

Interplay between diet-induced obesity and chronic stress in mice: potential role of FKBP51

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Abstract

While it is known that stress promotes obesity, the effects of stress within an obesogenic context are not so clear and molecular targets at the interface remain elusive. The FK506-binding protein 51 (FKBP51, gene: *Fkbp5*) has been identified as a target gene implicated in the development of stress-related psychiatric disorders and is a possible candidate for involvement in stress and metabolic regulation. The aims of the current study are to investigate the interaction between chronic stress and an obesogenic context and to additionally examine whether FKBP51 is involved in this interaction. For this purpose, male C57BL/6 mice were exposed to a high-fat diet for 8 weeks before being challenged with chronic social defeat stress. Herein, we demonstrate that chronic stress induces hypophagia and weight loss, ultimately improving features arising from an obesogenic context, including glucose tolerance and levels of insulin and leptin. We show that *Fkbp5* expression is responsive to diet and stress in the hypothalamus and hippocampus respectively. Furthermore, under basal conditions, higher levels of hypothalamic *Fkbp5* expression were related to increased body weight gain. Our data indicate that *Fkbp5* may represent a novel target in metabolic regulation.

Key Words

- ▶ glucocorticoid receptor
- ▶ obesity
- ▶ HPA axis
- ▶ gene expression

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Introduction

The global prevalence of obesity is rising (Yach *et al.* 2006), emphasizing the need to decipher the complex regulatory mechanisms underlying energy balance. Evidence indicates that chronic stress is a risk factor for obesity development (Tamashiro 2011). Nevertheless, the relationship between stress and metabolic regulation is very complex and not easily interpreted. For example, in response to stress, some individuals lose weight, whereas others gain weight (Kivimaki *et al.* 2006, Dallman 2010). The seemingly opposite stress-induced metabolic outcomes in humans are also observed in animal models (Weninger *et al.* 1999, Kim *et al.* 2003, Michel *et al.* 2005,

Moles *et al.* 2006, Kuo *et al.* 2008, Bartolomucci *et al.* 2009) and highlight the intricate relationship between stress and metabolic regulation. Stress and metabolic regulation share common regulatory pathways orchestrated by neural networks centered in the hypothalamus. The hypothalamic–pituitary–adrenal (HPA) axis is a central component of the stress response (de Kloet *et al.* 2005) and is also involved in energy balance (Akana *et al.* 1994).

In response to an acute stressor, changes in HPA axis activity are generally regarded as adaptive and aim to re-establish homeostasis. However, such changes may become maladaptive when the stressor persists

(McEwen 2007). For example, alterations in HPA axis function on account of chronic stress are considered a major risk factor for both psychiatric and metabolic disorders (Holsboer 2000, Tamashiro 2011). Similarly, perturbations in glucocorticoid receptor (GR) signaling, which plays an important role in the glucocorticoid-mediated negative feedback loop for the termination of the stress response (Ulrich-Lai & Herman 2009), have been implicated in the development of obesity (Bjorntorp & Rosmond 2000, Pasquali *et al.* 2006, Grun & Blumberg 2007). Furthermore, it has been shown that the energy status of an individual itself, reflected in levels of circulating metabolic hormones, is able to directly affect HPA axis activation (Bagdade *et al.* 1967, Akana *et al.* 1994, Considine *et al.* 1996, Hallschmid & Schultes 2009, Roubos *et al.* 2012). Indeed, it is evident that both chronic stress and diet have the potential to interact in order to modulate both metabolic and neuroendocrine phenotypes.

Research on the interaction between stress and obesity has focused primarily on the role of chronic stress in mediating obesity. Although these studies are important for understanding the role of chronic stress in development of obesity, research is also required to identify mechanisms responsible for the emergence of various pathophysiologies once obesity has already been established. Despite the rising rates of both obesity and chronic social stress, relatively little research has addressed the question as to how an obese individual responds to chronic stress. One study investigated the behavioral outcomes of chronic stress exposure in a mouse model of diet-induced obesity (DIO) (Finger *et al.* 2011). The authors found that diet-induced obese mice were resistant to selective stress-induced anxiety- and depressive-like symptoms. However, there is a paucity of data identifying molecular targets involved in the interplay between DIO and chronic stress.

Emerging literature on possible mechanisms underlying stress-induced psychiatric disorders have identified the *Fkbp5* gene, encoding the FK506-binding protein 51 (FKBP51), as a novel candidate gene (Binder *et al.* 2004, 2008, Ising *et al.* 2008, Binder 2009, Zimmermann *et al.* 2011). Interestingly, mice deficient in FKBP51 display a moderately lean phenotype under basal conditions (Hartmann *et al.* 2012, Sanchez 2012). In this regard, *Fkbp5* is an interesting candidate gene to study in the context of stress and metabolic regulation.

In the brain, *Fkbp5* is expressed ubiquitously, with high expression levels in the hippocampus and the hypothalamus (Scharf *et al.* 2011). FKBP51 is well recognized for its ability to regulate GR sensitivity and

HPA axis functioning (Touma *et al.* 2011, Hartmann *et al.* 2012). Most notably, FKBP51 acts as a negative regulator of GR by reducing nuclear translocation of the GR complex and ligand-binding sensitivity (Davies *et al.* 2002, Wochnik *et al.* 2005, Binder 2009). Interestingly, functional polymorphisms within *Fkbp5* interact with environmental cues, namely early life trauma, to predict the risk of developing various psychiatric disorders (Binder *et al.* 2008, Roy *et al.* 2010, Xie *et al.* 2010, Collip *et al.* 2013), indicating that *Fkbp5* is sensitive to the environment and may be an important mediator of other gene \times environment interactions. In this context, studies demonstrated that food deprivation (FD) induces *Fkbp5* expression in the brain (Scharf *et al.* 2011, Yang *et al.* 2012). In the study by Scharf and colleagues, FD was used as a stressor, but intuitively, changes resulting from FD may also represent a response to the metabolic challenge.

Taken together, there is evidence to suggest that FKBP51 may be involved in the interplay between stress and metabolic regulation. Thus, in this study, we examined the interaction between chronic stress and an obesogenic context. Furthermore, we examined whether a possible interaction exists between FKBP51 and chronic stress in order to modulate metabolic-related readouts.

Materials and methods

Animals and animal housing

Initially, 5-week-old male C57BL/6 mice (Charles River Laboratories, Maastricht, The Netherlands) were housed in groups for 6 weeks. Mice were maintained on a 12 h light:12 h darkness cycle, with controlled temperature (22 ± 2 °C) and humidity ($55 \pm 5\%$). Mice were allowed to access high-fat (58% kcal from fat, D12331) or control chow diet *ad libitum* (10.5% kcal from fat, D12329, Research Diets, Inc., New Brunswick, NJ, USA), which, except for the fat content, were identical. After 6 weeks, mice were housed individually for an additional 2 weeks, maintained on their respective diets, before the onset of testing. Body weight and food intake were measured weekly. The experiments were carried out in accordance with the European Communities' Council Directive 2010/63/EU. The protocols were approved by the committee for the Care and Use of Laboratory animals of the Government of Upper Bavaria, Germany.

Experimental design

After 8 weeks on the dietary regimen, each diet group was randomly divided into stressed and control groups,

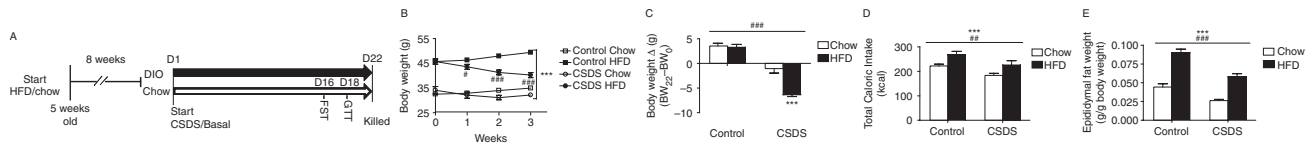


Figure 1

Experimental design and phenotypic features during CSDS. (A) Experimental time course. Mice were exposed to 21 days of CSDS or left under basal conditions following 8 weeks of exposure to a HFD or chow dietary regimen. Behavioral and metabolic readouts were carried out in the final week of the CSDS procedure. (B) Body weights of chow-fed and HFD-fed mice during the CSDS procedure. (C) Stress-induced changes in body weight and (D) total caloric intake in chow-fed and HFD-fed mice calculated as the

average total caloric intake throughout the entire CSDS procedure. (E) Effect of CSDS on relative epididymal fat pad weight in mice fed on chow or HFD. Body weight during CSDS was analyzed by repeated measures two-way ANOVA. All other data were analyzed by two-way ANOVA followed by Student's *t*-test. ****P*<0.001, ##*P*<0.01, ###*P*<0.001; *significant diet effect and #significant stress effect.

balanced for body weight (chronic stress: high-fat diet (HFD) (*n*=10), chow (*n*=10) and control: HFD (*n*=11), chow (*n*=10)) (Fig. 1A). Mice were challenged with 3 weeks of chronic social defeat stress (CSDS) or left under control (basal) conditions. Body weight and food intake were recorded daily. The forced swim test (FST) was performed on day 16 of the 21-day stress procedure, and on day 18, the glucose tolerance test (GTT) was performed.

home cages to recover. At 30-min (stress response) and 90-min (stress recovery) after the onset of the FST, blood samples were collected by tail cut (Flutter *et al.* 2000). Samples were collected in EDTA-coated microcentrifuge tubes (Kabe Labortechnik, Nümbrecht-Elsenroth, Germany) and later centrifuged at 6000 *g* at 4 °C for 15 min. Plasma was collected and stored at −20 °C.

CSDS procedure

The CSDS paradigm lasted for 21 days and was conducted as described previously (Wagner *et al.* 2011, 2012). Briefly, experimental mice were placed in the home cage of a dominant CD1 resident mouse. Interaction between the mice was permitted until the experimental mouse was attacked and defeated by the CD1 aggressor. Mice were subsequently separated by a wire mesh divider that prevented physical contact but maintained sensory contact for 24 h. Each day, for 21 days, the procedure was repeated with a different unfamiliar CD1 aggressor mouse. The control mice were housed in their home cages throughout the CSDS procedure. Both control and stressed mice were handled daily during the course of the stress procedure.

Intraperitoneal glucose tolerance test

Mice were fasted for 14 h and subsequently received intraperitoneal injections of 2 g/kg body weight of D-glucose. Blood glucose levels were assessed from blood collected by tail cut at 0, 30, 60, and 120 min intervals following the glucose load. Glucose levels were measured using a handheld Contour XT glucometer (Bayer Health Care, Basel, Switzerland).

Forced swim test

Mice were placed in a 2-l glass beaker filled with water (22±1 °C) to a height of 15 cm, so that the mouse could neither touch the bottom nor escape. The test lasted 6 min. Time immobile, time struggling, and latency to immobility were scored (Porsolt *et al.* 1977).

Tissue collection and processing

Mice were anesthetized with isoflurane and then killed by decapitation. Basal trunk blood was collected and processed (as described above). Brains were removed, snap frozen, and stored at −80 °C until use. Thymus and adrenal glands were removed, pruned from fat, and weighed. Epididymal fat was collected and weighed. All tissue weights are expressed as relative weights (weight (mg)/body weight (g)).

Acute stress response

The FST served as an acute stressor. At the conclusion of the FST, animals were towel dried and returned to their

In situ hybridization

Coronal whole-brain sections were cryosectioned at 18 μm thickness and directly thaw mounted onto Super Frost Plus Slides as eight sequential series. A single series of sections was selected for each ³⁵S UTP-labeled ribonucleotide probe (*Fkbp5*, *Gr (Nr3c1)*, and *Mc4r*) or ³⁵S ATP-labeled oligonucleotide probe (*Pomc*). The antisense riboprobe

was transcribed from a linear plasmid for *Fkbp5* (forward primer: 5'-CTTGACCACGCTATGGTTT-3'; reverse primer: 5'-GGATTGACTGCCAACACCTT-3'), *Gr* (forward primer: 5'-AGGTCGACCAGCCGTCCAGA-3'; reverse primer: 5'-AAGCTTGCCTGGCAATAAAC-3'), *Mc4r* (forward primer: 5'-GCAACAGCAGACTGGTCAA-3'; reverse primer: 5'-CACAGCCAGGCTACAGATGA-3'), and *Pomc* (forward primer: 5'-GGGTCCCTCCAATCTTGT-3'; reverse primer: 5'-ACGTTGGGTACACCTTCAC-3'). *In situ* hybridization was performed as described previously (Schmidt *et al.* 2007). For signal detection, the slides were exposed to a Kodak Biomax MR film (Eastman Kodak Co.) and developed. Autoradiographic densities were quantified using the NIH ImageJ Software (NIH, Bethesda, MD, USA). Regions of interest were traced from digitized autoradiograms and the mean optical density from two sections was calculated for each animal. The data were analyzed blindly, subtracting the background signal from the measurements.

Hormone quantification

Plasma corticosterone levels were determined by RIA using a commercially available kit (MP Biomedicals, Inc., Solon, OH, USA; sensitivity 12.5 ng/ml). Plasma insulin and leptin levels were determined using a mouse metabolic magnetic bead panel (Millipore Corp., Billerica, MA, USA; sensitivity: insulin 14 pg/ml and leptin 19 pg/ml).

Statistical analysis

All variables were evaluated using the IBM SPSS Statistics 18 Software (IBM SPSS Statistics). Body weight and glucose tolerance were analyzed by repeated measures two-way ANOVA with stress and diet conditions as the between-subject factors. Student's *t*-test was employed for comparison of two independent groups. All other data were analyzed by two-way ANOVA for significant overall effects. For instances where the initial test yielded a significant interaction, Student's *t*-tests (two-tailed) were conducted to locate the interaction effect using simple comparisons. Effect size was calculated for each significant effect. Cohen's *f*-statistic was used to estimate effect sizes for ANOVAs, in which an *f*-value of 0.10, 0.25, and 0.40 reflects a small, medium, and large effect size respectively (Cohen 1992). Cohen's *d*-statistic was similarly used to measure the effect size for Student's *t*-tests, using 0.20, 0.40, and 0.80 as a small, medium, and large effect size respectively. Finally, correlations between metabolic- and neuroendocrine parameters and mRNA expression were

analyzed with the Pearson's product-moment test under basal and CSDS conditions. Fisher's *z*-transformation was then used to compare correlation coefficients between basal and CSDS conditions. Statistical significance was set at $P < 0.05$. Data are expressed as mean \pm s.e.m.

Results

Effects of DIO and stress on body weight parameters

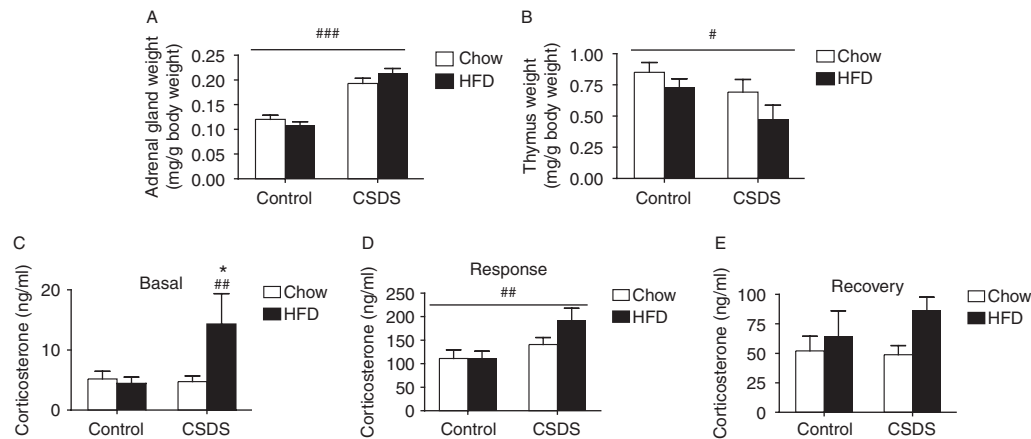
DIO had already been established in mice at the onset of the stress procedure ($T_{30.3} = -9.645$, $d = 3.05$, $P < 0.001$) on account of the preceding 8 weeks of HFD exposure. Over the course of the stress procedure, mice fed on a HFD were heavier than mice fed on chow (time \times diet \times stress: $F(1.3, 49.1) = 27.587$, $f = 0.86$, $P < 0.001$ and diet: $F(1, 37) = 124.982$, $f = 1.84$, $P < 0.001$) (Fig. 1B). At the end of the CSDS, all defeated animals exhibited a reduction in body weight gain (stress: $F(1, 36) = 127.7$, $f = 1.88$, $P < 0.001$), but the stress-induced weight loss was significantly greater among HFD-fed mice compared with chow-fed mice (stress \times diet: $F(1, 36) = 16.296$, $f = 0.67$, $P < 0.001$) (Fig. 1C). Energy intake was also lowered on account of CSDS (stress: $F(1, 37) = 10.177$, $f = 0.52$, $P = 0.003$) and increased on account of the HFD (diet: $F(1, 37) = 12.335$, $f = 0.58$, $P = 0.001$) (Fig. 1D).

The stress-induced body weight loss was recapitulated in the quantification of epididymal fat, which reflected a reduction in relative epididymal fat weight in all defeated animals (stress: $F(1, 37) = 40.988$, $f = 1.05$, $P < 0.001$) as well as a relative increase from a HFD, independent of stress condition (diet: $F(1, 37) = 97.818$, $f = 1.63$, $P < 0.001$) (Fig. 1E).

Effects of DIO and stress on organ weights and corticosterone levels

Increased adrenal gland weight (stress: $F(1, 37) = 94.535$, $f = 1.60$, $P < 0.001$) and reduced thymus weight (stress: $F(1, 33) = 5.240$, $f = 0.40$, $P = 0.029$) resulted from CSDS independent of dietary condition (Fig. 2A and B). Furthermore, mice fed on a HFD tended to present reduced thymus size compared with chow-fed mice (diet: $F(1, 33) = 3.453$, $f = 0.32$, $P = 0.072$).

CSDS elevated basal corticosterone levels only in mice fed on a HFD (stress \times diet: $F(1, 35) = 5.625$, $f = 0.40$, $P = 0.023$) (Fig. 2C). Furthermore, the response to an acute stressor was significantly elevated in mice exposed to chronic stress, regardless of the diet (stress: $F(1, 36) = 8.745$, $f = 0.49$, $P = 0.005$) (Fig. 2D). Corticosterone tended to remain elevated during stress recovery on account of

**Figure 2**

Physiological and neuroendocrine phenotypes in response to CSDS. (A) Relative adrenal gland weight and (B) thymus weight in chow-fed and HFD-fed mice after CSDS. (C) Basal morning corticosterone levels at the end of the CSDS procedure. (D) Corticosterone response and (E) recovery

the HFD regimen (diet: (1,36)=2.847, $f=0.28$, $P=0.100$) (Fig. 2E).

Effects of DIO and stress on depressive-like behavior

There was no effect of stress on any of the behavioral parameters analyzed in the FST (time spent struggling, floating, and latency to float) (Fig. 3). There was a significant effect of diet on the time spent struggling ($F(1,33)=5.066$, $f=0.39$, $P=0.031$) and the latency to float ($F(1,33)=5.069$, $f=0.39$, $P=0.031$), whereby mice fed on a HFD struggled less and began floating sooner compared with chow-fed mice.

Effects of DIO and stress on glucose tolerance and metabolic hormones

Under control conditions, the HFD significantly impaired glucose tolerance (time \times stress: $F(1.7,64.4)=8.648$,

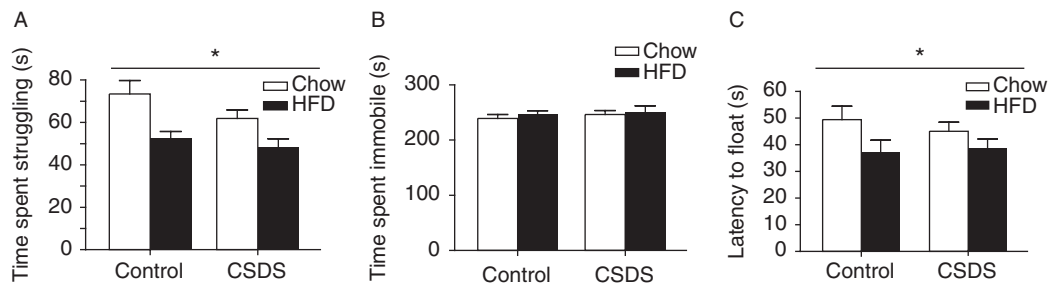
measured 30 and 90 min respectively, after the onset of the FST. All data were analyzed by two-way ANOVA followed by Student's *t*-test.

* $P<0.05$, # $P<0.05$, ## $P<0.01$, ### $P<0.001$, #### $P<0.001$; *significant diet effect and #significant stress effect.

$f=0.48$, $P=0.001$); diet \times stress: $F(1,37)=6.637$, $f=0.42$, $P=0.014$); however, chronic stress exposure counteracted these DIO-induced effects on glucose tolerance (Fig. 4A). Furthermore, CSDS resulted in decreased insulin (stress: $F(1,35)=7.313$, $f=0.46$, $P=0.010$) and leptin ($F(1,36)=15.758$, $f=0.66$, $P<0.001$), whereas exposure to a HFD increased both circulating insulin (diet: $F(1,35)=6.250$, $f=0.42$, $P=0.017$) and leptin levels (diet: $F(1,36)=13.926$, $f=0.62$, $P=0.001$) (Fig. 4B and C).

Effects of DIO and stress on hypothalamic gene expression

A HFD (obesogenic context) significantly increased *Fkbp5* mRNA expression in the ventromedial hypothalamic nuclei (VMH) (diet: $F(1,34)=5.685$, $f=0.41$, $P=0.023$), an effect that was most robust among mice exposed to CSDS (Fig. 5A and B). There was no effect of chronic stress exposure on hypothalamic *Fkbp5* expression.

**Figure 3**

Behavioral response in the forced swim test. (A) Time spent struggling in the FST. (B) Time spent immobile in the FST. (C) Latency to first float in the FST. Data were analyzed by two-way ANOVA. *Significant diet effect, $P<0.05$.

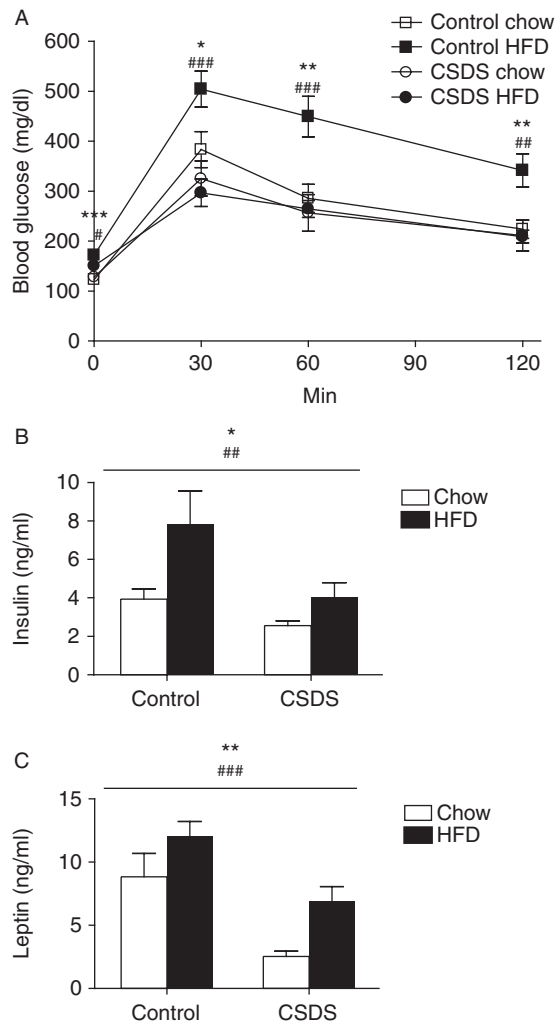


Figure 4 Metabolic profile in response to CSDS. (A) Blood glucose levels during an intraperitoneal glucose tolerance test performed after a 14 h fast. (B) Circulating levels of plasma insulin and (C) leptin collected after 3 weeks of CSDS. The glucose tolerance test was analyzed by repeated-measures two-way ANOVA. All other data were analyzed by two-way ANOVA followed by Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ### $P < 0.01$, #### $P < 0.001$; *significant diet effect and #significant stress effect.

As shown in Fig. 5C and D, VMH *Fkbp5* mRNA levels significantly correlated with total change in body weight ($r = 0.481$, $P = 0.032$) and total caloric intake ($r = 0.551$, $P = 0.001$) under basal conditions. In contrast, this association was lost or tended to reverse (body weight change: $r = -0.481$, $P = 0.070$) under conditions of CSDS (Fig. 5E and F) A Fisher's *z*-transformation revealed that the correlations between basal and CSDS condition differed significantly for total body weight change ($z = 2.51$, $P = 0.012$) but not for total caloric intake ($z = 0.723$, $P = 0.469$).

Gr expression was significantly decreased by an obesogenic environment in both the VMH (diet: $F(1,30) = 9.323$, $f = 0.56$, $P = 0.005$) and arcuate nucleus (diet: $F(1,29) = 4.346$, $f = 0.39$, $P = 0.046$) of the hypothalamus independent of CSDS exposure (Fig. 5G, H and I).

In the PVN, diet significantly elevated MC4R regardless of stress condition (diet: $F(1,36) = 4.518$, $f = 0.36$, $P = 0.040$), but there was no effect of either stress or diet on POMC expression (data not shown).

Effects of DIO and stress on hippocampal gene expression

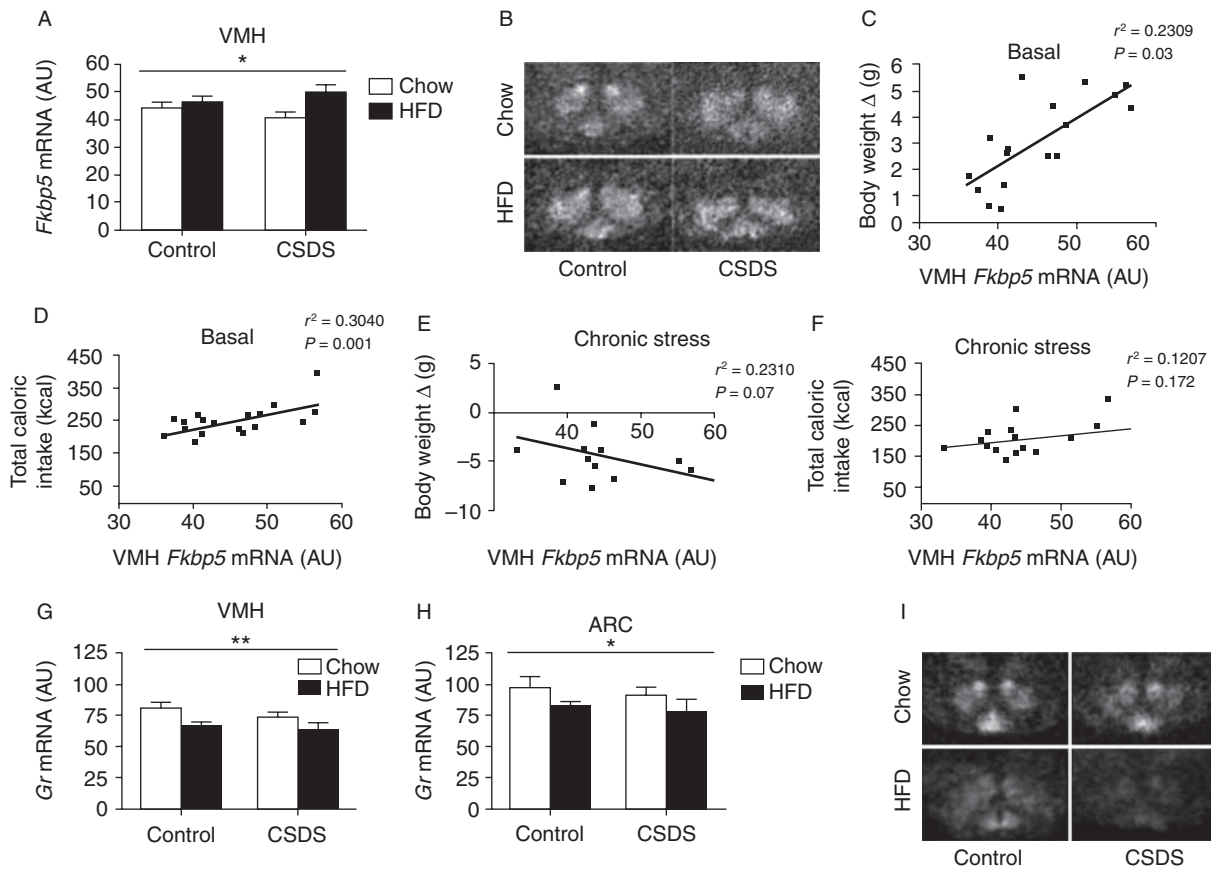
CSDS significantly increased *Fkbp5* mRNA expression in the CA3 region of the hippocampus (stress: $F(1,35) = 5.186$, $f = 0.38$, $P = 0.029$) (Fig. 6A and E). There was no effect of feeding regimen on hippocampal *Fkbp5* expression.

CA3 *Fkbp5* mRNA levels negatively correlated with total caloric intake ($r = -0.494$, $P = 0.037$) and mean caloric efficiency ($r = -0.472$, $P = 0.048$) under conditions of chronic stress (Fig. 6C and D). In contrast, this association does not exist under basal conditions (data not shown). Fisher's *z*-transformation was used to compare the correlation coefficients between basal and CSDS conditions. The analyses revealed that the correlations were not significantly different between conditions (total caloric intake: $z = 1.25$, $P = 0.210$ and mean caloric intake: $z = 0.787$, $P = 0.431$).

Finally, under stressful conditions, higher *Fkbp5* mRNA expression was associated with an improved corticosterone response reflected in the negative correlation between CA3 *Fkbp5* expression and the level of corticosterone measured 30 min after an acute stressor (response corticosterone level) ($r = -0.489$, $P = 0.046$) in the defeated mice (Fig. 6B). The Fisher's *z*-transformation revealed that the correlation between *Fkbp5* expression and corticosterone response differed significantly between basal and CSDS conditions ($z = 2.52$, $P = 0.011$).

Discussion

Little research has been carried out to improve understanding of the dynamic relationship between chronic stress and an obesogenic environment. This study aimed to investigate the direct effects of CSDS in an established diet-induced obese mouse model, and further to investigate FKBP51 regulation relative to metabolic regulation. We clearly demonstrated a body weight reduction on account of chronic stress exposure, which further led to improved glucose tolerance and lower levels of leptin and insulin. Additionally, *Fkbp5* expression was induced in

**Figure 5**

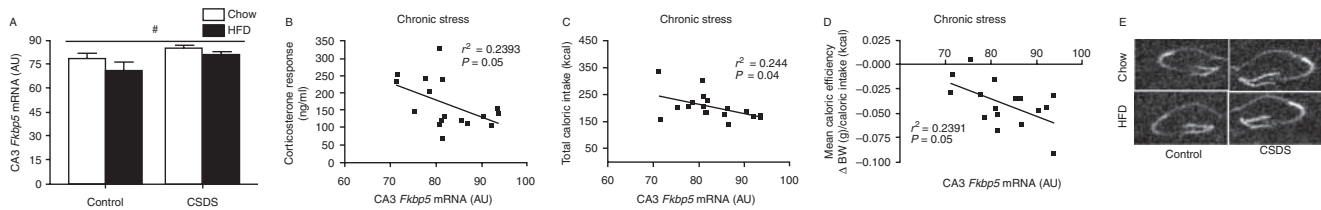
Hypothalamic gene expression and correlational data. Quantitative (A) and qualitative (B) *Fkbp5* mRNA expression in the VMH. (C) Correlational analysis under basal conditions between VMH *Fkbp5* mRNA expression and total body weight change as well as (D) total caloric intake. (E) Correlational analysis under chronic stress conditions between VMH *Fkbp5* mRNA expression and total body weight change as well as (F) total

caloric intake. (G) Quantitative *Gr* mRNA expression in the VMH and (H) ARC of the hypothalamus. (I) Qualitative *Gr* mRNA expression in the VMH and ARC. Expression data were analyzed by two-way ANOVA; correlations were analyzed with the Pearson's product moment. * $P < 0.05$, ** $P < 0.01$; *significant diet effect.

response to diet and stress in the hypothalamus and hippocampus respectively. Correlational analyses revealed that under non-stressed conditions, higher hypothalamic expression of *Fkbp5* is associated with increased body weight gain. Collectively, our data indicate that *Fkbp5* may represent a novel target in metabolic regulation.

We investigated the interaction between chronic stress exposure and obesity by subjecting DIO mice to CSDS. Mice challenged to CSDS presented adrenal hypertrophy and thymus involution, which supports the validity of our stress paradigm (Schmidt *et al.* 2007, Wang *et al.* 2011). Furthermore, chronic stress exposure led to hypophagia. The stress-induced hypophagia probably contributed to the stress-induced body weight loss. In parallel, chronic stress exposure reduced the relative epididymal fat pad mass regardless of the dietary condition.

Glucose intolerance is a clinical feature of the metabolic syndrome and is a feature present in mouse models of DIO (Surwit *et al.* 1988, Ahren & Pacini 2002). As expected, we found impaired glucose tolerance in DIO mice under basal conditions. Interestingly, CSDS was able to offset the effects of the HFD on glucose tolerance. In addition, CSDS lowered circulating levels of leptin and insulin, whereas a HFD led to increased levels of both. Previous studies have also demonstrated that leptin and insulin levels decrease in response to stress (Chuang *et al.* 2010, Finger *et al.* 2011, Solomon *et al.* 2011) and increase following a HFD (Scarpace & Zhang 2007, Chuang *et al.* 2010, Finger *et al.* 2011, Solomon *et al.* 2011). Although our data clearly indicate that CSDS offsets the DIO-induced effects on glucose tolerance and levels of leptin and insulin, it is practically impossible to decipher whether such downstream metabolic phenotypes are a

**Figure 6**

Hippocampal CA3 region gene expression and correlational data. (A) Quantitative *Fkbp5* mRNA expression in the hippocampal CA3 region. (B) Correlational analysis under chronic stress conditions between CA3 *Fkbp5* mRNA expression and corticosterone response. (C) Correlational analysis under chronic stress conditions between CA3 *Fkbp5* mRNA expression and total caloric intake (throughout the stress procedure) as

well as (D) mean caloric efficiency (calculated as the average daily caloric efficiency throughout the entire stress procedure). (E) Qualitative *Fkbp5* mRNA expression in the hippocampus. Expression data were analyzed by two-way ANOVA; correlations were analyzed with the Pearson's product moment. #Significant stress effect, $P < 0.05$.

direct outcome of stress or rather an indirect outcome of differences in body weight. Despite its limitations, this collection of phenotypes confirms stress-induced alterations in body weight regulation and related metabolic phenotypes.

In addition to examining metabolic phenotypes resulting from CSDS exposure in DIO mice, we assessed stress responsiveness and depressive-like behavior. In line with previous findings (Bartolomucci *et al.* 2005, Wagner *et al.* 2012), CSDS caused disturbances in HPA axis function, as reflected in the heightened corticosterone release in response to an acute stressor in mice exposed to CSDS. Although CSDS had no effect on basal corticosterone levels in our chow-fed mice, there was a significant increase in basal corticosterone levels from CSDS in the HFD-fed mice. It is difficult to determine whether the HFD aggravated disturbances in HPA axis function or whether stress-induced body weight loss accounts for the aggravated phenotype. A prominent theory of emotional behavior explains that consumption of calorically dense food actually offsets the negative emotional effects of chronic stress exposure and HPA axis activation (Pasquali *et al.* 2006, Dallman 2010). In this context, our data showing significant weight reduction on account of chronic stress exposure may be analogous to a state of starvation (Leibel *et al.* 1991), which is accompanied by neuroendocrine counter-regulatory adjustments to reestablish the non-reduced state (Bjorntorp & Rosmond 2000). Therefore, the aggravated HPA axis dysfunction in the stress-exposed HFD-fed mice may be driven by a state of weight loss rather than a direct effect of the HFD.

In the FST, a HFD decreased time spent struggling and the latency to float, which is interpreted as increased depressive-like behavior (Porsolt *et al.* 1977). Again this would oppose the protective role of calorically dense food in stress-induced emotional despair. Nevertheless,

the dietary effect observed in the FST may be a confounding effect of increased body fat, whereby the mice float more readily, because we observed no effect of stress on any depressive-like phenotype. Finally, it is difficult to disentangle whether the observed dietary effects are on account of an obesogenic (HFD) context or rather a starvation-like state (body weight differences, discussed above).

In addition to the P -statistics, we also reported effect sizes as proposed by Cohen (1992). Such analyses are useful to determine whether the observed effects are small, medium, or large and may give a stronger indication of the relevance of the observed data. Indeed, most of our results display large effect sizes, indicating that the effects are biologically meaningful.

It is important to identify markers mediating the crosstalk between stress regulation and energy balance. *Fkbp5* is a strong candidate gene for stress-related metabolic disorders on the basis that it has already been identified as a candidate gene in depression, which shares common overlapping pathways with obesity (Bornstein *et al.* 2006). Importantly, *Fkbp5* knockout (51KO) mice are less affected by CSDS and show a reduced body weight compared with WT mice (Hartmann *et al.* 2012). Therefore, we investigated the effects of HFD and CSDS on *Fkbp5* expression in the hypothalamus and hippocampus given their respective roles in metabolic and stress regulation. In the hypothalamus, chronic stress did not have any effect on *Fkbp5* expression, but HFD was found to have an effect selectively in hypothalamic nuclei. Specifically, we assessed *Fkbp5* expression in the arcuate nucleus, paraventricular nucleus, and VMH nucleus and found that *Fkbp5* expression was significantly regulated by diet within the VMH, whereby exposure to a HFD elevated *Fkbp5* expression. Additionally, we examined *Gr* mRNA expression on the basis that FKBP51 acts as a negative regulator of GR

(Davies *et al.* 2005, Wochnik *et al.* 2005, Binder 2009). A HFD resulted in decreased GR expression in both the VMH and arcuate nucleus (ARC). Collectively, the resulting reduced *Gr* expression and elevated *Fkbp5* expression from a HFD would indicate to reduced GR signaling.

This study also investigated mRNA expression of proopiomelanocortin (*Pomc*) and melanocortin 4 receptor (*Mc4r*), which are the components of the central melanocortin system. The melanocortin signaling system is well known for its role in food intake, body weight regulation, and regulation of the stress response (Seeley *et al.* 2004, Liu *et al.* 2007, 2013). We wanted to determine whether the stress-induced hypophagia and weight loss were reflected at the level of gene expression. We report that neither *Pomc* nor *Mc4r* mRNA expression were regulated by stress. However, mice fed on a HFD showed increased *Mc4r* expression in the PVN, which is well recognized for its anorexigenic effects (Hinney *et al.* 2013).

Although *Fkbp5* expression increased in response to a HFD in the VMH, there was no effect of dietary condition on hippocampal *Fkbp5* expression. In contrast, in the hippocampal CA3 region *Fkbp5* expression increased on account of CSDS. This corroborates results from an earlier study that demonstrated an increase in *Fkbp5* expression in the hippocampus in response to CSDS (Wagner *et al.* 2012). Moreover, the results of this study also indicated an association between higher hippocampal *Fkbp5* mRNA expression and an improved corticosterone response following chronic stress exposure. Interestingly, our data also reveal an association between higher CA3 hippocampal *Fkbp5* expression and lower response corticosterone levels following CSDS.

The specific spatial pattern of *Fkbp5* induction by either diet or stress conditions is in accordance with each region's respective function. The VMH is integrally involved in energy balance and is known to respond to metabolic signaling hormones (King 2006). The hippocampus on the other hand is well recognized for its role in the stress response, most notably in its termination (Ulrich-Lai & Herman 2009). Although we proposed the hypothesis that FKBP51 may be involved in the interplay between stress and metabolic regulation, the lack of interaction between diet and stress on *Fkbp5* expression and the spatially distinct regulation of *Fkbp5* expression by diet and stress does not support this. Nevertheless, the present data provide support for results from previous studies defining a role of FKBP51 in stress regulation and indicate a possible role in energy homeostasis. Further investigation involving genetic manipulation of *Fkbp5* in the brain is required to establish causality.

Finally, we used correlational analyses to assess the association between VMH and hippocampal *Fkbp5* expression and metabolic readouts. In the basal state, the expression level of VMH *Fkbp5* mRNA was correlated with both total body weight change and total caloric intake. Our data indicate that under basal conditions, increased expression of *Fkbp5* mRNA is associated with increased weight gain and increased total caloric intake. This agrees with the 51KO mouse model, which is leaner compared with WT littermates (Hartmann *et al.* 2012, Sanchez 2012). When we compared the correlation coefficients between basal and stressed conditions, there was in fact no difference for *Fkbp5* and caloric intake between conditions. However, the correlation coefficients for *Fkbp5* and body weight change were significantly different between stress conditions. Taken together, it appears that stress-induced changes interfere with the normal association between energy balance and FKBP51 function.

Strikingly, in the hippocampal CA3 region, there were no associations between *Fkbp5* mRNA levels and metabolic readouts under basal conditions. However, when challenged with CSDS, low *Fkbp5* expression in the hippocampus is associated with increased total caloric intake and mean caloric efficiency. Nevertheless, the correlation coefficients did not significantly differ between basal and stressed conditions, which may be due to the fact that the study was underpowered to address this question adequately. In contrast, the correlation coefficients for *Fkbp5* and corticosterone response were significantly different, revealing that higher corticosterone response correlates with lower *Fkbp5* expression exclusively under CSDS conditions, which is consistent with previously published data (Wagner *et al.* 2012). Our correlation analyses unveiled a highly complex association between *Fkbp5* expression and metabolic readouts, reflecting not only the aforementioned spatial regulation but also a strong dependence on the environmental conditions.

The stress-dependent relationship between *Fkbp5* mRNA levels and metabolic readouts may be observed in the regulation of FKBP51 by the stress response itself. FKBP51 modulates GR sensitivity and HPA activation, whereby lower FKBP51 expression reflects a state of higher GR sensitivity (Binder 2009). However, FKBP51 expression is also induced by GR activation through an ultrashort negative feedback loop in order to regulate GR sensitivity (Vermeer *et al.* 2003). Accordingly, higher FKBP51 induction would reflect an initial state of higher GR sensitivity corresponding to lower FKBP51 expression. In effect, it is very difficult to differentiate high initial FKBP51 expression (reflecting a lower GR sensitivity) from efficient

GR-induced FKBP51 expression (reflecting a higher GR sensitivity) even though they reflect two opposing conditions. Therefore, the seemingly contradictory results for FKBP51 expression under basal and chronic stress conditions may both represent the same initial condition, whereby high FKBP51 expression promotes metabolic phenotypes leading to a positive energy balance. In this case, the high initial FKBP51 expression following stress exposure would result in less efficient GR-activated FKBP51 induction, which would ultimately manifest as lower overall FKBP51 expression, masking the initial situation.

In summary, we show that adult exposure to CSDS results in hypophagia and weight loss. In parallel, FKBP51 is responsive to diet and stress conditions, as reflected in diet- and stress-induced *Fkbp5* expression changes. Moreover, higher levels of FKBP51 are closely related to higher food intake and body weight gain under basal conditions, which is consistent with the lean phenotype of 51KO mice. This collection of phenotypes indicates that there may be a novel role for FKBP51 in metabolic regulation. Nevertheless, genetic manipulation of *Fkbp5* in the brain is required to establish causality. Future investigation should focus on the exact role of FKBP51 in metabolism, which may have therapeutic implications for the treatment of metabolic disorders.

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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