Introduction

Obesity resulting from overeating is a universal problem; and restricted feeding is the best remedy to cure obesity-associated problems. This is also true in laboratory animals. Laboratory rodents are commonly fed ad libitum, e.g. food is available all the time. However, there is ample evidence that ad libitum feeding increases the incidence of kidney, heart diseases, and neoplasias and shortens lifespan in rats (Roe, 1994; Roe et al., 1995; Hubert et al., 2000).

Keenan et al. (1999) has stated that ad libitum feeding of rodents is the most poorly controlled experimental factor in animal-based research. In the long-
term, studies rats die prematurely due to malignancies and degenerative diseases, and this impairs the statistical sensitivity of the study and leads to more animals being needed.

Group housing is the preferred method, and indeed this is a regulatory requirement in Europe (Council of Europe 2007; European Union 2007). However, when animals are group housed, there is no practical or effective way to restrict evenly the food intake of all individuals within the group. Food consumption within the group may also vary, with the dominant animal eating more than the others. When animals are housed individually, restricted feeding is technically possible, but it may, depending how and when food is offered, change the diurnal rhythm. Furthermore, solitary housing is not practical because it requires more cages, and hence is costly. Rats are nocturnal animals and in their natural environment they forage for food and eat mainly during the dark phase because there is less risk posed by predators. In animal facilities, rats also eat predominantly during the dark period when the food is available ad libitum (Spiteri, 1982; Strubbe et al., 1986b; Strubbe & Alingh Prins, 1986), in fact eating during the dark is probably genetically determined (Ritskes-Hoitinga & Strubbe, 2004). It has been shown that when ad libitum feeding was reinstated after a restricted feeding schedule, the rats will immediately revert to their original feeding pattern (Spiteri, 1982; Strubbe et al., 1986b). Locomotion behaviour also increases if the food deprivation period is longer than six hours (Vermeulen et al., 1997); a probable consequence of food searching behaviour.

Daily feeding activity and other diurnal rhythms are controlled by the circadian oscillator, which is located in the suprachiasmatic nuclei in the hypothalamus (Stephan, 1984; Strubbe, et al., 1987; Ritskes-Hoitinga & Chwalibog, 2003). When rats are fed with restricted feeding they have access to food for a few hours, and in most cases this coincides with the housing facility’s working hours. In this kind of situation, they eat all the food immediately, which will impair both natural feeding patterns and gastrointestinal physiology. This can lead to a phase-shift of many biochemical and physiological functions in the gastrointestinal tract of nocturnally active rodents and further changes in serum insulin and glucose (Strubbe & Alingh Prins, 1986; Strubbe, 1987; Rubin et al., 1988), mucosal enzymes of small intestine (Saito et al., 1975) and bile flow (Ho & Drummond, 1975) in rats. Moreover, it has also been shown that an altered feeding schedule results in changes of blood pressure, heart rate and behavioural activity of rats (van den Buuse, 1999).

A decrease in rat food intake in the early studies was achieved with meal feeding; i.e. rats had access to food for only couple of hours a day (Saito et al., 1975; Stephan, 1984; Strubbe, & Alingh Prins, 1986; Roe et al., 1995; van den Buuse, 1999), or simply offering them a certain amount of food (Vermeulen et al., 1997; Markowska, 1999; Hubert et al., 2000). However, these methods necessitate solitary housing of rats.

There are studies trying to combine group housing and restricted feeding. Johnson et al. (2004) covered the feeding area except for a one cm wide slot, where the food was available to the rats. In the same study they also had a “foraging device”, where rats had to work, i.e. to move gravel for access to food. With the slot approach the rats spent more time feeding but consumed less food and with no effect on body weight. The rats preferred eating from the “foraging device”, and though they had to work for food, the body weights of these rats were even significantly higher than in ad libitum fed controls. A third approach that had been tried is the addition of largely indigestible sugar beet pulp fibre to the chow; there were reduced weight gain benefits, but also enlarged GI-track - especially caecum - in the increased fibre-fed group (Eller et al., 2004).

We hypothesized that rats will only work - in this case gnaw wood - for food they necessarily need, provided that the work intensity is correctly set. The aim of this study was to assess whether a novel system of food restriction would have any effect on weight gain over a short period, food utilisation and
amount of wood gnawed in adult rats and whether their time budget differs from *ad libitum* fed rats.

**Materials and Methods**

**Animals**
A total of 18 BN (BN/RijHsd) and 18 Fischer344 (F344/NHsd) male rats, all supplied from Harlan, (Horst, The Netherlands), were used in this study. 10 of which were fitted with a telemetric transponder (details below). The rats were 25 weeks old and weighed 280 - 370 g (BN) or 350 - 460 g (F344), respectively, at the beginning of the experiment.

**Animal housing and care**
Rats were housed in the same room either in open top polysulfone cages (Tecniplast, Buguggiate, Italy) or polysulfone individually ventilated cages (IVC) (Tecniplast, Buguggiate, Italy) (3 rats / cage). The cage type used was 1500U ***Eurostandard IV S (48.0 x 37.5 x 21.0 cm – floor area 1500 cm²) with a solid bottom and stainless steel wire lid; IVC cages had their own double lids. The cage floor was covered with 3.0 l aspen chip bedding (of size 4 x 4 x 1 mm, 4HP, Tapvei Oy, Kaavi, Finland). The cages were changed weekly. The room temperature was 21.2 ± 0.3°C and relative humidity (RH) 53.5 ± 7.7 %, but the temperature was 1 – 4 °C and RH 2 – 3 % higher in the IVCs than both in open cages and in the room. Artificial lighting with fluorescent tubes (light colour warm white) were on from 06.00 to 18.00 and the light intensity at 1 m above floor in the open cages was 16-18 lx compared to 6–9 lx in the IVC’s. The sound level adjusted with R-weighting in empty IVC cages was 20-25 dB(R) compared to 12-18 dB(R) in the empty open cages, with the corresponding adjusted A-weighting being 45-47 dB(A) and 46-49 dB(A), respectively. Tap water was provided in polycarbonate bottles and changed once a week and refilled once in between. For a more thorough description, see Kemppinen et al. (2008) preceding paper.

**Experimental procedure**
Animals were housed three animals per cage, one of them with telemetric transponder. The experiment utilized a crossover design with two week rounds and a rotational order. Within both strains there were two different kinds of mazes (diet board and plain board) made of two crossed aspen boards (34.0 x 14.7 x 3.2 cm; 21.1 x 14.7 x 3.2 cm), a rectangular aspen tube (20.0 x 12.0 x 12.0 cm), or controls without any addition (Figure 1). One maze included holes for food pellets, the diet board, where rats had to gnaw for food, the other was of plain board. The items were made out of aspen because this was the same material as the bedding presumably with the same emissions.

![Figure 1](image-url) Illustration of the study groups: A: diet board, B: plain board, C: tube, D: control. Both strains had one of each added item for two weeks in both the IVCs and open top cages.

Irradiated (25 kGy) pelleted feed (2016 Global Rodent Maintenance, Harlan Teklad, Bicester, UK) was offered to three groups (plain board, tube and control groups) *ad libitum*, while the diet board group had the food pellets embedded snugly in drilled holes (12 mm) of the aspen board. The feed was added once a week and weighed. The aspen boards were weighed before and after the food pellets were placed into the holes. These diet boards were changed once a week. After the change, the remaining food pellets were removed from the dietboards and weighed. Rats were weighed before and
after every study round. All the aspen items were weighed before use and at cage change. In addition, to assess the effect of the various feeding regimens on the rats’ physiological activity and heart rate, ten rats had been implanted with a radio telemetry transmitter (model TA11PA-C40; Data Sciences International, St. Paul, MN, USA). The cylinder shape transmitter body (3.0 cm long, Ø 1.5 cm) monitored pressure and activity via a fluid filled catheter (8 cm long) for sending the signals to an electronics module. The electronics module translated the signals into digitized form and transmitted them to the receiver plate located under the cage. The receiver detected the transmitted signal and converted it to a form readable by the computer.

The rats were anesthetized with the combination of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) + midazolam (Dormicum®, Hoffmann - La Roche AG, Grenzach-Wyhlen, Germany)(0.15 - 0.20 ml/100g SC). The abdominal area was clipped and then scrubbed with MediScrub®, 1% triclosan solution (Medichem International, Sevenoaks, UK) solution and disinfected with chlorhexidine solution (Klorohexol® 5 mg/ml, Leiras, Turku, Finland), and an ocular lubricant (Viscottears®, Novartis Healthcare, Copenhagen, Denmark) was applied on both corneas. A sterile drape was placed over the surgical area and a small area cut away to enable a 3 cm incision to be made through the skin along the abdominal midline. The sterile transmitter was pre-soaked in sterile saline for at least 20 min before the surgery and then placed into the abdominal cavity, and the catheter into the abdominal aorta. The transmitter was sutured into the abdominal wall with 4-0 Ethicon® Ethilon® II (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium) and the abdominal and skin incisions were closed with 5-0 Ethicon® Vicryl® (Johnson & Johnson Intl, St-Stevens-Woluwe, Belgium). After the surgery, the animals were given twice a day 0.01 – 0.05 mg/kg SC buprenorphine (Temgesic®; Schering-Plough Europe, Brussels, Belgium) and once a day a dose of 5 mg/kg SC carprofen (Rimadyl®; Vericore Ltd., Dundee, UK) and parenteral fluids for three days. The pain medication for each rat was titrated with individual response. All rats were given initially buprenorphine at the highest dose; this was continued for at least two days; and carprofen medication for at least three days. The animals were allowed to recover for ten days before the experiment was started.

Data processing and statistical analysis
Activity and heart rate were processed for time budget graphs from the telemetric signals for ten min periods on the first, third, seventh and 13th night and the following light period for each night for all instrumented rats. The number of ten minute periods without activity (activity = 0) were calculated from the graphs, and comparisons made between the groups during the 13th night, and between the days processed in the diet board and plain board group.

All data was assessed with Kolmogorov-Smirnov for normality of distribution. Mixed-model repeated measures ANOVA using strain and group as main effects and age as covariate was applied to weight, disappearance of food, wood gnawed and activity during the dark. Significance was set at p < 0.05. Results
Calculation on a rat basis showed that with respect to the weight gain, there was a significant interaction both in IVC (p = 0.005) and in open cages (p < 0.001) between strain and group (Figures 2A & 2B). In F344 rats, the diet board was more effective in controlling weight, but when combining the strains, all comparisons with diet board were significant (p < 0.05). When the calculation was done on a cage basis, then it seemed that only the rats with the open-cage type diet board displayed any significantly (p = 0.008) reduced weight gain as compared to the plain board group.
In terms of food consumption and in the IVC-system, there was a significant (p < 0.001) interaction between strain and group, with the effect being
clear in F344 rats (Figures 3A and 3B). In the open cage system, both strain and group were significant factors; all three comparisons with diet board were significant. When the strains were pooled, the difference was between 12 - 18 % less food eaten as compared to respective controls.

The amount of wood gnawed differed significantly from normal distribution; hence a mixed model was applied to the ranks. In terms of the amount of wood gnawed, there was a significant (p = 0.001 – 0.005) interaction between strain and group in both cage types. The rats gnawed more wood with diet board as compared to the plain board and tube groups in both caging systems. Furthermore, F344 rats gnawed wood more than BN rats (Figures 4A and 4B).

Typical activity and heart rate recordings for the last light and dark period of the two week round for both BN and F344 rats are shown in Figures 5A - 5D. Calculation from all diet board and plain board activities shows that in both cage types there was a significant interaction (p < 0.001) between the
strain and light, with both of the strains being more active during the dark. F344 rats were significantly (p < 0.05) more active in the dark phase than BN rats in both groups. There were no differences in the activity of the rats between the diet board and plain board groups.

**Discussion**

It has been demonstrated that rats prefer to work for food. Carder & Berkowitz (1970) and Neuringer (1969) reported that even if the rats had free access to food they would rather earn their food as long as the work demands were low. In a preference test, rats preferred to eat mostly from the foraging device which required digging gravel to achieve access (Johnson et al., 2004). This preference of the rats may reflect their need to perform foraging behaviour as they would in their natural environment.

All the rats with the diet board grew less than other groups in both cage types; especially in the F344 rats the diet board was effective in controlling weight. The F344 rats lost weight in the diet board group especially in the open cages, when the rats were older, but the magnitude of loss was marginal – only a few grams over two weeks, most likely fat tissue (Figure 2). The working hypothesis has been that rats should grow less on the restricted feeding (Roe et al., 1995; Hubert et al., 2000), but this has not been observed in all studies. With the “foraging device”, the weight gain of the rats was higher than in ad libitum fed controls; and when the rats had limited access to food, their body weights remained unchanged, both being indications that the approach had been unsuccessful (Johnson et al., 2004).

Eller et al. (2004) have tried to determine whether consuming sugar beet pulp fibre made from water-soluble polysaccharides would have any effect on the weight gain of rats. The rats indeed grew less with the fibre diet, but autopsy after the study revealed an enlarged digestive system in the rats that had received the fibre enriched diet – especially the caecum was enlarged. This may be attributa-
Figure 5. Typical single rat (A = BN – dark, B = BN – light, C = F344 – dark, D = F344 - light) activity and heart rate (HR) recording for the last 24 h of the two week round. There was a significant interaction (p = 0.000) between the strain and light, and both strains were more active during the dark. F344 rats were significantly (p < 0.05) more active in the dark phase than BN rats.
The F344 rats ate more than the BN rats in all of the groups. In the open cage system rats ate significantly less in the diet board group compared to the other three study groups. When the strain specific data was pooled the difference was between 12 - 18% less as compared to the respective controls. The rats in the open cages ate more in the plain board group than in the two other control groups – apparently because the plain board round followed the diet board round, and the animals regained their weight loss in that round (Figure 3). In the study of Johnson et al. (2004) the rats consumed less food when they had limited access to food, while the contrary was true with the “foraging device”, both as compared to controls.

The rats gnawed the wood most with the diet board as compared to plain board and tube groups in both cage types. This was unavoidable task if they wished to eat the food pellets. The F344 rats gnawed wood significantly more than the BN rats - this may relate to a difference in the natural behaviour of these two rat strains (Figure 4). Eskola et al. (1999) have shown that rats would spontaneously gnaw aspen blocks and tubes but this opportunity for gnawing combined with ad libitum feeding had no effect on the growth of Wistar rats, a situation similar to F344 rats in plain board and tube groups.

The F344 rats were significantly more active during the dark than the BN rats in both cage systems. There were no differences in the activity between the plain board and diet board groups suggesting that working for food was not overly strenuous to the rats. Furthermore, the activity of the rats at that time did not differ from their activity during ad libitum feeding. It has been shown that when rats have limited access to food they spend more time feeding, but with the "foraging device" the time spent feeding was markedly decreased. There were only negligible changes between the study groups in their relative total activity levels (Hawkins et al., 1999; Johnson et al., 2004). There were no changes in the social hierarchy of the rats and no increased fighting or stereotype behaviour when rats had limited access to food (Hawkins et al., 1998).

The rats eat most of their food in the dark. In the study of the Spiteri (1982) the rats consumed 94% of their food intake during the dark. The normal feeding activity of rats consists of two peaks during the dark, the first one at the beginning of the dark phase and the other at the end (Spiteri, 1982; Strubbe et al., 1986a). Light is a strong ‘Zeitgeber’ because it shifts the clock in a circadian time-dependent way ensuring synchrony with the external light-dark cycle. The feeding activity and other diurnal rhythms are controlled by the circadian oscillator of the suprachiasmatic nuclei in the hypothalamus. It has been claimed that there are more oscillators involved in the circadian system and this provides the flexibility needed for adaptation to different external and internal stimuli (Anglés-Pujolrás et al., 2006).

When the rats are given access to meals at set times for a few hours each day, they eat all the food almost instantaneously and spend the rest of the day without food; this impairs their natural feeding activity and associated gastrointestinal physiology. This study used the diet board for food restriction allowing rats to enjoy a natural feeding pattern and indeed feeding activity was similar to the plain board group (Figure 5).

The diet-board has clear advantages over previous methods of restricted feeding. The rats can eat at any time and in addition is unlikely to alter biochemical and physiological phenomena timed by circadian rhythm, as opposed to set meal times and most other, if not all, restricted feeding methods (Ho & Drummond, 1975; Saito et al., 1975; Stephan, 1984; Strubbe et al., 1986b; Strubbe et al., 1987; Rubin et al., 1988; van den Buuse, 1999). We conclude that the diet board seems to be a promising way to control obesity and health problems in laboratory rats. Challenging questions still need to be answered to determine whether this approach has refinement and reduction potential.
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