Genetic Differences in Hypothalamic-Pituitary-Adrenal Axis Activity and Food Restriction-induced Hyperactivity in Three Inbred Strains of Rats

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Abstract

We used three inbred rat strains known for significant differences in the activity and reactivity of their hypothalamic-pituitary-adrenal (HPA) axis to stress [Fischer 344 (F344), Brown Norway (BN) and Lewis (Lew) rats] to search for a strain difference in the paradoxical increase in running activity induced by food restriction and to explore the role of the HPA axis in this behaviour. Rats were randomly assigned to either an ad lib sedentary group (AL), a control wheel activity group (ACT), a food restriction-induced hyperactivity group (FR-ACT) group (1.5 h/day ad lib food, 22.5 h/day ad lib wheel access) or a pair-fed group (FR). The BN and Lew rats reached the 25% body weight-loss criterion of FR-ACT (strain effect: $F_{2,132} = 45.58$, $P < 10^{-6}$) faster than the F344 strain due to higher food restriction-induced running activity (strain effect: $F_{2,65} = 17.43$, $P = 0.00001$). FR and FR-ACT decreased thymus weight (marker of integrated HPA axis activation) in all strains. In Lew and BN strains, FR-ACT induced a further decrement on thymus weight compared to their FR group. Prefeeding corticosterone levels (15.00 h) increased during the study in BN and Lew FR-ACT rats, but not in F344. Total wheel turns were correlated to both final adipose weight ($r = 0.49$, $P = 0.002$) and thymus weight decrement ($r = 0.59$, $P = 0.0001$), emphasizing the relationship between fat mass and HPA axis activation in excessive running activity. Increased running in conditions of food restriction and HPA axis activation may be linked at the level of the central nervous system. However, the involvement of corticotrophin-releasing hormone, agouti-related peptide or cocaine- and amphetamine-regulated transcript in behavioural disturbances of FR-ACT rats was excluded (in situ hybridization). We propose that corticosterone may be the link between initial low levels of fat mass and/or rate of fat mass loss (peripheral energy stores) and increased wheel activity, favouring fueling through lipolysis and proteolysis and reinforcing the self starvation via reward mechanisms, thus establishing a deleterious vicious cycle.

An elevated physical activity in anorexia nervosa is present in 31–80% of patients suffering from this severe psychiatric disorder. Excessive exercise has been viewed as a strategy among anorectic patients to lose weight. However, subjective reports of patients’ experiences of elevated physical activity levels sometimes reveals a compulsive component, indicating that exercise might not be under cognitive control of the patient (1). Although not representative of the complexity of human anorexia nervosa, an experimental animal model referred to as semistarvation-induced hyperactivity by some authors (2, 3) or activity-based anorexia by others (4, 5) is associated with two behaviours seen in most patients suffering from anorexia nervosa (i.e. low food intake and excessive physical activity). The principle of this animal paradigm is: if rats are exposed to running wheels with food freely available, limited activity normally occurs. When rats with access to a running wheel are restricted to a fixed amount of food, presented once per day, consistent running occurs, which becomes excessive and associated rapidly with dramatically reduced food intake. If this vicious circle is not disrupted by the investigator, rats starve and run themselves to death (4). These two cases of decoupling between food intake and energy expenditure are associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis (5, 6), but the interactions between the HPA axis and increased physical activity associated with food restriction remain unidentified (2).

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Recent insights on the importance of glucocorticoids in feeding and energy balance have emphasized the role of the HPA axis in energy homeostasis (7). Glucocorticoids also have complex effects in the central nervous system acting in brain to increase the salience of activities associated with food-seeking such as wheel running (8). Indeed, Challet et al. (9) demonstrated the involvement of corticosterone in the rise in locomotor activity characteristic of the end of the fasting period in Sprague-Dawley rats submitted to fasting and wheel activity; the increase in locomotor activity was suppressed in fasted adrenalectomised rats and restored by corticosterone replacement. In the brain, glucocorticoids may exert positive hedonic effects because elevated glucocorticoids levels enhance dopamine release in the nucleus accumbens (10) thereby increasing the behaviourally reinforcing properties of stress (8) or increasing the compulsive nature of some activities, as clearly demonstrated for drug-taking behaviours (11). The pleasurable component of increased physical activity in both human anorexia nervosa and animal models of semistarvation-induced hyperactivity/activity-based anorexia may reinforce this behaviour, and inhibit food intake via reward mechanisms (3, 12). From this perspective, we hypothesized that rats with the highest corticosterone response to food restriction should also run more when food restriction is associated with free access to a running wheel. To test this hypothesis, we chose to use three inbred rat strains known for significant differences in the reactivity of their HPA axis to stress, Fischer 344 (F344), Brown Norway (BN) and Lewis (Lew) rats, to determine if genetic differences in HPA axis activity and reactivity to stress influence the rate of developing a food restriction-induced hyperactivity. Compared to the hyperactivity and the hyper-reactivity of the HPA axis of the F344 rats, BN and Lewis rats display an hypoactivity and an hyporeactivity of their HPA axis (13–15). F344 and Lewis also differ in their serotoninergic activity (16) and in the levels of tyrosine hydroxylase in dopaminergic brain reward regions (17). Of interest, metabolic comparisons show that, compared to Sprague-Dawley rats, F344 rats have both insulin and leptin resistance (18). Finally, for a similar body weight, F344, BN and Lew strains display different levels of fat mass. In rats of the same age, but with different energy reserves due to dietary manipulations, the locomotor activity during prolonged fasting increases to reach a similar loss of energy reserves, essentially lipids and not a proportion of body mass loss (19). Overall, the present study addressed the following specific questions: (i) is there a strain difference in running activity induced by food restriction between F344, Lew and BN rats and, if this is the case, (ii) is the HPA axis and/or fat mass levels related to these interstrain differences in food restriction-induced hyperactivity? In an attempt to characterize the relative contribution of various levels of the HPA axis to the corticosterone responses to food restriction and wheel activity, we measured plasma corticosterone levels, thymus and adrenal weights that reflect the integrated activity of the HPA axis and the expression of corticotrophin-releasing hormone (CRH) and of two of the four major neuropeptides involved in the control of food intake and in the HPA axis activation: agouti-related peptide (AgRP) and cocaine- and amphetamine-regulated transcript (CART) (20, 21). We chose to focus on AgRP and CART because

neuropeptide Y (NPY)/AgRP and pro-opiomelanocortin (POMC)/CART neurones in the arcuate nucleus are first-order neurones in the hypothalamic response to the circulating adiposity signals insulin and leptin (22). Moreover, whereas the responses of NPY and POMC to food restriction or to exercise have been previously reported (23), the present study is the first to investigate the effect of both food restriction and physical activity on AgRP and CART mRNA.

Materials and methods

Experiments

Two separate studies were carried out. Experiments were conducted on male Lewis (Lew), Fischer 344 (F344) and Brown-Norway (BN) rats (IFFA CREDO, Les Oncins, France). F344 and Lewis are two genetically related strains because they originate from the Wistar strain whereas BN originate from wild Normals. Although anorexia nervosa mainly affects women and there is a sexually dimorphic sensitivity to food restriction-induced hyperactivity group (FR-ACT) in rats (male rats are more resistant to the development of FR-ACT) (24), we chose to study only male rats to discard any confusing effect of variations of oestrogen and progesterin secretion during the estrus cycle. The animals were 5-week-old upon arrival at our laboratory, and were housed by strain four per cage (with food and water ad lib) in an animal quarter at constant temperature (23–25 °C) and under a 12 : 12 h light/dark cycle (lights on 07.00 h). They were left undisturbed for 2 weeks before the beginning of the experiments. This study was conducted in conformity with the French recommendations on animal experimentation. The rats were given unrestricted access to water throughout the experiment and fed on standard laboratory chow. When their body weight reached 170–180 g (7–8 weeks old), rats were either killed to determine their initial fat mass (n = 6 rats/strain) or subjected to experiment 1 or 2.

Experiment 1

On day 1 of the experiment, after 14 days of habituation to the animal colony, animals were placed in individual cages and randomly assigned to either an ad lib sedentary group (AL), a control wheel activity group (ACT), an FR-ACT or a pair-fed group (FR) (n = 6 per experimental group for each strain) (Table 1). AL rats were housed in individual cages where they had permanent access to food and no wheel access. ACT group had 24-h access to the wheel as well as to food. The FR-ACT paradigm involved a daily wheel access for 22.5 h when food-deprived. For the remaining 1.5 h (15.00–16.30 h) each day, the animals were locked out of the wheels and given free access to food. The fourth group of pair-fed rats had no wheel access and was food restricted, with the rats being given an allowance that was adjusted individually each day such that each rat’s meal remained equal to that of a previously assigned FR-ACT rat.

The rats were kept in a laboratory that housed all cages, wheels and recording equipment. Access to this room was limited to feeding and weighting times to prevent interference with activity. Wheel turns were automatically monitored by computer and stored at 60-s intervals for each wheel. Throughout the experiment, rats were weighed at 15.00 h. When an animal in the FR-ACT group lost 25% of its free feeding weight, it was considered to have met the weight-loss criterion for FR-ACT. At this time, the animals (including matched AL, ACT and FR rats) were removed from their cage and killed by decapitation in an adjacent room (15.00–15.20 h) within 30 s of removal from their home cage. Immediately after decapitation (15.00–15.20 h), trunk blood (4 ml) was collected in 10-ml polyethylene tubes containing 0.1 ml of 10% EDTA, and stored on ice until centrifugation. Plasma samples were stored at −80 °C until analysis. Adrenal glands were excised, trimmed of fat and weighed. The thymus gland was also removed and weighed as an indicator of peripheral glucocorticoid status. White adipose tissue [retroperitoneal, epididymal and subcutaneous (inguinal)] was dissected and weighed.

Experiment 2

The development of the response to food restriction is characterized by the appariation of a preprandial peak of corticosterone followed by an inhibition
Transcortin, [3H]corticosterone as the tracer and dextran-coated charcoal to protein binding assay (16) using rhesus monkey serum as the source of corticosterone measurements each strain). At 16.30 h, another blood sample was taken from each rat. Tail ¼ accomplished within 2 min of touching the cage to ensure low basal levels of effects on the day of sampling. Many of the procedures were similar to section of a meal. Before introduction of the feeding schedule, every day for the unique daily meal (25, 26). The objective of this second experiment was to determine if the marked differences in wheel activity between BN, Lew and F344 rats were due to intrinsic differences in plasma corticosterone anticipation of a meal. Before introduction of the feeding schedule, every day for the preceding 7 days, each rat was handled gently to minimize handling-stress effects on the day of sampling. Many of the procedures were similar to experiment 1; thus only differences in the methods are reported. Blood samples were taken from every animal each day between 15.00 h and 15.30 h. Rats were quickly removed from their home cage, gently wrapped in a towel in an adjacent room, and lightly restrained with this towel. The tail was exposed, a small nick was made with a scalpel (no. 15 blade) and blood (30 l) was collected in 1.5-ml polyethylene tubes containing 4 l of HPA axis activity by consummatory behaviour (25). To finely characterize the corticosterone responses to food restriction and to wheel activity, we conducted a second experiment to determine the day-to-day variation of corticosterone levels at a moment where large short-term variations of corticosterone are evidenced in food-restricted rats: before and after the unique daily meal (25, 26). The objective of this second experiment was to determine if the marked differences in wheel activity between BN, Lew and F344 rats were due to intrinsic differences in plasma corticosterone anticipation of a meal. Before introduction of the feeding schedule, every day for the preceding 7 days, each rat was handled gently to minimize handling-stress effects on the day of sampling. Many of the procedures were similar to experiment 1; thus only differences in the methods are reported. Blood samples were taken from every animal each day between 15.00 h and 15.30 h. Rats were quickly removed from their home cage, gently wrapped in a towel in an adjacent room, and lightly restrained with this towel. The tail was exposed, a small nick was made with a scalpel (no. 15 blade) and blood (30 l) was collected in 1.5-ml polyethylene tubes containing 4 l of 10% NaCl, 100 mM Tris-HCl containing 1 mM EDTA), washed in descending concentrations of SSC (2 l× 10 min; 1 l× 5 min; 0.1 l× 30 min at 60 °C) and dehydrated through graded concentrations of alcohol. After a 2-h period of vacuum drying, the slides were exposed on a X-ray film (Eastman Kodak, Rochester, NY, USA) for periods varying between 24 h and 72 h, depending upon the probes used. Once removed from the autoradiography cassettes, the slides were exposed from 7–21 days, before being developed in D19 developer (Eastman Kodak) for 5 min. Finally, tissues were rinsed in running distilled water for 1 or 2 h, counterstained with thionin (0.25%), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

**Table 1. Experimental Design.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Food access</th>
<th>Wheel access</th>
<th>Name of the experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis (Lew)</td>
<td>Ad lib</td>
<td>NO</td>
<td>Lewis ad libitum (Lew AL)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>NO</td>
<td>Lewis food-restricted (Lew FR)</td>
</tr>
<tr>
<td></td>
<td>Ad lib</td>
<td>22.5 h/day</td>
<td>Lewis control wheel activity (Lew ACT)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>22.5 h/day</td>
<td>Lewis food restricted and wheel activity (Lew FR-ACT)</td>
</tr>
<tr>
<td>Brown-Norway (BN)</td>
<td>Ad lib</td>
<td>NO</td>
<td>BN ad libitum (BN AL)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>NO</td>
<td>BN food-restricted (BN FR)</td>
</tr>
<tr>
<td></td>
<td>Ad lib</td>
<td>22.5 h/day</td>
<td>BN control wheel activity (BN ACT)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>22.5 h/day</td>
<td>BN food restricted and wheel activity (BN FR-ACT)</td>
</tr>
<tr>
<td>Fischer 344 (F344)</td>
<td>Ad lib</td>
<td>NO</td>
<td>F344 ad libitum (F344 AL)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>NO</td>
<td>F344 food-restricted (F344 FR)</td>
</tr>
<tr>
<td></td>
<td>Ad lib</td>
<td>22.5 h/day</td>
<td>F344 control wheel activity (F344 ACT)</td>
</tr>
<tr>
<td></td>
<td>Restricted to 15.00–16.30 h</td>
<td>22.5 h/day</td>
<td>F344 food restricted and wheel activity (F344 FR-ACT)</td>
</tr>
</tbody>
</table>

Abbreviations used in the text are provided in parenthesis.

Plasma corticosterone was measured after alcohol extraction by a competitive protein binding assay (16) using rhesus monkey serum as the source of transcortin, [3H]corticosterone as the tracer and dextran-coated charcoal to absorb the unbound fraction (sensitivity 4 ng/ml, specificity > 95%, interassay coefficient of variation 8.0%). Samples were analysed in duplicate.

**Brain preparation**

After decapitation, the brains of rats of experiment 1 were immediately removed and kept in paraformaldehyde for a period of at least 4 weeks. They were then transferred to a solution containing paraformaldehyde and sucrose (10%) before being cut 24 h later using a sliding microtome (Histoslide 2000; Reichert-Jung, Heidelberg, Germany). Brain sections were taken from the olfactory bulb to the brain stem. Thirty-micrometre thick sections were collected and stored at −30 °C in a cold sterile cryoprotecting solution containing sodium phosphate buffer (50 mM), ethylene glycol (30%) and glycerol (20%).

**In situ hybridization histochemistry**

**In situ** hybridization histochemistry was used to localize CRH, AgRP and CART mRNAs on tissue sections taken from the entire brain. The protocol used was largely adapted from the technique described by Simmons et al. (27). Briefly, one out of every five brain sections was mounted onto poly-L-lysine-coated slides and allowed to desiccate overnight under vacuum. The sections were then successively fixed for 20 min in paraformaldehyde (4%), digested for 30 min at 37 °C with proteinase K (10 μg/ml in 100 mM Tris-HCl containing 50 mM EDTA, pH 8.0), acetylated with acetic anhydride (0.25% in 0.1 μ triethanolamine, pH 8.0) and dehydrated through graded concentrations (50, 70, 95 and 100%) of alcohol. After vacuum drying for at least 2 h, the hybridization mixture (9 μl), which contains an antisense 35S-labelled cRNA probe (107 c.p.m.), was spotted on each slide. The slides were sealed under a coverslip and incubated overnight at 60 °C in a slide warmer. The next day, the coverslips were removed and the slides rinsed four times with 4 l× SSC (0.6× NaCl, 60 mM sodium citrate buffer, pH 7.0), digested for 30 min at 37 °C with RNaseA (20 μg/ml in 10 mM Tris–500 mM NaCl containing 1 mM EDTA), washed in descending concentrations of SSC (2 l× 10 min; 1 l× 5 min; 0.1 l× 30 min at 60 °C) and dehydrated through graded concentrations of alcohol. After a 2-h period of vacuum drying, the slides were exposed on a X-ray film (Eastman Kodak, Rochester, NY, USA) for periods varying between 24 h and 72 h, depending upon the probes used. Once removed from the autoradiography cassettes, the slides were exposed from 7–21 days, before being developed in D19 developer (Eastman Kodak) for 3.5 min at 14–15 °C and fixed in rapid fixer (Eastman Kodak) for 5 min. Finally, tissues were rinsed in running distilled water for 1 or 2 h, counterstained with thionin (0.25%), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

**Antisense 35S-labelled cRNA probes**

The CRH cRNA probe was generated from the 1.2-kb EcoRI fragment of a rat CRH cDNA (Dr K. Mayo, North-western University, Evanston, IL, USA) subcloned into a pgEM4 vector (Stratagene, La Jolla, CA, USA) and linearized with HindIII and EcoRI (Pharmacia Biotech Canada Inc., Baie-D’Urfé, Canada) for antisense and sense probes, respectively. The AgRP cRNA probe was generated from the 600-pb EcoRI fragment of a rat AgRP cDNA (Melissa Graham, Amgen Inc., Thousand Oaks, CA, USA) subcloned into a pGEM-T vector (Stratagene, La Jolla, CA, USA), linearized with HindIII and EcoRI (Pharmacia Biotech Canada Inc.) for antisense and sense probes, respectively. The CART cRNA probe was generated from the 222-pb AatII/NsiI fragment of a rat CART cDNA subcloned into a pGEM-T vector (Stratagene) and linearized with AatII and Sp6 and NsiI T7 for antisense and sense probes, respectively.

Radioactive riboprobes were synthesized with sense probes. The specificity of each probe was confirmed by the absence of a positive signal in sections hybridized with sense probes. Radioactive riboprobes were synthesized by incubation of 250 ng linearized plasmid in 10 mM NaCl, 10 mM dithiothreitol, 6 mM MgCl2, 40 mM Tris (pH 7.9), 0.2 mM ATP/GTP/CTP, α-35S-UTP, 40 U RNAsin (Promega, Madison, WI, USA) and 20 U SP 6 RNA polymerase for CRH, AgRP and CART antisense probes, for 60 min at 37 °C. The DNA templates were treated with 100 μl of DNase solution (1 μl DNase, 5 μl of 5 mg/ml rRNA, 94 μl of 10 mM Tris/10 mM MgCl2). Preparation of the riboprobes was completed through phenol–chloroform extraction and ammonium acetate precipitation.
Semi-quantitative Analysis of the hybridization signals

The hybridization signals revealed on NTB2 dipped nuclear emulsion slides were analysed and quantified under a light microscope (BX50; Olympus Corp., Tokyo, Japan) equipped with a black and white video camera coupled to a computer running Image software (version 1.55 non-FPU; Wayne Rasband, NIH, Bethesda, MD, USA). The optical density (OD) of the mRNA hybridization signal was determined from coronal brain sections under darkfield illumination at a magnification of ×25. The brain sections of the various rats were matched for rostrocaudal level as closely as possible. The OD determination was performed on each side (right and left) of the brain and the two OD values obtained were averaged. This average was included in the statistical analyses as the individual score of a rat. When no hybridization signal was visible under darkfield illumination, the brain structures of interest were outlined under brightfield illumination and then subjected to densitometric analysis under darkfield illumination. The OD for each specific region was corrected for the average background signal, which was determined by sampling unlabelled areas outside of the areas of interest.

Statistical analysis

Data were analysed initially independently for experiments 1 and 2. Because tail samplings have no significant effects on the parameters measured, results of experiments 1 and 2 were combined. In experiments 1 and 2, a 3 × 4 ANOVA was used to detect main and interaction effects of ‘strain’ (Lew, BN, F344) and ‘experimental group’ (AL, ACT, FR, FRACT), on the various dependent variables measured. Moreover, the effect of the delay before sacrifice (‘day’) within groups on body weight, food intake, wheel activity (experiments 1 and 2) and corticosterone (experiment 2) was assessed using ANOVA with repeated measures. In the case of a significant main effect or interaction, a posteriori comparisons were performed using Tukey’s HSD test. Relationships between total wheel activity, average wheel activity (total activity/number of days in the experiment) and corticosterone, relative thymus, adrenals and fat weights were quantified using the Pearson’s product-moment correlation coefficient or by multiple regression analysis. P < 0.05 was considered statistically significant.

Results

Body weight, fat mass, food intake and wheel activity

All rats began the experiments at the same weight. Cumulative weight variations for the 12 groups of rats during the experiment are shown in Fig. 1. Two-way ANOVAS (strain × group) with repeated measures showed strain (F2,132 = 4.77, P = 0.01), group (F3,132 = 393.11, P < 10⁻⁶) and day (F5,660 = 8.58, P < 10⁻⁶) effects with significant interactions between these parameters. Figure 1 shows that voluntary access to wheel activity resulted in a decrease in body weight gain (ACT versus AL groups, P = 0.03), except in the BN strain. Furthermore, food restriction alone (FR groups) or in combination with wheel running (FR-ACT) resulted in significant reduction of ponderal growth. The corresponding analysis on the day of the sacrifice (final weight or Δ weight) yielded a higher weight loss in FR-ACT versus FR groups (P = 0.001), whereas there was no difference in the total amount of weight loss between the three strains of rats (Table 2).

Although all rats began the experiment at the same weight, their initial total adipose tissue weight differed: F344 1.052 ± 0.05 mg/g of body weight, Lew: 0.872 ± 0.03 mg/g, BN: 0.699 ± 0.02 mg/g (P < 0.05 between each strain). To determine the respective effect of food restriction and wheel activity in fat depot, two-way ANOVAS (strain × group) were conducted across all groups. Strain (F2,132 = 20.72, P < 10⁻⁶), group (F3,132 = 220.97, P < 10⁻⁶) and group × strain (F6,132 = 6.65, P < 10⁻５) effects were revealed. Figure 2 shows that exercise alone (ACT) and food restriction alone (FR) significantly decreased final total adipose tissue weight (except in the BN ACT group). The combination of these two factors further decreased final adipose weight. No interstrain difference in final adipose tissue weight was depicted within the FR or within the FR-ACT groups. Separate analysis on each fat pad (subcutaneous, epididymal and retroperitoneal) yield similar results than the sum of the three adipose fat pads (total adipose tissue weight), except for retroperitoneal adipose tissue weight: in AL groups, the weight of this fat pad was similar to the total adipose weight in AL F344 rats compared to Lew is due to higher subcutaneous and inguinal fat depots. On the other hand, BN AL rats have less fat mass than F344 and Lew rats whatever the fat pad considered. Of interest, in food-restricted rats (FR and FR-ACT), the retroperitoneal adipose tissue was totally mobilized as its weight was null in the three strains. Although no interstrain difference in final adipose tissue weight was depicted in the FR and in the FR-ACT groups, because F344 AL rats have a significantly higher initial fat
The differences in BN and Lew rats compared to F344 rats to reach FR-ACT criterion were associated with differences in wheel activity (Fig. 3). Three-way ANOVAs with repeated measures revealed an effect of strain (F(2,65) = 17.43, \( P = 0.00001 \)) and day (F(5,325) = 47.45, \( P < 10^{-6} \)) with significant interactions between all these variables. Post-hoc tests showed that, in control conditions (ACT groups), the activity was low and similar across the three strains of rats. Exposure to food restriction progressively increased 24-h total running in all strains. As indicated by the total 24-h wheel turns for days 2–7 (Fig. 3a), a significant difference was found between the three strains in the rate of increase of running: FR-ACT BN group ran more than their ACT counterpart from day 3, Lew from day 4 and F344 from day 5. Moreover, in FR-ACT groups, an interstrain difference was also found, with the FR-ACT BN group running more than the F344 group from day 3 and more than the Lewis group on days 3 and 4 (but not after); from day 5, Lew rats ran significantly more than F344 rats. This order remained the same when more a detailed examination of wheel turns was carried out. This involved dividing the total 22.5 h of running into four time periods: the first 2.5 h following feeding (postprandial period), the next 12 h (night period); the first 5 h of the light period (early light period) and the 3 h immediately before the next feed (prefeeding period). The largest differences between the groups (FR-ACT versus ACT) emerged in the prefeeding
period corresponding to the food anticipatory period of 3 h before feeding (Fig. 3 B). Terminal wheel revolutions were also evaluated as a measure of activity levels on the day that the animals met the 25% weight-loss criterion for FR-ACT. There was no difference in 24-h activity levels for this measure between BN and Lewis rats, but F344 rats ran significantly less than the two other strains (F344 versus Lew: $P = 0.003$, F344 versus BN: $P = 0.04$). By contrast, on this last day, there was no difference in prefeeding activity levels between the three strains of rats.

Thymus and adrenals weight (Fig. 4)

To test for differences in adrenal and thymus weights as markers of integrated HPA axis activation during the whole experiment, two-way ANOVAs (strain × group) were conducted on adrenal and thymus weights across all groups. This analysis showed an effect of strain ($F_{2,132} = 26.98$, $P < 10^{-6}$) and group ($F_{3,132} = 24.18$, $P < 10^{-6}$) without interaction. Post-hoc tests showed an increase in adrenal weight with food-restriction but not with wheel activity (AL versus ACT: $P > 0.05$; AL versus FR: $P = 0.00002$; ACT versus FR: $P = 0.003$) and a further increase when wheel activity was added to food restriction (FR versus FR-ACT: $P = 0.01$). However, when each strain was considered separately, the statistical threshold for significance between adrenal weight in FR and FR-ACT rats was not reached (Fig. 4 A). With respect to thymus weight, a similar profile was observed (strain effect: $F_{2,132} = 56.07$, $P < 10^{-6}$; group effect: $F_{3,132} = 68.33$, $P < 10^{-6}$). In AL rats, as previously reported (14), F344 have a significantly lower relative thymus weight compared to the two other strains (Fig. 4B). Food restriction (FR) and FR-ACT decreased thymus weight in all strains of rats. In Lew and BN strains, FR-ACT induced a further decrement on thymus weight compared to their counterparts in the FR group.

Corticosterone

There was no overall effect of strain on corticosterone levels measured on the day of sacrifice (15.00 h) (Table 2). By contrast, there was a strong effect of experimental conditions ($F_{3,112} = 40.69$, $P < 10^{-6}$), with an overall stimulatory effect
of FR and FR-ACT conditions on plasma corticosterone compared to AL and ACT conditions. When FR and FR-ACT rats were compared, a synergistic effect of food restriction and activity on corticosterone was observed in the Lew strain (day of sacrifice: FR versus FR-ACT: \( P = 0.01 \)) but not in the two other strains.

**CRH, AgRP and CART mRNA in the brain (Fig. 5)**

The expression of CRH mRNA in the PVN did not vary significantly in relation to either experimental group or strain. The expression of AgRP mRNA in the ARC was significantly affected by experimental group \( (F_{3,60} = 11.17, \ P < 10^{-3}) \) and strain \( (F_{2,60} = 16.63, \ P < 10^{-5}) \). Food deprivation associated with free access to wheel activity (FR-ACT) increased the expression of AgRP mRNA in the ARC regardless of the strain considered. Under all experimental treatments, the expression of AgRP mRNA was significantly higher in the ARC of BN rats compared to F344 \( (P < 10^{-3}) \) and Lew \( (P < 10^{-4}) \) rats. The expression of CART mRNA in the ARC was significantly affected by experimental group \( (F_{3,60} = 7.88, \ P = 0.0005) \) and strain \( (F_{2,60} = 3.67, \ P = 0.03) \). Food deprivation associated or not with free access to wheel activity (FR-ACT and FR, respectively) decreased the expression of CART mRNA in the ARC regardless of the strain. By contrast, no significant difference was observed between FR and FR-ACT groups. Under all experimental treatments (with the exception of FR), the expression of CART mRNA was significantly lower in the ARC of BN rats compared to F344 \( (P = 0.02) \) and Lew \( (P = 0.02) \) rats.

**Day-to-day pre and postfeeding corticosterone levels**

BN and Lew rats differed from F344 in their rate to reach FR-ACT criterion (25% body weight loss): this was faster in BN and Lew due to higher wheel activity when food restricted. Collection at only one time point (time of sacrifice) of either plasma for determination of corticosterone levels or brain for the expression of CRH mRNA did not enlighten on the role of the HPA axis in such strain differences. By contrast, markers of integrated corticosterone secretion during the previous days such as thymus weight suggest a greater activation of the HPA axis in BN and Lew strains compared to F344 rats, with an additional decrease of thymus weight when wheel activity was added to food restriction (FR-ACT groups) in BN and Lew strains compared to F344 rats, with an additional decrease of thymus weight when wheel activity was added to food restriction (FR-ACT groups) in BN and Lew strains, whereas FR-ACT failed to further decrease thymus weight in F344 rats. Therefore, to finely characterize the corticosterone responses to food restriction and to wheel activity, we conducted a second experiment to determine the day-to-day variation of corticosterone levels at a moment where large short-term variations of corticosterone are evidenced in food-restricted rats: before and after the unique daily meal (25, 26).

On the first day of the experiment (day 1), two-way ANOVAs revealed an overall effect of strain \( (F_{2,120} = 20.80, \ P < 10^{-3}) \) and time \( (15.00 \text{ h versus } 16.30 \text{ h}; F_{1,120} = 56.75, \ P = 0.0005) \) and strain \( (F_{2,120} = 3.67, \ P = 0.03) \). Food deprivation associated or not with free access to wheel activity (FR-ACT and FR, respectively) decreased the expression of CART mRNA in the ARC regardless of the strain. By contrast, no significant difference was observed between FR and FR-ACT groups. Under all experimental treatments (with the exception of FR), the expression of CART mRNA was significantly lower in the ARC of BN rats compared to F344 \( (P = 0.02) \) and Lew \( (P = 0.02) \) rats.

**Fig. 6.** Means ± SEM plasma corticosterone concentrations in pair-fed group (FR) and ad lib sedentary group (AL) (left) and food restriction-induced hyperactivity group (FR-ACT) and control wheel activity group (ACT) (right) groups in Lewis (Lew), Brown Norway (BN) and F344 rats during each day of the protocol before food intake (15.00 h) and at the end of the 90-min daily access to food (16.30 h). The abscissa corresponds to the day of the experiment. Note that one BN FR-ACT (and one AL, ACT and FR) rat was sacrificed on day 6 and five on day 7, five Lew on day 7 and one on day 8 and four F344 on day 8 and one on day 9.

LEW

BN

F344

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Table 3. Summary of the Main Results.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Initial adipose tissue weight</th>
<th>Final adipose tissue [mean value] weight</th>
<th>Number of days necessary to reach the 25% weight loss criterion</th>
<th>Food intake/day</th>
<th>Total wheel activity</th>
<th>Thymus weight</th>
<th>Prefeeding cortisol levels (15.00 h): day of the sacrifice [Day effect during all the experiment]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>N</td>
<td>N</td>
<td>Depends on</td>
<td>N</td>
<td>N vs. S</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>ACT</td>
<td>N  ( \downarrow ) ( \downarrow )</td>
<td>the FR-ACT group</td>
<td>( \downarrow )</td>
<td>( \uparrow )</td>
<td>( \downarrow )</td>
<td>( \uparrow )</td>
<td>( \uparrow ) ( \uparrow ) [Yes]</td>
</tr>
<tr>
<td>FR</td>
<td>N</td>
<td>( \downarrow ) ( \downarrow ) ( \downarrow )</td>
<td>( \uparrow )</td>
<td>( \downarrow )</td>
<td>( \downarrow )</td>
<td>( \uparrow )</td>
<td>( \uparrow ) ( \downarrow ) ( \downarrow ) [Yes]</td>
</tr>
<tr>
<td>FR-ACT</td>
<td>NS ( \uparrow ) ( \mu )</td>
<td>( \downarrow ) ( \downarrow ) ( \downarrow )</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) [8.7 days]</td>
<td>( \downarrow )</td>
<td>( \uparrow ) ( \downarrow )</td>
<td>( \uparrow ) ( \uparrow )</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow ) ( \downarrow ) [Yes]</td>
</tr>
<tr>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) [6.7 days]</td>
<td>( \downarrow )</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow ) ( \uparrow )</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow )</td>
<td>( \uparrow ) ( \uparrow ) ( \uparrow ) ( \downarrow ) [Yes]</td>
</tr>
</tbody>
</table>

All rats start the experiment at the same weight but with different levels of adipose tissue (‘Initial adipose tissue weight’). When rats of the food restriction-induced hyperactivity group (FR-ACT group) lost 25% of their initial weight, all rats [including matched ad lib sedentary group (AL), control wheel activity group (ACT) and pair-fed group (FR) rats] were killed. For clarity, values of the AL group are considered as normal and labelled N. [Day effect during all the experiment]: prefeeding cortisol levels increased during the course of the study (FR and FR-ACT groups). NS, Non-significant between each group (column experimental group) or strain (column strain effect); S: P < 0.05 between each group (column labelled N. [Day effect during all the experiment]: prefeeding cortisol levels increased during the course of the study (FR and FR-ACT groups). NS, Non-significant between each group (column experimental group) or strain (column strain effect); S: P < 0.05 between each group (column experimental group) or strain (column strain effect); μ: except for Brown Norway (BN) (BN ACT versus BN AL: P > 0.05); *except for F344 (FR = FR-ACT).

P < 10⁻⁶) on plasma corticosterone with a strain × time interaction (F_{2,120} = 5.25, P = 0.006). Post-hoc analysis revealed that, as previously reported (14), at 15.00 h (i.e. in unstriressed conditions), F344 rats have a significantly greater plasma corticosterone level than BN and Lew strains (P = 0.0001 for both) (Fig. 6). After this basal sampling, all rats were randomly assigned to their experimental group, meaning that, during this period from 15.00 h to 16.30 h, the rats were housed in individual cages in the same colony room with free access to food but not to wheel for FR-ACT groups. At 16.30 h, all strains significantly increased their plasma corticosterone (time effect: P < 10⁻⁶) but the differences between the strains in the corticosterone response to novel environment remained in the same order (F344 versus BN: P = 0.001; F344 versus Lew: P = 0.0001; Lew versus BN: P > 0.05) (Fig. 6).

When each strain of rat was analysed separately with repeated measures (this strain by strain analysis was necessary due to the different duration of the experimental protocol according to the strain considered), BN and Lew strains differed again from F344 on the following points: (i) an experimental group effect was seen with FR and FR-ACT > AL and ACT groups (BN: FR-ACT versus ACT: P = 0.0004, FR versus AL: P = 0.001; Lew: FR-ACT versus ACT: P = 0.0005, FR versus AL: P = 0.002) and (ii) a day effect was noticeable (BN: F_{6,240} = 2.84, P = 0.01; Lew: F_{7,280} = 2.71, P = 0.01) with a significant day × food intake × experimental group interaction (BN: F_{18,240} = 2.67, P = 0.002; Lew: F_{21,280} = 2.46, P = 0.002) suggesting that, in FR and FR-ACT rats, prefeeding corticosterone levels (15.00 h) increased during the course of the study (Fig. 6A-B). None of these effects was seen in F344 rats (Fig. 6C). By contrast, in the three strains, an effect of feeding (15.00 h versus 16.30 h samplings) was observed, with corticosterone being significantly decreased after feeding in FR and FR-ACT groups. Indeed, in these experimental groups, food intake normalized plasma corticosterone to the values observed in ACT and AL rats, in all strains (Fig. 6).

**Relationships among wheel running, thymus and adipose tissue weights**

The initial difference in fat mass between the three strains of rats, not maintained at the end of the experiments in food-restricted rats, and their different rates to reach the 25% body weight loss criterion led us to investigate the relationship between final fat mass and wheel activity. When FR-ACT rats of experiments 1 and 2 were pooled, a significant correlation was found between total wheel activity and final adipose tissue weight (r = −0.49, P = 0.002), with these correlations being stronger with the subcutaneous adipose tissue weight (r = −0.57, P = 0.0002) than with the epididymal adipose tissue weight (r = −0.42, P = 0.01). However, it should be noted that no correlation was calculated with retroperitoneal adipose tissue because its weight was null at the end of the experiment, suggesting that this fat depot was the first mobilized in response to energy deficit. A strain by strain analysis showed some strain differences: whereas a significant correlation between total wheel activity and final total adipose tissue weight was depicted only in BN rats (r = −0.73, P = 0.009), a significant correlation remained in each strain between total wheel activity and subcutaneous adipose tissue weight: BN (r = −0.72, P = 0.01), Lew (r = −0.60, P = 0.03), F344 (r = −0.60, P = 0.03). Total wheel activity was also correlated with Δ thymus weight (thymus weight of AL groups minus thymus weight of
FR-ACT groups) used as a marker of the intensity of HPA axis activation (all strains confounded: \( r = 0.59, P = 0.0001 \); analysis strain by strain: BN: \( r = 0.72, P = 0.01 \); Lew: \( r = 0.55, P = 0.03 \); F344: \( r = 0.77, P = 0.002 \)). In addition, \( \Delta \) thymus weight was also negatively correlated with final adipose tissue weight in all strains confounded (\( r = -0.60, P = 0.00009 \)) but not in an analysis strain by strain where this correlation was only found in Lew rats (\( r = -0.75, P = 0.003 \)). Multiple regression analysis with total wheel activity as dependent variable indicated that this parameter was related to \( \Delta \) thymus weight independently of final adipose tissue weight (all strains confounded: \( r = 0.43, P = 0.009 \); analysis strain by strain: BN: \( r = 0.65, P = 0.01 \); Lew: \( r = 0.63, P = 0.01 \); F344: \( r = 0.55, P = 0.04 \)).

The main results of this study are summarized in Table 3.

Discussion

In the present study, we used three inbred rat strains known for significant differences in the activity and reactivity of their HPA axis to stress to search for a strain difference in running activity induced by food restriction and to explore its potential link with the HPA axis. BN and Lew FR-ACT rats, the leanest strains, were markedly more prompt to lose 25% of their initial body weight than F344 rats. These differences in the rate of weight loss were not related to differences in the amount of food intake but to higher wheel activity in BN and Lew rats as in ACT, no interstrain difference in wheel activity was depicted for differences induced by food restriction. Therefore, the question arises as to why a metabolic stress such as food restriction induces different levels of wheel hyperactivity in BN and Lew compared to F344 rats?

Different elements link the activation of the HPA axis and excessive running in the present study. In the three strains of rats, a prefeeding corticosterone peak developed under restricted feeding (FR and FR-ACT groups) followed, after food intake, by a dramatic decrease in plasma corticosterone such that corticosterone was normalized to control values observed at the same time in both AL and ACT groups. This is in agreement with the metabolic roles of corticosterone (28, 29). In the present study, at the beginning of the experiments, before animals have lost too much weight (day 2), corticosterone increases after the meal, helping insulin to store energy (28, 29), whereas after, the rats lost weight, corticosterone decreases after the meal, probably to prevent further protein utilization (9, 26). In parallel with the prefeeding corticosterone peak, wheel activity was increased in the period preceding the anticipated meal (30; present study) whereas, in the postprandial period, this activity was totally suppressed. The comparison of the three strains of rats emphasizes the cooccurrence of higher HPA axis activation and higher wheel running in food-restricted rats. Indeed, the amount of anticipatory wheel running and the prefeeding corticosterone levels increased from day to day in the BN and Lew strains whereas, in the F344 strain, no effect of day was observed on the prefeeding corticosterone level coinciding with the absence of the prefeeding increase in wheel activity. Moreover, when thymus weight of food-restricted sedentary rats (FR) and food-restricted running rats (FR-ACT) were compared on the day of sacrifice, a synergistic effect of food restriction and running activity on thymus weight was observed in the BN and Lew strains (thymus weight: FR-ACT < FR) but not in F344 rats. Because thymus is a glucocorticoid target tissue and thymus weight is sensitive to integrated corticosterone secretion in previous days (28), this suggests that the activation of the HPA axis is not exclusively linked to food restriction in FR-ACT BN and Lew rats. Finally, total wheel activity is correlated with \( \Delta \) thymus weight, an index of the intensity of the HPA axis activation and corticosterone secretion. Thus, our results, although only associative in nature rather than truly mechanistic, support the hypothesis of a link between higher HPA axis activation and higher running associated with food restriction.

An important issue that remains to be addressed is the direction of this link between the activation of the HPA axis and the increased wheel activity of FR-ACT rats. On the one hand, negative energy balance and physical activity by themselves activate the HPA axis (31, 32). On the other hand, chronic corticosterone administration has been shown to result in increased locomotor activity (33) and corticosterone is necessary for the occurrence of schedule-induced wheel running after adrenalectomy (34). Because total wheel activity is correlated with both the decrease in thymus weight and final adipose body mass (mainly subcutaneous fat pad), our data suggest that final fat mass and the relative increase in HPA axis activity (corticosterone increase and thymus weight decrease during FR-ACT: BN and Lew > F344), but not higher basal HPA axis activity, are major determinants of the vulnerability to excessive running in conditions of food restriction. The fact that F344 lost more fat mass than the other two strains but increased less their HPA axis activity does not support the role of the amount of fat mass loss in such behaviour. Although, initially, it may appear counterintuitive that F344 rats do not have the higher corticosterone increment compared to the two other HPA axis hyporeactive strains, this is not in contradiction with our results, but rather may imply that the amplitude of the circadian rhythm is probably blunted relative to mesor values because mean levels of corticosterone are normally higher in F344 (13, 14), which might explain the relative lack of development of food-anticipatory corticosterone elevations in the F344 (29). An alternative explanation is that initial higher fat mass in F344 may delay HPA axis activation.

A detailed analysis indicates that the BN rats have the least fat at the beginning of the experiment and initially ran the most, whereas the F344 have the most fat and ran the least. It also appears that the F344 which started the experiments fatter took longer to generate much anticipatory activity. Overall, these results emphasize the relationship between initial fat mass level and activity and are in agreement with the hypothesis of Scalfani and Rendel (19) that body fat mass must fall to some relatively critical level before locomotor activity increases during food-restriction. However, the results are to be interpreted with caution. Indeed, the regression model revealed that \( \Delta \) thymus weight (as a marker of integrated corticosterone secretion), and not final adipose weight, underlies the detected relationship with total wheel activity, suggesting that the activation of the HPA axis is

mainly related to wheel activity rather than to chronic energy deficiency.

We propose that initial low levels of fat mass or fast food restriction-induced lowering of adipose tissue may be the initial link between corticosterone and high running activity in food-restricted rats. Challet et al. (9) demonstrated that both the late increase in nitrogen excretion due to depletion of fat stores and the rise in locomotor activity during prolonged fasting are suppressed by bilateral adrenalectomy and restored by corticosterone replacement. Thus, corticosterone is the critical hormone suppressed by adrenalectomy and has a key role in these metabolic and behavioural processes. Indeed, corticosterone plays an important role in the mobilization of energy reserves during energy deficit from fasting or energy restriction or during prolonged exercise by stimulating lipolysis and increasing protein catabolism.

Excessive wheel running in conditions of low food intake and HPA axis activation may be linked at the level of the central nervous system. One potential mediator of such a relationship is CRH because it suppresses food intake and activates behaviours that compete with eating such as locomotor activity (35). The increased adrenal weight in food restricted groups (FR and FR-ACT) favours this hypothesis and suggests an activation at the level of pituitary or above because the weight of adrenal glands is a marker of chronic stimulation by ACTH. Heybach and Vernikos-Danellis (36) reported that the anticipatory corticosterone increase in daily food deprived rats is preceded by an increase in plasma ACTH concentrations, which probably results from an increased CRH secretion. However, in agreement with Wong et al. (37), our results do not favour a role of CRH at the PVN level because no overall alteration in the expression of CRH mRNA in the PVN was found in both FR and FR-ACT rats compared to AL and ACT rats. However, because brain tissues were collected at only one time point, this study does not rule out the possibility that abnormalities in neuropeptide gene expression might be present at other times during the course of activity-induced anorexia. An alternative explanation could be that the level of CRH mRNA expression in the present study results from the balance between the stimulatory tone exerted both by the metabolic drive (energy deficit as reflected by decreased fat mass) (38) and increased physical activity (31) and the inhibitory tone exerted by increased glucocorticoids levels (28). However, the use of the Lew strain argues against a major involvement of CRH in the higher activation of their HPA axis compared to F344 because Lew rats possess a hypothalamic gene defect that results in a global CRH hyporesponsiveness (relative deficiency in CRH biosynthesis and release) (39). We therefore hypothesized that disturbances in the hypothalamic AgRP and CART neurones activation pathways may link the paradoxically decreased feeding in the face of increased activity in food-restricted rats. In accordance with their physiological roles, the expression of the anorexigenic (CART) and the orexigenic (AgRP) mRNA were decreased and increased, respectively, in FR and FR-ACT rats, excluding the involvement of these neuropeptides in the physiopathology of unadapted food intake in FR-ACT rats. Although arcuate AgRP neurones project to the PVN and may therefore potentially affect the release of CRH (20), the absence of a strain by experimental group interaction in AgRP mRNA expression is a further argument against an important role of this neuropeptide in the strain difference in the HPA axis activation.

Prefeeding wheel activity has been characterized as foraging (i.e. to food-seeking behaviour) (40). Because the leptin-NPY/AgRP neuroendocrine system has been proposed to serve the purpose of directing attention to food acquisition when energy stores are depleted (41), it is tempting to speculate that the increased AgRP (directly or indirectly) might act to increase the drive for food and thus food-seeking behaviour. However, once again, the absence of a strain by experimental group interaction in AgRP mRNA expression, does not evoke an important role of this neuropeptide in the difference in wheel running between the three strains of rats.

NPY mRNA have not been assessed in the present study. Different elements favour the choice of AgRP instead of NPY. First, AgRP is coexpressed with NPY in most hypothalamic NPYergic arcuate nucleus neurones but, although NPY is described as the most potent orexigenic molecule when the feeding response is measured over a few hours, its effects are short-lived in comparison to those of AgRP (22). Second, NPY and AgRP appear to be differentially regulated only under particular circumstances such as treatment with fatty acid synthase inhibitors (42), but not during food restriction where hypothalamic peptide content and mRNA levels of both NPY and AgRP are increased (43), suggesting that the variations of NPY should parallel those of AgRP in the present study. Therefore, the involvement of NPY in the present strain behavioural differences remains improbable.

For these reasons, we propose that instead of AgRP, corticosterone is the link between food restriction-induced decrease in fat mass and increased wheel running.

Food restriction-induced decrease in fat mass triggers (probably via the fall in leptin levels) the neuroendocrine adaptation to starvation (44), including activation of the HPA axis. In accordance with this, we have shown that the faster food restriction-induced lowering of adipose stores (BN and Lew strains) is associated with the higher corticosterone increment in both FR and FR-ACT groups, suggesting an energy regulation process. On the other hand, because numerous studies have reported that corticosterone has a critical role in stimulant-induced behavioural sensitization (8), corticosterone sensitization of the dopaminergic and noradrenergic neurones of the limbic system may reinforce selfstarvation via reward mechanisms (8, 12). In this regard, we rely on the findings of Piazza et al. (10), who demonstrated that elevated glucocorticoids levels enhance dopamine release in the nucleus accumbens, thereby increasing the compulsive nature of some activities as clearly demonstrated for drug taking behaviours. Put another way, we propose that corticosterone may contribute to the physiopathology of excessive running in the face of reduced food intake, reinforcing the sensitization to running through its positive hedonic effects. Indeed, dopaminergic neurones express corticosterone receptors on their cell bodies (45) and dopamine has both HPA axis activation properties and anorectic and rewarding effects. Moreover, a higher release of this amine has been proposed to be responsible for anorexia and weight
loss as well as the hyperactivity present during the course of anorexia nervosa (46). Finally, numerous studies have shown that Lew, compared to F344 rats, represent an addiction-prone genotype due to difference in endogenous dopamine level (17). In human anorexia nervosa, a similar hypothesis has been advanced (12), where a combination of physical activity and reduced food intake is rewarding and directs attention to stimuli that subsequently control the self-starvation behaviour. Therefore, the combination of food restriction-induced higher relative increment in corticosterone secretion with the reinforcing properties of stress may make Lew and BN strains particularly willing to develop excessive running in conditions of food restriction. The signals that communicate such information to the brain and activate the HPA axis remain to be determined. The demonstration that leptin normalizes activity levels in food-restricted rats (3) favours the role of fat mass as the initial signal that communicates information about negative energy balance to the brain. Moreover, leptin inhibits the semistarvation-induced activation of the HPA axis (47). However, data on leptin effects on the HPA axis activity are conflicting. Chronic exogenous administration of leptin depresses CRH mRNA expression, pituitary ACTH production and inhibits corticosterone response to stress (48). On the other hand, leptin may be stimulatory on the HPA axis, stimulating CRH neurones that mediate the anorectic effects of CRH infusion and increasing secretion of ACTH and corticosterone (49).

Further studies will be necessary to identify the interactions between leptin, activation of the HPA axis and increased running activity during food restriction.

In conclusion, we used three strains of rats differing in their HPA axis activity and reactivity, and in their initial level of fat mass. Although a number of hypothetical relationships have been evoked between wheel running and food restriction, our data emphasize the major role of fat mass (initial levels of fat mass and/or rate of fat mass loss) and corticosterone in such behaviour, probably with a critical level of low fat mass stimulating running. We propose that corticosterone may be the link between food restriction-induced negative energy balance associated with initial low levels of fat mass (peripheral energetic) and increased physical activity, favouring fueling through lipolysis and proteolysis and reinforcing the self starvation via reward mechanisms, thus establishing a deleterious vicious cycle.

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