Chronic social stress lessens the metabolic effects induced by a high-fat diet

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Abstract
Stress has a major impact on the modulation of metabolism, as previously evidenced by hyperglycemia following chronic social defeat (CSD) stress in mice. Although CSD-triggered metabolic dysregulation might predispose to pre-diabetic conditions, insulin sensitivity remained intact, and obesity did not develop, when animals were fed with a standard diet (SD). Here, we investigated whether a nutritional challenge, a high-fat diet (HFD), aggravates the metabolic phenotype and whether there are particularly sensitive time windows for the negative consequences of HFD exposure. Chronically stressed male mice and controls (CTRL) were kept under (i) SD-conditions, (ii) with HFD commencing post-CSD, or (iii) provided with HFD lasting throughout and after CSD. Under SD conditions, stress increased glucose levels early post-CSD. Both HFD regimens increased glucose levels in non-stressed mice but not in stressed mice. Nonetheless, when HFD was provided after CSD, stressed mice did not differ from controls in long-term body weight gain, fat tissue mass and plasma insulin, and leptin levels. In contrast, when HFD was continuously available, stressed mice displayed reduced body weight gain, lowered plasma levels of insulin and leptin, and reduced white adipose tissue weights as compared to their HFD-treated non-stressed controls. Interestingly, stress-induced adrenal hyperplasia and hypercortisolemia were observed in mice treated with SD and with HFD after CSD but not in stressed mice exposed to a continuous HFD treatment. The present work demonstrates that CSD can reduce HFD-induced metabolic dysregulation. Hence, HFD during stress may act beneficially, as comfort food, by decreasing stress-induced metabolic demands.

Introduction
Metabolic syndrome and type II diabetes (DM2) prevalence increased during the last decades, taking on epidemic proportions (Lovic et al. 2020). Chronic psychological stress is recognized as an important risk factor for metabolic disorders (Scott et al. 2012). Thus, understanding how chronic stress affects metabolism represents an important research question in biomedical sciences that remains to be fully addressed.

Chronic stress challenges homeostatic processes with several maladaptive consequences, including the
development of the metabolic syndrome (Russell & Lightman 2019). Major risk factors for the metabolic syndrome include obesity and elevated blood glucose levels (Sherling et al. 2017). In agreement, epidemiological studies show that chronic stress is linked to weight gain, with the highest impact seen in males (Torres & Nowson 2007). Here, stress-induced eating is proposed to be an important contributing factor underlying the relation between chronic stress and obesity (Razzoli et al. 2017). Aside from stress-induced hyperphagia, stress also increases the preferential intake of hyper-caloric and palatable foods, such as high fat containing foods (Yau & Potenza 2013). Hence, the desire to consume high caloric food under stressful conditions could comprise an additional factor contributing to obesity. In line with this, poor dietary habits are known to contribute to the rapidly rising incidence of type 2 diabetes (Sami et al. 2017). In conclusion, overconsumption of hyper-caloric foods under stressful conditions could form the basis for the development of the metabolic syndrome.

Previously, we demonstrated that chronic social defeat (CSD) stress in male mice leads to hyperphagia and hyperglycemia (van der Kooij et al. 2018), physiological alterations seen with an emergent metabolic disorder. The hyperphagia occurred during CSD and persisted even after discontinuation of the stressor. Hyperglycemia was first observed in the peripheral blood 2 days post-CSD and persisted for the following 2 weeks. Hyperglycemia extended to the brain and was associated with an overall reduction in cerebral glucose uptake. In contrast to the changes in food intake and glucose homeostasis, peripheral insulin and leptin levels were unaffected, insulin resistance was not observed and body weights did not change throughout the experiment. Still, social subordination stress has been identified as a major risk factor for insulin resistance and type 2 diabetes (Kelly & Ismail 2015). Therefore, we hypothesize that the observed metabolic disturbances induced by CSD represents a prodromal phase where the contribution of a nutritional challenge, in the form of a high-fat diet, could lead to the progression of the metabolic disorder. Accordingly, there are studies showing a stress-mediated increase in high-caloric preference (Tamashiro et al. 2006) and worsening of HFD-induced obesity under stressful conditions (Kuo et al. 2007, 2008). However, other studies reported protective effects of HFD from CSD (Buwalda et al. 2001, MacKay et al. 2017, Coccurello et al. 2018).

Previously, it was demonstrated that the interplay between social stress and a high-fat diet is dynamic and that temporal aspects need to be taken into account (Finger et al. 2012). To investigate the putative interactions and dynamics between CSD and a nutritional challenge, mice were provided with a HFD, which commenced the day following CSD (named: HFD-post) or supplied HFD continuously (during as well as after CSD; named: HFD-last). Adipose tissue plays a crucial role in metabolic homeostasis, and its dysfunction in obese humans is linked to disrupted metabolic homeostasis, insulin resistance and diabetes (Kusminski et al. 2016). We, thus, assessed the potential synergistic effects of CSD and HFD for signs of obesity in mice by measuring animals’ food intake, plasma leptin and insulin levels, overall body and fat tissue weights as well as glucose homeostasis.

Material and methods

Animals

Adult male C57BL/6j mice (Janvier, France) arrived at the animal facility at 8 weeks of age and were habituated to housing conditions (temperature: 22 ± 2°C, humidity: 50 ± 5%) for at least 1 week before experiments commenced. Mice were single-housed with food/water in a 12 h light: 12 h darkness cycle ad libitum. C57Bl/6j mice were randomly assigned in a two-by-two design to CTRL or CSD and to HFD-post or HFD-last. Adult male CD-1 retired breeders (Janvier, France) were used as aggressors in the CSD paradigm and were trained and preselected based on their aggressive behavior (latency to attack <60 s), as described previously (Golden et al. 2011). Experiments were performed in accordance with the European Directive 2010/63/EU for animal experiments and approved by the local authorities (license No G17-1-005, Animal Protection Committee of the State Government, Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany).

Chronic social defeat paradigm

The CSD paradigm was conducted for 10 days, as published previously (van der Kooij et al. 2018). Briefly, on each day, male C57Bl/6j mice were sequentially exposed to three social defeats (physical attack) lasting 10 s each in the home cage of three different, unknown, male CD-1 aggressor mice. In between the aggressive encounters, animals were separated for 15 min by a metal grid partition to avoid further physical contact but allowing sensory contact. Following the three daily social defeats, intruder and aggressor mice were housed
in the same cage but were physically separated by the grid partition until the next day. CTRLs were exposed to a novel clean cage (90 s) for 10 consecutive days and their home cages were also equipped with a grid partition to mimic the conditions for stressed animals. Following CSD, mice were single-housed for further experimentation.

**Diets**

Animals were fed with either a standard diet (SD) containing 13.9 kJ/g (caloric contribution: 11% fat, 36% protein, 53% carbohydrate; Altromin, C1090/10) or a high-fat diet (HFD) containing 21.1 kJ/g (caloric contribution: 60% fat, 17% protein, 23% carbohydrate; Altromin, C1090/60) (Ruiz de Azua et al. 2017). HFD-last treatment persisted over the course of 10 days social defeat and continued until sacrifice. HFD-post treatment commenced the day after CSD and continued until sacrifice.

**Open field test**

The open field consisted of a rectangular arena (45 × 45 × 41 cm) that was illuminated with dimmed lights (37 lx). Mice were introduced near the wall of the arena and allowed to explore for 10 min. Locomotor activity was analyzed during the open field test using EthoVision tracking software (Version XT, Noldus, Wageningen, The Netherlands).

**Physiological measurements**

**Peripheral blood glucose measurement**

To exclude postprandial rise of blood glucose, animals were fasted for 1 h prior to the glucose measurement. Blood was obtained by a small tail incision under stress-free unrestrained conditions (Fluttert et al. 2000, Jene et al. 2018). The first drop was always discarded and morning blood glucose levels were measured three times successively using an electronic glucometer (Accu-Chek®, Roche, Switzerland) and averaged to obtain reliable values.

**Measurement of body weight and energy intake**

Individual body weight and food intake were measured every 5 days. Caloric intake was calculated based on the amount of individual food intake using the energy content of the standard diet (13.9 kJ/g) and the high fat diet (21.1 kJ/g), respectively.

**Dissection of fat tissues and adrenal glands**

Mice were sacrificed by decapitation at either 2 or 8 weeks post-CSD. The adrenal glands were dissected, the surrounding fat tissue was carefully removed using forceps and micro-scissors and adrenal weights were taken. We also collected white (epididymal (eWAT) and s.c. (sWAT)) and brown adipose tissue (BAT) and measured tissue weight.

**Gene expression analyses**

White epididymal adipose tissue (eWAT) was homogenized in TRIzol (Sigma-Aldrich, 12183-555), the aqueous phase was collected and RNA was isolated using an RNA kit (QIAGEN, 74106) (Ruiz de Azua et al. 2017). cDNA transcription was performed using the RT2 first strand kit (iScript Transcription Supermix for RT-qPCR, BioRad), following manufacturer’s protocol. Real time RT-PCR was carried out with QuantStudio 3 (Applied Biosystems) using SYBR select master mix (4472903, Applied Biosystems). The following primer sequences were used: TNF-alpha FW: GCGGTGCCTATGTCTCAG REV: GCCATTTGGGAACTTCTCATC, IL-1 beta FW: CAACCAACAATGTATAATCCAG REV: GATCCACACCTCTCCAGCA, IL-6 FW: TCTAATTCATATCTTACATG REV: TGGTCCTTAGACACTCCTC, GAPDH FW: TGAAGCAGGTCATCTGAGGG REV: CGAAGGTGGACATCTGGGAG, β-actin FW: AGAGGGAAATCGTGGATGAC REV: CAATAGTGATGACCTGGCCGT. Mean expression of GAPDH and β-actin were used for normalization of the data.

**Plasma corticosterone, insulin and leptin measurements**

Blood for corticosterone measurements was taken 5–6 days post-CSD between 09:00 h and 13:00 h through a small tail incision (Fluttert et al. 2000, Jene et al. 2018). Blood for insulin and leptin measurements was obtained from trunk blood at sacrifice 8 weeks post-CSD. Animals were not fasted prior to taking these blood samples. Blood was collected in EDTA tubes, and spun in a precooled centrifuge at 10,000 g for 10 min at 4°C, after which the plasma was extracted and frozen at −80°C until processing. Plasma for leptin measurement was diluted 10×. Morning corticosterone, insulin and leptin were measured using the ELISA corticosterone kit (Enzo Life Sciences, catalog no. ADI-901-097), Ultra-Sensitive Mouse Insulin ELISA Kit (catalog no.90080; Crystal Chem, Inc.) and the Mouse Leptin ELISA Kit (catalog no. 90030; Crystal Chem, Inc.) respectively, according to the manufacturer’s instructions.
Glucose tolerance test (GTT) and insulin tolerance test (ITT)

GTT and ITT were performed as described previously (Ayala et al. 2010, van der Kooij et al. 2018). Animals were subjected to food deprivation for 15 h in GTT and for 6 h in ITT before glucose measurement. Peripheral blood glucose measurements were taken at baseline and at 30-min intervals for 2 h following i.p. injection of a glucose bolus (2 g/kg) or insulin bolus ((0.5 U/kg), Sigma-Aldrich), respectively. GTT was performed on day 12/13 post-CSD and ITT on day 22/23 post-CSD. Statistical analyses for GTT and ITT were performed on the area under the curve (AUC).

Statistical analysis

Values are expressed as mean ± s.e.m. Statistical outliers were defined as values ± twice the s.d. from the mean and were excluded from further analysis. Unpaired two-tailed Student's t tests were used to compare sets of data obtained from two independent groups of animals, two-way ordinary ANOVA or two-way repeated measures ANOVA followed by Sidak’s post hoc tests were used when appropriate. P-values are reported in figure legends, statistical significance was considered at the P < 0.05 level; a P < 0.1 was considered as a trend. All data were analyzed using Prism version 6 (GraphPad Software Inc.).

Results

First, we examined separately the effects of HFD given post-CSD (HFD-post) vs the impact of HFD given lastingly (during as well as after CSD: HFD-last) in blood glucose levels at 48 h after conclusion of the CSD paradigm (Fig. 1A).

HFD-induced hyperglycemia is lower in conjunction with chronic stress exposure

CSD increased blood glucose levels 48 h after stress-induction in CSD-exposed mice under SD conditions, as before (Fig. 1B) (van der Kooij et al. 2018). Moreover, HFD induced an increase of blood glucose in non-stressed control mice, regardless the time point of HFD intervention. Unexpectedly, CSD completely suppressed the HFD-induced increases in blood glucose levels in both groups of mice (Fig. 1B), independently of the starting point of HFD treatment. Next, we investigated the interaction of CSD and HFD on the metabolic phenotype.

CSD does not interfere with HFD-post-induced alterations in glucose clearance and does not affect insulin-mediated blood glucose reductions

Glucose breakdown was reduced in HFD-post-treated mice as compared to SD-treated mice, and CSD-exposed mice displayed a trend (P = 0.07) to facilitate glucose breakdown (Fig. 2A and B). In the ITT, HFD-post treatment tended to reduce the recovery to normoglycemia after an insulin bolus whereas CSD exposure did not affect the outcome (Fig. 2C and D).
Metabolic effects of high fat diet and stress

Both CSD and HFD-post boost caloric intake but only HFD-post leads to an increase in body weight

Caloric intake was higher in HFD-post treated mice compared to SD-treated mice and within HFD-post-treated mice; CSD-exposed mice consumed more calories than their respective controls (Fig. 3A). HFD-post treatment also increased animals’ body weights, similarly for CTRL-HFD-post and CSD-HFD-post mice, as compared to regular chow-fed mice (Fig. 3B).

HFD-post increases leptin and fat tissue independent of stress-exposure

Plasma insulin levels were unaffected by HFD-post and CSD-exposure (Fig. 4A). Plasma leptin levels were increased by HFD-post, independent of stress exposure (Fig. 4B). HFD-post increased eWAT, sWAT and BAT in both groups (Fig. 4C, D and E). Whereas CSD had no effect on eWAT (Fig. 4C) and sWAT (Fig. 4D), CSD had an overall decreasing effect on BAT weight (Fig. 4E). As previously mentioned (van der Kooij et al. 2018), CSD-treated mice exhibited higher adrenal gland weights whereas HFD-post non-stressed mice presented lower adrenal weights (Fig. 4F).

HFD-last-induced effects on glucose tolerance are less pronounced in CSD-exposed mice

Glucose homeostasis in the GTT was perturbed in CTRL-HFD-last mice, as these animals exhibited significantly increased glucose levels after an i.p. glucose bolus. Additionally, this impaired glucose homeostasis...
was diminished when HFD-last mice were also exposed to CSD (Fig. 5A and B). The response to insulin in the ITT was not affected by either diet or CSD-exposure (Fig. 5C and D).

**Stressed mice display reduced HFD-last-induced obesity, despite similar caloric intake**

Energy intake between CTRL-HFD-last and CSD-HFD-last mice was unaltered but a higher caloric intake was found for HFD-last-treated animals compared to SD-exposed animals (Fig. 6A). Similar to our results pertaining HFD-post (Fig. 3B), HFD-last treated mice exhibited significantly increased body weight over the course of approximately 8 weeks as compared to both regular chow-fed mice (Fig. 6B). However, HFD-induced increases in body weight were lower for CSD-HFD-last as compared to CTRL-HFD-last mice. In contrast, CTRL-SD and CSD-SD did not differ in body weight.

**HFD-last induced effects on insulin, leptin and fat tissue weights are curtailed in stress-exposed mice**

Pertaining to plasma insulin, we observed an overall reducing effect of CSD on insulin levels (Fig. 7A). HFD-last increased peripheral insulin levels, but this increase was dampened in CSD-exposed mice. CSD exhibited an overall reduction on leptin levels, while HFD-last induced leptin levels in CTRLs were significantly decreased in combination with CSD exposure (Fig. 7B). The changes in body weight in response to HFD and CSD (Fig. 6B) were reflected by
similar changes in adipose tissue weight. eWAT and sWAT did not differ between CTRL-SD and CSD-SD mice (Fig. 7C and D). HFD-last induced increases of eWAT (Fig. 7C) and sWAT (Fig. 7D) in CTRL mice, but these increases were lessened in CSD-exposed mice. BAT-weights were lower in response to CSD but higher after HFD-last (Fig. 7E). CSD exhibited an overall increasing effect on adrenal gland weights with no additional effect of HFD-last treatment (Fig. 7F).

**HFD-last prevents the stress response seen after chronic social defeat**

In a new group of animals, we investigated the effects of CSD and HFD on behavioral activity, stress activation and inflammatory processes in adipose tissue 1–2 weeks post-CSD. Locomotor activity was decreased in CSD-treated animals as compared to their respective controls, but post-testing did not reveal statistically significant differences for the individual treatment groups (SD: $-7.2\%$; HFD-post: $-12.5\%$; HFD-last: $-12.7\%$, Fig. 8A). Plasma corticosterone levels were increased and adrenal glands were enlarged for CSD-SD and CSD-HFD-post, but these effects were absent for CSD-HFD-last (Fig. 8B and C). mRNA levels of IL-6 in the eWAT were not affected by stress exposure but were reduced in response to HFD-post and HFD-last (Fig. 8D). mRNA levels of IL-1β and TNFα in eWAT were not affected by either stress exposure or diet (Table 1).

**Discussion**

When stressed, humans tend to shift their food-intake in favor of a calorically dense diet. Similarly, rodents exposed to chronic stress also increase the intake of highly calorically dense food in favor of a calorically dense diet. Similarly, rodents exposed to chronic stress also increase the intake of highly calorically dense food in favor of a calorically dense diet.
Palatable food, such as diets containing high content of fat and/or glucose (Pecoraro et al. 2004, La Fleur et al. 2014a). The consumption of hyper-caloric food under emotional stress as comfort food contributes to stress relief. Joining stress-induced hyperphagia, however, a desire for comfort food may accelerate the risk for the metabolic syndrome. Dietary effects on stress-sensitivity in animal models are inconsistent where some studies support the hypothesis that a high-fat diet decreases stress impact but others find an aggravation of stress-effects (Eudave et al. 2018). Since CSD exposure leads to lasting hyperglycemia, already on a normal diet (Kumar et al. 2013, van der Kooij et al. 2018), we found it particularly interesting to investigate the synergistic consequences of CSD and HFD on glucose homeostasis and associated metabolic parameters. We hypothesized that stress-induced hyperphagia and hyperglycemia combined with a HFD would accelerate the progression toward the metabolic syndrome. Contrasting with our expectations, however, HFD availability during CSD prevented HFD-related metabolic disturbances.

This conclusion is based on two observations. First, most of the HFD-last induced metabolic alterations, including a reduced glucose tolerance, elevated insulin and leptin concentrations; obesity as well as increases in white and brown adipose tissue were absent or less pronounced in the stress-exposed animals (CSD-HFD-last). Second, we did not observe a similar interaction between CSD and HFD when the dietary change took place alone.

Figure 7
HFD-last induced increases in insulin, leptin and fat tissues were reduced in CSD-exposed mice. (A) CSD had an overall decreasing effect on plasma insulin levels. HFD-last-induced an overall rise of insulin levels, but these were dampened in CSD-HFD-last (diet: $F_{1,31} = 30.16$, $P < 0.001$; stress: $F_{1,31} = 7.21$, $P = 0.01$; $n = 8–10/group$; CTRL-SD vs CSD-SD: $t = 0.58$, $df = 31$, $P = n.s.$; CTRL-SD vs CTRL-HFD-last: $t = 5.27$, $df = 31$, $P = 0.001$; CTRL-HFD-last vs CSD-HFD-last: $t = 3.26$, $df = 31$, $P < 0.05$). (B) CSD reduced plasma leptin and HFD-last increased leptin levels; HFD-last-induced increase of leptin in CTRL was reduced in stressed mice (interaction: $F_{1,31} = 4.4$, $P = 0.04$; diet: $F_{1,31} = 58.33$, $P < 0.001$; stress: $F_{1,31} = 9.88$, $P = 0.004$; $n = 8–10/group$; CTRL-SD vs CSD-SD: $t = 0.71$, $df = 33$, $P = n.s.$; CTRL-SD vs CTRL-HFD-last: $t = 7.0$, $df = 33$, $P < 0.001$; CSD-SD vs CSD-HFD-last: $t = 3.87$, $df = 33$, $P < 0.01$; CSD-HFD-last vs CSD-HFD-last: $t = 3.87$, $df = 33$, $P < 0.01$). (C) While SD did not induce changes between CTRL and CSD, HFD-last-induced increase in CTRL was reduced in CSD concerning eWAT weight (diet: $F_{1,32} = 106.4$, $P < 0.001$; stress: $F_{1,32} = 8.46$, $P < 0.007$; $n = 8–10/group$; CTRL-SD vs CSD-SD: $t = 1.09$, $df = 32$, $P = n.s.$; CTRL-SD vs CTRL-HFD-last: $t = 8.04$, $df = 32$, $P < 0.001$; CSD-SD vs CSD-HFD-last: $t = 6.5$, $df = 32$, $P < 0.001$; CTRL-HFD-last vs CSD-HFD-last: $t = 3.02$, $df = 32$, $P < 0.05$) and (D) sWAT weight (interaction: $F_{1,30} = 16.25$, $P < 0.001$; diet: $F_{1,30} = 258.0$, $P < 0.001$; stress: $F_{1,30} = 28.71$, $P < 0.001$; $n = 7–9/group$; CTRL-SD vs CSD-SD: $t = 0.97$, $df = 30$, $P = n.s.$; CTRL-SD vs CTRL-HFD-last: $t = 13.76$, $df = 30$, $P < 0.001$; CSD-SD vs CSD-HFD-last: $t = 8.81$, $df = 30$, $P < 0.001$; CTRL-HFD-last vs CSD-HFD-last: $t = 6.43$, $df = 30$, $P < 0.001$). (E) CSD exhibited an overall reducing effect on BAT weight, while HFD-last increased BAT fat mass (diet: $F_{1,31} = 14.3$, $P < 0.001$; stress: $F_{1,31} = 10.66$, $P < 0.01$; $n = 7–10/group$; CTRL-SD vs CSD-SD: $t = 1.68$, $df = 31$, $P = n.s.$; CTRL-SD vs CTRL-HFD-last: $t = 3.2$, $df = 31$, $P < 0.05$; CTRL-HFD-last vs CSD-HFD-last: $t = 2.92$, $df = 31$, $P < 0.05$). (F) While CSD induced adrenal hyperplasia, adrenal gland weights were not affected by HFD-last treatment (stress: $F_{1,34} = 8.31$, $P < 0.01$; diet: $F_{1,34} = 0.45$, $P = 0.51$; $n = 9–10/group$; CTRL-SD vs CSD-SD: $t = 2.91$, $df = 34$, $P < 0.05$; CTRL-HFD-last vs CSD-HFD-last: $t = 1.22$, $df = 34$, $P = n.s.$). Data are mean ± s.e.m.; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; two-way ANOVA with Sidak’s multiple comparisons posttest. A full color version of this figure is available at https://doi.org/10.1530/JOE-20-0633.
place immediately after stress (HFD-post). HFD-post treated mice, both stressed and controls, shared many of the metabolic changes seen in CTRL-HFD-last treated mice. The diminished metabolic response of CSD-exposed mice to HFD-last cannot be explained by adjustments in caloric intake, as both groups showed similar caloric intake. Importantly, the adrenal glands were enlarged and plasma corticosterone levels were increased in stressed mice, suggesting that our stress paradigm was effective.

Early after chronic stress (48 h), we measured glucose levels in the blood and replicated the CSD-induced hyperglycemia reported previously in regular chow-fed mice (van der Kooij et al. 2018). Glucose levels were also elevated in HFD-fed mice, but unexpectedly, this HFD-induced hyperglycemia (both HFD-post and HFD-last) was dampened in stressed mice, conceivably reflecting heightened energy expenditure during stress exposure, owing to a negative energy balance, as previously reported (Coccurello et al. 2018). Under HFD-post conditions, caloric intake was consistently increased for CSD-exposed mice, as compared to their respective controls. Possibly, this CSD-induced augmented food consumption, seen with CSD-HFD-post, represents compensatory adjustments aimed at coping with a negative energy balance resulting directly from CSD. The absence of a comparable intensified caloric intake for CSD-HFD-last, compared to CTRL-HFD-last, may be important in explaining why stressed mice in the HFD-last cohort are partially protected from HFD-induced metabolic disturbances. We found that altered locomotor activity cannot explain this stress-associated negative energy balance, since stress-exposed mice did not display hyperactivity in the open field test. Previously, a CSD paradigm lasting 21 days was found to induce hypophagia and weight loss (Balsevich et al. 2014). Here it was found that CSD-treated mice were protected from metabolic disturbances by an 8 weeks lasting HFD regime, preceding the stressor, these findings thus resemble some of the aspects seen with CSD-HFD-last treated mice in the current study.

Figure 8
HFD-last blocks the hypercortisolemia and adrenal hypertrophy typically seen after CSD. (A) Overall, locomotor activity was decreased in CSD-treated animals (F(2,56) = 10.96, P < 0.01; n = 10–11/group) but these differences were not significant at group level after post-testing (CTRL-SD vs CSD-SD: t = 1.2, df = 56, P = n.s.; CTRL-HFD-post vs CSD-HFD-post: t = 2.2, df = 56, P = n.s.; CTRL-HFD-last vs CSD-HFD-last: t = 2.3, P = n.s.). (B) Blood plasma corticosterone concentrations were increased for CSD-SD and CSD-HFD-post, but not for CSD-HFD-last as compared to their respective controls (stress: F(2,56) = 16.66, P < 0.001; CTRL-SD vs CSD-SD: t = 2.84, df = 56, P < 0.05; CTRL-HFD-post vs CSD-HFD-post: t = 3.25, df = 56, P < 0.01; CTRL-HFD-last vs CSD-HFD-last: t = 0.91, df = 56, P = n.s.). (C) Adrenal weights were increased for CSD-SD and CSD-HFD-post, but not for CSD-HFD-last as compared to their respective controls (diet: F(2,57) = 9.34, P < 0.01; stress: F(2,57) = 24.89, P < 0.001; CTRL-SD vs CSD-SD: t = 3.37, df = 57, P < 0.01; CTRL-HFD-post vs CSD-HFD-post: t = 3.67, df = 57, P < 0.01; CTRL-HFD-last vs CSD-HFD-last: t = 1.56, df = 57, P = n.s.). (D) mRNA levels of IL-6 were not affected by stress exposure, but were reduced in response to HFD (stress: F(1,49) = 0.01, P = 0.93; diet: F(2,49) = 7.45, P < 0.01, n = 9–10/group; SD vs HFD-post: t = 3.67, df = 49, P < 0.01; SD vs HFD-last: t = 2.96, df = 49, P < 0.05; HFD-post vs HFD-last: t = 0.73, df = 49, P = 0.85). Data are mean ± s.e.m.; *P < 0.05, **P < 0.01; two-way ANOVA with Sidak’s multiple comparisons posttest. A full color version of this figure is available at https://doi.org/10.1530/JOE-20-0633.
Our present findings suggest that a coupling between caloric intake and metabolic demands during stress may protect from HFD-induced obesity and, therefore, HFD availability during stress-induced hyperphagia in CSD may be beneficial. This metabolic coupling, between HFD-last and CSD, is absent in HFD-post and may explain its inability to address the augmented energy demand during CSD. On a related note, we reported previously that caloric restriction during CSD, in an attempt to counteract stress-induced hyperphagia, was not beneficial in restoring CSD-induced hyperglycemia or cognitive impairments (van der Kooij et al. 2018). Metabolic coupling between the energetic demands during stress and dietary availability is probably important in explaining our findings, particularly those pertaining to the differences seen in CSD-HFD-last vs CTRL-HFD-last. Herein, a differential HPA axis activation probably plays a central role.

Specifically, we observed stress-induced adrenal hyperplasia as well as hypercortisolemia in SD-treated and HFD-post-treated mice but not in HFD-last-treated animals. Hence, we argue that HFD may have acted as comfort food, where its availability during CSD may have reduced the effects of stress itself by dampening HPA-axis activation (Pecoraro et al. 2004, Ulrich-Lai et al. 2007). Although CSD did not prevent metabolic disturbances under a HFD-post regime, an exacerbation of metabolic-related disturbances was not seen either. That the metabolic profile of CSD-HFD-post treated mice did not worsen, as compared to CTRL-HFD-post, could be related to the short delay between the end of CSD and the start of HFD. In that sense, HFD-post may still have retained some residual effect as comfort food in CSD-stressed mice and accordingly, we hypothesize that a further delay in HFD-treatment onset, relative to CSD conclusion, would have aggravated metabolic disturbances.

Inflammatory processes have been linked to stress-associated functional impairments, with a distinct role for IL-6, which can infiltrate the brain in response to CSD (Menard et al. 2017). Additionally, HFD can promote the central as well as peripheral inflammatory tone (Guillemot-Legris et al. 2016, Suárez et al. 2019). Considering the pronounced effects of our dietary treatments on peripheral stress activation and the well-known anti-inflammatory properties of glucocorticoids, we decided to investigate the effects of HFD and CSD on mRNA levels of inflammatory cytokines (IL-1β, TNFα and IL-6) in eWAT. We did not observe any effect of CSD on the gene expression levels of these inflammatory markers, which may be connected the measurements taking place early post-CSD (Guillemot-Legris et al. 2016). Regardless of stress-exposure, mRNA levels of IL-6, but not IL-1β or TNFα, were reduced for HFD-treated mice (both HFD-post and HFD-last). Seeing the particular involvement of IL-6 in CSD-associated pathologies, it will be interesting to investigate whether a HFD has the potential to halt a CSD-induced IL-6 cerebral influx.

Overall, HFD-treated mice (both HFD-last and HFD-post) featured a number of physiological hallmarks indicative of the metabolic syndrome. CTRL-HFD-last presented most signs of the metabolic syndrome evidenced by obesity, enlarged adipose tissue, impaired glucose homeostasis, hyperinsulinemia and enhanced leptin levels. However, even in CTRL-HFD-last, insulin sensitivity was not affected. Hence, despite all metabolic changes, diabetes did not develop.

Circulating plasma insulin levels were higher for HFD-last treated animals and this elevation was stronger for CTRL-HFD-last than for CSD-HFD-last. The most parsimonious explanation for this difference in insulin levels is related to body weight. Obesity is namely associated with hyperinsulinemia (Lichtenstein & Schwab 2000, de Ferranti & Mozaffarian 2008) and insulin levels at sacrifice are reflected by animals’ body weights with CTRL-HFD-last being the heaviest, followed by CSD-HFD-last. In general, SD-treated mice exhibited much lower body weights than HFD-treated mice and we observed no additional effects of CSD on body weight. In line, insulin levels did not differ between SD-treated controls and CSD-exposed mice. We do not have a clear explanation as to why insulin levels for HFD-post treated mice in both groups, controls and stressed, were unaltered. The briefier HFD-availability for HFD-post, as compared

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**Table 1** The effects of chronic social stress and HFD on relative mRNA levels in eWAT of the pro-inflammatory cytokines IL-1β and TNFα.

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th></th>
<th>CSD</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>HFD-post</td>
<td>HFD-last</td>
<td>HFD-post</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.56 ± 0.03</td>
<td>1.59 ± 0.01</td>
<td>1.55 ± 0.02</td>
<td>1.54 ± 0.01</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.52 ± 0.02</td>
<td>1.50 ± 0.01</td>
<td>1.48 ± 0.01</td>
<td>1.49 ± 0.01</td>
</tr>
</tbody>
</table>

CSD, chronic social defeat; eWAT, epididymal white adipose tissue; HFD, high fat diet; IL-1β, interleukin-1 beta; SD, standard diet; TNFα, tumor necrosis factor α.
to HFD-last, may partially explain the absence of hyperinsulinemia in HFD-post mice. Congruently, glucose levels and body weight are known to be increased already 1 week after HFD while circulating insulin levels increased progressively with time (Winzell & Ahrén 2004). Compatible with the idea that differences in HFD-exposure could have led to the diverging circulating insulin levels is the observation that body weights at sacrifice were higher for HFD-last than HFD-post.

Leptin impacts food intake and plays an important role in energy balance including glucose metabolism and diabetes (Klok et al. 2007, Meek & Morton 2016). Since insulin and glucocorticoids promote leptin secretion from adipocytes (Wabitsch et al. 1995), we surmised that CSD-HFD interactions in our study may be mirrored by changes in leptin levels. Circulating leptin levels at sacrifice were increased in HFD-treated mice (both HFD-last and HFD-post) and these elevations coincided with adipose tissue weight. Hence, seeing that obesity, body weight gain and adipose tissue content in CSD-HFD-last treated mice was less pronounced than in CTRL-HFD-last, the lower leptin levels in CSD-HFD-last were in line with expectations.

Our findings are limited as several mediators other than glucose, insulin and leptin are also important in the interaction between stress and metabolism, which we did not investigate in this study. Ghrelin, for example, is stress-inducible, promotes appetite and adiposity and is thought to be key in meeting energetic demands imposed by chronic stress (Patterson et al. 2013). Additionally, interactions between hypothalamic signaling and the liver have been identified as pivotal in energy metabolism by regulating food intake, glucose homeostasis and circadian rhythms (La Fleur et al. 2014b, García-Cáceres et al. 2016). Herein, neuropeptide Y has emerged as an important mediator between stress and food intake (Kuo et al. 2007, Wu et al. 2011, Reichmann & Holzer 2016). Studying the potential involvement of these mediators in future research will doubtlessly lead to novel insights and may be instrumental in explaining the differential metabolic effects of HFD-last vs HFD-post in our stressed mice.

Our data suggest that interactions between CSD and HFD, affecting whole body metabolism, may critically depend on sensitive time windows. The impact of chronic stress, including CSD, on energy metabolism is well recognized (Picard et al. 2018, van der Kooij et al. 2020). In the current study, HFD-availability during CSD paradoxically ameliorated the development of HFD-induced metabolic disturbances and may have acted as comfort food by compensating for energetic demands during stress.

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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