Divergent Metabolic Effects of Acute Versus Chronic Repeated Forced Swim Stress in the Rat

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Objective: This study sought to examine divergence regarding the impact of acute versus chronic repeated stress on energy balance.

Methods: Rats were exposed to either chronic repeated forced swim (FS) stress for 7 days or an acute stress (a single FS). Body weight and food intake were measured daily. Metabolic parameters explored included brown adipose tissue (BAT) weight and activity.

Results: Chronic repeated FS stress decreased body weight and caloric efficiency. It also increased the relative weight of BAT. The same stressor delivered only once did not alter adrenal or BAT weight, but it did increase the metabolic activity of BAT. In stress-naive rats, acute FS stress induced an anorexigenic response during the first day after the stressor that caused a reduction in body weight (that persisted for 4 days). By contrast, the chronic FS rats did not show an anorexigenic response after the final stressor, and there was no change in body weight during the following 4 days.

Conclusions: Rats exposed to chronic repeated FS stress adapt to the stressor over time; they become less sensitive to its anorexigenic effects and its metabolic effects in BAT, adaptations that ultimately reduce sensitivity to the weight-lowering effects of an acute stressor.

Introduction

Amid the current obesity pandemic, there is much need for clarity regarding the relationship between stress and metabolic health and the mechanisms involved. The body weight and metabolic consequences of stress are highly dependent on the type, intensity, and duration of the stressor involved and show much individual variability. In general terms, chronic mild stressors that are often linked to psychological threats to well-being (such as those associated with financial worries, work problems, family responsibilities, or health concerns) commonly lead to increased food intake and obesity development (1-5), whereas acute intense stressors are more likely to reduce food intake and cause weight loss (6-8).

In rodents, acute stress induces an anorexigenic response leading to weight loss; the more intense the stressor, the bigger the effect (9). Interestingly, although weight loss can persist for many days after acute stress, food intake recovers almost immediately (10,11). With repeated exposure to the stressor, the body weight-lowering effects can persist for months, again, despite recovery of caloric intake (12). Thus, in situations of chronic repeated exposure to stress, rodents appear to be able

to defend a lower set point for energy homeostasis, likely involving metabolic adaptations that result in increased energy expenditure.

As shown in recent reviews (13,14), to understand the consequences of stress on energy balance, it is necessary to consider the stress effects on both the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Stimulation of the HPA axis under acute stress culminates in an increased release of glucocorticoids, thereby promoting mobilization of the body's energy stores to allow a rapid response that favors survival under acute threatening situations. However, when glucocorticoid levels are chronically increased, detrimental metabolic effects are observed that lead to a positive energy balance in the long term. Conversely, activation of the sympathetic nervous system that causes the release of catecholamines is linked to increased thermogenesis in brown adipose tissue (BAT) (15,16), decreased food intake, and decreased body weight (17). Recent reports (14) have suggested that BAT thermogenesis could be a key factor in determining whether a given stressor causes weight gain or weight loss.

The aim of the present study was to explore the metabolic changes underpinning the weight loss that occurs as a result of moderate stress

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exposure in the rat. Because this stress-induced weight loss and associated anorexia adapt over time (18), we compared the impact of acute and chronic repeated forced swim (FS) stress on caloric efficiency, on parameters linked to energy expenditure in BAT (including BAT weight and uncoupling protein 1 [UCP1] activation), and also on parameters linked to glucocorticoid signaling in white adipose tissue (WAT).

Methods

Animals and general procedure

Adult male Sprague-Dawley rats (9-10 weeks; Charles River Laboratories, Sulzfeld, Germany) were individually housed in a 12-hour light/dark cycle (lights on at 6 AM), with regular chow and water available *ad libitum* in their home cages. All animal procedures were carried out with ethical permission and in accordance with the University of Gothenburg Institutional Animal Care and Use Committee guidelines. The experimental treatments were always carried out in the morning, between 8 AM and 1 PM.

Experimental design

To reduce baseline stress, starting at least 1 week after their arrival, all rats were handled three times on different days for approximately 2 min/d. In experiment 1, rats were divided into three different groups and matched at outset according to their body weight: (1) a chronic stress group (chronic exposure to FS stress at 36°C repeated for 1 hour every day for 7 days and euthanasia 1 hour after the last FS session; $n = 7$), (2) an acute stress group (exposure to 1 hour of a single FS [acute FS] and euthanasia 1 hour later; $n = 7$), and (3) a control never-stressed group (7 days with handling only and euthanasia 1 hour after last handling; $n = 6$). In experiment 2, the same design was used, but the rats were euthanized 4 days after the last FS session under basal conditions ($n = 8$ per group). In experiment 1, the gonadal WAT, BAT, adrenal glands, and thymus were rapidly extracted and frozen in liquid nitrogen. Body weight and food intake measurements were determined daily during the experiments, always at the same time of day. The caloric efficiency was calculated as follows: caloric efficiency = (body weight gain/food intake) × 100.

In experiment 2, the same samples were taken except for the adrenal glands and the thymus because we noted, in other experiments, that the ability of chronic FS stress to alter the weight of these tissues is not observed when rats are not euthanized immediately after stress (Figure 1).

FS as a stressor

Rats were placed in transparent cylindrical plexiglass tanks (height: 40 cm, internal diameter: 19 cm) containing water at 36°C to a depth of 24 cm for 1 hour. The temperature of the water bath for this stress protocol is based on a previous study (19).

RNA isolation and mRNA expression

Total RNA from BAT and WAT samples was extracted with the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) or RNeasy Micro Kit (Qiagen) according to the guidelines of the manufacturer. The RNA quantity and quality of the samples were checked with the NanoDrop (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). First-strand complementary DNA (cDNA) synthesis was prepared with 500 ng of total RNA and the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, California). Gene expression profiling was performed by using Custom TaqMan Assays (Life Technologies, Stockholm, Sweden) and the 7900HT Fast Real-Time PCR System (Life Technologies). Data were normalized as described previously (20), and the mean values of *Actb* and *Hmbs* expression were used as endogenous controls. The genes assessed included the glucocorticoid receptor (*GR*), mineralocorticoid receptor (*MR*), and 11β-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*; also known as *HSD11B1*) in WAT as well as *UCP1* in BAT.
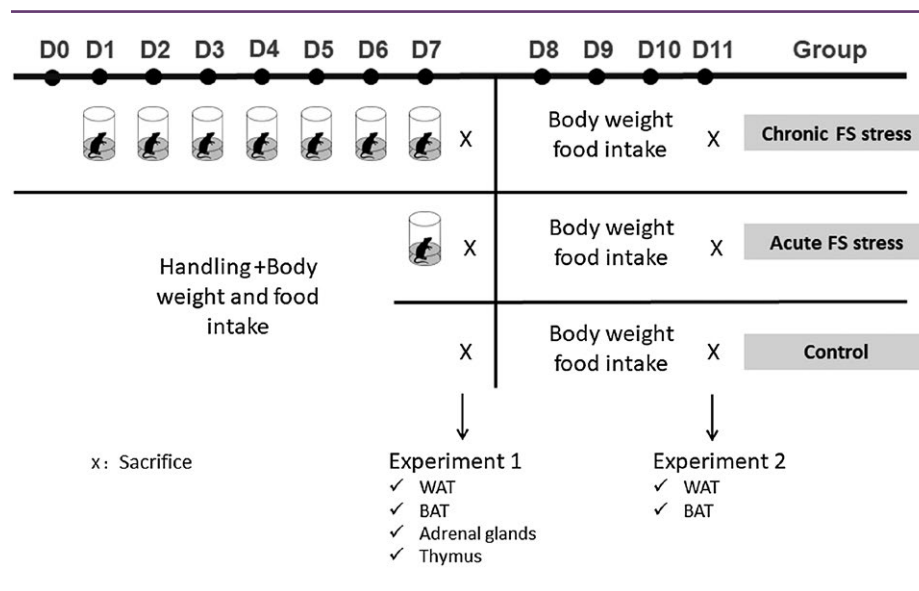

Figure 1 Schematic representation of the experimental design used in experiments 1 and 2. BAT, brown adipose tissue; D, day; FS, forced swim; WAT, white adipose tissue.

Western blot

BAT samples were homogenized in ice-cold radioimmunoprecipitation assay buffer (Sigma-Aldrich, St. Louis, Missouri) supplemented with Complete Mini Protease Inhibitor Tablets (Roche Diagnostics, Mannheim, Germany) and PhosSTOP Phosphatase Inhibitor Cocktail Tablets (Roche Diagnostics). Total protein was collected from the fat pads after being centrifuged at 12,000 rpm at 4°C for 30 minutes. After determining total protein by the Bradford protein assay, equal amounts (30 μg) of protein for each group were resolved on 4% to 20% TGX stain-free gels (Bio-Rad Laboratories GmbH, Munich, Germany) and transferred onto polyvinylidene difluoride membranes. The membranes were probed with the primary antibody UCP1 (U6382; Sigma-Aldrich) in 0.01M Tris-buffered saline supplemented with Triton X-100 (Sigma-Aldrich Sweden AB, Stockholm, Sweden) containing 5% nonfat dry milk overnight followed by horseradish peroxidase-conjugated secondary antibody. Ultraviolet activation of the stain-free gel on a ChemiDoc MP Imaging System (Bio-Rad Laboratories) was used to control for proper loading as previously described (21,22). Band densitometry and quantification were performed using Image Laboratory (version 5.0; Bio-Rad Laboratories AB, Solna, Sweden). The protein band densities were normalized to the total-protein loading control. The analysis was performed as described previously (23).

Statistical analysis

The statistical analysis was performed using SPSS Statistics (version 21; IBM Corp., Armonk, New York). The generalized linear model with group (control and chronic FS stress) as a between-subjects factor was used to analyze the average changes in body weight, food intake, and caloric efficiency (experiments 1 and 2) and relative weight of the adrenal gland, thymus, and BAT during chronic treatment (experiment 1). Note that in these analyses, the control group included the rats exposed to acute FS on the last day of the experiment because that single experience with stress would not be expected to change their response from the control never-exposed rats at the time of the analysis. The same analysis was used to study the changes in gene expression in BAT and WAT immediately after stress (experiment 1), relative BAT weight, and expression of UCP1 in BAT (experiment 2). But in the later cases, three levels were included in the variable group (control, acute FS stress, and chronic FS stress). The analyses were always followed by post hoc Bonferroni comparisons. Finally, in experiment 2, to analyze the changes in body weight, food intake, and caloric efficiency after the last stress session, a generalized linear model with repeated measures (generalized estimating equations) was used (24). We included the between-subjects variable group (three levels: control, acute FS stress, and chronic FS stress) and the within-subjects-factor day (four levels). This provided analysis of the daily changes in the groups in these variables after the last stress session. The caloric efficiency and the changes in UCP1 (protein expression) in BAT in experiment 1 did not fit normality; in these cases, the Mann-Whitney U test or Kruskal-Wallis nonparametric test was used followed by Mann-Whitney U comparisons in the last case.

Results

Impact of chronic repeated FS stress on body weight gain, caloric intake, and caloric efficiency

Analysis of body weight gain, food intake, and caloric efficiency during the stress period demonstrated that rats exposed to chronic repeated FS stress gained less weight, reduced their food intake, and reduced their caloric efficiency compared with the control rats. In this case, the control rats can also include those in the acute FS group before exposure to the stressor (experiment 1; Figure 2A-2C) (weight
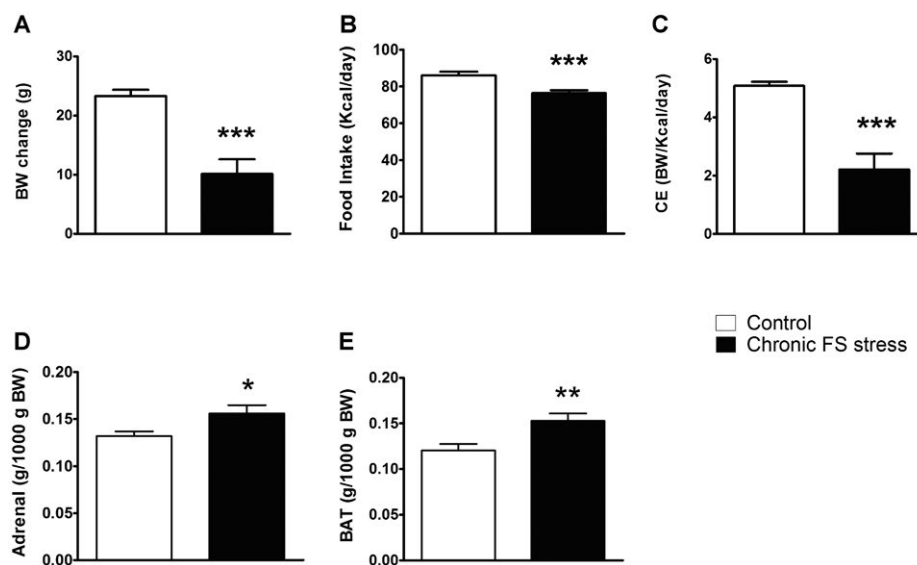

Figure 2 Metabolic consequences of exposure to chronic repeated stress (experiment 1). (A) BW change, (B) average daily food intake (kcal/d), and (C) average daily CE during the chronic stress in rats exposed to chronic repeated FS (chronic FS stress) or rats never exposed to stress during the first 6 days of the experiment (i.e., control and acute FS groups combined). On the euthanasia day: relative weight (grams of tissue per 1000 g of BW) of (D) adrenal glands and (E) BAT. Mean and SEM are represented ($n = 7$-13 rats per group). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus control group. BAT, brown adipose tissue; BW, body weight; CE, caloric efficiency; FS, forced swim.

gain: Wald $\chi^2_1 = 55.6$; food intake: Wald $\chi^2_1 = 11.6$; caloric efficiency: $U = 1.0$; all: $P < 0.001$).

Impact of chronic repeated FS stress versus acute FS stress on weight of dissected organs and expression of relevant genes

On day 7 of experiment 1, the chronic FS stress rats and half of the control rats (from this moment on, called acute FS stress rats) were exposed to 1 hour of FS and euthanized 1 hour later, whereas the rest of the control rats were euthanized without any stress experience. The analysis of the tissue showed a group effect, demonstrating that chronic FS stress rats had an increase in the size of the adrenal glands (Figure 2D: Wald $\chi^2_2 = 7.6$; $P < 0.05$) and in BAT weight (Figure 2E: Wald $\chi^2_2 = 9.7$; $P < 0.01$) relative to the body weight compared with control and acute FS stress rats (hereafter combined in a single group under the name control). No differences between groups were observed regarding thymus or WAT weight (data not shown).

The study of relevant stress genes in WAT and BAT revealed differences between groups in the level of expression of *GR* (Wald $\chi^2_2 = 10.13$; $P < 0.01$), *MR* (Wald $\chi^2_2 = 6.04$; $P < 0.05$), and *11β-HSD1* (Wald $\chi^2_2 = 7.0$; $P < 0.05$) (Figure 3A). The comparison between groups showed that only the acute FS rats had reduced expression of *GR* compared with controls and that the differences observed in *MR* and *11β-HSD1* mRNA expression were between those of the acute and chronic FS groups, not the control rats. The data for *UCP1* gene expression in BAT showed the opposite results. Differences between groups were observed (Wald $\chi^2_2 = 8.14$; $P < 0.05$), although the acute FS rats were the group showing higher expression of *UCP1* compared with both the control and chronic FS stress groups (Figure 3A).

When the protein levels of UCP1 in BAT were analyzed (Figure 3B), a trend to significance was observed in the comparison between groups ($P = 0.092$); relative to the control rats euthanized under basal conditions, the rats exposed to acute FS or chronic FS stress showed an increased fold change (1.916- and 1.896-fold, respectively), although only the acute FS group reached statistical significance ($P = 0.041$).

Impact of chronic repeated FS stress and acute FS stress on body weight gain, caloric intake, and caloric efficiency, measured up until 4 days after stress

As was the case in experiment 1, rats exposed to chronic FS stress gained less weight and showed reduced caloric efficiency during the stress period than the controls (experiment 2; Figure 4A: Wald $\chi^2_1 = 7.34$; $P < 0.01$; Figure 4E: Wald $\chi^2_1 = 6.52$; $P < 0.05$), although in this experiment, the reduction in food intake did not reach statistical significance (Figure 4C). The daily analysis of these parameters beyond the last day of stress (i.e., the only stress day for the acute FS stress group) revealed group and day effects for the body weight gain (Wald $\chi^2_2 = 6.25$; $P < 0.05$ and Wald $\chi^2_3 = 255.5$; $P < 0.001$, respectively) without interaction between these variables. The acute FS stress rats showed reduced body weight gain compared with the controls and the chronic FS stress group (in both cases, $P < 0.05$), suggesting that chronically stressed rats were protected from the anorexigenic effects of the stress (Figure 4). The food intake data showed a day effect and interaction between group and day (Wald $\chi^2_3 = 11.88$; $P < 0.01$ and Wald $\chi^2_6 = 22.3$; $P < 0.001$, respectively), with differences observed in the control and acute FS stress groups on days 2 and 4 post stress, respectively, compared with their own values on day 1 post stress (Figure 4D). Finally, the analysis of the caloric efficiency revealed significant effects of day and interaction between group and day (Wald $\chi^2_3 = 33.55$; $P < 0.001$ and Wald $\chi^2_6 = 16.17$; $P < 0.05$, respectively). Further study of this interaction showed that, at 24 hours after the last stress exposure, the acute FS stress group, but not the chronic FS stress group, showed a robust reduction in caloric efficiency (Figure 4F). These findings are in line with the observed results for body weight gain and support the idea that chronically stressed rats are protected against the anorexigenic effects of stress.
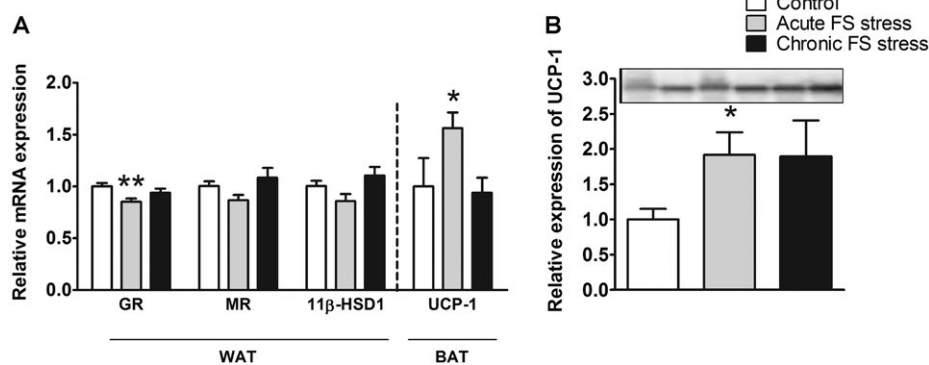

Figure 3 Impact of chronic repeated stress on expression of relevant genes in WAT and BAT as well as on UCP1 in BAT. Changes in gene expression depending on stress treatment in adipose tissue taken at euthanasia in experiment 1: (A) *GR*, *MR*, *11β-HSD1* in WAT and changes in the gene expression of *UCP1* in BAT. Gene expression was analyzed with RT-PCR. (B) Protein levels of UCP1 in BAT were analyzed with Western blot. Mean and SEM are shown (*n* = 6-7 rats per group). *$P < 0.05$ and **$P < 0.01$ represent the difference compared with the signaled group. *11β-HSD1*, 11β-hydroxysteroid dehydrogenase type 1; BAT, brown adipose tissue; *GR*, glucocorticoid receptor; *MR*, mineralocorticoid receptor; RT-PCR, real-time polymerase chain reaction; *UCP1*, uncoupling protein 1; WAT, white adipose tissue.
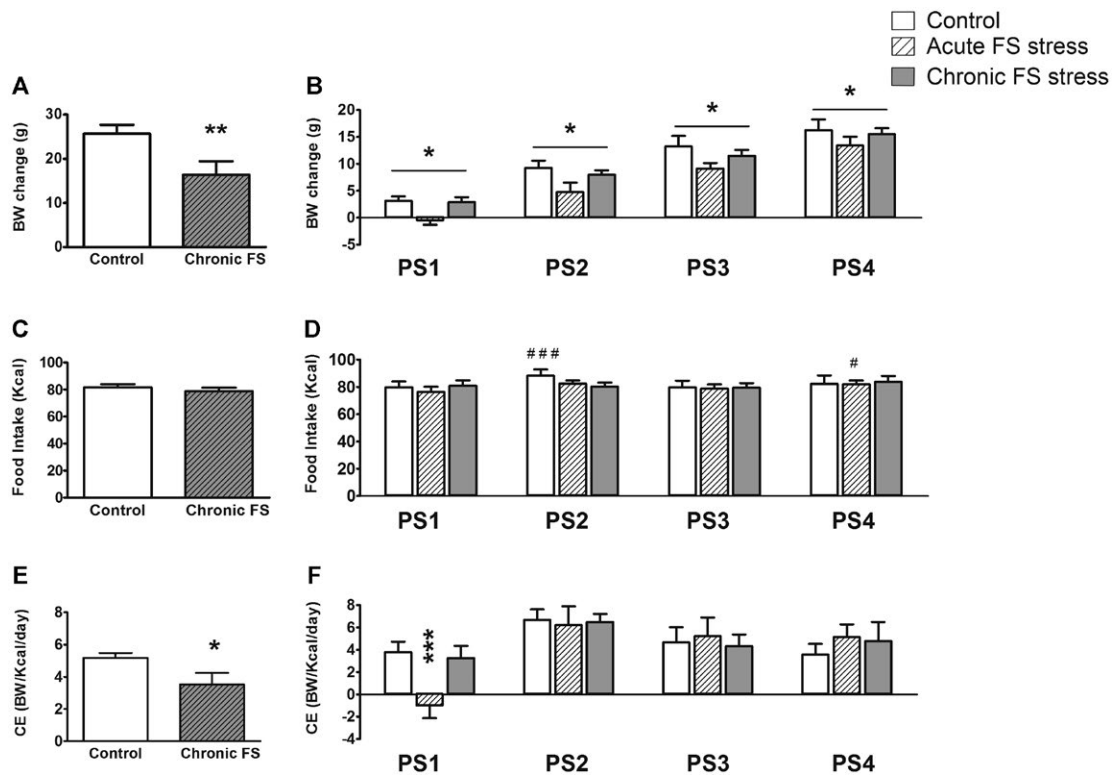
Figure 4 Metabolic consequences during and after exposure to chronic repeated stress (experiment 2). (A) BW gain, (C) average daily food intake (kilocalories), and (E) average CE during chronic stress in rats exposed to repeated FS (chronic FS) or rats never exposed to stress during the first 6 days of the experiment (i.e., control and acute FS groups combined). (B) Accumulated BW gain, (D) daily food intake, and daily CE are represented until the day of the euthanasia, 96 hours after the last stress session on day 7. Mean and SEM are represented ($n = 8$ rats per group). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus control group (Figure 3A, Figure 3E-3F), and *$P < 0.05$ represents general group effect in Figure 3B; #$P < 0.05$ and ###$P < 0.001$ represent differences versus day 1 in the corresponding group. BW, body weight; CE, caloric efficiency; FS, forced swim.
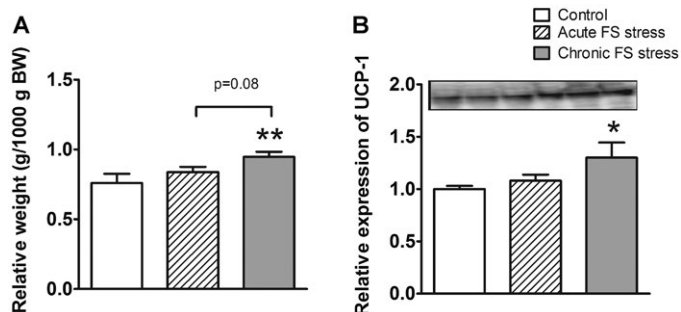

Figure 5 Impact of chronic repeated FS stress or exposure to a single FS stress on the relative weight of WAT and BAT, taken 4 days after the last stressor (experiment 2). On the euthanasia day: (A) relative weight (grams of tissue per 1,000 g of BW) of BAT and (B) relative expression of the UCP1 protein in BAT of control rats never exposed to stress, rats acutely exposed to a single acute FS on day 7, or chronically stressed rats exposed to repeated FS (chronic FS) for 7 days. Mean and SEM are represented ($n = 8$ rats per group). *$P < 0.05$ and **$P < 0.01$ versus control group. BAT, brown adipose tissue; BW, body weight; FS, forced swim; UCP1, uncoupling protein 1; WAT, white adipose tissue.

Impact of chronic repeated FS stress versus acute FS stress on weight of dissected organs and expression of relevant genes, measured 4 days after exposure to stressor

The study of BAT at the moment of euthanasia demonstrated that, even at 4 days after the last stressor, the rats previously exposed to chronic FS stress had an increase in the relative weight of BAT (Figure 5A; Wald $\chi^2_6 = 7.46$; $P < 0.05$). No differences were observed in the gene expression of *UCP1* in BAT. However, a trend to significance was observed in the protein levels ($P = 0.071$), in which proper comparisons showed that rats exposed to chronic FS stress showed an increased fold change compared with control rats ($P = 0.021$). No changes were observed in the amount of WAT or in the studied genes at the moment of euthanasia in experiment 2.

Discussion

The results of the present study indicate that the metabolic adaptations to chronic repeated stress that occur over time include a recovery in caloric efficiency and a reduced activation of BAT. In particular, we found that the effects of an acute stressor on caloric efficiency and

BAT activation in rats were lessened over time with repeated exposure to the same stressor.

The results of the present study indicate that acute stress reduces body weight gain and caloric efficiency, which are associated with increased gene and protein expression of UCP1 in BAT. However, in rats chronically exposed to FS, caloric efficiency was not reduced compared with controls when we measured it at 24 hours after the last stress exposure. Still, an increase in UCP1 (protein) was observed up to 4 days after the chronic stressor. Our results suggest that increased expression of UCP1 (that is linked to increased energy expenditure) might play a role in the initial reduction of body weight gain and caloric efficiency that is observed during exposure to FS stress. Given the persistence of these effects of stress on caloric efficiency and BAT activation in the chronic stress model, we may infer that additional mechanisms must explain the lessening of anorexigenic and weight loss effects that result from chronic repeated stress.

In the chronic stress paradigm (repeated FS for 7 days), rats gained less weight, ate less, and had reduced caloric efficiency relative to control unstressed rats. These data are in line with the results from other repeated stress models for which the most commonly reported result in rodents is that stress reduces food intake and body weight in a manner that is directly related to the stress severity (9,25). A single exposure to restraint (10) or immobilization (11) induces a reduction in body weight that can persist for >10 days, even if the food intake is quickly normalized (10,11). In the chronic repeated stress model used here, the reduction in body weight was most dramatic on the first day of stress exposure, after which the body weight began to recover despite the fact that food intake was not increased. This would suggest that adaptations occur during repeated exposure to stress, increasing caloric efficiency with chronic experience. Supportively, it was demonstrated that prior chronic unpredictable or repeated stress exposure can protect rats from the anorexigenic effects of a subsequent acute and intense stressor-like immobilization (18). Therefore, although intense stress seems to induce immediate body weight loss, compensatory mechanisms were shown to rapidly activate to offset the negative energy balance, and they can be independent of food intake (26). To our knowledge, this is the first study to compare the short- and long-term metabolic effects of a given stressor in rats with a history of stress (the chronic repeated stress group) versus those never exposed to stress (the acute stress group). Differences between these two groups provide not only information about how the stress response adapts with repeated exposure to the same stressor, but also further insight regarding the mechanisms that might contribute to the body weight loss in the chronic repeated stress paradigm.

First, we studied the effects of repeated and acute FS on the gene expression of glucocorticoid receptors I (*MR*) and II (*GR*) and on *11β-HSD1* in WAT. There is a growing interest in the role of GRs in WAT on negative feedback of the HPA axis induced by stress because they could be important in the interactions between stress and metabolism (27,28). Unexpectedly, no differences were observed between controls and repeated FS rats, suggesting that, if they ever existed, the differences in gene expression between these two groups vanished with repeated exposure. We did observe a small but significant reduction in *GR* mRNA expression in the acute FS group compared with the controls. This is probably the result of a high glucocorticoid increase after experiencing FS for the first time because the stress model used here was shown to induce a strong corticosterone release that adapted with repeated exposure (19). High concentrations of glucocorticoids downregulate GRs in the central nervous system and peripheral areas (29,30), and it is therefore

possible that this downregulation extends to WAT. Given that glucocorticoids were shown to be elevated during chronic stress paradigms similar to those used here (19), it is perhaps surprising that we did not detect a downregulation of *GR* mRNA in WAT in the chronic FS group.

We also explored the activation of BAT (a tissue that is involved in energy expenditure and thermogenesis) involving increased UCP1 (a mitochondrial uncoupling protein) expression. Studies linking stress to the activation of BAT date back to the early 1980s (31), and it now seems clear that the type of stress and its severity and duration are important for increasing the thermogenic capacity of BAT (14,26). We found that an acute stress caused a rapid activation of BAT, reflected not only by an increase in *UCP1* mRNA, but also by an increase in UCP1 protein levels. The fact that *UCP1* mRNA in BAT was increased in the acute stress group but returned to control values in the chronic repeated stress group suggests that, although the stressor FS can activate BAT, the response adapts (lessens) with repeated exposure to the same stressor. The effects of chronic repeated FS stress appear to differ from those obtained after chronic immobilization (26). With the chronic stress model used by Gao et al. (26), an increase in the mRNA expression of *UCP1* was observed after chronic immobilization, while the expression did not differ from that in the controls when the rats were acutely exposed to the stressor. However, the use of different techniques to measure mRNA together with the fact that immobilization is a more intense and longer stressor than ours (3 h/d over 4 weeks), which might impact secondary mechanisms like changes in the glucocorticoid capacity to inhibit UCP1 (32,33), could explain the discrepancies between the results. A rapid induction of *UCP1* mRNA that occurs within hours is commonly used as a marker of BAT activation, but it should be noted that the full recruitment of the UCP1 protein can take much longer (even weeks) as the protein accumulates (34), and this could be especially relevant when considering the impact of a stress of differing durations (acute vs. chronic) on BAT activation. Although *UCP1* mRNA returned to control values by day 7 of repeated stress exposure, there are indications that UCP1 protein levels accumulated during this period; UCP1 protein levels (per 30 mg of protein in dissected BAT) were almost double that of the control rats (although this difference did not reach significance because of variability in the chronic FS group). We also detected an increase in the absolute (data not shown) and relative amount of BAT in the chronic repeated stress group, which could contribute to increased thermogenesis and body weight loss. On the other hand, in the second study, we found that the effects of chronic repeated stress on BAT weight and UCP1 protein levels could still be detected 4 days after the end of the stress period. These results demonstrate that the activation of UCP1 in BAT remained elevated when the rats in the chronic FS group were gaining weight exactly as in the controls. It may be that the increased amount of BAT and UCP1 expression (and presumably increased BAT-induced thermogenesis) in chronic FS is insufficient to impact the body weight gain. Alternatively, it could be that chronic repeated stress triggers compensatory mechanisms that compensate for the effects of increased BAT-induced thermogenesis effects on energy balance. Therefore, any effect of UCP1 on body weight gain would lose relevance as adaptations occur during the chronic repeated stress.

The second study was undertaken to determine whether the metabolic adaptations persist during a 4-day period after the end of a period of chronic repeated stress. In this study, we were first able to confirm the observation that rats that were repeatedly exposed to FS gained less weight and had reduced caloric efficiency compared with the control rats during the stress exposure, although we could not detect a significant decrease in food intake, which was probably explained by different

sensitivities to stress in different groups of rats. These data confirm that other mechanisms than food intake contribute to body weight changes under stress (14). During the 4-day period immediately after the 7 days of chronic repeated stress, there was no difference in body weight, food intake, or food efficiency relative to the never-stressed control group. This full recovery of the rats exposed to chronic stress diverged from the response of those exposed to an acute single stress, which reduced the body weight for up to 4 days and was coupled, at least initially, to a reduction in caloric efficiency. Collectively, these data highlight the fact that rats are better protected from the weight-lowering effects of the stressor when they have been exposed to it many times, providing further evidence that metabolic adaptations have occurred.

Conclusion

The early effects of chronic repeated stress exposure on body weight involved a reduction in caloric efficiency, but this effect was rather short-lived, recovering already during repeated stress exposure. By contrast, BAT activation (reflected by the amount of BAT and the amount of UCP1 protein) was increased at the end of the chronic stress period, an increase that could still be detected at 4 days after the last exposure to FS, when chronically stressed rats did not show any difference from the controls. Consequently, the possible effects of stress on BAT-induced thermogenesis and body weight gain are likely acute. Further studies are needed to know which mechanisms are activated under chronic stress to counteract the negative energy balance initially observed in chronic stress models.**O**

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