

# Glucocorticoids decrease body weight and food intake and inhibit appetite regulatory peptide expression in the hypothalamus of rats

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**Abstract.** The aim of the present study was to investigate the effects of glucocorticoids (GCs) on appetite and gene expression of the hypothalamic appetite regulatory peptides, neuropeptide Y (NPY), agouti-related protein (AGRP) and cocaine and amphetamine-regulated transcript (CART), in non-obese and obese rats. Both non-obese and obese rats were randomly assigned to three groups: normal saline, low- and high-dose GC groups (NSG, LDG and HDG, respectively), which received an intraperitoneal injection with normal saline (0.2 ml/100 g) or hydrocortisone sodium succinate at 5 and 15 mg/kg, respectively, for 20 days. The expression levels of NPY, AGRP and CART mRNA in the hypothalamus were measured by real-time quantitative PCR. Non-obese and obese rats were found to undergo weight loss after GC injection, and a higher degree of weight loss was observed in the HDG rats. The average and cumulative food intakes in the obese and non-obese rats injected with high-dose GC were lower compared to that in the NSG ( $p < 0.05$ ). mRNA expression levels of the orexigenic neuropeptides, NPY and AGRP, and the anorexigenic neuropeptide, CART, were significantly lower in the HDG than levels in the NSG for both the obese and non-obese rats ( $p < 0.05$ ). GC treatment decreased appetite and body weight, induced apparent glucolipid metabolic disturbances and hyperinsulinemia, while down-regulated mRNA expression levels of the orexigenic neuropeptides, NPY and AGRP, and anorexigenic neuropeptide, CART, in the hypothalamus in the rats. The mechanism which induces this neuropeptide expression requires further study.

## Introduction

The balance between energy intake and expenditure is of critical importance to maintain normal body weight, and obesity is the most common state associated with energy imbalance. At present, studies on the mechanisms of obesity involve many factors, and the hypothalamus is a 'hot spot', since it plays a key role in the regulation of appetite and energy homeostasis. Previous studies found that appetite and body weight decreased after damage to the lateral hypothalamic area (LHA) and increased after damage to the ventromedial hypothalamic nucleus (VMH). In view of these findings, a dual center theory was suggested, i.e., the 'LHA hunger center' and 'VMH repletion center' (1). Recent studies have reported that specific neural pathways and several neuropeptide signaling pathways control energy metabolism homeostasis, which are crucial to the regulation of appetite.

Neuropeptide Y (NPY) is an important neurotransmitter in the hypothalamus which regulates appetite and energy homeostasis, and is closely related to the activities of many other appetite regulatory factors (2-6). The mRNA expression level of NPY and its quantity of release are increased during fasting, while they are decreased after food intake. Central administration of NPY has been found to stimulate food intake, reduce energy expenditure and brown fat lysis, inhibit sympathetic nervous activity and thyroid axis activity, and increase serum leptin, insulin and cortisol levels (7-9).

NPY gene knockout mice exhibited decreased refeeding in response to 24 or 48 h fasting (10), although they had normal body weight and adipose tissue. It is possible that a compensation mechanism exists, and other signaling pathways aid to partially up-regulate appetite, for example, agouti-related protein (AGRP).

The endogenous melanocortin receptor antagonist AGRP and NPY coexist in the arcuate nucleus (ARC), and both exert orexigenic effects (11-14). The AGRP mRNA level is up-regulated in fasting. The AGRP C-terminal fragment inhibits the anorectic effect of  $\alpha$ -MSH and increases nighttime food intake. AGRP not only alters food intake, but also decreases energy expenditure. Central administration of AGRP and transgenic animals overexpressing AGRP both result in increased food intake (15,16). Chronic intracerebroventricular

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(i.c.v.) administration of AGRP was found to increase food intake, body weight and body adiposity, resulted in a profound suppression of brown adipose tissue uncoupling protein 1 (BAT UCP-1) and decreased plasma TSH, and these metabolic effects were independent of food intake (17,18).

Cocaine and amphetamine-regulated transcript (CART) peptides play a role in the control of food intake by the brain, and exhibit functional interaction with NPY. CART and  $\alpha$ -MSH coexist in the ARC, and are also expressed in nerve cells in the LHA and paraventricular nucleus (PVN). Administration of the CART peptide fragment (i.c.v.) was found to inhibit feeding in rats both at night and in response to fasting. Injection of the CART peptide before NPY attenuated the increase in feeding caused by the injection of NPY alone (11-14).

Elevated circulating level of glucocorticoids (GCs) or hypersensitivity to these hormones has long been thought to play a role in the development and maintenance of obesity. In obese humans, particularly those with abdominal obesity, increased production of GCs, increased concentrations of tissue GC and GC receptors, and over-responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to different neuropeptides and stress tasks have been reported. Clinically, patients treated with GCs or who exhibit GC over-secretion consistently show increased body weight. Animals administered GCs (i.c.v.) reportedly display increased food intake and body weight (19,20), while other studies have reported that GCs inhibit food intake and body weight.

Thus, paradoxical findings exist on the effect of GC on food intake, body weight and appetite regulatory peptides in rats. In the present study, we investigated the effect of a GC (hydrocortisone sodium succinate, 20-day continuous intraperitoneal infusion) on food intake, body weight and the hypothalamic appetite regulatory peptides, NPY, AGRP and CART, in non-obese and obese rats.

## Materials and methods

**Materials.** The Superscript II RT kit was purchased from Invitrogen (USA). The RNase-free DNase kit was purchased from Qiagen (Germany). The PCR primers were synthesized by the Shanghai Sangon Factory (Shanghai, China). TaqDNA polymerase, isopropyl alcohol and dehydrated alcohol were also purchased from the Shanghai Sangon Factory. One hundred and twenty-five healthy male SPF SD rats were purchased from the Experimental Animal LLC (license no. 2003-0003; Shanghai, China). Hydrocortisone sodium succinate was purchased from Tianjin Biochemistry Pharmaceutical Factory (batch no. 20060314; Tianjin, China).

**Animals and diets.** One hundred and twenty-five healthy male SFP SD rats, weight 180-200 g, were randomly assigned into groups of 38 chow-diet rats (CD; containing water 9.2%, crude protein 22.1%, crude fat 5.28%, crude ash content 5.20%, crude cellulose 4.12%, no nitrogen extract 52.0%, calcium 1.24%, phosphorus 0.92%, calcium/phosphorus 1.35, lysine 1.34%, methionine + cystine 0.72%; total energy content 352 kcal/100 g), and 87 high-fat diet rats [HF; containing fat 20%, granulated sugar 4%, whole milk powder (Yili Brand) 2%, cholesterol 1%, chleolate 0.5%, basic animal feeds 73%; total energy content

Table I. Primers used for real-time RT-PCR.

Name (organism/Genbank)	Primers (5'-3')
$\beta$ -actin (rats/NM_031144)	GACGGTCAGGTCATCACTATCG ACGGATGTCAACGTCACACTTC
NPY (rats/NM_012614)	CAAGAGATCCAGCCCTGAGACA CATCACCACATGGAAGGGTCTTC
AGRP (rats/XM_574228; AF_206017)	GGCCATGCTGACTGCAATG CGGTCTGCTGCTGTCTTGTTTC
CART (rats/NM_017110)	GATGCGTCCCATGAGAAGGA TCGGAATGCGTTTACTCTTGAG

493 kcal/100 g, made by Shanghai Experimental Animal LLC and stored at -20 to 4°C]. All rats were provided with water (pH 2.5-2.8) and feed freely. After 80 days of HF feed, the rats with a body weight greater than the mean weight + 1.5 standard deviations, and a Lee's index greater than the mean Lee's index + 1.5 standard deviations, were characterized as obese. Rats fed the CD were characterized as non-obese. All animal handling procedures and the protocol were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**GC treatment.** Non-obese and obese rats were randomly assigned to a normal saline group (NSG; 0.2 ml/100 g/day), low-dose GC group (LDG; hydrocortisone sodium succinate 5 mg/kg/day) or high-dose GC group (HDG; hydrocortisone sodium succinate 15 mg/kg/day), and received a peritoneal injection at 8:00 a.m. for 20 consecutive days (deviation  $\leq 10$  min). Hydrocortisone sodium succinate was freshly prepared before the injection every day.

**Observation index.** Vitality, activity, fur, appetite, diachorema, weight, stem length (from nose to anus), Lee's index {Lee's index = [weight (g)/stem length (cm)]<sup>1/3</sup> x 10}, serum glucose and serum lipids were examined.

**Sample preparation.** Food and drink deprivation was carried out  $\geq 8$  h, starting at 7:00-9:00 a.m. Serum samples were collected from the tail (to avoid hemolysis), stored for 30 min at room temperature, centrifuged at 5,000 rpm for 20 min to separate serum, and then stored at -80°C.

**Serology assay method.** Serum glucose and lipids were detected by a fully automatic biochemistry assay instrument (Olympus AV2700).

**Total RNA extraction and identification.** Total RNA was extracted using TRIzol (Gibco/Brl) according to the manufacturer's instructions.

**Real-time PCR.** The primers used for real-time RT-PCR experiment were synthesized as shown Table I. Opticon Monitor 3 software was used to analyze the results, and thus obtained the  $C_T$ . The housekeeping gene  $\beta$ -actin was used as the control

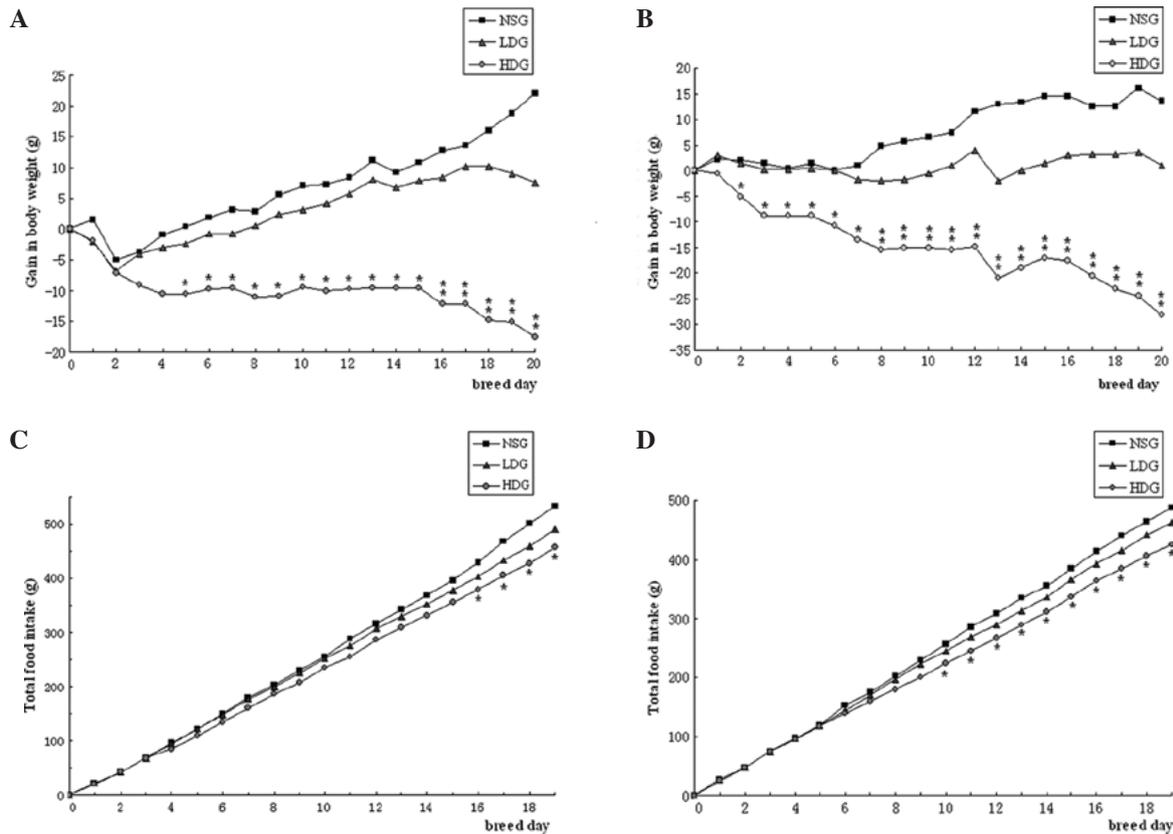


Figure 1. Effect of GC on body weight and cumulative food intake in rats. High-dose GC (15 mg/kg/day) reduced body weight in both (A) non-obese rats from the 5th day of injection and (B) obese rats from the 2nd day, and these differences lasted until the end of treatment. High-dose GC inhibited food intake in both (C) non-obese rats from the 16th day and (D) obese rats from the 10th day, and the significance of the effect lasted to the end of the treatment. After low-dose GC treatment (5 mg/kg/day), neither body weight nor cumulative food intake was significantly different from that of the NSG (\* $p < 0.05$ , \*\* $p < 0.01$ ). NSG, normal saline group; LDG, low-dose glucocorticoid group (5 mg/kg/day); HDG, high-dose glucocorticoid group (15 mg/kg/day).

to attain  $\Delta C_T$  after verifying  $C_T$ . The difference between the treated and control groups was expressed as  $2^{-\Delta\Delta C_T}$ .

$$\Delta C_T = C_{T(\text{target})} - C_{T(\beta\text{-actin})} \text{ and } \Delta\Delta C_T = \Delta C_{T(\text{treated})} - \Delta C_{T(\text{control})}$$

**Statistical methods.** Measurement data are presented as the mean  $\pm$  standard error. The Student's *t*-test was used for comparisons between the means of two groups after homoscedasticity was verified by homogeneity test for variance with the SPSS Base 11.5 software package. A value of  $p < 0.05$  was considered statistically significant.

## Results

**GC-related decrease in the body weight of the rats.** The mean weight decreased by  $25.69 \pm 23.05$  g after high-dose GC treatment in the non-obese rats, while it increased by  $5.08 \pm 30.68$  g ( $t = 4.09$ ,  $p < 0.01$ ) after normal saline treatment, while the body length did not exhibit any significant difference ( $0.58 \pm 0.95$  vs.  $0.54 \pm 0.54$  cm,  $t = 0.56$ ,  $p > 0.05$ ). In the obese rats, the mean weight decreased by  $33.07 \pm 30.01$  g after high-dose GC treatment, while it increased by  $12.00 \pm 30.08$  g ( $t = 4.19$ ,  $p < 0.01$ ) after normal saline treatment, and the body length was unchanged ( $0.68 \pm 1.22$  vs.  $0.50 \pm 0.71$  cm,  $t = 0.47$ ,  $p > 0.05$ ).

Rats in the LDG had no obvious difference in body weight or length change compared to rats in the HDG and NSG for either the non-obese or obese rats ( $p > 0.05$ ).

The body weight of the non-obese rats after high-dose GC treatment was evidently decreased from the 5th day to the end of treatment compared to the NSG rats ( $t = 2.08$ ,  $p < 0.05$ ), and the difference was significantly enhanced from the 16th day ( $t = 3.06$ ,  $p < 0.01$ ) (Fig. 1A). For the obese rats, the decrease began from the 2nd day ( $t = 2.54$ ,  $p < 0.05$ ) and was augmented from the 8th day ( $t = 4.53$ ,  $p < 0.001$ ) (Fig. 1B). These results revealed that body weight decreased after high-dose GC treatment in both the non-obese and obese rats, and decreased earlier in the obese rats.

**GC decreases the appetite in the rats.** To understand the possible mechanism of the weight decrease which occurred after the high-dose GC injection, the mean food intake in the rats was analyzed. In the non-obese rats the mean food intake was found to be significantly decreased after 20 days of continuous high-dose GC treatment compared to the mean food intake level in the NSG rats ( $23.91 \pm 1.89$  vs.  $28.02 \pm 3.22$  g/day,  $t = 3.85$ ,  $p < 0.05$ ), while there was no significant difference in food intake between the LDG and NSG rats ( $25.61 \pm 4.52$  vs.  $28.02 \pm 3.22$  g/day,  $t = 1.55$ ,  $p > 0.05$ ) (Fig. 1C). In the obese rats, the mean food intake also decreased after high-dose GC treatment compared to the NSG rats ( $22.64 \pm 2.86$  vs.  $25.87 \pm 3.56$  g/day,  $t = 2.60$ ,  $p < 0.05$ ), and again there was no significant difference in mean food intake between rats in the LDG and NSG ( $24.64 \pm 2.95$  vs.  $25.87 \pm 3.56$  g/day,  $t = 0.95$ ,  $p > 0.05$ ) (Fig. 1D).

Table II. Effects of GC on serum glucose, lipids and insulin in rats.

Group (N)	Glu (mmol/l)	TG (mmol/l)	CHO (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	INS ( $\mu$ IU/ml)
Non-obese rats						
NSG (12)	5.28 $\pm$ 0.74	1.02 $\pm$ 0.26	2.45 $\pm$ 0.37	0.44 $\pm$ 0.09	1.65 $\pm$ 0.33	19.42 $\pm$ 2.90
LDG (13)	6.21 $\pm$ 0.97 <sup>a</sup>	1.87 $\pm$ 0.62 <sup>c</sup>	3.10 $\pm$ 0.81	0.65 $\pm$ 0.30 <sup>a</sup>	1.33 $\pm$ 0.31 <sup>a</sup>	24.31 $\pm$ 3.76 <sup>b</sup>
HDG (13)	6.80 $\pm$ 0.80 <sup>c</sup>	2.12 $\pm$ 0.61 <sup>c</sup>	3.70 $\pm$ 1.25 <sup>b</sup>	0.80 $\pm$ 0.14 <sup>c</sup>	1.16 $\pm$ 0.26 <sup>c</sup>	38.58 $\pm$ 5.38 <sup>c</sup>
F	10.19	14.50	6.00	10.10	8.52	72.16
p-value	<0.001	<0.001	<0.01	<0.001	<0.01	<0.001
Obese rats						
NSG (13)	6.53 $\pm$ 1.72	1.67 $\pm$ 0.73	3.00 $\pm$ 0.85	0.93 $\pm$ 0.18	1.12 $\pm$ 0.15	33.56 $\pm$ 3.12
LDG (13)	7.77 $\pm$ 1.19 <sup>a</sup>	2.30 $\pm$ 0.71	3.79 $\pm$ 0.86 <sup>a</sup>	1.73 $\pm$ 0.94 <sup>b</sup>	0.90 $\pm$ 0.17 <sup>b</sup>	40.69 $\pm$ 3.57 <sup>c</sup>
HDG (14)	8.16 $\pm$ 0.87 <sup>b</sup>	2.84 $\pm$ 0.95 <sup>b</sup>	4.13 $\pm$ 1.01 <sup>b</sup>	1.86 $\pm$ 0.73 <sup>b</sup>	0.86 $\pm$ 0.16 <sup>c</sup>	53.62 $\pm$ 5.69 <sup>c</sup>
F	5.50	6.95	5.17	6.85	10.32	75.09
p-value	<0.01	<0.01	<0.05	<0.01	<0.001	<0.001

Glu, glucose; TG, triglycerides; CHO, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein, INS, insulin. NSG, normal saline group; LDG, low-dose glucocorticoid group (5 mg/kg/day); HDG, high-dose glucocorticoid group (15 mg/kg/day). <sup>a</sup> $p$ <0.05, <sup>b</sup> $p$ <0.01, <sup>c</sup> $p$ <0.001, both compared to NSG. Data are represented as the mean  $\pm$  SE.

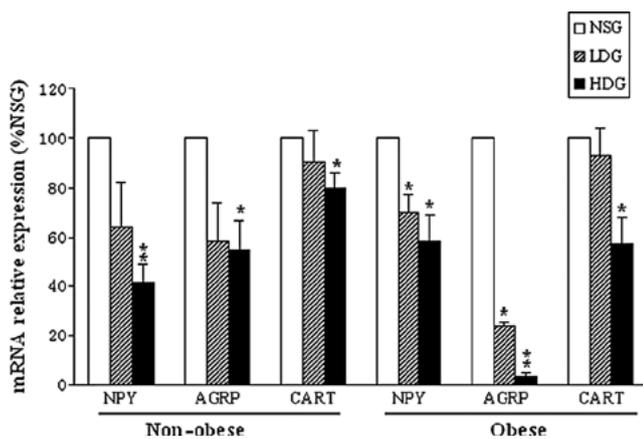


Figure 2. Effect of GC on NPY, AGRP and CART gene expression in the hypothalamus of rats. Non-obese rats (NSG, n=12; LDG and HDG, n=13), Obese rats (NSG and LDG, n=13; HDG, n=14). NSG, normal saline group; LDG, low-dose glucocorticoid group (5 mg/kg/day); HDG, high-dose glucocorticoid group (15 mg/kg/day). Data are expressed as the means  $\pm$  SEM of the NSG. <sup>\*</sup> $p$ <0.05, <sup>\*\*</sup> $p$ <0.01.

Cumulative food intake in the HDG rats became significantly less than that of the NSG rats on the 16th day after treatment for the non-obese rats (380.00 $\pm$ 35.00 vs. 430.42 $\pm$ 57.53 g,  $t$ =2.31,  $p$ <0.05) (Fig. 1C). For the obese rats, cumulative food intake decreased earlier than that in the non-obese rats, which began on the 10th day after treatment (232.79 $\pm$ 21.45 vs. 257.15 $\pm$ 38.66 g,  $t$ =2.42,  $P$ <0.05) (Fig. 1D). After low-dose GC treatment, cumulative food intake did not exhibit any significant difference compared to the NSG rats ( $p$ >0.05).

*GC treatment elevates serum glucose and insulin concentrations resulting in dislipidemia.* Serum glucose, triglycerides, total cholesterol, low-density lipoprotein and insulin increased ( $p$ <0.01), while high-density lipoprotein decreased significantly ( $p$ <0.001) after the 20-day continuous high-dose GC

treatment compared to rats in the NSG for both the non-obese and obese rats (Table II).

For the non-obese rats, after 20 continuous days of low-dose GC treatment, total cholesterol was not significantly different than total cholesterol in the NSG rats ( $p$ >0.05), while serum glucose, insulin, triglyceride and low-density lipoprotein were significantly elevated compared to the control ( $p$ <0.05). However, in the obese rats, after low-dose GC treatment, triglycerides were not significantly different compared to the rats in the NSG ( $p$ >0.05), while serum glucose, insulin, total cholesterol and low-density lipoprotein were significantly elevated ( $p$ <0.05).

*GC treatment decreases the NPY, AGRP and CART gene expression levels in the hypothalamus of the rats.* After 20 continuous days of high-dose GC treatment, mRNA gene expression levels of the appetite peptides NPY, AGRP and CART decreased to 58 $\pm$ 11, 3 $\pm$ 1.7 and 57 $\pm$ 11%, respectively, compared to levels in the NSG for the obese rats; while in the non-obese rats, these levels dropped to 41 $\pm$ 8, 55 $\pm$ 11 and 80 $\pm$ 6%, respectively, compared to those of the NSG rats (Fig. 2).

However, after the 20-day continuous low-dose GC treatment, the mRNA expression levels of the appetite peptides NPY and AGRP and CART decreased to 70 $\pm$ 7, 24 $\pm$ 1.5 and 93 $\pm$ 11%, respectively, compared to levels in the NSG for the obese rats. For the non-obese rats, these levels declined to 64 $\pm$ 18, 58 $\pm$ 16 and 90 $\pm$ 13%, respectively, compared to those in the NSG (Fig. 2).

We observed that after high-dose GC treatment, mRNA gene expression levels of the orexigenic neuropeptides NPY and AGRP were lower than levels in the NSG ( $p$ <0.05), and also for the mRNA expression level of the anorectic neuropeptide CART, in both the non-obese and obese rats (Fig. 2).

There were certain differences in the appetite-regulation peptide expression patterns between the obese and non-obese rats after low-dose GC treatment (Fig. 2). The NPY, AGRP and CART mRNA expression levels did not exhibit any significant differences compared to the NSG for the non-obese rats. For

the obese rats after low-dose GC treatment, the NPY and AGRP mRNA expression levels were significantly decreased compared to those of the NSG rats ( $p < 0.05$ ), while CART mRNA did not exhibit a significant decrease.

## Discussion

The present data revealed that a 20-day continuous intraperitoneal infusion of the synthetic glucocorticoid, hydrocortisone sodium succinate, to non-obese and obese rats decreased their body weight and food intake. Twenty-day consecutive intraperitoneal injection of GC at a dose of 5 and 15 mg/kg both decreased weight and food intake in the rats (180-200 g), significantly at the latter dose. It was also found that weight and food intake decreased earlier and the extent of the decrease was markedly greater in the HDG compared to the LDG rats. This effect of GC on food intake and weight in rats is in agreement with previous studies. De Vos *et al* treated rats once daily for 20 days with a subcutaneous pharmacological dose of hydrocortisone (1, 10 and 100  $\mu\text{g/g/day}$ ) and reported that glucocorticoids caused a decrease in body weight and food consumption (21). Zakrzewska *et al* (19) found that intraperitoneal infusion of dexamethasone (Dex; 0.025 mg/kg/day) for 3 days in normal rats resulted in a significant decrease in food intake and body weight. Konno *et al* (22) reported that a 10-day subcutaneous injection of Dex (2 mg/kg/day) resulted in a significant decrease in weight and food intake in Wistar rats. Coll *et al* (23) reported a significant decrease in body weight and length in *Pomc*<sup>-/-</sup> mice after receiving corticosterone-supplemented drinking water (25  $\mu\text{g/ml}$  final concentration). These results indicate that GCs have an inhibitory effect on body weight and food intake in rodents.

However, divergent findings have been reported on the effect of GCs on body weight and food intake. Zakrzewska *et al* (19) found that i.c.v. infusion of Dex (0.025 mg/kg/day) for 3 days in normal rats resulted in a significant increase in food intake relative to vehicle-infused control animals. Germano *et al* (24) reported weight and daily food intake were significantly reduced 14 days after adrenalectomy (ADX) compared to a sham group. Devenport *et al* found that continuous infusion of corticosterone yielded a bitonic dose-response curve for body weight and feeding efficiency (25). The effects appear to occur at approximately 1  $\mu\text{g/dl}$ , and reached peak anabolic effect at approximately 2  $\mu\text{g/dl}$ , then decreased with the increase in serum levels.

In the present study NPY, AGRP and CART mRNA expression was evaluated in the hypothalamus in non-obese and obese rats after GC treatment using real-time quantitative PCR. At the end of the experiment, NPY and AGRP mRNA expression had significantly decreased in the obese rats after 20 consecutive days of a 5 mg/kg/day GC injection, while no significant decrease was observed in non-obese rats (5 mg/kg/day). The mRNA expression of the orexigenic neuropeptides NPY and AGRP decreased in both the non-obese and obese rats after 20 consecutive days of 15 mg/kg/day GC injection. This result may account, at least in part, for the decreased food intake and weight after GC treatment in rats, since the orexigenic neuropeptides NPY and AGRP are important in promoting appetite and maintaining energy homeostasis. The finding that GC treatment decreased NPY and AGRP mRNA

expression in the hypothalamus of non-obese and obese rats is consistent with the result that intraperitoneal Dex infusion (0.025 mg/kg/day for 3 days) resulted in a significant decrease in the arcuate nucleus NPY levels (19). However, there are several contradictory reports. Konno *et al* reported a higher NPY mRNA level in the hypothalamus of Wistar rats after 10 consecutive days of a subcutaneous injection of Dex (2 mg/kg/day) (22). Makimura *et al* (26) found that ADX completely blocked the elevation of NPY and AGRP mRNA in diabetic mice. Hypothalamic AGRP mRNA was reportedly induced by a GC implant, whereas the NPY mRNA was not significantly influenced. In addition, Drazen *et al* (27) found that ADX altered the sensitivity of the central melanocortin system to the effect of the melanocortin antagonist AGRP, with the orexigenic effect of this peptide being absent in ADX rats, but restored with GC supplementation (2.7 mg/ml corticosterone in their drinking water).

CART is co-localized within the majority of proopiomelanocortin (POMC) neurons in the hypothalamus and inhibits food intake. CART expression is regulated by GCs, and CART, in turn, appears to regulate hypothalamic-pituitary-adrenal (HPA) axis activity via a direct effect on corticotrophin-releasing hormone (CRH) signaling (28-31). Germano *et al* reported that Wistar rats following long-term ADX displayed a decrease in CART expression in the hypothalamus under *ad libitum* feeding conditions (24). Hunter *et al* reported that ADX in rats significantly reduced CART expression in the hypothalamus and this effect was blocked by corticosterone (400  $\mu\text{g/ml}$  in their drinking water) (32). However, we found that GC treatment decreased CART mRNA expression in the hypothalamus in both non-obese and obese rats.

There is a GC response element upstream of the rat NPY gene, and GC receptors have been shown to be highly expressed in all NPY-containing neurons in the arcuate nucleus (33-35). Since all arcuate AGRP neurons express NPY, there are GC receptors in AGRP neurons (11). We believe that centrally administered GCs may exert a direct effect on appetite peptide neurons, while peripherally administered GCs exert a more complex and indirect effect.

Arc neurons, including POMC and AGRP/NPY neurons, have been shown to respond to changes in ambient glucose concentrations, either as being glucose excited (increase in glucose leads to increase in firing) or glucose inhibited (increase in glucose leads to decrease in firing) (36-38).

Arumugam *et al* found that glucose deprivation (2 mmol/l) stimulated an increase in NPY mRNA levels in INS-1 cells, since INS-1 cells have certain functional similarities with hypothalamic neurons (39). Conversely, glucose excess (11 mmol/l) inhibited expression of NPY mRNA, but transiently increased the expression of CART. They also found that 1  $\mu\text{M}$  Dex stimulated a 5.2-fold increase in NPY mRNA, while it reduced the levels of CART mRNA by 65%. Singh *et al* demonstrated that the maternal diabetic state associated with fetal hyperglycemia in the absence of fetal insulin changes led to a 40% decline in fetal brain NPY mRNA and a 50% decline in protein levels (40).

Circulating insulin may regulate food intake and body weight as the result of its effects in the central nervous system (CNS). Circulating insulin delivery into the CNS is facilitated by an insulin receptor-mediated transport process (41). Insulin

was found to reduce food intake and body weight in a dose-dependent manner when administered directly into the CNS (42). Central insulin administration attenuates the increase in hypothalamic NPY mRNA levels associated with both fasting and insulin-deficiency diabetes (43), and insulin has been shown to transport across the endothelial cells of the blood-brain barrier (41). Combined with the evidence that receptors for insulin are concentrated in the ARC (44,45), these results suggest that the hypothalamic NPY system is normally inhibited by the negative feedback provided by insulin. In our study, the increase in the serum insulin level after GC treatment may in part explain the decrease in food intake and body weight in rats. In another study, Sahu *et al* reported that in the adult rat, streptozotocin-induced diabetes associated with hyperglycemia and hypoinsulinemia caused an increase in hypothalamic NPY levels (46).

In our study, we found that 20 consecutive days of intraperitoneal injection of GC at doses of 5 and 15 mg/kg led to hyperglycemia and hyperinsulinemia in obese and non-obese rats, and we think that this may be part of the reason for the decrease in NPY in the hypothalamus.

We suspect that hyperglycemia and hyperinsulinemia exert a synergistic effect on the inhibition of NPY expression in the hypothalamus after long-term GC treatment. Short-term GC treatment is not always accompanied by hyperglycemia and hyperinsulinemia, which may be the reason for the contradictory effects on NPY expression between long- and short-term GC treatments.

In normal rats, leptin administration blunts the effect of fasting by increasing the hypothalamic NPY mRNA levels (44). Systemic administration of leptin inhibits NPY gene overexpression through a specific action in the arcuate nucleus and exerts a hypoglycemic action that is partly independent of its weight-reducing effects (47). A majority of both NPY/AGRP and POMC/CART neurons have been found to coexpress leptin receptors (48,49).

Ma *et al* found that the effects of leptin depend on the ambient glucose concentration (50). When glucose is low (5 mM or less), AGRP neurons are more important for mediating the anorectic effects of leptin than POMC cells. However, at high glucose concentrations (11 mM), activation of POMC cells may contribute to the appetite-suppressing effects of leptin.

De Vos *et al* treated rats once daily for 20 days with a subcutaneous pharmacological dose of hydrocortisone (1, 10 and 100  $\mu\text{g/g/day}$ ), and found that GCs induce overexpression in rat adipose tissue dose-dependently (21). The glucose-dependent effects of leptin may also help explain why GCs display a bitonic dose-response curve for body weight and feeding efficiency. Short-term or low-dose GC treatment may increase the serum glucose level slightly, and in this condition leptin may exert an orexigenic effect by inhibiting POMC function, while long-term, high-dose GC treatment always increases the serum glucose level significantly and excites the leptin anorexigenic effect.

We presume that the difference between our study and previous reports may be due to the low-dose GC and/or the short-term GC treatment increasing NPY and AGRP mRNA expression through a direct effect. However, high-dose GC and/or long-term GC treatment resulted in an inhibitory effect on NPY and AGRP mRNA expression, which may be an

indirect effect of GCs. It is known that hyperglycemia, lipid metabolic disturbance, hyperinsulinemia and hyperleptinemia are associated with lower expression levels of NPY and AGRP mRNA. High-dose GC and/or long-term GC treatment may induce hyperglycemia, lipid metabolic disturbance, hyperinsulinemia and hyperleptinemia first, and these metabolic disturbances inhibit NPY and AGRP mRNA expression in the hypothalamus. In the present study, we found that weight and food intake decreased earlier, and the extent of the decrease being more obvious after high- than low-dose GC treatment is also consistent with this hypothesis. High-dose GC treatment causes earlier and more serious glucose and lipid disturbances, which could result in a decrease in NPY and AGRP mRNA expression, followed by decreased food intake and weight.

The current understanding of the central appetite regulating NPY, AGRP and CART signaling pathways is not able to explain the decrease in CART expression, and the decrease in food intake and weight, concurrently after GC treatment. There is still much more to explore with regards to how GCs affect appetite and body weight. Whether the dosage, administration route (i.c.v. or intraperitoneal injection) or duration of treatment is responsible for the contradictory outcome of appetite and body weight change after GC treatment, or via some as yet unidentified mechanism or target, remains to be determined.

In conclusion, GC treatment in rat decreased appetite and body weight, induced apparent glucolipid metabolic disturbances and hyperinsulinemia, while down-regulated orexigenic neuropeptides NPY and AGRP and anorexigenic neuropeptide CART mRNA expression levels in the hypothalamus in rats. High-dose GC and/or long-term GC treatment may exert an inhibitory effect on body weight, food intake and appetite peptide expression in the hypothalamus through an indirect effect secondary to metabolic changes. Elucidation of the mechanism requires further study.

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