The CRF/urocortin system regulates white fat browning in mice through paracrine mechanisms

Lu B [*], Diz-Chaves Y[*], Markovic D, Contarino A, Penicaud L, Fanelli F, Clark S, Lehnert H, Cota D, Gramatopoulos DK[*], Tabarin A[*,#]

[*] **These authors contributed equally to the study**

#Corresponding author: Dr A. Tabarin (antoine.tabarin@chu-bordeaux.fr)

tel : +33 557656810 ; fax : + 33557656529

(BL, DM, DKG) Division of Metabolic and Vascular Health, Warwick Medical School, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK

(YDC) Laboratory of Endocrinology, Center for Biomedical Research, Campus As Lagoas-Marcosende, University of Vigo, Vigo, Spain.

(AC) Université de Bordeaux 2, INCIA, CNRS UMR 5287, 33000 Bordeaux, France

(LP) Centre des Sciences du Goût et de l'Alimentation, UMR 6265 CNRS, UMR 1324 INRA, Université de Bourgogne, 21000 Dijon, France

(FF) Endocrinology Unit and Centro di Ricerca Biomedica Applicata, Department of Clinical Medicine, S.Orsola-Malpighi Hospital, Alma Mater University of Bologna, I-44138 Bologna, Italy

21 (HL) Department of Internal Medicine I, University of Luebeck, Ratzeburger Allee 160, D-
22 23538 Luebeck, Germany

23 (SC, DC) INSERM, Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale,
24 U862, F-33000 Bordeaux, France; Université de Bordeaux, F-33000 Bordeaux, France

25 (AT) INSERM, Neurocentre Magendie, Physiopathologie de la Plasticité Neuronale, U862, F-
26 33000 Bordeaux, France; Université de Bordeaux and Dpt of Endocrinology, USN Haut
27 Leveque, CHU of Bordeaux, 33604 Pessac, France

28

29

30

31 **Running tittle :** CRF/Urocortin system regulates fat browning.

32

ABSTRACT

Objectives

The corticotrophin-releasing factor (CRF)/urocortin system is expressed in the adipose tissue of mammals but its functional role in this tissue remains unknown.

Methods

Pharmacological manipulation of CRF-Receptors, CRF_1 and CRF_2, activity was performed in 3T3L1 white pre-adipocytes and T37i brown pre-adipocytes during *in vitro* differentiation. The expression of genes of the CRF/Urocortin system and of markers of white and brown adipocytes was evaluated along with mitochondrial biogenesis and cellular oxygen consumption. Metabolic evaluation of corticosterone-deficient or supplemented Crhr null (*Crhr*$^{-/-}$) mice and their wild-type controls was performed along with gene expression analysis carried out in white (WAT) and brown (BAT) adipose tissues.

Results

Peptides of the CRF/Urocortin system and their cognate receptors were expressed in both pre-adipocyte cell lines. *In vitro* pharmacological studies showed an inhibition of the expression of the CRF_2 pathway by the constitutive activity of the CRF_1 pathway. Pharmacological activation of CRF_2 and, to a lesser extent, inhibition of CRF_1 signaling induced molecular and functional changes indicating transdifferentiation of white pre-adipocytes and differentiation of brown pre-adipocytes. Crhr$^{-/-}$ mice showed increased expression of CRF_2 and its agonist Urocortin 2 in adipocytes that was associated to brown conversion of WAT and activation of BAT. Crhr$^{-/-}$ mice were resistant to diet-induced obesity and glucose intolerance. Restoring physiological circulating corticosterone levels abrogated molecular changes in adipocytes and the favorable phenotype of Crhr$^{-/-}$ mice.

Conclusions

Our findings suggest the importance of the CRF_2 pathway in the control of adipocyte plasticity. Increased CRF_2 activity in adipocytes induces browning of WAT, differentiation of BAT and is associated with a favorable metabolic phenotype in mice lacking CRF_1. Circulating corticosterone represses CRF_2 activity in adipocytes and may thus regulate adipocyte physiology through the modulation of the local CRF/Urocortin system. Targeting CRF-receptor signaling specifically in the adipose tissue may represent a novel approach to tackle obesity.

Keywords: CRF, urocortin, white adipose tissue, brown adipose tissue, CRF_1, CRF_2, obesity,

adipocyte plasticity

Introduction

The corticotrophin-releasing factor (CRF)/urocortin system is a complex neuroendocrine system that includes four structurally related peptides [CRF, and urocortins -1, -2 and -3] and two seven-transmembrane domain receptors, CRF_1 and CRF_2, which mediate the physiological effects of these peptides (1). CRF is a preferential CRF_1 ligand, while urocortin 1 has high affinity for both receptors and urocortin 2 and -3 are specific for CRF_2. Apart from its pivotal role in orchestrating responses to stress, the CRF/urocortin system also regulates energy balance (1-5). Proposed effects include modulation of energy expenditure, fuel partitioning and metabolism through various mechanisms, including the regulation of the sympathetic nervous system (SNS) activity and of glucocorticoid secretion, as well as regulation of food intake (1-3; 5).

The distinct anatomical distributions of CRF_1 and CRF_2 imply diverse physiological functions. Although the relative contribution of the two receptors in energy homeostasis remains debatable, emerging evidence suggests an independent and prominent role of the CRF_2 pathway in the CNS to regulate feeding, glucose metabolism and thermoregulation (1; 3; 5; 6). CRF_2 pattern of expression suggests that this receptor may also participate in the regulation of energy balance in key peripheral tissues involved in energy metabolism and modulate fuel utilization by acting locally through paracrine mechanisms at the level of pancreatic β- and skeletal muscle cells (7-9).

CRF, urocortins and CRF-R are expressed in the white adipose tissue of various species, including human subcutaneous and visceral white adipocytes (1; 10-13). The functional role of CRF-R in the adipose tissue remains unknown. Only one pharmacological study found that activation of the CRF_2 reduces lipolysis in mature human subcutaneous white

adipocytes (14). Therefore, the purpose of the present study was to investigate the functional relevance of CRF-R pathways in the adipose tissue.

MATERIALS AND METHODS

Total RNA extraction and quantitative RT-PCR (qPCR)

Samples (inguinal white adipose tissue, interscapular brown adipose tissue, 3T3-L1 and T37i cells) were homogenized with lysis buffer and total RNA was extracted using Qiagen RNeasyTM Lipid Tissue Mini Kit, according to the manufacturer's instructions. Extracted total RNA was reverse-transcribed into cDNA by two-step reverse transcription PCR using SuperScriptTM II Reverse Transcriptase. QPCR was carried out using a Light Cycler^{TM} system (Roche Molecular Biochemicals Germany). The qPCR primers are listed in Supplemental Table 1. The reaction was carried out in a 10 µl of reaction mixture containing 5 µl PCR 2 x Mastermix with 2 mM MgCl2, 0.5 µl Light Cycler DNA Master SYBER®Green I, 1 µl of each primer (2 µg/ µl), and 1 µl cDNA. The qPCR protocol consisted of a denaturation step at 95°C for 15 sec, following by 40 cycles of amplification at 95°C for 5 sec, 58°C for 10 sec, 72°C for 15 sec, and finally by a melting curve analysis step at 95°C for 10 sec, 56°C for 15 sec, and 99°C for 10 sec. Quantitative amounts of gene of interest were standardized against the housekeeping genes β-actin and GAPDH. Preparations lacking RNA or reverse transcriptase were used as negative controls. RNA expression was tested in 4 independent experiments. mRNA relative level of expression was calculated using the comparative ($2^{-\Delta\Delta CT}$) method.

3T3-L1 and T37i cell cultures

3T3-L1 cells were differentiated in growth medium (DMEM/F12 medium containing 10% BCS, 100 U/ml penicillin and 100 mg/ml streptomycin) and 15 nM HEPES with 1 g/l glucose. Differentiation was induced by incubation with 10% FBS with 5 µg/ml insulin , 0.25 µM dexamethasone, and 0.5 mM IBMX for 2 days before return to growth medium. 3T3-L1 fully differentiated within 6-10 days. T37i cells were cultured in DMEM/F12 medium with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin, and 15 nM HEPES with 1 g/l glucose. Differentiation was achieved by incubating sub-confluent undifferentiated T37i cells with 2nM triiodothyronine and 20 nM insulin for 8-12 days. In some experiments, 3T3L1 preadipocytes were exposed to CRF (100 nM), urocortin 2 (100 nM), and/or the CRF_1 antagonist NBI 27914 (1 µM) for various time periods (2, 4, 6 or 8 days). At the end of the required period, cells were washed with ice-cold PBS and lysed in RNA extraction buffer. Extracted RNA was further processed by qPCR.

Immunocytochemistry

Adipose tissue samples were fixed for 16-24 hr in 4% paraformaldehyde (PFA) at 4°C, paraffin embedded and cut at 7µm using a microtome (Leica Microsystems, USA). Fixed tissues were washed with filter-sterilized PBS. Non-specific banding was blocked with 3% BSA in PBS-Triton X-100 (0.01%) for 1h.

For cell fixation, round glass cover slips (25 mm) were treated with acetic acid for 30 min, then with 70% ethanol for 30 min, and acetone containing 200 µl 3-(aminopropyl) triethoxy saline (APES). Prior to use, the plates were sterilized by UV radiation for 30 min. The cover slips were coated with 100 µg/ml poly-D-lysine in PBS. After 10 min soaking, cover slips were washed with filter sterilized PBS. Confluent cells were trypsinized, and resuspended in 15 ml of media. 100-150 µl of cells were left on a cover slip for 20 min, and 4 ml medium was added. When appropriate, media were removed from the wells, and cells were briefly

washed with PBS and fixed with 0.5 ml of 4% PFA in PBS for 30 min. After washing with PBS, cells were processed for immunostaining.

After 3 washes with PBS, slides were incubated overnight at 4°C with primary antibodies (1:50 and 1:100) for COXIV, (Invitrogen, Paisly, UK) or UCP1 (Abcam, Cambridge, UK). Then slides were washed with PBS and incubated with secondary antibodies (donkey anti-rabbit Alexa-Fluor®488) for 1h at RT. Sections were mounted with VectaShield® Hard Set™ mounting medium. Samples were examined under an oil immersion objective using Leica model DMRE laser scanning confocal microscope with TCS SP2 scan head. Between 30 to 35 individual cells in 6 random fields of view were selected and analyzed. The scan speed was 400 Hz, and the format was 1024 x 1024 pixels. No specific fluorescence was observed in cells treated only with the secondary antibody. The images were manipulated with Leica and Image J (National Institute of Health, Bethesda, Maryland, USA) software.

Cellular respiration assay

3T3L1 preadipocytes were exposed to CRF (100 nM), Urocortin 2 (100 nM), isoproterenol (1 µM) and NBI 27914 (1 µM). On days 4 and 8 of cell differentiation, measurements of the oxygen concentration were made over 1 to 2 min using the Seahorse XF24 instrument (Seahorse Bioscience) and the rates of oxygen consumption were determined. DMSO was used as vehicle throughout the Seahorse respiration assays. Cells were equilibrated in the medium at 37°C for 30 min, and then baseline metabolic rates were measured over the next 30 min and were reported in nM/min of the oxygen consumption rate. Results were normalized to total protein level.

Animal procedures

All experiments involving animals were conducted in strict compliance with the European Union recommendations (2010/63/EU) and were approved by the French Ministry of Agriculture and Fisheries (animal experimentation authorization n° 3309004).

Housing and Diets

Crhr deficient mice (*Crhr*$^{-/-}$) on a C57BL / 6Jx129Sv-Ter genetic background were generated and genotyped as previously described (15) and their WT (*Crhr*$^{+/+}$) littermates used as controls. Experiments were performed in 7-8 months individually housed male mice under a 12/12h light/dark cycle and controlled temperature (23°C). The regular chow diet contained 9.5% Kcal as fat with an energy density of 2.9 Kcal/g (AO4, UAR). The HF diet contained 45% Kcal as fat with an energy density of 4.73 Kcal/g (N° 12450B, Research Diets). Food intake and body weight were recorded and feed efficiency calculated as body gain weight (g)/total caloric intake (100/Kcal). Corticosterone (SIGMA-Aldrich) was supplemented at 5µg/ml in drinking water while *Crhr*$^{+/+}$ mice received the vehicle only (0.2% ethanol in drinking water).

Body Composition

Whole body composition was evaluated by dual energy X-ray absorptiometry (Piximus, General Electric).

Locomotor activity

Locomotor activity of *Crhr*$^{-/-}$ and *Crhr*$^{+/+}$ littermates was evaluated using individual locomotor activity cages with two levels photocell beams allowing recording of both horizontal (locomotion) and vertical (rearing) behaviour (Imetronic). Mice were housed for 22h a day for 3 days for habituation and then locomotor activity was recorded.

Plasma measurements

Blood samples were collected by tail bleeding in heparinized capillary tubes. Blood samples for corticosterone measurement were obtained 1 h before the onset of the dark phase within 1 min of removal of mice from their cage. Corticosterone, leptin and insulin were measured using immunoassays (ICN Pharmaceuticals and Linco). Triglycerides were measured using an enzymatic kit (PAP 150 kit, bioMerieux). Plasma catecholamines were measured by HPLC as previously described (16). Whole β-hydroxybutyrate was measured using the β-hydroxybutyrate deshydrogenase method (17). For the glucose tolerance test (GTT), mice were tested in the morning after an overnight fast. Glucose (2 g/kg in saline) was administered ip and tail blood collected immediately before and 30, 60, 90 and 120 min after injection. Glucose was measured using a Lifescan One Touch glucometer (Johnson and Johnson). HOMA-IR was calculated using the formula [insulin (mU/L) x glucose (mg/dl)]/405.

Statistics

Data are presented as mean ± SEM. Data were tested for homogeneity and comparison between groups was performed by Student's unpaired *t*-test with Prism software (GraphPad). For multiple comparison tests, ANOVA followed by Dunnett test was used. For data with non-normal distribution, the Kruskal-Wallis ANOVA followed by Bonferroni test was used. $P<0.05$ was considered significant.

RESULTS

Regulation of the adipocyte CRF/urocortin system in vitro.

To determine the role of the CRF/urocortin system in adipocytes, we first investigated the expression of CRF_1 and $-R_2$ and the impact of their pharmacological manipulation in 3T3L1 white pre-adipocytes or T37i brown pre-adipocytes (18; 19). CRF_1 and CRF_2 as well as

CRF, Urocortin 1 and Urocortin 2 mRNAs were detected in both cell lines (Figures 1A and 2A). Treatment of 3T3L1 cells with CRF increased CRF_1 mRNA expression only (Figure 1A). Differently, treatment with the specific CRF_2 agonist Urocortin 2 during differentiation stimulated mRNA expression of CRF and of the CRF_2 pathway components Urocortin 2 and CRF_2 (Figure 1A). Inhibition of the endogenous CRF_1 activity by the specific CRF_1 antagonist NBI-27914 mimicked the effects of Urocortin 2, suggesting that activity of the CRF_1 pathway spontaneously represses the CRF_2 pathway. Roughly similar changes were also induced in the brown adipocyte precursors T37i cells (Figure 2A).

CRF_1 and CRF_2 pathways differently regulate in vitro the transcriptional machinery promoting the brown adipocyte phenotype.

During 3T3L1 cells differentiation, activation of the CRF_2 pathway by Urocortin 2 dramatically increased mRNA expression of PRDM16 and BMP7, two key factors inducing brown adipocyte phenotype and able to stimulate beige adipocyte differentiation (20-22) (Figure 1B). Furthermore, Urocortin 2 increased the mRNA expression of PGC1-α and of UCP1, two markers of brown or beige adipocyte activation (20; 21) (Figure 1B). Similar effects, albeit less potent, were induced by the β-receptor agonist isoproterenol, a strong activator of BAT thermogenesis that induces ectopic expression of UCP1 in WAT (23).

While simulating the expression of brown fat-promoting genes, Urocortin 2 inhibited the differentiation-dependent induction of the white adipocyte gene markers leptin, Wdnm1, resistin and chemerin (Figure 1B). Conversely, exposure of 3T3L1 cells to the preferential CRF_1 agonist CRF during differentiation did not induce expression of brown fat-promoting genes, but enhanced by 4 to 20 fold the expression of the aforementioned white adipocyte gene markers (Figure 1B). Blockade of CRF_1 by NBI-27914 mimicked, although less potently, the

effects of Urocortin 2 (Figure 1B). Similarly, Urocortin 2 induced the expression of molecular markers characteristic of brown adipocyte differentiation in T37i cells (Figure 2B).

Activation of the CRF_2 pathway and inhibition of the CRF_1 pathway induces functional changes consistent with the browning of white preadipocytes

To further investigate the opposing actions of CRF_1 and CRF_2 pathways on the white pre-adipocyte transcriptional machinery, we studied the functional consequences of pharmacological manipulations of CRF-Rs on mitochondrial biogenesis in 3T3L1 cells. Urocortin 2 and, to a lesser extent, NBI-27914 increased protein expression of COXIV, a marker of mitochondrial biogenesis in a time-dependent manner (Figure 1C). To determine whether these molecular changes were associated with changes in cellular metabolism, we measured oxygen consumption rate (OCR) in stimulated 3T3L1 cells. Treatment with Urocotin 2 during differentiation enhanced OCR, whereas CRF had no effect (Figure 1D). NBI-27914 mimicked to a lesser extent the effects of Urocortin 2 (Figure 1D). Whereas the combination of these two drugs had no additive effect over Urocortin 2-induced OCR (data not show). These results are therefore consistent with increased mitochondrial respiration confirming the transdifferentiation of white preadipocytes towards metabolically activated "beige" adipocytes (20; 21; 24; 25) as the result of the activation of the CRF_2 pathway and, to a lesser extent, to the inhibition of the CRF_1 pathway.

These findings therefore identify divergent roles for CRF_1 and -R_2 pathways in pre-adipocyte differentiation and pinpoint the importance of the local interplay between the CRF_1 and CRF_2 pathways regulating adipocyte precursors fate *in vitro*.

Increased CRF_2 activity in $Crhr^{-/-}$ mice induces browning of WAT in vivo that is reversed by corticosterone.

To confirm the hypothesis that unimpeded CRF_2 activity induces brown conversion of WAT *in vivo*, we performed complementary studies in *Crhr*$^{-/-}$ and their *Crhr*$^{+/+}$ littermates. Deletion of *Crhr* decreases ACTH and corticosterone secretion (15). We therefore also studied *Crhr*$^{-/-}$ mice supplemented with corticosterone in drinking water.

As expected, plasma corticosterone at the time of the diurnal peak in *Crhr*$^{-/-}$ mice was decreased compared to that of *Crhr*$^{+/+}$ mice (3.9 ± 0.5 ng/ml vs. 23.8 ± 3.85 ng/ml respectively, $p < 0.001$) while it was restored to physiological levels in steroid-supplemented *Crhr*$^{-/-}$ mice (*Crhr*$^{-/-}$ Cort) (27.3 ± 5.6 ng/ml). Since *Crhr* deletion induces variable alterations in the expression of other components of the CRF/urocortin system within tissues (11; 26), we carried out gene expression analysis in the adipose tissue of *Crhr*$^{-/-}$ mice. Molecular changes in the inguinal WAT of *Crhr*$^{-/-}$ mice were similar to those induced by the pharmacological inhibition of the CRF_1 pathway in 3T3L1 cells, including a 3 to 4 fold increase in CRF, Urocortin 2 and CRF_2 mRNA expression (Figure 3A). Urocortin 2 and CRF_2 protein expression was also increased (Figure 3A, right panels). Similar changes were observed in the BAT (Figure 4A). Interestingly, mRNA levels of CRF, Urocortin 2 and CRF_2 in WAT and BAT were comparable between *Crhr*$^{+/+}$ and *Crhr*$^{-/-}$ Cort mice, suggesting that the upregulation in urocortin 2 and CRF_2 expression induced by the lack of CRF_1 signaling *in vivo* is secondary to the reduced levels of circulating corticosterone.

Similarly to our *in vitro* findings, genes involved in determining the beige phenotype were upregulated in the inguinal WAT of *Crhr*$^{-/-}$ mice, while the expression of WAT-specific genes was strongly decreased (Figure 3B). These changes were associated with a dramatic increase in the expression of UCP1 and COXIV proteins, confirming the browning of WAT (Figure 3C). Up-regulation of brown-adipocyte gene markers was also observed in the BAT of *Crhr*$^{-/-}$ mice (Figure 4B). Importantly, β3-adrenergic receptor expression in inguinal WAT and BAT (data not shown) and plasma levels of norepinephrine (*Crhr*$^{+/+}$: 13.3 ± 1.5 µg/l vs. *Crhr*$^{-/-}$:

14.9 ± 1.2 µg/l, p=N.S.) did not differ between genotypes. As already reported elsewhere (27), plasma epinephrine levels were lower in *Crhr*$^{-/-}$ than in *Crhr*$^{+/+}$ mice (3.2 ±0.4 µg/l vs. 15.5 ± 2.9 µg/l respectively; p<0.001). Corticosterone supplementation in *Crhr*$^{-/-}$ mice completely prevented the molecular changes suggestive of WAT browning (Figure 3, B and C) and activation of BAT (Figure 4B).

***Crhr*$^{-/-}$ mice display a lean phenotype and resistance to high-fat (HF) diet that is reversed by physiological circulating levels of corticosterone.**

To determine the *in vivo* metabolic impact of the above-described changes, we further characterized *Crhr*$^{-/-}$ mice. Adult *Crhr*$^{-/-}$ mice maintained on regular chow displayed similar weight as compared to their *Crhr*$^{+/+}$ littermates, but had a decrease in fat mass and an associated increase in lean mass (supplementary figure 1, A-D). The reduced adiposity could not be explained by differences in food intake or locomotor activity (supplementary figure figure 1, E and F). *Crhr*$^{-/-}$ mice displayed reduced fasting plasma insulin concentrations, although glucose tolerance was similar between genotypes (supplementary figure figure 1, G and H). *Crhr*$^{-/-}$ mice maintained on a HF diet for 50 days showed reduced body weight gain, adiposity and leptin levels compared to *Crhr*$^{+/+}$ mice (Figure 5, A-D). Locomotor activity and caloric intake were similar between genotypes (Figure 5, E-F) while *Crhr*$^{-/-}$ mice had decreased feed efficiency (Figure 5G), suggesting an increase in energy dissipation. Accordingly, *Crhr*$^{-/-}$ mice had increased plasma hydroxybutyrate levels (Figure 6H), characteristic of increased fatty acid oxidation. *Crhr*$^{-/-}$ mice were also protected from diet-induced metabolic alterations and had significantly lower fasting HOMA index, lower plasma triglycerides and improved glucose tolerance as compared to *Crhr*$^{+/+}$ mice (Figure 5, H-J). Conversely, the replacement of physiological levels of corticosterone abolished the protection against the deleterious effects of a HF diet (Figure 5, A-K).

DISCUSSION

This study demonstrates that, *in vitro*, the CRF/urocortin system critically contributes to regulate the differentiation fate and function of preadipocytes cell lines and, more specifically, that increased activity of the CRF_2 pathway, through local mechanisms, induces transdifferentiation of white pre-adipocytes to metabolically active beige adipocytes and promotes differentiation of BAT. These pharmacological results were corroborated *in vivo* using *Crhr*$^{-/-}$ mice in which CRF_2 activity is unimpeded and that show molecular evidence of browning of WAT, activation of BAT and resistance to diet-induced obesity. Our study also identifies a previously unknown role of circulating corticosterone in hampering the browning of WAT and activation of BAT through the inhibition of the CRF_2 pathway in adipocytes.

In accordance with previously described expression of the CRF/urocortin system in the adipose tissue of humans and various animal species (1; 10-13), we demonstrate that white and brown preadipocytes cell lines express the mRNAs of CRF_1 and -R_2 and their ligands CRF, Urocortin-1 and Urocortin-2. The increased expression of Urocortin 2 and CRF_2 mRNAs after Urocortin 2 treatment suggests that activation of the CRF_2 pathway establishes a positive feedback loop potentially favoring further auto-activation. Conversely, the increased expression of the components of the CRF_2 pathway observed after treatment with the CRF_1 antagonist NBI-27914 implies that the constitutive activity of the CRF_1 pathway limits the expression and function of the CRF_2 pathway. Although we did not measure the CRF/urocortin family peptides in the cell culture media, the local expression of the members of the CRF system on the one hand and the results of the *in vitro* pharmacological studies including use a receptor antagonist on the other suggests a paracrine regulation of the CRF/urocortin system within the adipocytes.

Our *in vitro* experiments demonstrate the ability of the CRF/urocortin system to regulate the transcriptional machinery governing the differentiation of preadipocyte cell lines. Divergent roles for CRF_1 and $-R_2$ pathways were identified. Activation of CRF_2 signaling stimulated the transcriptional machinery characteristic of the differentiation and activation of brown adipocytes in both 3T3L1 and T37i cell lines, while inhibiting the induction of white adipocytes gene markers in white preadipocytes, presumably through the induction of key transcriptional factors such as BMP7 and PRDM16 (20; 21; 28). Inhibition of CRF_1 mimicked, although to a lesser extent, the consequences of the activation of the CRF_2 pathway. The transformation of white preadipocytes into activated "beige" adipocytes suggested by the increase in PGC-1α and UCP1 mRNAs and consistent with a transdifferentiation process (24; 25; 29) was confirmed by the increased mitochondrial biogenesis and cellular respiration induced by CRF_2 activation and, to a lesser degree, CRF_1 inhibition. Altogether, these *in vitro* data suggest that the balance between the CRF_1 and CRF_2 intracellular signaling in pre-adipocytes play an important role in determining, through paracrine mechanisms, cell commitment towards divergent differentiation. More specifically in white preadipocyte cell lines, the CRF_2 pathway strongly stimulates the differentiation towards a brown adipocyte phenotype while activation of the CRF_1 pathway by endogenous CRF prevents it, allowing the expected programmed differentiation towards a white adipocyte phenotype. Notably, several studies have stressed the importance of the balance between the activity of the CRF_1 and CRF_2 pathways in the regulation of gastro-intestinal motility, behavioral responses to stressors and SNS activity (2; 5; 26; 30).

In agreement with our *in vitro* results, *Crhr*$^{-/-}$ mice exhibited features suggesting an unrestrained CRF_2 activity, including an increased expression of Urocortin 2 and CRF_2 mRNA levels in inguinal WAT and interscapular BAT. In agreement with the *in vitro* results observed after pharmacologically-induced upregulation of CRF_2 mRNA expression or direct activation

of the CRF_2 pathway, these changes were associated with an increased expression of the transcriptional machinery characteristic of brown adipocytes in both WAT and BAT and decreased expression of the white adipocytes gene markers.

The CRF_1 pathway stimulates the activity of the SNS (1; 2; 31). *Crhr*$^{-/-}$ mice had similar levels of β3-adrenergic receptor expression in adipocytes and similar plasma levels of norepinephrine compared to *Crhr*$^{+/+}$ mice. Thus, it is unlikely that the browning of WAT and activation of BAT observed in *Crhr*$^{-/-}$ mice results from a local or systemic increase in SNS activity. However, whether the changes observed in the WAT of *Crhr*$^{-/-}$ mice represents recruitment of beige adipocytes or transdifferentiation of white adipocytes deserves further studies.

In accordance with a functional activation of brown and beige adipocytes through heightened CRF_2 activity, *Crhr*$^{-/-}$ mice were obesity resistant and showed features typical of increased energy dissipation, overall suggesting an important role for the CRF_2 pathway in the regulation of energy balance *in vivo*. Interestingly, peripheral chronic administration of a CRF_2 agonist in rats reduces white fat mass while inducing expression of typical muscle genes in the WAT (32). Elsewhere, transgenic expression of the CRF_2 agonist Ucn3, or *in vitro* stimulation of the CRF_2 pathway with Urocortin 2 activates energy dissipating substrate cycles in the muscle and up-regulates UCP2 and UCP3 mRNAs (9; 33). Taking into account that myocytes and brown adipocytes are derived from a common mesenchymal precursor (28; 34), we might speculate for a broader role of the CRF_2 pathway in promoting mitochondrial thermogenesis in peripheral tissues, such as the adipose tissue and skeletal muscle. We therefore cannot exclude at present the involvement of additional mechanisms to the modification of adipocyte activity to account for the favorable metabolic phenotype of *Crhr*$^{-/-}$ mice including increased lipid oxidation in the liver (13). Since our in vitro studies involved pre-adipocyte cell lines and whole animal studies involved loss-of-function since birth, the effects of manipulation of the

CRF system after differentiation of adipocytes or during adulthood remain to be determined. Complementary studies using chronic infusion of Ucn2 or selective and inducible knockdown of Crhr1 as well as inducible over expression of Crhr2 or Ucn2 in the adipose tissue will allow addressing these important mechanistic issues".

Although the importance of glucocorticoids in the differentiation of white preadipocytes is well acknowledged (18; 19; 35), our experiments also identify a previously unknown role of corticosterone in white adipocyte biology and energy balance.

Glucocorticoids influence the expression of components of the CRF/urocortin system in a tissue-selective manner (11; 36; 37). Indeed, corticosterone administration inhibits the overexpression of Urocortin 2 and CRF_2 in the skin (11) and hypothalamus (36) of *Crhr*$^{-/-}$ and adrenalectomized mice. Accordingly, our data suggest a repression of CRF_2 activity in white adipocytes by physiological levels of corticosterone, which allow the expected white adipocyte differentiation. Conversely, corticosterone deficiency in *Crhr*$^{-/-}$ mice results in unrestrained CRF_2 activity that promotes the browning of WAT (Figure 6). Concordantly, *in vivo* reduction of active glucocorticoids specifically in the adipose tissue of 11β-hydroxysteroid dehydrogenase type 2 transgenic mice promotes the expression of brown adipocyte markers in the subcutaneous WAT, decreases the expression of white adipocytes gene markers and is associated with increased thermogenesis, leading to resistance to diet-induced obesity (38). Thus, corticosterone should be considered as one of the secreted molecules that is able to modulate the plasticity of adipose tissue and the induction of beige adipocytes (21). Complementary studies focusing on the expression of components of the CRF/Ucn system in the adipose tissue of adrenalectomized mice clamped with various doses of corticosterone and of mice treated with molecules targeting the 11β-hydroxysteroid dehydrogenase in the adipose tissue are mandatory in order to further dissect the interactions between circulating corticosterone and adipocytes plasticity

Finally, it should be mentioned that recent studies have shown the presence of functional brown and beige adipocytes in adult humans (24; 39; 40). Stimulating the thermogenesis of adipose tissue represents a promising strategy to tackle obesity and type 2 diabetes (20; 21; 41-43). In this perspective, our study suggests that the adipocyte CRF_2 pathway could be a specific target for the pharmacological treatment of metabolic disease.

Acknowledgments. Supported by INSERM (DC, AT), Region Aquitaine (DC, AT) and EquipEx OptoPath ANR-10-EQPX-08 (DC).

Conflict of Interest

The authors wish to confirm that there are no know conflict of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome**.**

Supplementary information is available at IJO's website.

References

1. Fekete EM, Zorrilla EP: Physiology, pharmacology, and therapeutic relevance of urocortins in mammals: ancient CRF paralogs. Frontiers in neuroendocrinology 2007;28:1-27

2. Carlin KM, Vale WW, Bale TL: Vital functions of corticotropin-releasing factor (CRF) pathways in maintenance and regulation of energy homeostasis. Proceedings of the National Academy of Sciences of the United States of America 2006;103:3462-3467

3. Chen P, Van Hover C, Lindberg D, Li C: Central urocortin 3 and type 2 corticotrophin-releasing factor receptor in the regulation of energy homeostasis : critical involvement of the ventromedial hypothalamus. Front Endocrinol 2013;3:1-12

4. Richard D, Lin Q, Timofeeva E: The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. Eur J Pharmacol 2002;440:189-197

5. Zorrilla EP, Tache Y, Koob GF: Nibbling at CRF receptor control of feeding and gastrocolonic motility. Trends Pharmacol Sci 2003;24:421-427

6. Tabarin A, Diz-Chaves Y, Consoli D, Monsaingeon M, Bale TL, Culler MD et al.: Role of the corticotropin-releasing factor receptor type 2 in the control of food intake in mice: a meal pattern analysis. Eur J Neurosci 2007;26:2303-2314

7. Chen A, Brar B, Choi CS, Rousso D, Vaughan J, Kuperman Y, et al.: Urocortin 2 modulates glucose utilization and insulin sensitivity in skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America 2006;103:16580-16585

8. Li C, Chen P, Vaughan J, Lee KF, Vale W: Urocortin 3 regulates glucose-stimulated insulin secretion and energy homeostasis. Proceedings of the National Academy of Sciences of the United States of America 2007;104:4206-4211

9. Jamieson PM, Cleasby ME, Kuperman Y, Morton NM, Kelly PA, Brownstein DG, et al.: Urocortin 3 transgenic mice exhibit a metabolically favourable phenotype resisting obesity and hyperglycaemia on a high-fat diet. Diabetologia 2011;54:2392-2403

10. Boorse GC, Denver RJ: Widespread tissue distribution and diverse functions of corticotropin-releasing factor and related peptides. General and comparative endocrinology 2006;146:9-18

11. Chen A, Blount A, Vaughan J, Brar B, Vale W: Urocortin II gene is highly expressed in mouse skin and skeletal muscle tissues: localization, basal expression in corticotropin-releasing factor receptor (CRFR) 1- and CRFR2-null mice, and regulation by glucocorticoids. Endocrinology 2004;145:2445-2457

12. Seres J, Bornstein SR, Seres P, Willenberg HS, Schulte KM, Scherbaum WA, et al.: Corticotropin-releasing hormone system in human adipose tissue. The Journal of clinical endocrinology and metabolism 2004;89:965-970

13. Sakamoto R, Matsubara E, Nomura M, Wang L, Kawahara Y, Yanase T, et al.: Roles for corticotropin-releasing factor receptor type 1 in energy homeostasis in mice. Metabolism: clinical and experimental 2013;62:1739-1748

14. Friedberg M, Zoumakis E, Hiroi N, Bader T, Chrousos GP, Hochberg Z: Modulation of 11 beta-hydroxysteroid dehydrogenase type 1 in mature human subcutaneous adipocytes by hypothalamic messengers. The Journal of clinical endocrinology and metabolism 2003;88:385-393

463 15. Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, et al.:
464 Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired
465 stress response, and aberrant neuroendocrine development. Neuron 1998;20:1093-1102

466 16. Grossi G, Bargossi AM, Lucarelli C, Paradisi R, Sprovieri C, Sprovieri G: Improvements
467 in automated analysis of catecholamine and related metabolites in biological samples by
468 column-switching high-performance liquid chromatography. Journal of chromatography
469 1991;541:273-284

470 17. Byrne HA, Tieszen KL, Hollis S, Dornan TL, New JP: Evaluation of an electrochemical
471 sensor for measuring blood ketones. Diabetes care 2000;23:500-503

472 18. Ntambi JM, Young-Cheul K: Adipocyte differentiation and gene expression. The Journal
473 of nutrition 2000;130:3122S-3126S

474 19. Viengchareun S, Penfornis P, Zennaro MC, Lombes M: Mineralocorticoid and
475 glucocorticoid receptors inhibit UCP expression and function in brown adipocytes. American
476 journal of physiology Endocrinology and metabolism 2001;280:E640-649

477 20. Harms M, Seale P: Brown and beige fat: development, function and therapeutic potential.
478 Nat Med 2013;19:1252-1263

479 21. Wu J, Cohen P, Spiegelman BM: Adaptive thermogenesis in adipocytes: is beige the new
480 brown? Genes & development 2013;27:234-250

481 22. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al.: Prdm16
482 determines the thermogenic program of subcutaneous white adipose tissue in mice. The
483 Journal of clinical investigation 2011;121:96-105

484 23. Cannon B, Nedergaard J: Brown adipose tissue: function and physiological significance.
485 Physiol Rev 2004;84:277-359

486 24. Bartelt A, Heeren J: Adipose tissue browning and metabolic health. Nature reviews
487 Endocrinology 2014;10:24-36

488 25. Rosenwald M, Perdikari A, Rulicke T, Wolfrum C: Bi-directional interconversion of brite
489 and white adipocytes. Nature cell biology 2013;15:659-667

490 26. Bale TL, Picetti R, Contarino A, Koob GF, Vale WW, Lee KF: Mice deficient for both
491 corticotropin-releasing factor receptor 1 (CRFR1) and CRFR2 have an impaired stress
492 response and display sexually dichotomous anxiety-like behavior. The Journal of
493 neuroscience: the official journal of the Society for Neuroscience 2002;22:193-199

494 27. Yoshida-Hiroi M, Bradbury MJ, Eisenhofer G, Hiroi N, Vale WW, Novotny GE, et al.:
495 Chromaffin cell function and structure is impaired in corticotropin-releasing hormone receptor
496 type 1-null mice. Molecular psychiatry 2002;7:967-974

497 28. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al.: PRDM16 controls a
498 brown fat/skeletal muscle switch. Nature 2008;454:961-967

499 29. Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, et al.: The
500 emergence of cold-induced brown adipocytes in mouse white fat depots is determined
501 predominantly by white to brown adipocyte transdifferentiation. American journal of
502 physiology Endocrinology and metabolism 2010;298:E1244-1253

503 30. Bale TL, Anderson KR, Roberts AJ, Lee KF, Nagy TR, Vale WW: Corticotropin-releasing
504 factor receptor-2-deficient mice display abnormal homeostatic responses to challenges of
505 increased dietary fat and cold. Endocrinology 2003;144:2580-2587

506 31. Cullen MJ, Ling N, Foster AC, Pelleymounter MA: Urocortin, corticotropin releasing
507 factor-2 receptors and energy balance. Endocrinology 2001;142:992-999

508 32. Argiles JM, Fontes-Oliveira CC, Fuster G, Ametller E, Figueras M, Olivan M, et al.:
509 Patterns of gene expression in muscle and fat in tumor-bearing rats: effects of CRF2R agonist
510 on cachexia. Muscle & nerve 2010;42:936-949

511 33. Solinas G, Summermatter S, Mainieri D, Gubler M, Montani JP, Seydoux J, et al.:
512 Corticotropin-releasing hormone directly stimulates thermogenesis in skeletal muscle possibly
513 through substrate cycling between de novo lipogenesis and lipid oxidation. Endocrinology
514 2006;147:31-38

515 34. Timmons JA, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, et al.:
516 Myogenic gene expression signature establishes that brown and white adipocytes originate
517 from distinct cell lineages. Proceedings of the National Academy of Sciences of the United
518 States of America 2007;104:4401-4406

519 35. Tomlinson JJ, Boudreau A, Wu D, Atlas E, Hache RJ: Modulation of early human
520 preadipocyte differentiation by glucocorticoids. Endocrinology 2006;147:5284-5293

521 36. Chen A, Perrin M, Brar B, Li C, Jamieson P, Digruccio M, et al.: Mouse corticotropin-
522 releasing factor receptor type 2alpha gene: isolation, distribution, pharmacological
523 characterization and regulation by stress and glucocorticoids. Mol Endocrinol 2005;19:441-
524 458

525 37. Chen A, Vaughan J, Vale WW: Glucocorticoids regulate the expression of the mouse
526 urocortin II gene: a putative connection between the corticotropin-releasing factor receptor
527 pathways. Mol Endocrinol 2003;17:1622-1639

528 38. Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, Flier JS: Adipocyte-specific
529 glucocorticoid inactivation protects against diet-induced obesity. Diabetes 2005;54:1023-1031

530 39. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, et al.: Functional
531 brown adipose tissue in healthy adults. N Engl J Med 2009;360:1518-1525

532 40. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, et al.: Beige adipocytes are a
533 distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366-376

534 41. Whittle A, Relat-Pardo J, Vidal-Puig A: Pharmacological strategies for targeting BAT
535 thermogenesis. Trends Pharmacol Sci 2013;34:347-355

536 42. Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, et al.: Recruited
537 brown adipose tissue as an antiobesity agent in humans. The Journal of clinical investigation
538 2013;123:3404-3408

539 43. Bartelt A, Heeren J: The holy grail of metabolic disease: brown adipose tissue. Current
540 opinion in lipidology 2012;23:190-195

541

542

543

544 **REFERENCES**

545 **Figure Legend**

546 **Figure 1**

547 **Modulation of CRF receptors activity during 3T3L1 differentiation induces brown**
548 **adipocyte characteristics. (**A-B) Activation of CRF_2 by 100nM Ucn2 or inhibition of CRF_1
549 by 1μM NBI-27914 or ß-adrenergic receptor activation with 1μM isoproterenol induced
550 changes in mRNA expression of CRF receptors, cognate agonists and brown-adipocytes genes.
551 *p<0.05 treatment vs. day 0 of differentiation without any treatment, n =4 independent
552 experiments in triplicate (C-D) Activation of CRF_2 with Urocortin 2 or inhibition of CRF_1 with
553 NBI-27914 stimulated mitochondrial biogenesis and cellular respiration as determined by
554 Cytochrome c oxidase (COX) IV immunostaining (in green; blue: nuclear DAPI staining) and
555 O_2 consumption analysis. *p<0.01 treatment vs. day 0 of differentiation without any treatment,
556 n =3 independent experiments in triplicate. Black boxes denote genes relevant to white
557 adipogenesis.

558 **Figure 2**

559 **Inhibition of CRF_1 or activation of CRF_2 in T37i pre-adipocytes promotes brown**
560 **adipocyte characteristics.** (A) Inhibition of CRF_1 by NBI-27914 (1μM) or activation of
561 CRF_2 by Urocortin 2 (100nM) induced changes in mRNA expression of CRF receptors and
562 cognate agonists. (B) Treatment with Urocortin 2 or NBI-27914 induced transcription of key
563 genes promoting T37i differentiation into brown adipocytes. *p<0.05 treatment vs. day 0 of
564 differentiation without any treatment, n =4 independent experiments in triplicate.

565

566 **Figure 3**

***Crhr* deletion induces brown-fat characteristics within the white adipose tissue (WAT).** (A) Increased mRNA expression of CRF_2 and related ligands as well as increased immunostaining of Urocortin 2 and CRF_2 (in green; blue: nuclear DAPI staining) in the WAT of *Crhr*$^{-/-}$ mice. (B) Changes in the mRNA expression of key genes involved in white or brown adipocyte differentiation in the WAT of *Crhr*$^{-/-}$ mice. Restoration of corticosterone levels in *Crhr*$^{-/-}$ mice reversed these changes. (C) COXIV and UCP1 protein expression (in green; blue: nuclear DAPI staining) in *Crhr*$^{+/+}$, *Crhr*$^{-/-}$ and corticosterone-supplemented *Crhr*$^{-/-}$ mice. **p*<0.05, ***p*<0.01, vs. *Crhr*$^{+/+}$ or corticosterone supplementation. n =5 animals for each condition.

Figure 4

Lack of CRF_1 alters transcriptional levels of key genes in brown adipose tissue. (A) Brown adipose tissue from *Crhr*$^{-/-}$ mice shows increased mRNA expression of CRF_2 receptors and cognate agonists. (B) Increased mRNA expression of key genes involved in brown adipocyte differentiation in the BAT of *Crhr*$^{-/-}$ mice. Restoration of corticosterone levels in *Crhr*$^{-/-}$ mice reverses changes described in A and B. **p*<0.05, **, *p*<0.01 vs. *Crhr*$^{+/+}$or corticosterone-supplemented *Crhr*$^{-/-}$ mice; n =5 independent experiments in triplicate.

Figure 5

Deletion of *crhr* induces resistance to diet-induced obesity, an effect reversed by corticosterone supplementation. (A-L) *Crhr*$^{+/+}$, *Chrh*$^{-/-}$ and *Crhr*$^{-/-}$ mice supplemented with corticosterone (*Crhr*$^{-/-}$ Cort) were fed with a HF diet for 50 days (n=5-6 animals per group). (A) Body weight gain (% above baseline weight on regular chow diet). $^{\&}$ *p* <0.05, $^{\&\&}$ *p*<0.01, Crhr $^{-/-}$ vs. *Crhr*$^{+/+}$ mice; $^{\#\#}$*p*< 0.01; $^{\#\#\#}$*p*< 0.001, *Crhr*$^{-/-}$ Cort vs. *Crhr*$^{+/+}$ mice. (B) Fat mass evaluated by DEXA. (C) Weight of inguinal (PG), mesenteric (MES) and retroperitoneal (RET) fat pads. (D) Plasma leptin concentration, (E) locomotor activity, (F) cumulative food intake, (G) feed

591 efficiency, (H) plasma beta-hydroxybutyrate concentration, (I) fasting homeostatic model
592 assessment (HOMA), (J) plasma triglycerides concentration, and (K) intraperitoneal glucose
593 tolerance test (Area under curve analysis: *p<0.05). *p<0.05, **p <0.01, *** p <0.001 vs.
594 *Crhr*$^{+/+}$ and *Crhr*$^{-/-}$ Cort groups.

595

596 **Figure 6**

597 Proposed model illustrating the role of the CRF/urocortin system and of circulating
598 corticosterone in white adipocytes differentiation. CRF_1 and CRF_2 intracellular signaling in
599 white adipocytes determines cell commitment towards divergent differentiation through
600 autocrine mechanisms. Activation of the CRF_2 pathway by local Urocortin stimulates the
601 differentiation of white adipocytes towards a "brown-like" phenotype, whereas activation of
602 the CRF_1 pathway by local CRF prevents it, thus allowing the expected differentiation
603 towards a white adipocyte phenotype. CRF_1 signaling in the central nervous system stimulates
604 the activity of the hypothalamo-pituitary-adrenal (HPA) axis and results in corticosterone
605 secretion. Physiological levels of circulating corticosterone dampen the activation of the CRF_2
606 pathway in adipose tissue and repress the browning of WAT through endocrine mechanisms.

607
608 **Supplementary Figure 1**

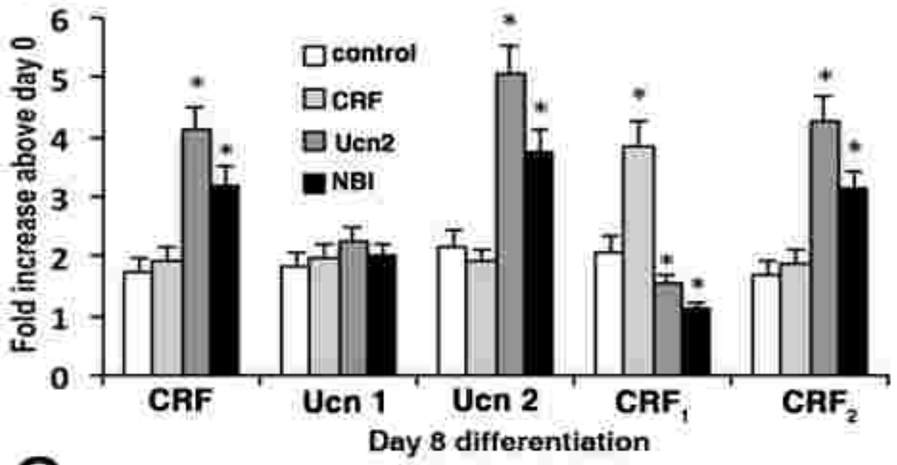
609 **Adult male Crhr$^{-/-}$ mice fed a regular chow diet have a lean phenotype**. (A) Body weight
610 of *Crhr*$^{+/+}$ (black circles) and *Crhr*$^{-/-}$ mice (white circles). (B) Fat mass and (D) lean mass
611 measured by DEXA. (C) Weight of inguinal (PG), mesenteric (MES) and retroperitoneal
612 (RET) fat pads. (E) Cumulative food intake. (F) 24-hours locomotor activity during dark and
613 light phases. (G) Fasting plasma insulin levels and (H) plasma glucose changes in response to

614 an intraperitoneal glucose tolerance test. *p <0.05, **p <0.01 vs. *CRFr1*$^{+/+}$ mice, n = 6 – 14

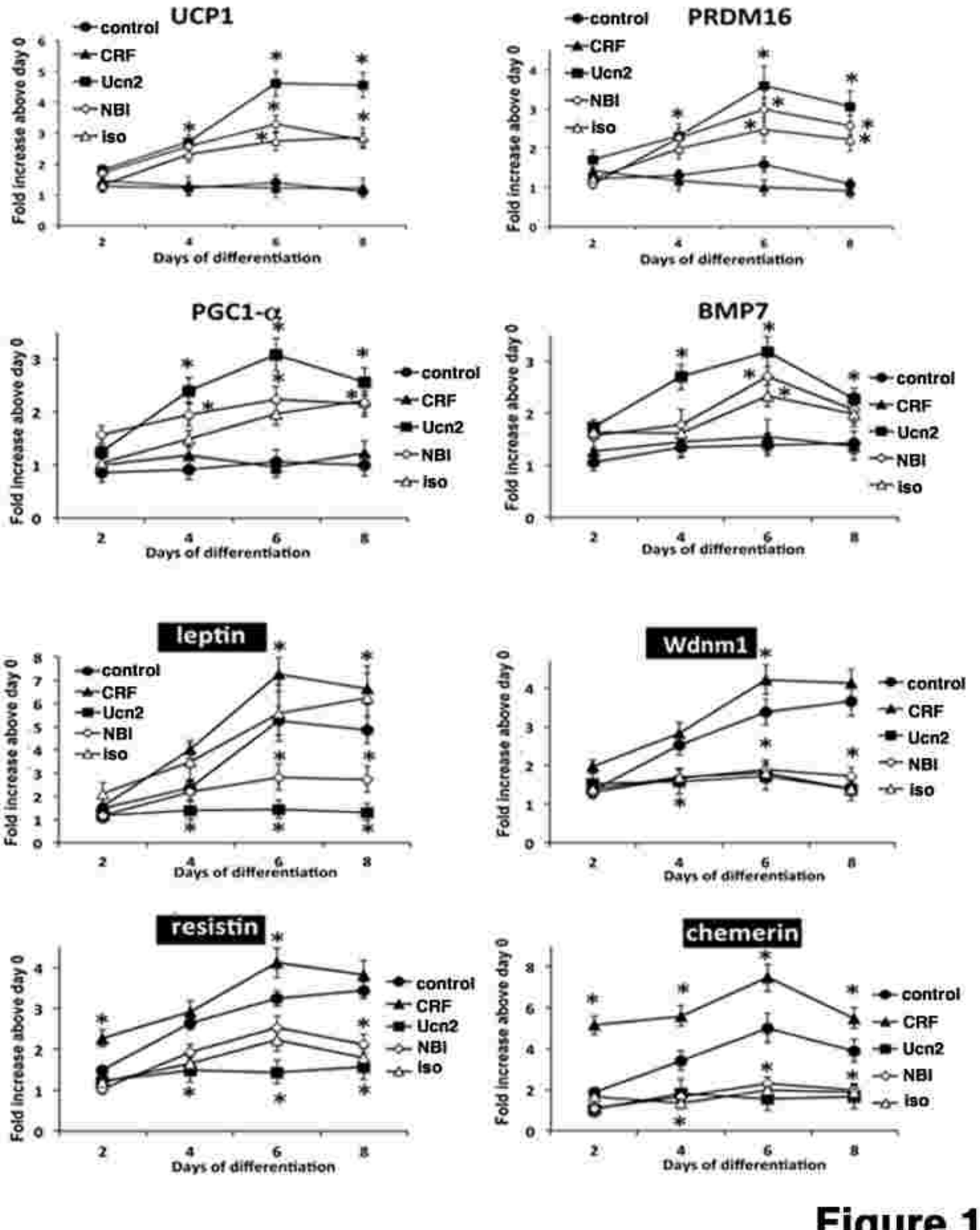
615 animals per group.

616
617
618

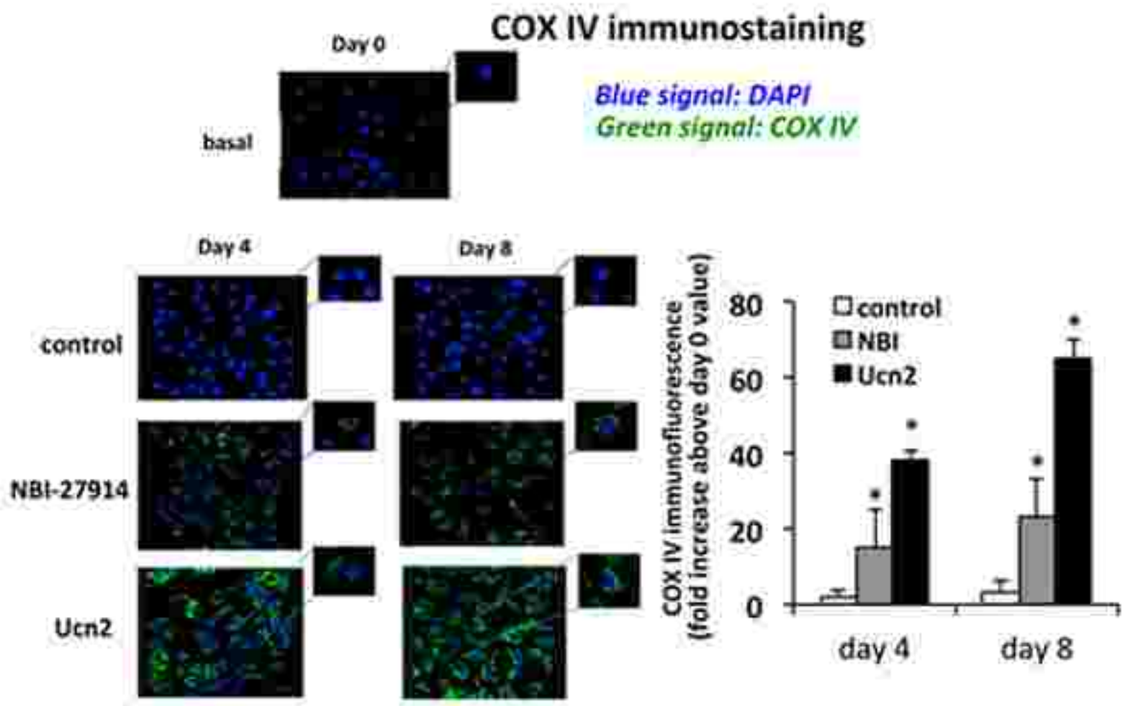
A



B



C



D

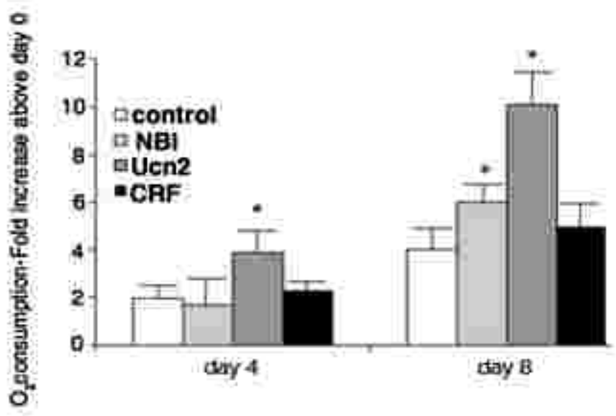


Figure 1

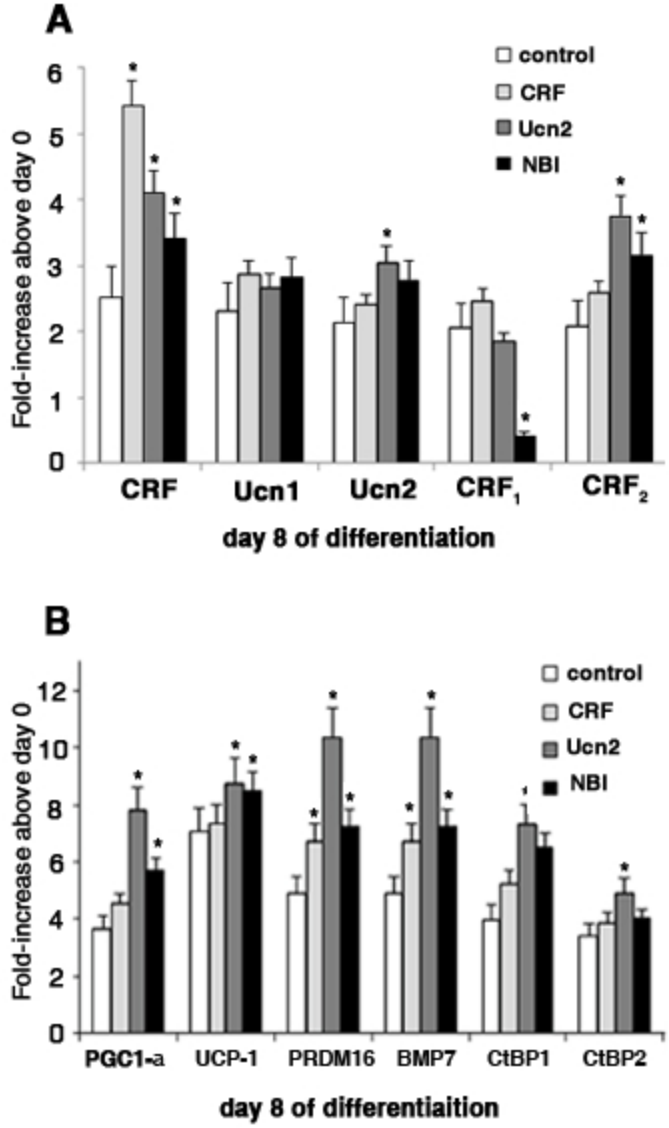
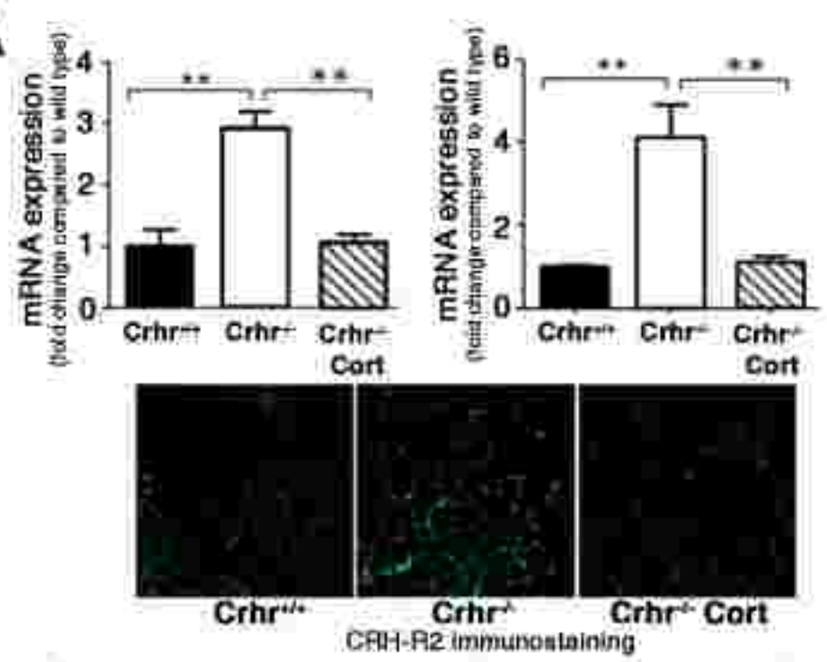
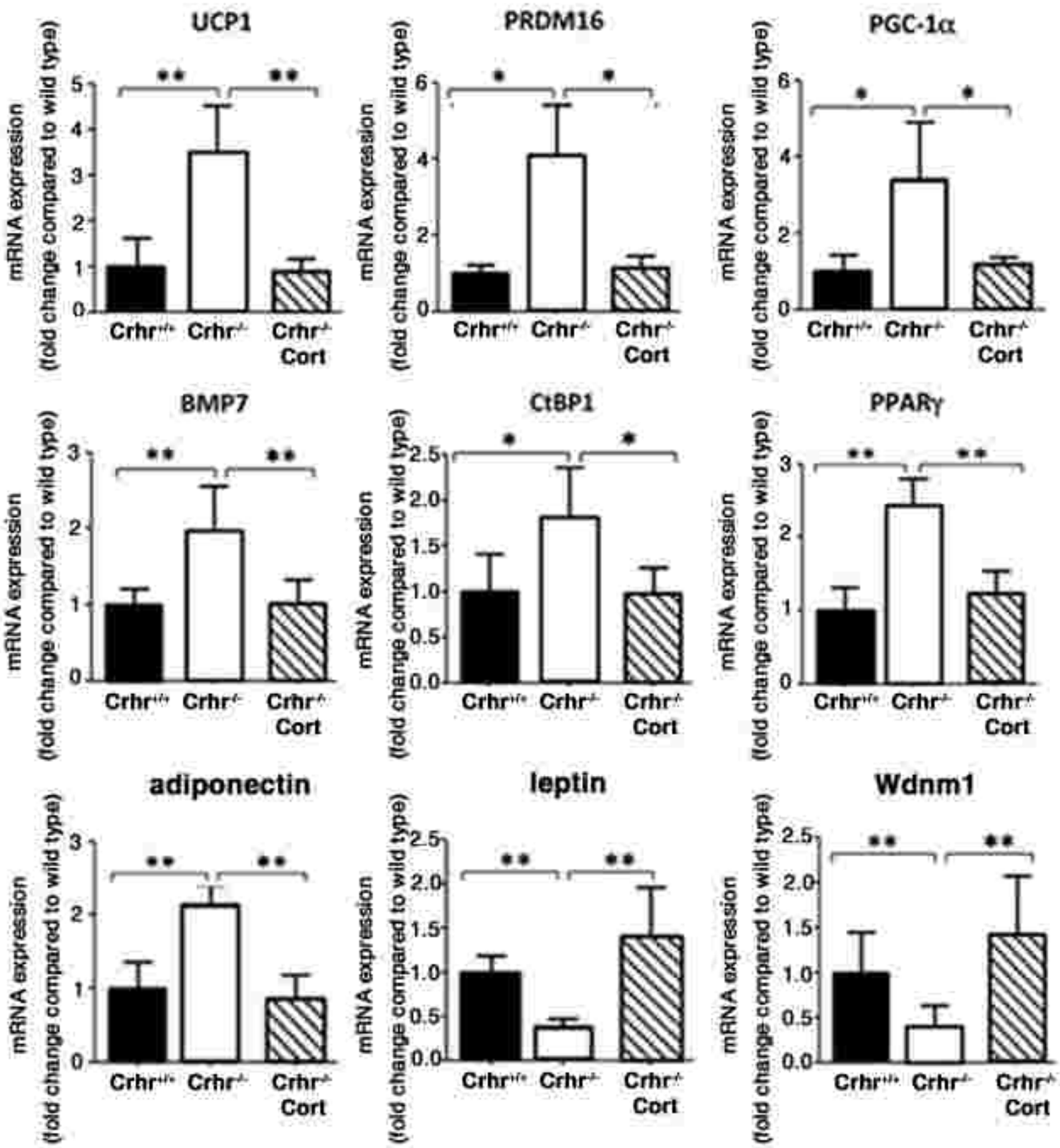

A
control
CRF
Ucn2
NBI
Fold-increase above day 0
0
1
2
3
4
5
6
CRF
Ucn1
Ucn2
CRF1
CRF2
day 8 of differentiation
B
control
CRF
Ucn2
NBI
Fold-increase above day 0
0
2
4
6
8
10
12
PGC1-a
UCP-1
PRDM16
BMP7
CtBP1
CtBP2
day 8 of differentiaition

Figure 2

A


mRNA expression
(fold change compared to wild type)
Crhr+/+
Crhr-/-
Crhr-/- Cort
CRH-R2 immunostaining
Ucn2 immunostaining

B


UCP1
PRDM16
PGC-1α
BMP7
CtBP1
PPARγ
adiponectin
leptin
Wdnm1
aP2
chemerin
resistin
mRNA expression
(fold change compared to wild type)
Crhr+/+
Crhr-/-
Crhr-/- Cort

C

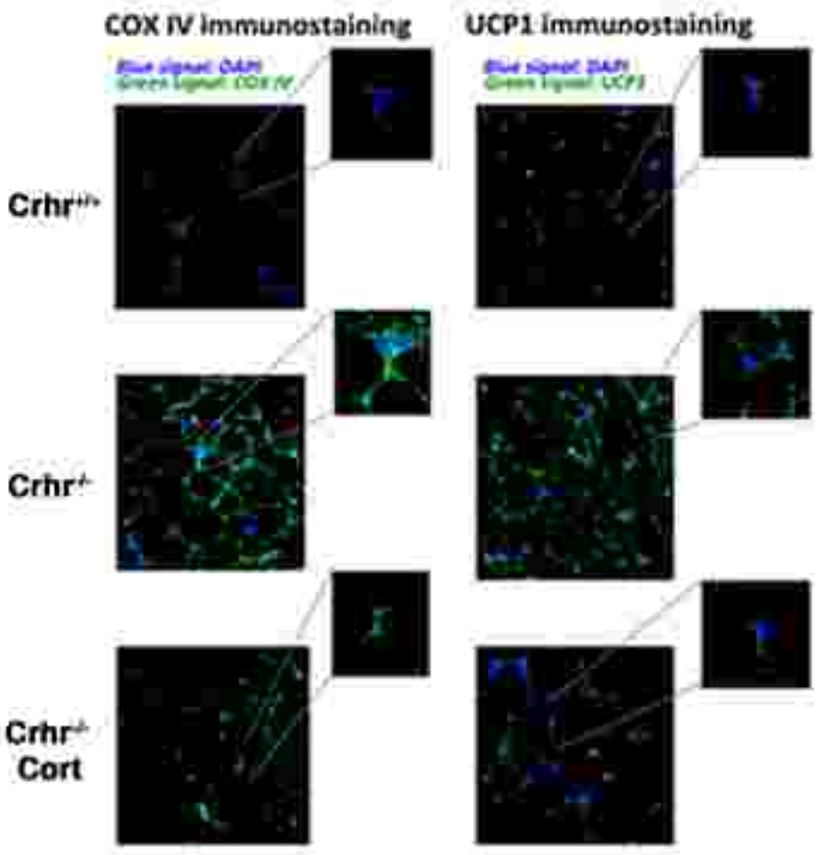

COX IV immunostaining
UCP1 immunostaining
Crhr+/+
Crhr-/-
Crhr-/- Cort

Figure 3

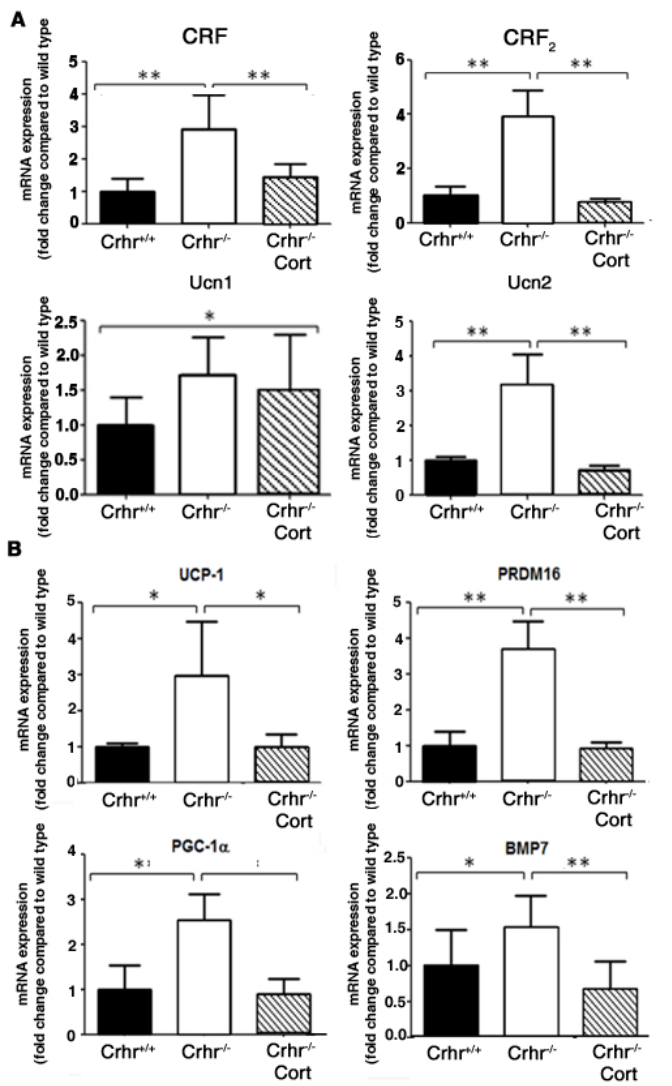
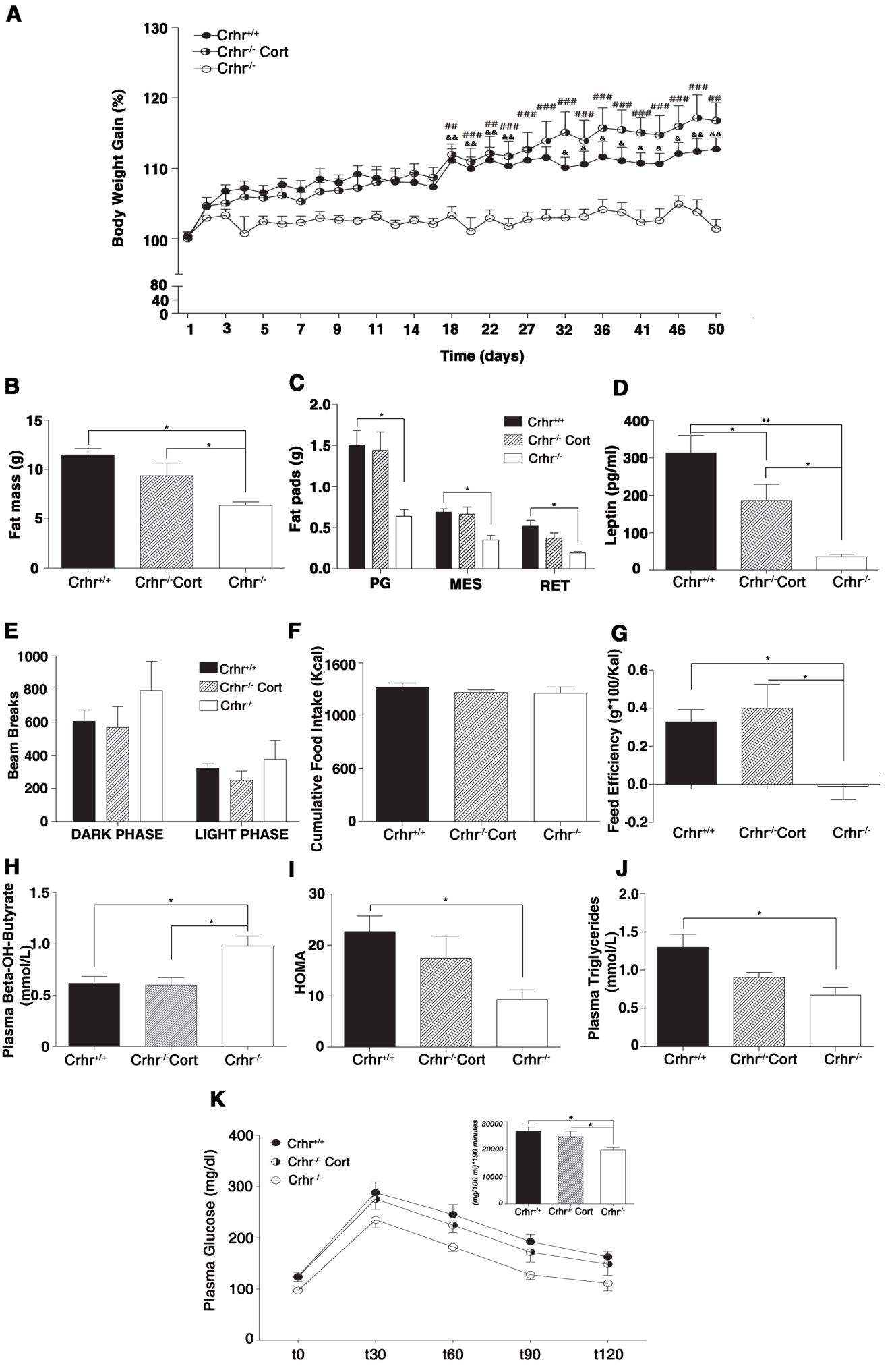
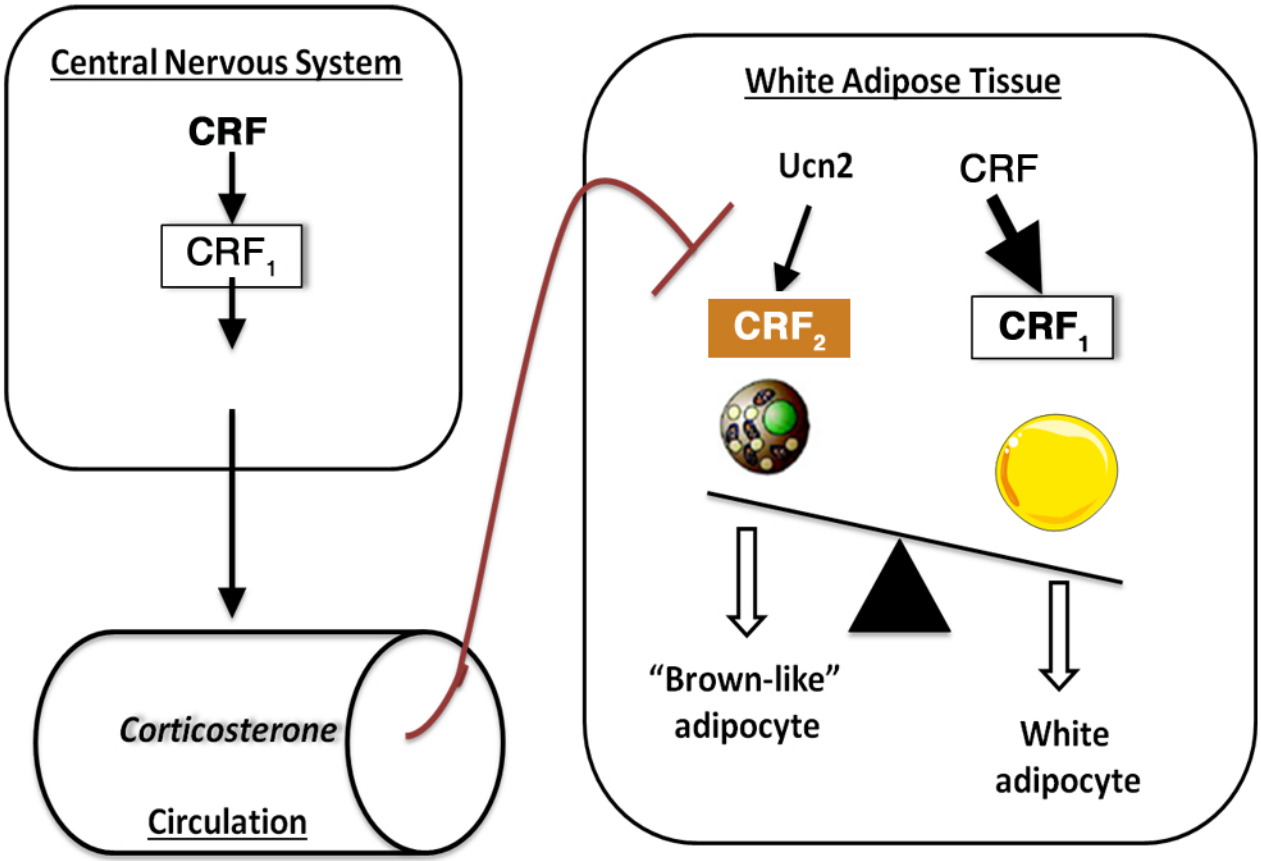

A
CRF
CRF2
Ucn1
Ucn2
mRNA expression
(fold change compared to wild type
Crhr+/+
Crhr-/-
Crhr-/-
Cort
B
UCP-1
PRDM16
PGC-1α
BMP7

Figure 4


A
Body Weight Gain (%)
Time (days)
Crhr+/+
Crhr-/- Cort
Crhr-/-
B
Fat mass (g)
C
Fat pads (g)
PG
MES
RET
D
Leptin (pg/ml)
E
Beam Breaks
DARK PHASE
LIGHT PHASE
F
Cumulative Food Intake (Kcal)
G
Feed Efficiency (g*100/Kal)
H
Plasma Beta-OH-Butyrate (mmol/L)
I
HOMA
J
Plasma Triglycerides (mmol/L)
K
Plasma Glucose (mg/dl)
(mg/100 ml)*180 minutes
t0
t30
t60
t90
t120


Central Nervous System
CRF
CRF1
Corticosterone
Circulation
White Adipose Tissue
Ucn2
CRF
CRF2
CRF1
"Brown-like" adipocyte
White adipocyte