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Simulated weightlessness alters the nycthemeral distribution of energy expenditure in rats

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Summary

The energy metabolism adaptations to simulated weightlessness in rats by hindlimb tail suspension are unknown. 12 male rats were assigned to 7 days of isolation, 7 days of habituation to the suspension device, 10 days of simulated weightlessness, and 3 days of recovery. The 24-hour energy expenditure was measured by continuous indirect calorimetry. We calculated the 12-hour energy expenditure during the active (night) and inactive (day) periods, the minimal observed metabolic rates with the day values taken as an index of the basal metabolic rate, and the non-basal energy expenditure representing the cost of physical activity plus the diet-induced thermogenesis. Suspension did not change the mean 24-hour energy expenditure ($360.8\pm15.3\,J\,min^{-1}\,kg^{-0.67}$), but reduced the night/day difference by 64 % ($P<0.05$) through a concomitant drop in night-energy expenditure and increase in day values. The difference between night and day minimal metabolic rates was reduced by 81 % ($P<0.05$), and the transient rise in day values suggests an early and moderate basal metabolic rate increase (9 %). An overall 19 % reduction in non-basal energy expenditure was observed during simulated weightlessness ($P<0.05$), which was mainly attributable to a reduction in the cost of physical activity. 3 days of recovery restored the night/day differences but increased the 24-hour energy expenditure by 10 % ($P<0.05$). In conclusion, hindlimb tail suspension in rats did not alter the 24-hour energy expenditure, but it transiently increased the basal metabolic rate, and altered both the energy expended on physical activity and the nycthemeral distribution of motor activity. These data suggest that the circadian rhythms of energy expenditure are affected during simulated weightlessness.

Key words: indirect calorimetry, microgravity, rat, energy expenditure, physical activity.

Introduction

Weightlessness is an uncommon environmental situation that induces well-described deleterious physiological adaptations, amongst which bone demineralization, muscle atrophy and cardiovascular deconditioning have been the subjects of numerous studies (for a review, see Vernikos, 1996). Surprisingly, the role of nutrition and related energy metabolism adaptations are not well understood despite their straightforward implications in the maintenance of whole body homeostasis. To our knowledge, only two studies in humans (Lane et al., 1997; Stein et al., 1999a) and one in monkeys (Stein et al., 1996), investigated energy expenditure (EE) during space flight using the doubly labeled water method. The results of these experiments show ongoing loss of body mass due to a negative energy balance in-flight. The subjects oxidized fat mass and lost lean body mass (Stein et al., 1999a). Psychological and metabolic stresses resulting from isolation, confinement and responsibilities have been implicated in this negative energy balance (Stein et al., 1999b), but the mechanisms are still poorly understood. Obviously, a better understanding of energy metabolism during space flight is essential for making accurate estimates of the energy requirements of astronauts on long-term missions.

Since the opportunities for in-flight experiments are few and are not easily dissociable from numerous confounding factors, ground-based models of weightlessness simulation have been developed in both humans and animals. Head-down bed rest (HDBR) in humans and hindlimb tail suspension in rats are the most commonly used models (for reviews, see Vernikos, 1996; Musacchia and Fagette, 1997). These models reproduce the hypokinesia, the hypodynamia and the cardio–thoracic fluid shift observed in space. Although scanty, recent experiments have investigated the human EE adaptations to HDBR to ensure the validity of the model and to give new insights (Gretebeck et al., 1995; Blanc et al., 1998), no data are

available on suspended rats. Interestingly, Mekaouche (1995) reported that the circadian rhythms of body temperature and motor activity were not altered during 7 and 14 days of suspension. However, by examining the active and quiescent phases, the author showed a strong reduction in the amplitude of the circadian rhythm of both body temperature and motor activity. Thermoregulation and physical activity are important components of the total EE, so these results suggest that energy metabolism is affected by simulated weightlessness in rats.

To gain a better understanding of the hindlimb tail-suspended rat model as a reliable tool for studying human adaptations to space, we used continuous indirect calorimetry recording to investigate the extent to which simulated microgravity in rats affects the 24-hour EE and its distribution throughout the nycthemere (day *versus* night). The long-term objectives are to provide accurate estimates of astronauts' energy requirements, to ensure their good health during the long-term missions foreseen by the International Space Station (ISS).

Materials and methods

Animals

A group of 12 male Wistar rats weighing 290±3 g (mean ± S.E.M.) (Iffa Credo, les Oncins, France) were housed in controlled conditions of 21±1 °C at a humidity of 60±10 % with a 12 h:12 h, light:dark cycle (20.00 h/08.00 h). They were fed chow containing 23.5 % proteins, 5 % lipids, 49.8 % carbohydrates, 12 % moisture, 4 % fibers and 5.7 % minerals (UAR Epinay sur Orge, France). Food and tapwater were provided *ad libitum*. All protocols and procedures described below were conducted in accordance with the guiding principles of the American Physiological Society and the Veterinary Board of the French Space Agency.

Experimental protocol

The experimental schedule ran for 27 days and comprised four successive periods: 7 days of isolation, 7 days of attachment, 10 days of simulated weightlessness and 3 days of recovery. The attachment period allowed us to minimize the stress experienced by the animals during the first days of suspension. During this period, the rats were kept in a horizontal position with their tails attached to the suspension device. Energy expenditure was measured continuously over the four periods by indirect calorimetry.

Simulated weightlessness model

The Morey tail-suspension model modified by Chen et al. (1993) was used to simulate weightlessness. Briefly, the tails were cleaned and dried. Tinctures of benzoin and resin were successively sprayed on the tail to protect the skin from irritation and to form a sticky surface. A suitable width strip of adhesive tape was then attached laterally along the proximal portion of the tail. The tape was then secured by wrapping the tail in three tail-width tape strips that wound separately around the tail. The rats were attached *via* a plastic bar in the tape to a fish swivel fixed to the top of a special cage. The animals were maintained individually in a –35 ° to –40 ° head-down tilt position and were able to use their forelimbs to move freely in a 360 ° arc.

Indirect calorimetry

The rates of O_2 consumption and CO_2 production were assessed by an indirect calorimeter consisting of an open-flow system using gas analyzers. Oxygen and carbon dioxide concentrations in downstream exhaust gases were successively measured in five different cages. To avoid errors resulting from the sequential changes from one cage to another, common parts of the system were rinsed for 90 s, after which gas exchanges were measured for 40 s. The final value is a mean of 10 values obtained every 4 s. A computer-controlled system of three-way valves allowed sequential analysis of the five cages, which were sampled every 11 min. One cage was left vacant and served as a reference for measuring ambient O_2 and CO_2. Air samples were pumped at a constant flow rate, controlled within strict limits by a mass flow meter (accuracy ±1 % of full scale; Tylan General, FM 380, San Diego, CA, USA), and were directed to a paramagnetic oxygen analyzer (range 0–100 %, time delay <3 s; Klogor, Lannion, France) and an infrared carbon dioxide analyser (range 0–1 %, time delay <3 s; Gascard I Edinburgh Sensors Ltd, UK), after being dried through a Permapure® system and calcium chloride, both of which were changed twice daily. The system was calibrated daily with pure nitrogen to establish the zero level of the analyzers and with a standard gas mixture (CFPO) containing 20.5 % O_2 (accuracy 20.44–20.56 %), 0.5 % CO_2 (accuracy 0.495–0.505 %) and 79 % nitrogen to set up the sensitivity. The measuring system was found to be accurate to within ±1 % by bleeding known rates of CO_2 and N_2, as well as known rates of O_2 and CO_2. Analog signals from the analyzers and mass flow meter were digitized with an interface card and stored in a desktop computer. O_2 and CO_2 concentrations were measured continuously over approximately 23.5 h per day, 30 min being required to calibrate the system, clean the cages, change food and water and weigh the animals. We recently observed a perfect match between EE values derived from the indirect calorimeter used and the doubly labeled water method during both isolation and simulated microgravity in rats (Blanc et al., 2000a). Moreover, the system has been found reliable for studying different physiological and pathological conditions in rodents (Cimmino et al., 1996; Mion et al., 1996; Atgié et al., 1998).

Calculations

The respiratory quotient (RQ) was calculated as the $\dot{V}_{CO_2}$ to $\dot{V}_{O_2}$ ratio, and the EE was calculated from the Depocas and Hart formula (Depocas et al., 1957) and expressed in $J\,min^{-1}\,kg^{-0.67}$ to normalize for individual variations (Heusner, 1985).

The 24 h EE can be divided into basal metabolic rate (BMR), diet-induced thermogenesis (DIT) and the cost of physical activity called the activity energy expenditure (AEE) (Fig. 1). The sum of the DIT and AEE is commonly referred to as the

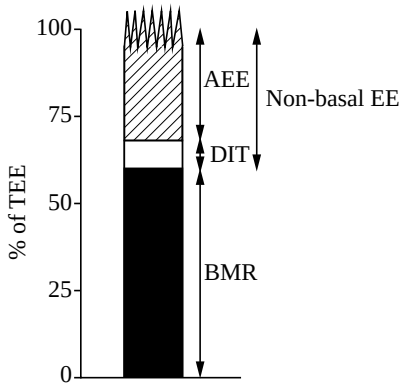

Fig. 1. Theoretical division of the total energy expenditure (TEE) into its principal components: basal metabolic rate (BMR), diet-induced thermogenesis (DIT) and the activity energy expenditure (AEE). The non-basal energy expenditure is commonly referred as the sum of DIT and AEE. Note, as represented, that AEE is the most variable part of the daily energy expenditure

non-basal EE. In rats it is not easy to separate these components; DIT cannot clearly be dissociated from AEE and BMR may include DIT.

In this study, the 24 h EE records were divided into energy expended during quiescent (day EE) and active (night EE) phases of the animals. In both these periods, for each measurement, the mean of the ten lowest values was calculated to determine the minimal observed metabolic rate (MOMR). The MOMR of the quiescent phase was considered an indirect estimate of the basal metabolic rate (Gordon, 1993). The differences between the EEs and the MOMR of the quiescent phase were computed as the non-basal EEs of each period.

Statistical analysis

The means of each period were calculated and the isolation was considered as the control period. The suspension period was divided into two periods of 5 days (Susp. 1 and Susp. 2 in the figures) to facilitate comparison with the other periods. A repeated-measures analysis of variance (RM–ANOVA) was used to detect any effects of the periods on the measured variables. A paired *t*-test was used to identify any differences between the day and night EEs, the day and night MOMRs, and the day and night non-basal EEs. The protected least-significant difference (PLSD) Fisher's test was used for *post-hoc* comparisons. Statistical analyses were performed with STATVIEW 5.01 (SAS Institute, CA, USA). All values are means ± S.E.M. and $P<0.05$ was considered statistically significant.

Results

Body weight

As shown in Fig. 2, rat body weight increased significantly during the five experimental periods. Interestingly, although suspension attenuated the rate of body-mass gain, 3 days of recovery restored it.

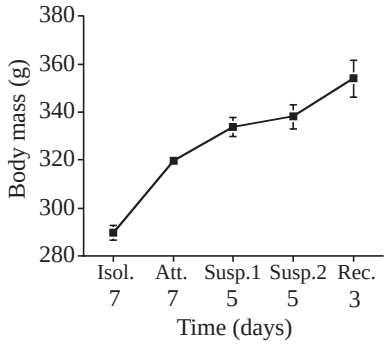

Fig. 2. Body mass evolution of rats during the four experimental periods: isolation (Isol.), attachment (Att.), suspension (Susp.) and recovery (Rec.) Values are means ± S.E.M. (N=12).

Daily and day/night energy expenditures

Fig. 3 represents the progression of the 24 h EE and the day and night EEs. Time over the five experimental periods had a significant effect on the 24-hour energy expenditure; however, PLSD Fisher's test showed that suspension did not modify the 24 h EE compared to isolation (358.7±18.3 *versus* 361.8±15.3 J min^{-1} $kg^{-0.67}$). In fact, the 24 h EE during recovery (394±11.5 J min^{-1} $kg^{-0.67}$) was the only one that was significantly different compared to the isolation period.

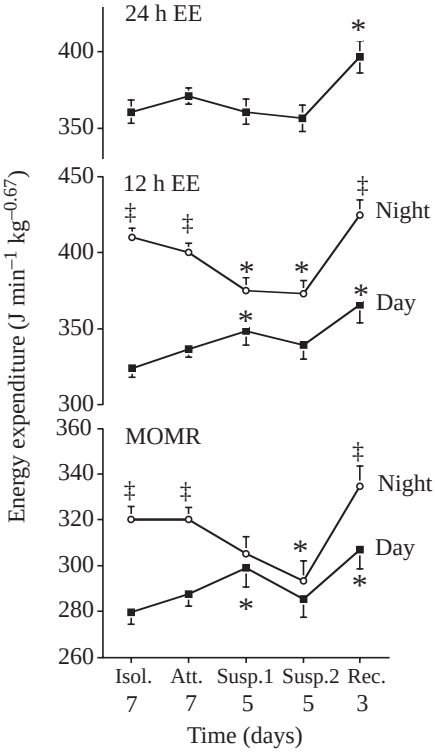

Fig. 3. Average 24 h EE and 12 h EE during day or night and MOMRs throughout the four experimental periods (N=12). Statistics are the results of the protected least-significance Fisher's test following a significant repeated-measures analysis of variance: *$P<0.05$ *versus* isolation; ‡$P<0.05$ *versus* day EE. Abbreviations as in Fig. 2.

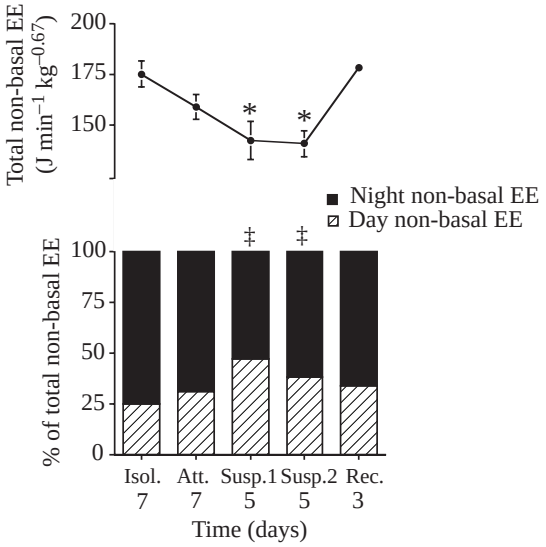

Fig. 4. Non-basal energy expenditure (EE) (diet-induced thermogenesis plus activity energy expenditure) during a nycthemere and during the day and night periods (N=12). Results of the protected least significance Fisher's test following a significant repeated measures analysis of variance: *P<0.05 *versus* isolation (top); ‡a significant (P<0.05) change in the day/night distribution of the non-basal EE (bottom). Abbreviations as in Fig. 2.

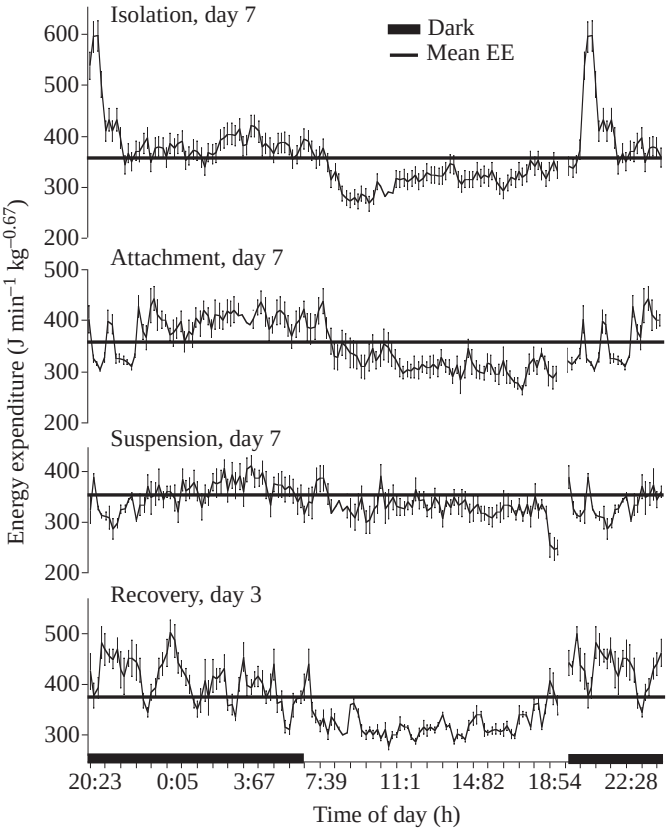

Fig. 5. Daily EE throughout a nycthemere on day 7 of isolation, attachment, suspension and on day 3 of recovery (N=12).

The EEs during the night and day periods were also affected by time. 2 weeks significantly reduced night EE of suspension compared to isolation (374±17.8 *versus* 410.7±13.5 J min^{-1} kg$^{-0.67}$). Conversely, day EE increased significantly during the first week of suspension (348.3±19.0 J min^{-1} kg$^{-0.67}$) and during recovery (363.7±8.9 J min^{-1} kg$^{-0.67}$), compared to isolation (325.6±15.1 J min^{-1} kg$^{-0.67}$). Interestingly, during the 2 weeks of suspension, the day and night EEs were not significantly different, unlike the situation during isolation and attachment. 3 days of recovery restored the difference between day and night EEs to isolation values.

MOMRs and index of BMR

Night MOMR was significantly reduced by the second week of suspension compared to the isolation period (293.9±14.7 *versus* 318.1±13.6 J min^{-1} kg$^{-0.67}$, respectively) (Fig. 3). Conversely, the day MOMR increased significantly only during the first week of suspension and during the recovery period when compared to the isolation period (299.0±18.0 and 305.1±8.9 *versus* 280.7±13.5 J min^{-1} kg$^{-0.67}$, respectively), suggesting increases in the BMR at these times. As in the case of the day and night EEs, the difference between day and night MOMRs was abolished during the two periods of suspension and restored during the short recovery period.

Non-basal EE

The non-basal energy expenditures are shown in Fig. 4. The 24-hour non-basal EE decreased significantly during the first and second weeks of suspension compared to the isolation period (142.8±9.4 and 141.0±6.5 J min^{-1} kg$^{-0.67}$ *versus* 175.3±6.1 J min^{-1} kg$^{-0.67}$, respectively). 3 days of recovery restored the isolation values. Day and night non-basal EEs expressed as a percentage of the total 24 h non-basal EE are also shown in Fig. 4. 2 weeks of simulated microgravity significantly reduced night non-basal EE compared to the isolation period (76.5±4.1 and 87.5±5.3 *versus* 131.5±4.1 J min^{-1} kg$^{-0.67}$, respectively). The opposite pattern was observed for the day non-basal EE, which increased significantly during the 22 weeks of suspension (66.7±8.1 and 53.5±5.4 *versus* 43.8±2.6 J min^{-1} kg$^{-0.67}$, respectively). Calculated as a percentage of 24 h non-basal EE, the day non-basal EE increased from 25 % during isolation to 39 % during suspension and the night non-basal EE dropped from 75 % to 61 %.

Throughout the experimental periods, the respiratory quotient did not change significantly from its value at isolation (0.94±0.02).

Time course of EE throughout a nycthemere

In support of the above results, four examples of EE recorded throughout a nycthemere, for each period, are shown in Fig. 5. The days shown in this figure were the last day of isolation and attachment, the equivalent day of suspension, i.e. suspension day 7, and the last day of the recovery period. This representation strongly corroborates the above results and

provides evidence that the nycthemeral variations of EEs are strongly reduced during simulated weightlessness in rats.

Discussion

Nutrition has never been considered a priority in space medicine research because the flights are relatively short. With the development of the ISS and its foreseen long-term missions, interest in nutrition research has resurfaced. It has long been known that energy intake is reduced in astronauts but the mechanisms for this are poorly understood. The only two available studies about EE levels in space show a strong negative energy balance that affects body composition (Lane et al., 1997; Stein et al., 1999a). This is of particular importance since energy homeostasis is a primary function of the body, and any alterations may have consequences for all bodily functions. An example of such an interaction is cardiovascular deconditioning (Blanc et al., 2000c). Moreover, while a negative energy balance may be acceptable for short-term missions because of the high energy density of fat stores, it is not permissible for long-term spaceflight. Therefore, a better understanding of the adaptations of energy requirements in space is a prerequisite to any planned long-term mission.

Total EE comprises principally basal metabolism, diet-induced thermogenesis and cost of physical activity. The latter is the most variable of these components (Saltzman et al., 1995). During space flight, the level of daily EE is unchanged compared to ground-based measurements (Lane et al., 1997). Despite this, body composition is strongly altered, lean body and fat masses are lost, and the subjects enter into severe negative energy balance (Lane et al., 1997; Stein et al., 1999a). Stress and hormones have been implicated (Stein et al., 1999b). By contrast, during simulation, humans demonstrate a positive energy balance and the total EE is decreased by 20 %, mainly due to a reduced AEE (Gretebeck et al., 1995; Blanc et al., 1998). This is fundamentally different to what is observed in space. Even if lean body mass is lost during bed rest and a transient metabolic stress is implied, as in space, the mechanisms cannot be similar since one occurs in a state of negative energy balance and the other occurs in a state of positive energy balance. In support of this, lipogenesis at the whole body level has been observed in humans during HDBR (Ritz et al., 1998; Blanc et al., 2000b). Given these theoretical considerations, one may consider the validity of the bed-rest model dubious, at least for nutrition-related problems. Validity is more dubious for the rat model since energy metabolism has not been measured during space flight or during simulation. Therefore, the validity of the rat hindlimb tail suspension model for simulating adaptation of the rat to space, as well as the validity of the rat model as a useful tool for studying human space deconditioning syndromes, are questions that remain unanswered. As classically observed in space, rat growth in the present study continued during suspension at a lower rate than during isolation, a finding which may, in terms of nutrition-related problems, answer the first question. Yet, this finding remains different from those abtained by human bed rest, where body weight drops (Ritz et al., 1998; Blanc et al., 1998). Our study gives new insights into the regulation of energy metabolism of rats during tail suspension; however, it clearly shows that any extrapolation to humans, during either actual or simulated weightlessness, needs to be treated with caution.

The main result of our study is that simulated weightlessness induces a strong reduction in the nycthemeral variations of EE without modifications of the mean value. More specifically, the difference between active and quiescent periods, i.e. night *versus* day, decreased by 64 % for EE and by 81 % for MOMR. For both variables, these changes are explained by an increase in the day and a decrease in the night components. Taken together, these findings show that simulated weightlessness alters the nycthemeral variation in EE, so that night and day EEs tend to be similar. This phenomenon is clearly visible in the 24 h representation. The MOMRs increased transiently by 10 % during the first week of suspension. This rise is interesting since we can consider it an index of the BMR. In humans, BMR has been shown to increase after short bed rest of 3 days (Acheson et al., 1995). After 7 or 42 days of simulations, differences in BMR were no longer noted, suggesting that the increase after 3 days is due to an acute adaptation phase (Ritz et al., 1998; Blanc et al., 2000b). In the present study the same modest transient increase is observed and may reflect initial perturbations in thermoregulation. First, the hindlimb tail-suspended rats cannot undergo thermotropic behaviour and, therefore, expose a large skin surface to temperature loss. Second, one key thermoeffector organ, the tail (Gordon, 1993), is non-functional due to the suspension system. Third, a hypothermia has been well described during suspension (Musacchia, 1992). Thomason and Booth (1990) reported an increase in corticosterone excretion during the first week of simulation. If such a phenomenon occurred in our experiment, we could speculate that the transient increase in BMR was partly attributable to a higher hypothalamo–pituitary–corticoadrenal system activity. The last confounding factor affecting the measurement of the BMR is the fact that BMR measurements in animals can be associated with dietary-induced thermogenesis (DIT). This is because food was provided *ad libitum* and we cannot be sure of the feeding state of the animals. While these observations show the need for further investigations, they strongly demonstrate that fuel homeostasis is altered during simulated weightlessness in rat.

Mekaouche et al. (1995) have studied the consequences of simulated weightlessness on the circadian rhythms of body temperature and motor activity in rats. They demonstrated that, despite an unchanged 24 h profile, there was a strong drop in the rhythms for both of these variables. These results underlie the implication of low physical activity in the energy adaptations to hindlimb tail suspension. As observed in humans, therefore, inactivity plays a key role in the body's response to simulated weightlessness; however, the mechanisms seem different because the total EE drops by 20 % in bed rest models (Gretebeck et al., 1995; Blanc et al., 1998), but according to our results, EE is unchanged during suspension. The differences between the 24 h or 12 h EE

recordings, together with the day MOMR, allowed us to determine the non-basal energy expenditure. Given our experimental design, this non-basal EE included changes in AEE as well as changes in DIT. However, the decreased body weight growth during suspension associated with the lack of changes in the 24-h EE implies that energy intake dropped during simulation, presumably because from a thermodynamic point of view, metabolized energy intake = 24 h EE + energy stored. Energy intake was not measured in this study because the first version of the cages, constructed to allow both suspension and gas-exchange measurements, did not allow for the waste of energy intake to be measured. These cages have now been modified to correct this weakness and a similar protocol effectively showed that rats decrease their energy intake while suspended (Blanc et al., 2000a). Thus, it is reasonable to argue that DIT was decreased during suspension. The drop in non-basal EE can therefore be seen as a cumulative decrease in both DIT and AEE. However, in mammals, DIT represents only 10 % of the 24 h EE and changes greater than ±5 % in a control environment are clearly unrealistic. Thus the 19 % lower non-basal EE induced by suspension may include DIT changes but is mainly driven by changes in AEE.

During the overall nycthemere, we observed a decrease in the AEE. More specifically, this reduction was due to a decrease in AEE during the night, the normal active period of rats. On the other hand, the day AEE increased. This pattern of adaptation has been reported by Mekaouche et al. (Mekaouche et al., 1995) at the whole body activity level through the telemetric system and also by Blewett and Elder (1993) through quantitative electro-myogram activity. Taken together, these results suggest that simulated weightlessness alters the rat activity patterns which, in turn, affect the nycthemeral variations of energy metabolism. The redistribution of activity from the night to the day period also suggests sleep perturbations that have been well-described in humans (Gundel et al., 1997). Consequently, several other physiological systems dependent on circadian drag should be perturbed. It is interesting to note that attachment to the suspension device, which is assumed to habituate the rat to the suspension device, induces (although not significantly) the observed changes. The study by Mekaouche et al. (1995) provided evidence for the role of attachment in the suspension device. It is therefore difficult to dissociate the effects of attachment from those of suspension *per se*. A previous report from our laboratory noted the importance of restraint in the suspension model (Bouzeghrane et al., 1996). Lastly, a stress response cannot be excluded.

As mentioned above, while these findings suggest that circadian rhythms are altered, more defined studies are warranted. This is of importance since circadian rhythms serve to coordinate the physiology and behaviour of an animal so that they are in synchrony with environmental needs. It is well known that there are daily, monthly and even annual rhythms that, without in-phase circadian rhythms, lead to chronic fatigue and impaired performance on the ground (Czeiler, 1995). There is some evidence that actual weightlessness affects circadian rhythms (Alpatov, 1994; Gundel et al., 1993; Hahn et al., 1971; Sulzman et al., 1992). This is potentially of serious concern because it could lead to avoidable 'human' errors. The monkey has proved to be a useful model for studying circadian rhythms. Fuller et al. (1996) studied the effects of a space flight environment on the circadian rhythms of male rhesus monkeys flown on the Russian Bion Missions. During flight, animals were maintained on a 24 h cycle. This enabled the animals to maintain normal heart-rate cycling and motor activity, though the actual heart rate was decreased, presumably because of the lower rate of energy expenditure. In contrast, several studies have now documented delays in the phasing of body temperature rhythms in monkeys and rats (Fuller, 1985; Fuller et al., 1996; Hahn et al., 1971). This discrepancy probably reflects the presence of more than one pacemaker in the body. Perturbed circadian rhythms are not unique to monkeys, since a free-running activity rhythm of beetles was decreased on flight Cosmos-1887 (Alpatov, 1994). A better understanding of the related changes in the circadian rhythms is important because, quite apart from performance and psychosocial effects, there may be other unrecognized effects of out-of-phase circadian rhythms.

In conclusion, we observed that simulated weightlessness did not change the mean EE but reduced the normal nycthemeral variations in rats. Despite an overall reduction in energy expended on physical activity, there was a redistribution of activity from the active period (night) to the quiescent one (day). While this study is the first to demonstrate how energy metabolism in rats adapts to simulated microgravity, there remain a number of questions regarding hindlimb suspension as a valid model of space flight for rats or humans, at least for nutrition-related problems. The absence of any in-flight measurements of energy expenditure is a weakness of this model. Therefore, additional experiments are needed under actual microgravity to ensure its validity. Hopefully, the development of the ISS will offer such opportunities.

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