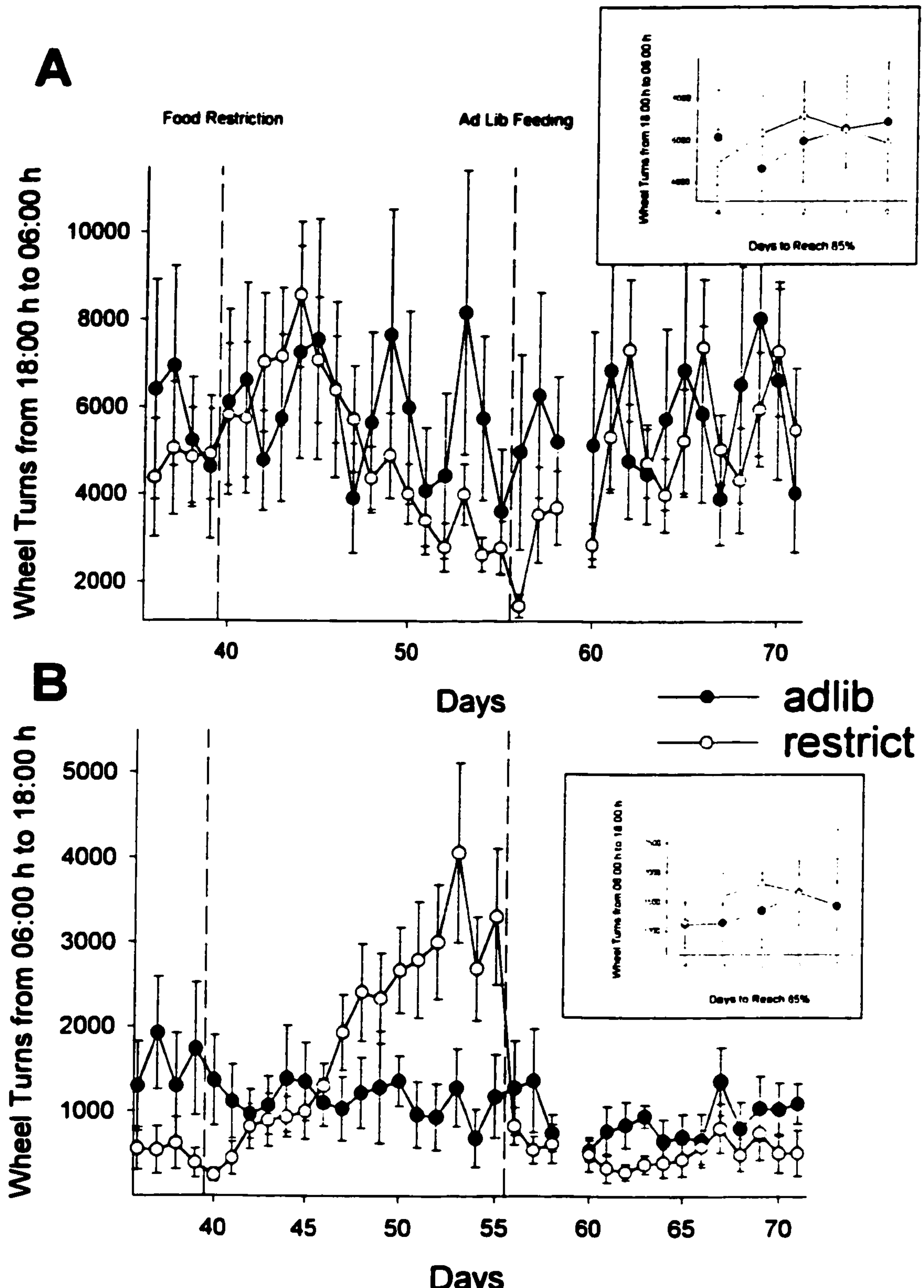
A
Food Restriction
Ad Lib Feeding
Wheel Turns from 18:00 h to 06:00 h
10000
8000
6000
4000
2000
40
50
60
70
Days
Days to Reach 85%
B
Wheel Turns from 06:00 h to 18:00 h
5000
4000
3000
2000
1000
40
45
50
55
60
65
70
Days
adlib
restrict

Figure 12