

# 11 $\beta$ -Hydroxysteroid Dehydrogenase Type 1 Induction in the Arcuate Nucleus by High-Fat Feeding: A Novel Constraint to Hyperphagia?

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11 $\beta$ -Hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) catalyzes regeneration of active intracellular glucocorticoids in fat, liver, and discrete brain regions. Although overexpression of 11 $\beta$ -HSD1 in adipose tissue causes hyperphagia and the metabolic syndrome, male 11 $\beta$ -HSD1 null (11 $\beta$ -HSD1<sup>-/-</sup>) mice resist metabolic disease on high-fat (HF) diet, but also show hyperphagia. This suggests 11 $\beta$ -HSD1 may influence the central actions of glucocorticoids on appetite and perhaps energy balance. We show that 11 $\beta$ -HSD1<sup>-/-</sup> mice express lower hypothalamic mRNA levels of the anorexigenic cocaine and amphetamine-regulated transcript and melanocortin-4 receptor, but higher levels of the orexigenic melanin-concentrating hormone mRNAs than controls (C57BL/6J) on a low-fat diet (11% fat). HF (58% fat) diet promoted transient (~8 wk) hyperphagia and decreased food efficiency in 11 $\beta$ -HSD1<sup>-/-</sup> mice and decreased melanocortin-4 receptor mRNA expression in control but not 11 $\beta$ -HSD1<sup>-/-</sup> mice. 11 $\beta$ -HSD1<sup>-/-</sup> mice showed a HF-mediated up-regulation of the orexigenic agouti-related peptide (AGRP) mRNA in the arcuate nucleus which paral-

leled the transient HF hyperphagia. Conversely, control mice showed a rapid (48 h) HF-mediated increase in arcuate 11 $\beta$ -HSD1 associated with subsequent down-regulation of AGRP. This regulatory pattern was unexpected because glucocorticoids increase AGRP, suggesting an alternate hyperphagic mechanism despite partial colocalization of 11 $\beta$ -HSD1 and AGRP in arcuate nucleus cells. One major alternate mechanism governing selective fat ingestion and the AGRP system is endogenous opioids. Treatment of HF-fed mice with the  $\mu$  opioid agonist DAMGO recapitulated the HF-induced dissociation of arcuate AGRP expression between control and 11 $\beta$ -HSD1<sup>-/-</sup> mice, whereas the opioid antagonist naloxone given with HF induced a rise in arcuate AGRP and blocked HF-diet induction of 11 $\beta$ -HSD1. These data suggest that 11 $\beta$ -HSD1 in brain plays a role in the adaptive restraint of excess fat intake, in part by increasing inhibitory opioid tone on AGRP expression in the arcuate nucleus. (*Endocrinology* 147: 4486–4495, 2006)

THE CLOSE PHENOTYPIC similarities between the prevalent metabolic syndrome (visceral obesity, insulin resistance/type 2 diabetes, dyslipidaemia, and hypertension) and rare Cushing's syndrome of circulating glucocorticoid excess have long been remarked. However, in metabolic syndrome/obesity, plasma cortisol levels are not raised and, in simple obesity, are lower than in healthy lean control subjects (1, 2). In explanation of this paradox, increased tissue sensitivity to glucocorticoids has been advocated. Specifically, the key intracellular glucocorticoid metabolizing enzyme, 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) is elevated in adipose tissue in obese humans and certain rodent models of obesity/metabolic syndrome (3–9). Although bidirectional in tissue homogenates, 11 $\beta$ -HSD1 predominantly catalyzes the reactivation of active cortisol and corticosterone from inert 11-keto steroids (cortisone, 11-dehydrocorticosterone) in intact cells and organs

(10–13). This increases intracellular levels of active glucocorticoids thus amplifying local action without altering circulating levels.

As well as the known peripheral effects of elevated adipose 11 $\beta$ -HSD1 to promote visceral obesity, insulin resistance/glucose intolerance, dyslipidemia, and hypertension in mice (6, 14), transgenic aP2–11 $\beta$ -HSD1 mice exhibit hyperphagia, consuming approximately 10% more calories per gram of body weight (6). Conversely, ectopic expression of 11 $\beta$ -HSD type 2, a potent glucocorticoid inactivating isozyme, in adipose tissue (aP2–11 $\beta$ -HSD2) produces the opposite phenotype with insulin sensitization, improved glucose tolerance, improved lipid profiles, and reduced food intake, notably on high-fat (HF) diet (15). Consistent with the metabolic phenotype of aP2–11 $\beta$ -HSD2 mice, global 11 $\beta$ -HSD1 deficiency (11 $\beta$ -HSD1<sup>-/-</sup>) in mice causes increased insulin sensitivity, improved glucose tolerance, and lipidaemia and resistance to HF diet-induced visceral obesity (16–18). However, paradoxically, 11 $\beta$ -HSD1<sup>-/-</sup> mice are hyperphagic when fed a HF diet (18); hyperphagia occurs despite reduced intra-adipose corticosterone levels and normal plasma corticosterone concentrations (18). Glucocorticoids modulate expression of several key appetite and energy balance regulatory peptides in the hypothalamus (19–22), acting, at least in part, directly in the CNS (23). 11 $\beta$ -HSD1 is also expressed in the CNS (24, 25). Therefore, the discordance in appetitive responses between fat-specific glucocorticoid ma-

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Abbreviations: AGRP, Agouti-related peptide; CART, cocaine and amphetamine-regulated transcript; HF, high fat; HF24 h, 24 h of HF diet; 11 $\beta$ -HSD1, 11 $\beta$ -hydroxysteroid dehydrogenase type 1; ISH, *in situ* hybridization histochemistry; LF, low fat; MCH, melanin-concentrating hormone; MC4R, melanocortin-4 receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin.

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nipulations and whole-body 11 $\beta$ -HSD1 deficiency led us to hypothesize that intracellular glucocorticoid regeneration by 11 $\beta$ -HSD1 within phagic/energy control centers of the brain might predominate over peripheral fat-derived signals in the control of HF-induced hyperphagia and energy conservation. In this study, we have determined whether 11 $\beta$ -HSD1 was expressed in the hypothalamic arcuate nucleus in the mouse and the contrasting hypothalamic neuropeptide responses induced by HF diet in C57BL/6J and 11 $\beta$ -HSD1<sup>-/-</sup> mice.

## Materials and Methods

### Animals

Mice homozygous for targeted disruption of the 11 $\beta$ -HSD1 gene, congenic on the C57BL/6J genetic background (18), and control C57BL/6J mice were pair-housed under controlled lighting (12-h light, 12-h dark) and temperature (23 ± 2 C) and provided *ad libitum* access to water and rodent chow (Special Diet Services, Essex, UK; product 801190) except as otherwise noted. All experiments were performed according to Home Office guidelines under the auspices of the UK Animals (Scientific Procedures) Act, 1986, and had ethical committee approval.

### In vivo experiments

To determine the effects of HF diet on food intake and expression of appetite neuropeptides in the arcuate nucleus, 6-wk-old male 11 $\beta$ -HSD1<sup>-/-</sup> and C57BL/6J control mice (25–30 g start weight) were randomly assigned to four groups (n = 5–6/group) and provided *ad libitum* access to HF diet (58% calories as fat, Research Diets D12331) or low-fat diet (LF) (11% calories as fat, Research Diets D12328) for 2 or 18 wk. Food intake and body weight were measured weekly. To determine whether the HF-induced hyperphagia was sexually dimorphic, female 11 $\beta$ -HSD1<sup>-/-</sup> and age-matched female C57BL/6J controls (24 month) were randomly assigned to *ad libitum* access to HF or LF diet for 9 wk (n = 5–6/group). Food intake and body weight were measured three times per week. Food efficiency was determined as body weight gained (grams) per week divided by total energy (kilocalories) consumed over this period. All animals were killed in the morning (0800–1000 h) after *ad libitum* access to food (LF or HF), trunk blood was taken, and plasma was separated and stored at –85 C for subsequent hormone assays.

### Effect of acute HF on expression of arcuate 11 $\beta$ -HSD1

Male wild-type mice were randomly assigned to three groups (n = 4/group) and fed LF or HF *ad libitum*. All diets were administered for 2 d, and food intake and body weight were measured on both days.

### Effects of the opioid manipulations on arcuate neuropeptide mRNAs and 11 $\beta$ -HSD1

Because opioids modulate appetite behavior, the  $\mu$ -opioid receptor agonist DAMGO (0.1 mg/kg body weight) or saline vehicle was administered *sc* to randomly assigned male control and 11 $\beta$ -HSD1<sup>-/-</sup> mice daily for 5 d. After the final injection, animals in each group (n = 4–6 per group and diet) were randomized to chow or HF diet overnight (24 h) and killed the next morning and brains were collected.

To examine this system further, control mice were randomly assigned to two groups (n = 6/group). During the first week, animals were fed standard chow followed by 2 wk of HF. Naloxone (Sigma-Aldrich, St. Louis, MO) or vehicle was diluted to 300  $\mu$ g/liter in the drinking water to supply a final dose of 1 mg/kg body weight. Fresh drinking water containing naloxone or vehicle was replaced three times per week. After 2 wk of treatment, animals were killed and brains were collected for later *in situ* hybridization histochemistry (ISH).

### Tissue preparation

Male animals were killed by decapitation. Terminal blood samples were collected in Microvette collection tubes (Sarstedt, Numbrecht, Ger-

many), and plasma was separated by centrifugation and stored at –20 C until assay. Whole brain was dissected out, rapidly frozen on dry ice, and stored at –80 C until being sectioned (10  $\mu$ m) in the coronal plane using a cryostat (Bright Instruments Co Ltd, Huntingdon, UK). Eight sections, 100  $\mu$ m apart, were mounted on 1% silane (Sigma-Aldrich)-coated glass slides (BDH, Dorset, UK).

### In situ hybridization

ISH was used to determine the effect of diet on mRNA expression in the hypothalamus. The procedure involved the use of <sup>35</sup>S-labeled antisense or control sense riboprobes to mouse agouti-related peptide (AGRP), cocaine and amphetamine-regulated transcript (CART), neuropeptide Y (NPY), proopiomelanocortin (POMC), melanin-concentrating hormone (MCH), melanocortin-4 receptor (MC4R), or 11 $\beta$ -HSD1. Riboprobes were generated from RT-PCR products amplified from mouse hypothalamic RNA and subcloned into PGEM –T Easy Vector (Promega, Southampton, UK). PCR primers were designed using the NCBI database and GeneRunner software (www.genrunner.com) to amplify the majority of each respective coding region while maintaining a high GC ratio, and were as follows: AGRP (5'-ATGCTGACTGCAATGTTG-3' and 5'-TAGGTGCGACTACAGAGG-3'), CART (5'-TCA-CAAGCACTTCAAGAGG-3' and 5'-TACTGCTACCTTTGCTGG-3'), NPY (5'-TAGGTAACAAGCGAATGG-3' and 5'-AACAAACAAGGGA-AATGGG-3'), POMC (5'-AACTCGACCTCTCGCTG-3' and 5'-ATGATGGCGTTCTTGAAG-3'), MCH (5'-GGTCCGCAACATCCTTACA-3' and 5'-CTCCAAATTACGTGTGCAAGT-3'), MC4R (5'-CATGGCATGTATACTTCCC-3' and 5'-GCTGTCCGAGTAAATGATG-3'), and 11 $\beta$ -HSD1 (5'-CAGCAATGTAGTGAGCAGAGGC-3' and 5'-TGTTGGTATGACTGCCAGGTCG-3'). Identity of subcloned PCR products was confirmed by DNA sequencing (The Sequencing Service, University of Dundee, Dundee, UK; www.dnaseq.co.uk).

Twenty-four coronal sections, 100  $\mu$ m apart, spanning the hypothalamus from the optic chiasm to the caudal arcuate nucleus were cut for each animal. ISH was performed using antisense or sense riboprobes generated from the subcloned PCR products, as previously described (26), except for omission of 30 min incubation in proteinase K. After ISH, sections were apposed to Kodak Biomax MR film (Sigma-Aldrich) for 4 d, whereas the hybridization signal remained in the linear response range of the film. For quantitation, the autoradiographs were uniformly transilluminated using a Northern Light illuminator (Imaging Research Inc., St. Catharines, Ontario, Canada) and the images were captured using a CCD camera (CCD-72S; Dage MTI, Michigan City, MI) equipped with a zoom lens. They were digitized using a frame grabber and analyzed using MCID Image analysis software (Imaging Research Inc.). Hybridization signal was analyzed using the single-pixel selector tool, and the mean optical density was calculated after subtracting background hybridization measured in adjacent brain areas that lacked specific hybridization signal. For each animal, the mean optical densities from all sections containing the arcuate nucleus were averaged and then combined according to genotype and diet to give an overall group mean ( $\pm$ SEM). Double ISH was performed using <sup>35</sup>S-UTP and digoxigenin-UTP (DIG RNA labeling mix; Roche Diagnostics, East Sussex, UK) labeled probes to 11 $\beta$ -HSD1 and AGRP, respectively, as previously described (27).

### Blood analyses for corticosterone, insulin, leptin, and thyroid hormones

Plasma corticosterone levels were determined by RIA, as described (10). Plasma insulin was measured by using the Ultra Sensitive Insulin ELISA kit (Crystal Chem, Downers Grove, IL). Plasma leptin was assayed using the Mouse Leptin ELISA kit (Crystal Chem). Thyroid hormones were measured by specific immunoassays.

### Assay of 11 $\beta$ -HSD1 activity

Adipose and CNS subregion 11 $\beta$ -HSD1 activities were determined, generally as previously described (28), but with 25 nM [<sup>3</sup>H]cortisone as substrate and 200 nM NADPH as cosubstrate. Assays were in the linear part of the relationship between protein concentration and time. Steroids were separated by TLC and quantified with a phosphorimager tritium screen (Fujifilm, Tokyo, Japan).

### Statistical analysis

All statistical analyses were performed using Systat, Version 10 (Systat Software Inc., Richmond, CA). To determine significances of difference in food intake, we used repeated-measures ANOVA followed by Student-Neuman-Keuls as a *post hoc* analysis when ANOVA indicated  $P \leq 0.05$ . To analyze gene expression, two-way ANOVA was used, and Student-Newman-Keuls was applied *post hoc*, where appropriate. To determine interactions between mRNA levels and plasma hormone values, Pearson's correlation analysis was used. Values are means  $\pm$  SEM.

### Results

#### Male and female $11\beta$ -HSD1<sup>-/-</sup> mice fed HF diet develop transient hyperphagia but show attenuated weight gain

We previously found that male  $11\beta$ -HSD1<sup>-/-</sup> mice develop hyperphagia and increased body temperature on HF diet (18). To clarify whether HF diet specifically produces hyperphagia in  $11\beta$ -HSD1<sup>-/-</sup> mice or whether this is merely a response to alteration of diet, male  $11\beta$ -HSD1<sup>-/-</sup> and congenic C57BL/6J controls were changed from normal chow to either LF (11%, similar fat content to normal chow) or HF (58%), carbohydrate-matched diets. Both genotypes maintained a similar calorie to body weight ratio (kilocalories per gram) when fed chow (data not shown). Calorie intake was not altered when either genotype was changed to LF diet, but male  $11\beta$ -HSD1<sup>-/-</sup> mice increased caloric intake by approx-

imately 30% within 2 wk of initiating HF diet, whether expressed as gross intake (Fig. 1A) or as intake per gram of body weight (data not shown). The hyperphagia was not permanent; male  $11\beta$ -HSD1<sup>-/-</sup> mice reduced calorie intake to LF control levels approximately 8 wk after starting HF and then maintained control intake thereafter up to 18 wk of HF. Despite hyperphagia, male HF-fed  $11\beta$ -HSD1<sup>-/-</sup> mice gained significantly less weight relative to caloric intake than HF-fed controls (Fig. 1C), as previously described (18), and thus showed decreased food efficiency (body weight gain per kilocalorie ingested) (Fig. 1E). Hyperphagia on initiation of HF diet also occurred in (aged) female  $11\beta$ -HSD1<sup>-/-</sup> mice (Fig. 1B), which indeed resisted weight gain entirely (Fig. 1D) and also exhibited a decrease in food efficiency (Fig. 1F).

#### Changes in plasma corticosterone, insulin, leptin, and thyroid hormones are unlikely to account for HF hyperphagia in $11\beta$ -HSD1<sup>-/-</sup> mice

We measured plasma corticosterone, insulin, and leptin as candidate modulators of hypothalamic appetitive neuropeptides. On LF diet,  $11\beta$ -HSD1<sup>-/-</sup> and control mice showed no significant differences in insulin, leptin, and corticosterone levels (Fig. 2). In control mice, HF for 2 wk decreased plasma corticosterone and increased plasma insulin and leptin levels (Fig. 2); and 18 wk HF led to a rise above basal levels in

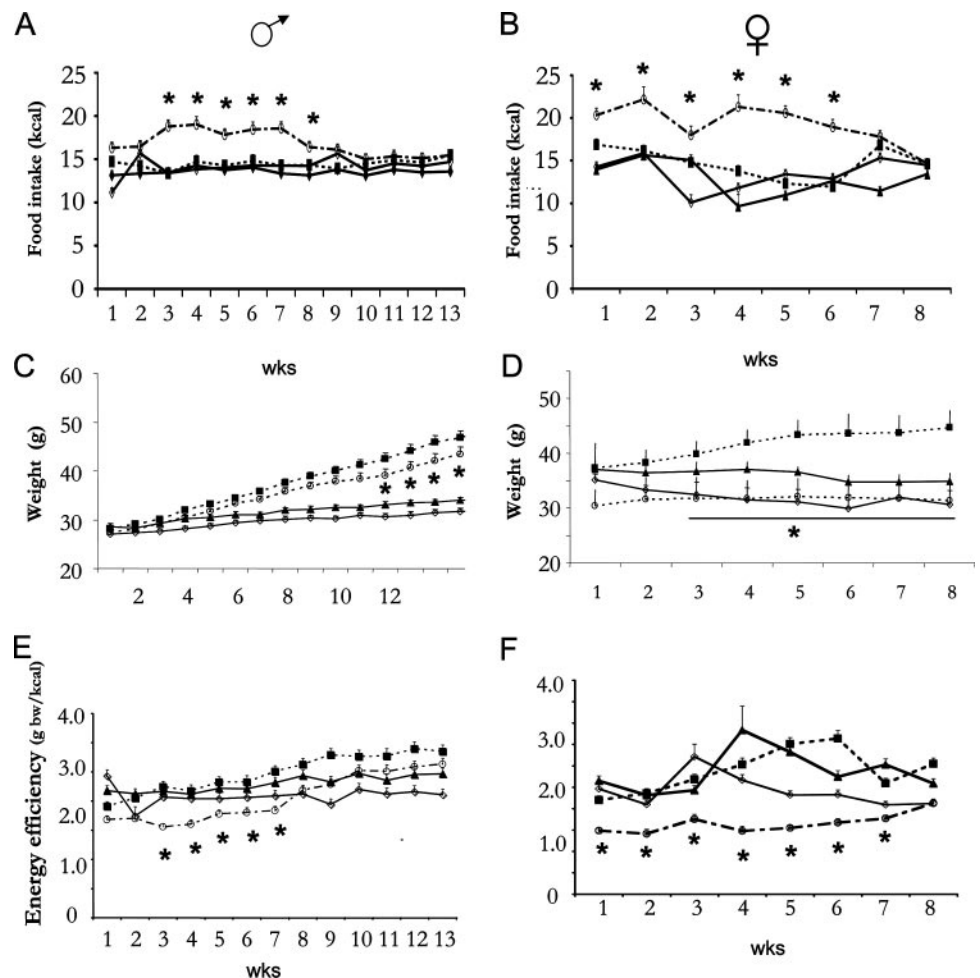


FIG. 1. Food intake and body weight of mice deficient for  $11\beta$ -HSD1. Male (A, C, and E) and female (B, D, and F)  $11\beta$ -HSD1<sup>-/-</sup> mice (open symbols) develop significant hyperphagia (A and B) 1–2 wk after starting HF diet (open circles, dotted line), which persists for 8 wk only. Hyperphagia does not occur when the  $11\beta$ -HSD1<sup>-/-</sup> mice are given a novel LF diet (open diamonds, solid line). Control C57BL/6J mice (+/+) are shown as filled black symbols (dotted line, HF; solid line, LF). Despite the increased caloric intake, male (C and E) and female (D and F)  $11\beta$ -HSD1<sup>-/-</sup> mice on HF diet show reduced weight gain and reduced food efficiency compared with controls. \*,  $P < 0.05$  compared with HF-fed congenic C57BL/6J controls.

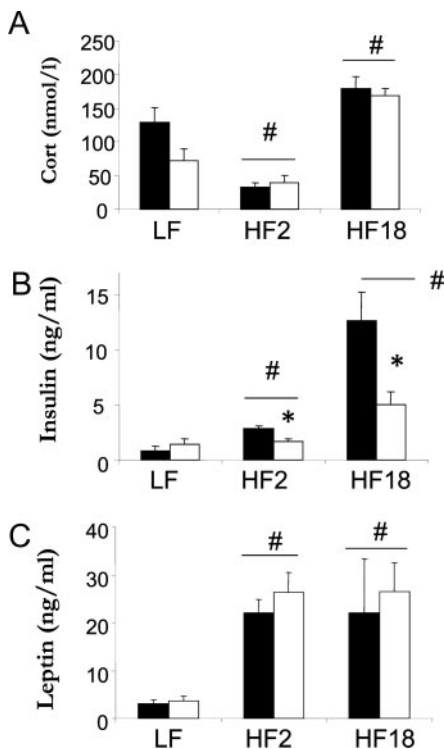


FIG. 2. Histograms depicting circulating corticosterone (A), insulin (B), and leptin (C) in control (black) and  $11\beta$ -HSD1<sup>-/-</sup> (white) mice. #,  $P < 0.05$  effect of diet, compared with LF; \*,  $P < 0.05$   $11\beta$ -HSD1<sup>-/-</sup> compared with the respective control. Corticosterone and leptin levels varied with diet but not genotype. In contrast, insulin levels varied with both diet and genotype with  $11\beta$ -HSD1<sup>-/-</sup> mice showing a lesser increase in insulin on HF.

corticosterone, a further elevation in insulin, and maintained elevated leptin levels. In  $11\beta$ -HSD1<sup>-/-</sup> mice, HF caused similar changes in corticosterone and leptin to controls and somewhat lesser rises in plasma insulin (Fig. 2B), consistent with peripheral insulin sensitization in this model (17, 18). Elevated thyroid hormones cause increased appetite but resistance to weight loss. Basal levels of  $T_4$  were similar in control ( $47 \pm 5 \mu\text{g/ml}$ ) and  $11\beta$ -HSD1<sup>-/-</sup> ( $46 \pm 6 \mu\text{g/ml}$ ) mice. HF reduced  $T_4$  levels in both genotypes ( $P < 0.05$ ) with a greater reduction in  $11\beta$ -HSD1<sup>-/-</sup> (to  $28 \pm 2 \mu\text{g/ml}$ ) than control ( $36 \pm 3 \mu\text{g/ml}$ ) mice. Plasma  $T_3$  levels did not vary with genotype or diet.

#### Effects of $11\beta$ -HSD1 deficiency on hypothalamic orexigenic peptide mRNAs

We used ISH to look at arcuate NPY and AGRP as well as MCH in the lateral hypothalamus under LF and two time points of HF feeding: 2 (HF2) and 18 wk (HF18), representing an early adaptive phase and a chronic adaptive phase, respectively (Fig. 3, A–C). NPY mRNA was unchanged by genotype at any time point, although there was a trend for HF feeding (HF2) to down-regulate NPY in both genotypes that returned to LF values by 18HF (Fig. 3A), paralleling previous findings in mice (29). AGRP was not different between the genotypes on LF (Fig. 3B). However, there was a divergent response between genotypes at HF2, wherein  $11\beta$ -HSD1<sup>-/-</sup> mice significantly up-regulated arcuate AGRP

mRNA, thus paralleling the contemporaneous transient hyperphagia, whereas control mice down-regulated AGRP (Fig. 3B), as previously noted in HF-fed control mice (29). By 18 wk HF, both genotypes reverted to AGRP mRNA levels comparable to LF (Fig. 3B). There was a significant effect of genotype but not diet on MCH, which was consistently higher in  $11\beta$ -HSD1<sup>-/-</sup> mice (Fig. 3C).

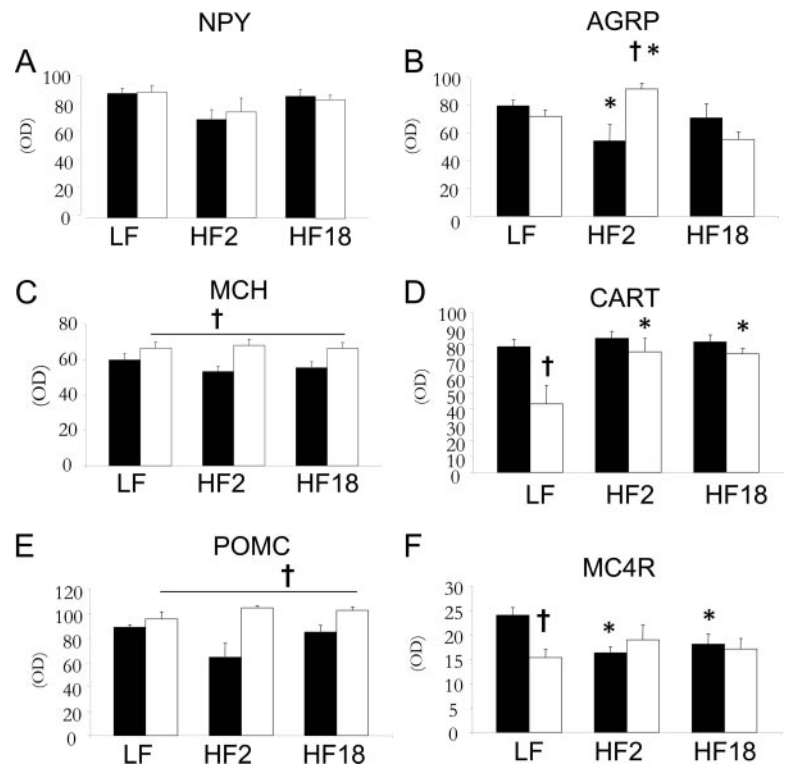
#### Effects of $11\beta$ -HSD1 deficiency on hypothalamic anorexigenic peptides

To address the role of tonic inhibitory influences on food intake, we measured arcuate mRNAs encoding CART and POMC, which generates the anorexigenic peptide  $\alpha$ -MSH (Fig. 3, D–F). We also measured MC4R receptor levels in the paraventricular nucleus (PVN). MC4R mediates the inhibitory appetitive and the energy balance effects of  $\alpha$ -MSH and integrates the antagonistic orexigenic effects of AGRP simultaneously. CART mRNA was lower in LF-fed  $11\beta$ -HSD1<sup>-/-</sup> mice and was elevated by HF to control levels (Fig. 3D). Diet did not affect CART in controls (Fig. 3D). In contrast, there was a significantly higher overall POMC level in the arcuate nuclei of  $11\beta$ -HSD1<sup>-/-</sup> mice, which might underlie increased thermogenesis, but there were no significant effects of diet on POMC mRNA in either genotype (Fig. 3E). MC4R levels were lower in LF-fed  $11\beta$ -HSD1<sup>-/-</sup> mice (Fig. 3F), suggesting lower target levels for the presumably higher  $\alpha$ -MSH (POMC-derived, Fig. 3E) and higher AGRP (Fig. 3B) in  $11\beta$ -HSD1<sup>-/-</sup> mice. HF diet reduced MC4R levels in control mice to levels comparable to those in  $11\beta$ -HSD1<sup>-/-</sup> mice (HF2 and HF18). Overall on control LF diet,  $11\beta$ -HSD1<sup>-/-</sup> mice had lower levels of mRNAs encoding CART (Fig. 4D) and MC4R in the PVN (Fig. 4F) and increased MCH (Fig. 4C), a pattern anticipated to promote food intake particularly for HF diets (30–32). However, none of the anorexigenic peptide mRNA changes with diet mirrored the transient hyperphagia seen in HF-fed  $11\beta$ -HSD1<sup>-/-</sup> mice.

#### $11\beta$ -HSD1 is expressed in arcuate nucleus and is increased with HF diet

Since lowering glucocorticoid levels selectively in adipose tissue reduces intake of HF diet (15), the hyperphagia in global  $11\beta$ -HSD1<sup>-/-</sup> mice implies a possible central “orexigenic” action of  $11\beta$ -HSD1 deficiency, which apparently prevails over the putative adipose tissue-derived anorectic factor(s) in these mice. Because circulating corticosterone levels were similar in control and  $11\beta$ -HSD1<sup>-/-</sup> mice on this genetic background, we examined expression of  $11\beta$ -HSD1 in the control mouse hypothalamus to determine which critical regions may be additionally modulated by local changes in glucocorticoid amplification.  $11\beta$ -HSD1 mRNA and oxidoreductase activity were found in C57BL/6J mouse arcuate nucleus where  $11\beta$ -HSD1 mRNA levels were higher than in other hypothalamic regions (Fig. 4A). In control mice, 2 wk HF diet elevated arcuate nucleus  $11\beta$ -HSD1 mRNA (Fig. 4B) and  $11\beta$ -reductase activity (LF diet  $1.5 \pm 0.6$  pmol cortisol generated per milligram of protein per hour; 2 wk HF  $11.4 \pm 2.1$  pmol cortisol per milligram of protein per hour,  $P < 0.01$ ). This rise was reversed with chronic (18 wk) HF. Although the

FIG. 3. Hypothalamic mRNAs in LF and after 2 (HF2) and 18 (HF18) wk of isocaloric HF diet in C57BL/6J control (*black columns*) and 11 $\beta$ -HSD1<sup>-/-</sup> (*white columns*) mice. A, NPY; B, AGRP; C, MCH; D, CART; E, POMC; F, MC4R mRNAs. \*,  $P < 0.05$  compared with LF; †,  $P < 0.05$  11 $\beta$ -HSD1<sup>-/-</sup> compared with C57BL/6J controls under same conditions. Note that 11 $\beta$ -HSD1<sup>-/-</sup> mice show reduced CART and MC4R and elevated MCH mRNAs under basal LF conditions. After 2 wk HF, AGRP mRNA levels decrease in control but increase in 11 $\beta$ -HSD1<sup>-/-</sup> mice. POMC and MCH mRNAs are elevated in 11 $\beta$ -HSD1<sup>-/-</sup> mice but do not vary with diet. MC4R mRNA falls with HF in controls but not 11 $\beta$ -HSD1<sup>-/-</sup> mice. CART mRNA in 11 $\beta$ -HSD1<sup>-/-</sup> mice rises to control values with HF. NPY mRNA is unaffected by diet or genotype.



hippocampus and neocortex also express 11 $\beta$ -HSD1, HF diet did not alter 11 $\beta$ -HSD1 mRNA or activity in these sites (data not shown).

*AGRP and 11 $\beta$ -HSD1 mRNAs are colocalized and negatively correlated within arcuate neurons in C57BL/6 mice*

In control mice, ISH on consecutive sections showed that 11 $\beta$ -HSD1 mRNA correlated negatively with AGRP mRNA in the arcuate nucleus ( $r = -0.52$ ;  $P < 0.05$ ; Fig. 5A). 11 $\beta$ -HSD1 and POMC mRNAs were also negatively correlated ( $r = -0.61$ ;  $P < 0.05$ ). No other neuropeptides correlated with 11 $\beta$ -HSD1 (data not shown). Arcuate AGRP mRNA was not correlated with plasma corticosterone levels (data not shown), suggesting that the levels of circulating steroid are not directly related to AGRP mRNA variation under the circumstances examined (diet varies but time of day was

constant). Double-ISH (Fig. 5B) showed 53% of arcuate nucleus cells expressing 11 $\beta$ -HSD1 mRNA also expressed AGRP mRNA, suggesting a neuroanatomical basis for a possible interaction. Thus it is conceivable that local glucocorticoid regeneration by 11 $\beta$ -HSD1 in arcuate neurons may regulate and/or be regulated by AGRP [which is not colocalized with POMC (33)]. Interestingly, shorter term HF diet (48 h) in control mice also induced a rise in 11 $\beta$ -HSD1 mRNA in the arcuate nucleus (Fig. 6A), but there was no accompanying change in AGRP mRNA (Fig. 6B) or change in food intake (Fig. 6D). This rapid induction of 11 $\beta$ -HSD1 by HF was selective to the arcuate nucleus as 48 h HF did not alter 11 $\beta$ -HSD1 in adipose tissue (data not shown) where longer exposures ( $>1$  wk) cause a down-regulation of 11 $\beta$ -HSD1 expression (34). Forty-eight-hour HF down-regulated MC4R (Fig. 6C), to a similar extent seen with HF2 (Fig. 3C). Thus induction of 11 $\beta$ -HSD1 in the arcuate precedes changes in

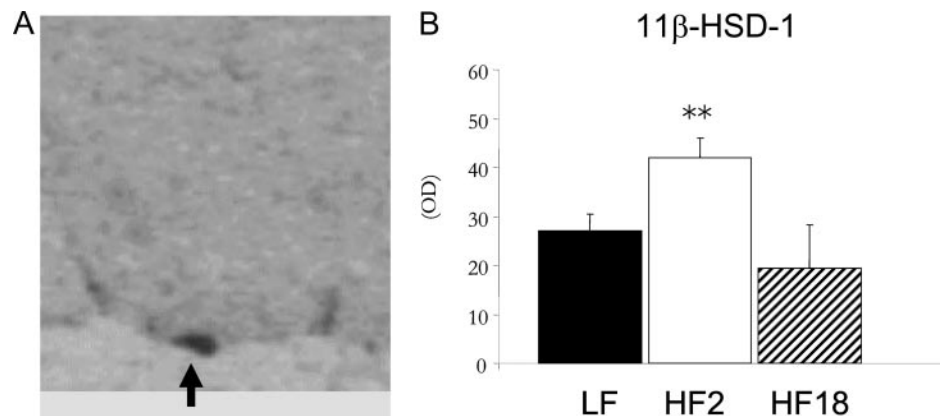
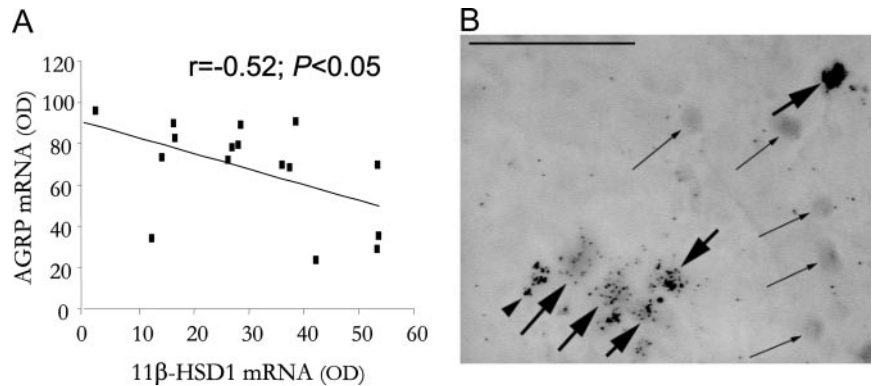


FIG. 4. A, *In situ* hybridization showing 11 $\beta$ -HSD1 mRNA in a typical coronal section of the mouse hypothalamus; arrow shows high expression in the arcuate nucleus. B, Compared with LF diet (*black column*), arcuate nucleus 11 $\beta$ -HSD1 mRNA levels are significantly increased after 2 wk of HF diet (*white column*), and then return to control with 18 wk HF (*hatched column*); \*\*,  $P < 0.01$  compared with LF.

FIG. 5. A, 11 $\beta$ -HSD1 and AGRP mRNAs are negatively correlated in the arcuate nucleus in control mice. B, Representative brightfield photomicrograph demonstrating approximately 53% of arcuate nucleus cells expressing 11 $\beta$ -HSD1 also express AGRP mRNA. 11 $\beta$ -HSD1 mRNA was detected as silver grains overlying the plane of the slide. AGRP mRNA was detected by nonradioactive ISH with anti-biotin antisera against hybridized biotinylated cRNA probe. Double-labeled cells are indicated by *thick arrows*. Cells expressing only 11 $\beta$ -HSD1 are indicated by *arrowheads*, and cells expressing only AGRP are indicated by *thin arrows*. Scale bar, 1 mm.



AGRP (Fig. 3B) and coincides with changes in MC4R mRNA in PVN.

#### Possible opioid mechanism for HF diet induction of arcuate nucleus 11 $\beta$ -HSD1 expression

Because changes in AGRP mRNA in the arcuate nucleus most closely paralleled the hyperphagia seen in HF-fed 11 $\beta$ -HSD1<sup>-/-</sup> mice, yet lower glucocorticoid levels would be expected to reduce AGRP expression, this suggests a dominant indirect mechanism linking these two elements despite the colocalization. Opioids have potent multifaceted actions on food intake (35). One such effect is that opioid blockade antagonizes AGRP-mediated fat appetite (36, 37). To test whether 11 $\beta$ -HSD1 interacts with this mechanism, control mice were given the  $\mu$ -receptor agonist DAMGO. This had no effect on 11 $\beta$ -HSD1 mRNA in LF-fed mice (Fig. 7A). Although 24 h of HF diet (HF24 h) did not increase arcuate 11 $\beta$ -HSD1 mRNA, DAMGO, together with HF24 h diet, increased ARC 11 $\beta$ -HSD1 mRNA (Fig. 7A). Strikingly, on both LF and HF24 h, DAMGO increased AGRP mRNA in 11 $\beta$ -HSD1<sup>-/-</sup> mice (Fig. 7B). Conversely, DAMGO with HF24 h caused a fall in AGRP mRNA in control mice, paralleling the change in AGRP seen after 2 wk of HF feeding (HF2; Fig. 3B) but not 2 d (HF48 h; Fig. 6B) in controls. Thus, administration

of the  $\mu$ -receptor agonist induces opposing changes in arcuate AGRP in 11 $\beta$ -HSD1<sup>-/-</sup> and control mice, apparently mimicking that observed with 2 wk HF feeding.

Conversely, the nonselective opioid antagonist naloxone prevented the HF increase in arcuate 11 $\beta$ -HSD1 mRNA expression (Fig. 7C). Moreover, whereas vehicle and HF diet reduced arcuate AGRP mRNA, naloxone not only prevented this but led to up-regulation of AGRP expression (Fig. 7D) similar to changes seen in 11 $\beta$ -HSD1<sup>-/-</sup> mice, although this dose of naloxone did not alter HF food intake or body weight over the 2 wk of treatment (data not shown). 11 $\beta$ -HSD1<sup>-/-</sup> mice had two additional orexigenic characteristics, less MC4R mRNA in the PVN and more MCH mRNA in the lateral hypothalamus, but the response of MC4R to HF and the unresponsiveness of MCH to HF were not altered by naloxone (not shown).

#### Discussion

Here we show that 11 $\beta$ -HSD1<sup>-/-</sup> mice develop transient hyperphagia and reduced food efficiency on HF diet. The effect is unexpected because glucocorticoid deficiency in adipose tissue associates with reduced food intake (15), whereas glucocorticoid excess in adipose tissue increases food intake and promotes metabolic dysfunction (6). The

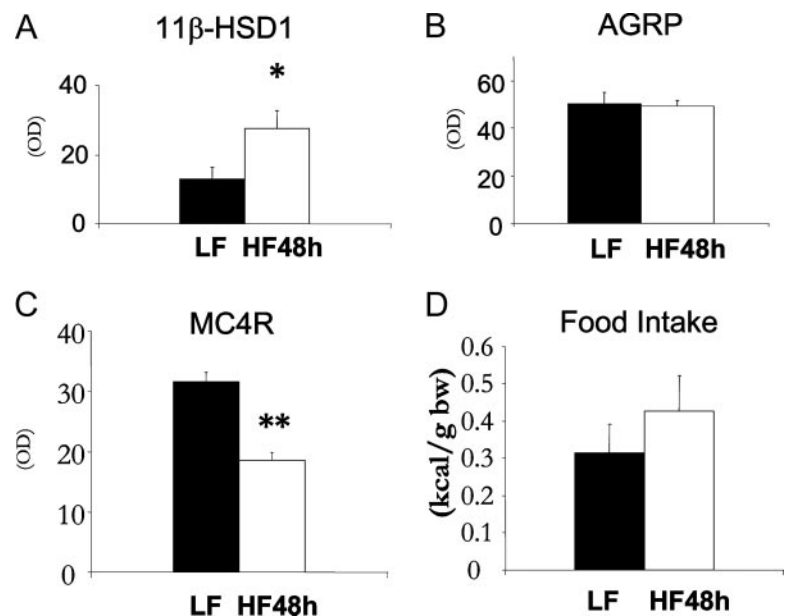
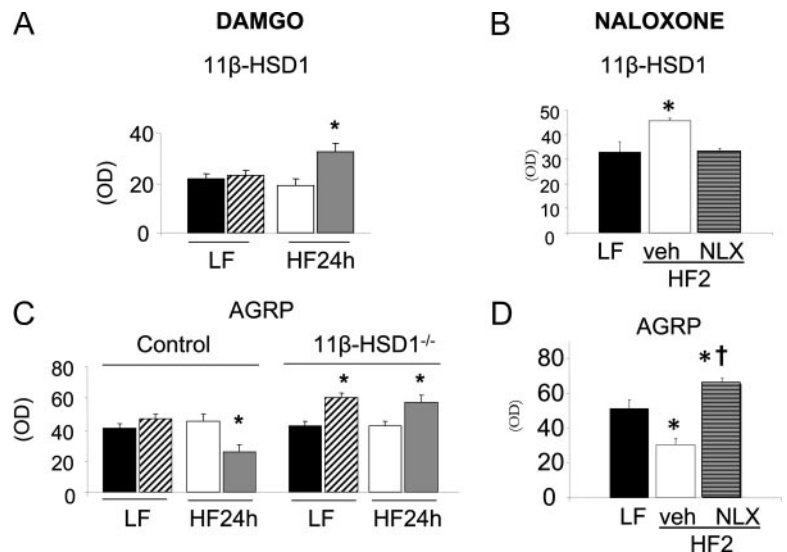


FIG. 6. A, 11 $\beta$ -HSD1 mRNA increased significantly in the arcuate nucleus after only 48 h HF diet (HF48 h; *white column*) compared with LF (*black column*). B, Arcuate nucleus AGRP mRNA was unchanged by 2 d of HF diet. C, MC4R mRNA in the PVN was down-regulated after HF diet. D, Despite differences in 11 $\beta$ -HSD1 and MC4R mRNAs with 2 d HF diet, caloric intake (kilocalories per gram of body weight) did not differ between the groups. \*,  $P < 0.05$  compared with LF; \*\*,  $P < 0.01$ .

FIG. 7. Effect of the opioid manipulation. A and B, Effects of the  $\mu$ -opioid receptor agonist DAMGO on arcuate nucleus levels of 11 $\beta$ -HSD1 mRNA in control mice on LF and 24 h HF (HF24 h) diet (A) and on arcuate AGRP mRNA in control and 11 $\beta$ -HSD1<sup>-/-</sup> mice (B) (black columns, LF with vehicle; diagonally striped columns, LF + DAMGO; white columns, HF with vehicle; gray columns, HF + DAMGO). C and D, Effect of naloxone (NLX) in control mice on arcuate 11 $\beta$ -HSD1 mRNA in controls on LF and 2 wk (HF2) with naloxone or vehicle in drinking water (C) and on arcuate AGRP mRNA (D) (black columns, LF with vehicle; white columns, HF2 with vehicle; horizontally striped columns, HF2 plus naloxone). \*,  $P < 0.05$  compared with vehicle control on same diet; †,  $P < 0.05$  compared with vehicle on HF diet.



implication is that central 11 $\beta$ -HSD1 deficiency overrides the effects of adipose deficiency, because overexpression of 11 $\beta$ -HSD1 in liver, the other major site of expression, has no effect on food intake (38). Indeed, the basal hypothalamic appetitive mRNA profile (reduced CART and MC4R, increased MCH) suggests 11 $\beta$ -HSD1<sup>-/-</sup> mice might be susceptible to early onset HF-induced hyperphagia, although their food intake on chow or LF is similar to controls. With HF, the main orexigenic peptide which parallels the transient hyperphagia in 11 $\beta$ -HSD1<sup>-/-</sup> mice is AGRP which shows opposite regulation in 11 $\beta$ -HSD1<sup>-/-</sup> and control mice. The early induction of 11 $\beta$ -HSD1 in arcuate nucleus and the reversal of arcuate AGRP responses to HF can be mimicked by  $\mu$ -receptor agonism, suggesting indirect relationships between arcuate 11 $\beta$ -HSD1 and AGRP and thus a novel control of appetite within the CNS.

#### 11 $\beta$ -HSD1 deficiency reveals transient HF-hyperphagia and decreased food efficiency

Some rodent strains initially overeat HF diet but soon reduce food intake to the caloric equivalent of less energy-dense chow (32), thus constraining accelerated weight gain (39). Basal intake of LF diet was similar in 11 $\beta$ -HSD1<sup>-/-</sup> and controls, as has been remarked in other lines with a genotypic predisposition to overeat (40) such as CART (31) and MC4R knock-out mice (32). However, in these models HF diets induce sustained hyperphagia. In contrast, HF hyperphagia in 11 $\beta$ -HSD1<sup>-/-</sup> mice was transient and did not accelerate weight gain, indicating perhaps that CNS 11 $\beta$ -HSD1 is involved in a process regulating the initial intake of excess calories. Indeed, the normal transient up-regulation of arcuate 11 $\beta$ -HSD1 with HF in controls supports this contention and suggests this may be an early adaptive response in arcuate neurons to moderate intake in the first wk of HF feeding.

#### 11 $\beta$ -HSD1<sup>-/-</sup> mice fed HF paradoxically up-regulate AGRP

HF-fed control mice reduced hypothalamic AGRP and MC4R and tended to reduce NPY and POMC mRNA levels,

effects seen in some previous studies of HF feeding in rodents (41, 42). These changes might be anticipated to maintain food intake and reduce energy expenditure (less MC4R) allowing weight gain (32). Although the primary driver(s) of these changes are uncertain, HF-induced elevation of leptin levels are proposed to be involved as these suppress arcuate AGRP (43–45) and, in some studies, POMC (41, 46). Moreover, many of these peptides are under partial glucocorticoid regulation; thus arcuate CART but not POMC levels fall with adrenalectomy (47), which agrees with the anticipated lowering of arcuate glucocorticoid levels in 11 $\beta$ -HSD1<sup>-/-</sup> mice. However the transient change in AGRP, also observed in previous studies of HF feeding in mice (29), imply either other pathways supervene and/or the onset of leptin resistance after 18 wk HF. Leptin is an unlikely candidate because 11 $\beta$ -HSD1<sup>-/-</sup> and C57BL/6J controls had similar plasma leptin levels. In striking contrast to controls, 11 $\beta$ -HSD1<sup>-/-</sup> mice increased AGRP mRNA expression in concert with increased food intake. AGRP and 11 $\beta$ -HSD1 were negatively correlated and partially colocalized in the arcuate nucleus, indicating that 11 $\beta$ -HSD1 could be involved in AGRP regulation, directly or indirectly. This speculation is supported by the earlier induction of arcuate 11 $\beta$ -HSD1 than AGRP by HF. Other neuropeptides changed with HF and indeed POMC mRNA also correlated negatively with 11 $\beta$ -HSD1 in arcuate. However, POMC mRNA did not vary with diet or intake. Moreover, POMC encodes anorexigenic  $\alpha$ -MSH and its generally elevated levels in 11 $\beta$ -HSD1<sup>-/-</sup> mice are not anticipated to contribute to their transient HF-associated hyperphagia although increased POMC might contribute to the decreased food efficiency by increasing metabolic rate (40); indeed 11 $\beta$ -HSD1<sup>-/-</sup> mice are mildly hyperthermic compatible with this notion (18).

Adrenalectomy reduces and glucocorticoids (endogenous (diurnal) or exogenous) induce arcuate AGRP (19, 20, 22, 42, 48, 49). Indeed, corticosterone is required for central orexigenic actions of AGRP (20). Yet in the current study, HF feeding in controls increased local arcuate 11 $\beta$ -HSD1, but was consistently associated with reduced AGRP mRNA. Additionally, 11 $\beta$ -HSD1 deficiency led to transient increases of

AGRP mRNA with HF diet. There are several possible explanations of this apparent paradox.

First, arcuate  $11\beta$ -HSD1 levels may be too low to contribute significantly to intra-arcuate glucocorticoid action. However,  $11\beta$ -HSD1 expression in the arcuate is among the highest in the rodent CNS (Fig. 2 and Ref. 25), and, in other CNS regions,  $11\beta$ -HSD1 inhibition or deficiency has clear cellular and functional consequences *in vitro* and *in vivo* (50, 51). It is unlikely that circulating corticosterone levels drives the higher control arcuate AGRP because plasma levels actually fell with 2 wk HF and were similar in the two genotypes, yet the AGRP expression profiles were opposite in the genotypes. Second, arcuate  $11\beta$ -HSD1 might act as a dehydrogenase, inactivating corticosterone, as proposed to occur in human preadipocytes lacking enzyme cofactor (NADPH) generation systems (52, 53). However, this reverse reaction is not apparent in primary neurons from other CNS regions (50);  $11\beta$ -HSD1<sup>-/-</sup> mice have reduced corticosterone levels in CNS regions that otherwise express the enzyme (51) and arcuate extracts from HF-fed controls have increased  $11\beta$ -reductase activity *in vitro*. Therefore, it seems most plausible that with 2 wk of HF, control mouse intra-arcuate glucocorticoid levels are elevated by increased  $11\beta$ -HSD1, while AGRP levels fall. In contrast, in  $11\beta$ -HSD1<sup>-/-</sup> mice, AGRP increases despite reduced arcuate glucocorticoid levels. Third, other peripheral signals may overcome glucocorticoid control. Plasma leptin, which at high levels can overcome glucocorticoid up-regulation of AGRP (54), varied similarly in the two genotypes and, therefore, is unlikely to be involved, but insulin levels were lower in HF-fed  $11\beta$ -HSD1<sup>-/-</sup> mice, as previously reported (18). Intracerebroventricular insulin decreases AGRP immunoreactivity in the hypothalamus (55). Thus lower circulating insulin levels might explain the hyperphagia of  $11\beta$ -HSD1<sup>-/-</sup> mice. However,  $11\beta$ -HSD1<sup>-/-</sup> mice are also insulin-sensitized, at least in peripheral tissues (17, 18), making the central effects of reduced peripheral insulin levels difficult to predict. Moreover, blood-brain barrier insulin transport is saturated at physiological serum insulin levels (56) and, therefore, intracerebral insulin concentrations may be similar in the genotypes. Another possibility is that increased thermogenic drive in brown adipose tissue of  $11\beta$ -HSD1<sup>-/-</sup> mice (18) could engender increased peripheral energy use and cause a compensatory increase in food intake via increased arcuate AGRP expression. Fourth, the effects of local intracerebral glucocorticoids may act differently to the effects of peripheral corticosterone, a concept weakly supported by the absence of an obvious glucocorticoid response element in the AGRP gene promoter (57), although these are not always necessary for glucocorticoid regulation. Fifth, other neural mechanisms affected by  $11\beta$ -HSD1 deficiency may override the influence of  $11\beta$ -HSD1 in the arcuate. For example, exogenous opioids promote fat ingestion (35, 58–61), perhaps in part through effects upon the nucleus accumbens reward system (62). AGRP-mediated fat appetite requires opioid receptor activity (36, 37), although this may be predominantly a local intrahypothalamic effect. The HF-induced increase in arcuate  $11\beta$ -HSD1 was accelerated by the  $\mu$ -receptor agonist DAMGO and blocked by naloxone, suggesting that arcuate  $11\beta$ -HSD1 induction under HF conditions in-

volves an opioid-mediated process. Interestingly, POMC encodes  $\beta$ -endorphin, an endogenous  $\mu$  ligand, so elevated POMC expression in  $11\beta$ -HSD1<sup>-/-</sup> arcuate may reflect a locally increased opioid tone. That arcuate  $11\beta$ -HSD1 did not respond to DAMGO or naloxone under LF indicates a likely indirect control dependent upon palatable diet. It seems plausible to suggest that the opposing effects of HF on AGRP in wild-type and  $11\beta$ -HSD1 knock-out mice may reflect differences in the actions of endogenous opioids upon the AGRP cells in the arcuate nucleus. Thus, the apparent paradoxical reciprocal relationships between arcuate  $11\beta$ -HSD1 and AGRP may reflect their predominant control by an opioid tone that is stimulated by HF feeding and normally acts to constrain excess food intake.  $11\beta$ -HSD1 appears necessary for the early adaptive opioid constraint of AGRP and hyperphagia upon HF feeding.

$11\beta$ -HSD1 uniquely increases local tissue levels of active glucocorticoids (16) without altering systemic corticosterone levels. Mice intrinsically resistant to HF diet-induced metabolic disease suppress adipose tissue  $11\beta$ -HSD1 to a greater degree than strains prone to metabolic disease (34). In this study, we show that HF diet selectively up-regulates arcuate nucleus  $11\beta$ -HSD1 which associates with prevention of AGRP induction and hyperphagia. In wild-type mice, this apparently overcomes HF-induced decreases in MC4R expression which should favor hyperphagia and decreased energy expenditure (32). Consistent with this,  $11\beta$ -HSD1-deficiency reveals HF induction of AGRP and hyperphagia, but decreased food efficiency. Thus, local up-regulation of arcuate  $11\beta$ -HSD1 may represent one of a series of adjustments of this enzyme system to constrain the adverse effects of excess fat intake. We suggest arcuate nucleus  $11\beta$ -HSD1 helps maintain a melanocortin balance that favors normophagia and is induced to modulate HF effects on the melanocortin system. Indeed, previous studies using adrenalectomy models would mask an adaptive response to diet by  $11\beta$ -HSD1-generated hypothalamic glucocorticoids as both corticosterone and the  $11\beta$ -HSD1 substrate 11-dehydrocorticosterone are absent.  $11\beta$ -HSD1 activity is a potential target to treat obesity. These findings illustrate the importance of intracellular glucocorticoid signaling in regulating energy homeostasis and have implications for the CNS effects of therapeutic inhibition of  $11\beta$ -HSD1.

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