Proopiomelanocortin-Deficient Mice Are Hypersensitive to the Adverse Metabolic Effects of Glucocorticoids

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Proopiomelanocortin (POMC) is a polypeptide precursor that undergoes extensive, tissue-specific posttranslational modification to generate a range of smaller, biologically active peptides (1). These include ACTH and α-, β-, and γ-melanocyte-stimulating hormone, collectively known as the melanocortins. Hypothalamic neurons expressing POMC are critically involved in the integration of nutritional and hormonal signals and the regulation of both appetite and energy expenditure (2). ACTH produced in the anterior pituitary is essential for adrenal steroidogenesis (3), and local production of melanocortins in the skin and hair follicles is critically involved in control of pigmentation (4,5). As expected, inactivating mutations of the POMC gene in humans and mice therefore result in a complex phenotype involving hyperphagia, obesity, glucocorticoid deficiency, and altered pigmentation (6–9).

Among murine models of obesity, Pomp-null mice are unusual in that obesity and hyperphagia develop in the absence of circulating glucocorticoid. Glucocorticoids have pleiotropic effects on metabolism, and in particular, changes in circulating levels of glucocorticoid can impact on the melanocortin system. For example, adrenalectomy is able to reverse the obese phenotype and restore hypothalamic melanocortin tone in leptin-deficient ob/ob mice (10). In addition, adrenalectomy alters the sensitivity of the central melanocortin system to the effects of the melanocortin antagonist agouti-related protein (AgRP), with the orexigenic effect of the latter being absent in adrenalectomized rats but restored with glucocorticoid supplementation (11).

To further investigate the interaction between glucocorticoids and the melanocortin system, we have conducted two studies. In the first, to examine acute effects, we have treated 8-week-old POMC-deficient mice with corticosterone for 10 days. In the second, we have administered glucocorticoids from weaning and examined the longer-term effects of glucocorticoid treatment in the presence and absence of POMC-derived peptides.

RESEARCH DESIGN AND METHODS

POMC-deficient mice were generated on a 129/SvEv background as previously described (8). Genotypes were determined by PCR of DNA from tail tissue as previously described (8). Mice were maintained under controlled temperature (22°C) and light (12 h of light from 0700 to 1900) and had ad libitum access to water and standard diet (4.5% fat diet; Special Diet Services, Witham, U.K.). Animals were individually caged throughout the duration of the experiment. Eight-week-old male mice were used throughout unless otherwise stated. All blood was collected in the first 2 h of the light cycle and collected within 1 min of initial handling in all experiments. All animal protocols used in these studies were approved by the U.K. Home Office.

Glucocorticoid replacement. Corticosterone replacement was given as supplemented drinking water (CORT) at a final concentration of 25 μg/ml. Corticosterone was purchased from Sigma-Aldrich (Poole, U.K.).

Body weight measurement and food/fluid consumption. Mice were weighed on day 1 and after 10 days of treatment. Food intake was calculated
as the mean consumed over the 10-day treatment period. Fluid intake was measured daily during the 10-day treatment study.

**Oxygen consumption measurements.** Oxygen consumption was simultaneously determined for multiple animals by indirect calorimetry by using an Oxymax 4.4 System (Columbus Instruments, Columbus, OH). The experimental animals (all 8-week-old females) were allowed to acclimatize to the chambers for 2 h, and measurements were taken subsequently for 3 h during the light cycle (1100–1600). Samples were recorded every 4 min, with the room air reference taken every 16 min and the air flow to chambers, 500 ml/min. Data represent the mean oxygen consumption per minute over the 3-h testing period.

**Body composition.** Fat and lean body mass were determined by using dual-energy X-ray absorptiometry (Lunar PIXImus2 mouse densitometer; General Electric Medical Systems) as described by the manufacturer.

**Blood glucose.** Glucose analysis was done on tail vein blood using Lifescan One-Touch Ultra Glucometer (Lifescan, High Wycombe, U.K.).

**Hormone assays.** Plasma corticosterone (ImmunoDiagnostics, Tyne and Wear, U.K.), leptin (R&D Systems, Abingdon, U.K.), and insulin (Crystal Chem, Chicago, IL) were determined using commercially available kits according to the manufacturers’ protocols.

**In situ hybridization.** Coronal hypothalamic sections (16 μm) were cut on a cryostat and immediately stored at −80°C until hybridization. In situ hybridization was performed as described previously (12) on brain sections from 3-month-old male mice by using antisense oligonucleotide probes designed for AgRP, corticotropin-releasing hormone (CRH), melanin-concentrating hormone (MCH), and neuropeptide Y (NPY) mRNA (Table 1). For quantification of mRNA, sections were placed in X-ray cassettes and then exposed to autoradiographic film. The duration of exposure to the X-ray film varied according to the mRNA transcript under investigation. All sections were scanned, and the specific hybridization signal was quantified by densitometry using a digital imaging system (Image 1.33; National Institutes of Health, Bethesda, MD). The optical density of the hybridization signal was determined and subsequently corrected by the optical density of its adjacent background value. The optical densities were obtained in 16–20 consecutive sections per mouse, and the average value for each animal was used to calculate group means.

**Statistics.** All data are reported as means ± SE. All data sets were analyzed for statistical significance by using Student’s t test, with the exception of body weight, body length, and plasma insulin from weaning, which were analyzed by repeated-measures ANOVA. The PRISM software package (GraphPad, San Diego, CA) was used for all analyses. Results were considered statistically significant at *P* < 0.05.

**RESULTS**

After 10 days of glucocorticoid supplementation, Pomc-null and wild-type mice have comparable plasma corticosterone. Within each genotype, CORT treatment did not significantly increase mean daily fluid intake (wild type, control vs. CORT, 7.6 ± 0.6 vs. 6.8 ± 0.4 ml/day, respectively, NS: Pomc−/−, control vs. CORT, 9.0 ± 0.8 vs. 9.5 ± 0.9 ml/day, respectively, NS). However, CORT-treated Pomc−/− mice drank significantly more than CORT-treated wild-type mice (9.5 ± 0.9 vs. 6.8 ± 0.4 ml/day, respectively, *P* < 0.05).

Eight-week-old male Pomc−/− mice given unsupplemented water (control) had undetectable plasma corticosterone levels. However, after 10 days of CORT (25 μg/ml), plasma corticosterone concentrations in wild-type and Pomc−/− mice were nearly identical (25.5 ± 5.1 vs. 28.6 ± 5.3 ng/ml, respectively, NS) (Fig. 1A). We also assessed the impact of CORT treatment on the hypothalamo-pituitary-adrenal axis by measuring CRH mRNA expression within the paraventricular nucleus by in situ hybridization. As expected, CRH expression was threefold higher in control glucocorticoid-deficient Pomc−/− mice compared with control wild-type mice (percentage of wild-type vehicle level, 325.8 ± 21.3 vs. 100.0 ± 14.5%, respectively, *P* < 0.001) (Fig. 1B). CORT treatment to wild-type and Pomc−/− reduced expression of CRH to identical levels (50.9 ± 4.3 vs. 51.5 ± 5.0%, respectively, NS).

It is noteworthy that plasma corticosterone and CRH expression in CORT-treated wild-type mice were both lower than the respective measurements in control wild-type mice, indicating that the corticosterone treatment strategy was sufficient to suppress an intact hypothalamo-pituitary-adrenal axis.

Corticosterone treatment increases body weight and fat mass in Pomc−/− mice only. CORT-treated Pomc−/− mice were significantly heavier than age-matched control Pomc−/− mice (total body wt, 38.7 ± 0.6 vs. 35.5 ± 1.1 g, respectively, *P* < 0.05) (Fig. 2A). Over the 10-day period, CORT-treated Pomc−/− mice gained more than twice as much weight as control Pomc−/− mice (absolute change in weight, 4.9 ± 0.2 vs. 2.1 ± 0.4 g, respectively, *P* < 0.001). In contrast, the effect of glucocorticoid supplementation in the wild-type mice was negligible, with control and CORT-treated animals having similar final body weights (23.9 ± 0.5 vs. 23.1 ± 0.7 g, respectively, NS) (Fig. 2A).

We have previously shown Pomc−/− mice to have an

![FIG. 1. Plasma corticosterone levels (A) and CRH mRNA levels (B) in the paraventricular nucleus in wild-type and mutant mice, either control or treated for 10 days with corticosterone (cort). CRH levels are expressed as means ± SE percentage of wild-type vehicle-treated levels (n = 6 in each group; *P* < 0.05, **P* < 0.01, and ***P* < 0.001; n.d., not detectable).](image-url)
increase in both lean and fat mass when compared with wild-type littermates. To determine how CORT treatment affected body composition, all mice underwent dual-energy X-ray absorption scanning, and regional fat depots were dissected. The increase in total body weight seen in CORT-treated PomC−/− mice was entirely due to an increase in fat mass (9.0 ± 0.5 vs. 12.8 ± 0.4 g, control vs. CORT; *P < 0.01) (Fig. 2B). This increase was seen in all anatomical depots, with a 66% increase in inguinal (435 ± 51 vs. 720 ± 41 mg, control vs. CORT; *P < 0.05), a 48% increase in mesenteric (461 ± 60 vs. 684 ± 49 mg, P < 0.05), a 34% increase in retroperitoneal (270 ± 16 vs. 362 ± 30 mg, P < 0.05), and a 26% increase in epididymal (1,061 ± 77 vs. 1,336 ± 59 mg, P < 0.05) fat depots. Lean mass was unaffected by CORT treatment (23.4 ± 0.8 vs. 24.4 ± 0.7 g, vehicle vs. CORT, NS) (Fig. 2C). However, CORT made no difference to the body composition of wild-type mice, with fat and lean mass identical to that of vehicle-treated animals (Fig. 2B).

Mutant mice given CORT treatment increased their plasma leptin levels fourfold compared with control PomC−/− mice (63.1 ± 6.9 vs. 14.2 ± 1.8 ng/ml, respectively, *P < 0.001) (Fig. 2D). In addition, despite CORT treatment causing no change in fat mass in wild-type animals, there was a significant increase in plasma leptin levels compared with vehicle-treated wild-type animals (6.9 ± 1.5 vs. 2.6 ± 0.2 ng/ml, respectively, P < 0.05) (Fig. 2D).

FIG. 2. Effect of CORT treatment on body composition. Total body weight (A), total fat and lean mass as determined by dual-energy X-ray absorption (B), weight of individually dissected fat depots (ing, inguinal; meso, mesenteric; retro, retroperitoneal; epi, epidymal) (C), and plasma leptin levels (D) in wild-type and mutant mice, either control or treated for 10 days with corticosterone (cort) (n = 6 in each group; *P < 0.05, **P < 0.01, and ***P < 0.001).

Glucocorticoid treatment exacerbates the hyperphagia seen in PomC-null mice. CORT treatment significantly increased mean daily food intake in PomC−/− mice compared with control mutant mice (6.4 ± 0.2 vs. 4.9 ± 0.1 g, respectively, P < 0.001) (Fig. 3A). However, CORT-treated wild-type mice had similar daily food intake to control-treated wild-type mice (4.4 ± 0.3 vs. 4.2 ± 0.2 g/day, respectively, NS).

We assessed oxygen consumption via indirect calorimetry to investigate the impact glucocorticoid treatment had

FIG. 3. Effect of CORT treatment on food intake and energy expenditure. Mean daily food intake (A), oxygen consumption per animal (B), and oxygen consumption corrected for total body mass (C) in wild-type and mutant mice, either control or treated for 10 days with corticosterone (cort) (n = 6 in each group; *P < 0.05 and **P < 0.01).
on energy expenditure. Although absolute oxygen consumption per animal was similar across treatment groups and genotypes (Fig. 3B), when corrected for total body mass, Pomp−/− mice had a reduced energy consumption compared with wild-type mice (0.055 vs. 0.073 ml·g⁻¹·min⁻¹, respectively, P < 0.01), which was not influenced by CORT treatment (Fig. 3C).

**Plasma insulin levels in CORT-treated Pomp-null mice are 23 times those seen in CORT-treated wild-type mice.** Pomp-null mice had higher fed blood glucose levels than their wild-type littermates. CORT treatment to Pomp−/− mice did not significantly alter blood glucose when compared with control Pomp-null mice (10.1 ± 0.4 vs. 8.5 ± 0.7 mmol/l, respectively, NS). Similarly, blood glucose levels in wild-type mice were also unaffected by CORT treatment (6.8 ± 0.2 vs. 5.8 ± 0.5 mmol/l, NS) (Fig. 4A).

Analysis of paired insulin samples demonstrated that CORT treatment resulted in insulin resistance in wild-type and Pomp-null mice. CORT treatment in wild-type mice increased plasma insulin levels 14-fold (CORT vs. control, 2.2 ± 0.7 vs. 0.16 ± 0.05 ng/ml, P < 0.05) (Fig. 4B). However, the same treatment caused insulin levels in mutant mice to be 50-fold higher than those seen in control Pomp−/− animals (51.0 ± 13.5 vs. 1.00 ± 0.1 ng/ml, respectively, P < 0.001) (Fig. 4B). Thus, with comparable levels of plasma corticosterone, CORT-treated Pomp-null mice have plasma insulin levels that are 23 times those seen in CORT-treated wild-type mice.

**Pomp−/− mice have a significant reduction in AgRP that is restored to wild-type levels with corticosterone treatment.** To investigate what might be driving the hyperphagia and weight gain seen with CORT treatment in mutant mice, we looked at how CORT treatment affected the expression levels of key orexigenic neuropeptides within the hypothalamus. Pomp−/− mice had a significant

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**FIG. 4.** Plasma glucose (A) and insulin levels (B) in wild-type and mutant animals, either control or treated for 10 days with corticosterone (cort) (n = 6 in each group; *P < 0.05 and **P < 0.001).

**FIG. 5.** Hypothalamic expression of AgRP mRNA (A and B) and NPY mRNA (C and D) in wild-type and mutant animals, either control or treated for 10 days with corticosterone (cort). Data are shown as representative dark-field photomicrographs (A and C) (magnification ×10) and as optical density expressed as means ± SE percentage of wild-type vehicle-treated levels (B and D). 3V, third ventricle (n = 6 in each group; *P < 0.05 and **P < 0.001).
reduction in arcuate AgRP mRNA levels compared with control wild-type mice (percentage of wild-type expression, 44.4 ± 3.3 vs. 100.0 ± 15.4%, respectively, \( P < 0.001 \)) (Fig. 5A and B). However, CORT administration more than doubled AgRP expression in Pomc–/– mice (Pomc–/–, control vs. CORT, 44.4 ± 3.3 vs. 101.8 ± 6.7%, respectively, \( P < 0.001 \)) (Fig. 5A and B). CORT treatment also increased AgRP expression in wild-type animals (wild-type control vs. CORT, 100.0 ± 15.4 vs. 140.8 ± 11.3%, respectively, \( P < 0.05 \)).

NPY mRNA levels in the arcuate were not significantly different between control wild-type and Pomc–/– mice (percentage of wild-type expression, 100 ± 13.1 vs. 77.7 ± 8.4%, respectively, NS) and within genotype were unaffected by CORT administration (control vs. CORT; wild type, 100 ± 13.1 vs. 87.8 ± 10.7%; mutant, 77.7 ± 8.4 vs. 71.8 ± 8.8%, NS) (Fig. 5C and D). As previously described, MCH mRNA levels were increased in mutant compared with wild-type mice (8), but CORT treatment did not affect expression level in either genotype (data not shown).

**Pomc–/– mice given corticosterone from weaning develop severe hyperglycemia.** In a separate cohort of male wild-type and Pomc–/– mice, we administered glucocorticoid from weaning at 4 weeks of age, again using CORT (25 μg/ml). In neither genotype did this significantly increase adult body weight. In wild-type mice, CORT treatment caused a significant fall off in weight gain over time (Fig. 6A). Furthermore, although the growth curve of CORT-treated Pomc–/– mice began at 8 weeks to rise above that of control mutant littermates, by 10–12 weeks, CORT-treated mice were losing weight.

In addition, CORT treatment from weaning significantly reduced the body length of both genotypes. CORT-treated Pomc–/– mice were significantly shorter than control mutants, only attaining a body length at 12 weeks comparable with that of wild-type mice (Fig. 6B).

Over the subsequent 8 weeks from weaning, CORT treatment had no effect on blood glucose in wild-type mice. However, the fall off in body weight and body length seen in CORT-treated Pomc–/– mice was accompanied by a progressive rise in blood glucose such that by 10–12 weeks of age, animals became frankly diabetic (Fig. 7C), with polyuria and ketonuria. By 12 weeks, CORT-treated Pomc–/– mice were also markedly polydipsic, drinking more than three times as much as CORT-treated wild-type mice (daily fluid intake at 12 weeks, wild-type, CORT-treated wild-type, Pomc–/– mice, CORT-treated Pomc–/– mice; 11.3 ± 0.7 vs. 10.7 ± 1.3 vs. 13.3 ± 0.7 vs. 34.7 ± 5.7 ml/day, respectively, \( P < 0.01 \)). Their coat condition deteriorated, exacerbated by the excess volume of urine produced, and proximal myopathy was visible macroscopically (Fig. 7F). The overall condition of the animals necessitated killing at 12 weeks. Hepatic steatosis was clearly visible macroscopically and confirmed microscopically (Fig. 7H and J).

Plasma samples taken at this time demonstrated that, as in the short-term study, CORT treatment resulted in severe insulin resistance, with the fold increase in insulin levels seen in Pomc–/– mice significantly higher than that seen in wild-type mice (insulin levels at 12 weeks: wild type, control vs. CORT; 0.77 ± 0.17 vs. 1.98 ± 0.36 ng/ml, respectively, \( P < 0.05 \); Pomc–/–, control vs. CORT; 3.01 ± 0.33 vs. 107.7 ± 22.58 ng/ml, respectively, \( P < 0.01 \)) (Fig. 7D). This is likely to be due to the difference in the corticosterone levels seen in CORT-treated mice at 12 weeks. The marked polydipsia seen in CORT-treated Pomc–/– mice inevitably led to ingestion of excess glucocorticoid with corticosterone levels significantly higher than those found in CORT-treated wild type (176.7 ± 43.2 vs. 63.8 ± 21.4 ng/ml, respectively, \( P < 0.05 \)) (Fig. 7K).

**DISCUSSION**

We administered corticosterone to wild-type and Pomc–/– mice for 10 days, achieving comparable plasma corticosterone and hypothalamic CRH expression levels. Only in the Pomc–/– mice did this cause increased body weight and fat mass. An increase in food intake was also only seen in Pomc-null mice, with this exacerbation of preexisting hyperphagia likely to be as a result of a corticosterone-dependent increase in the expression of the orexigenic neuropeptide, AgRP.

Ten days of glucocorticoid treatment did not increase blood glucose levels but did increase plasma insulin levels in both wild-type and Pomc–/– mice. However, the absolute plasma insulin levels measured and fold increase from control were markedly higher in Pomc-null mice. Furthermore, corticosterone treatment to mice from weaning resulted in a progressive rise in blood glucose and frank diabetes by 10–12 weeks only in Pomc–/– mice, having no such effect in wild-type animals.

Two other groups have investigated the impact of glucocorticoids on aspects of the phenotype exhibited by Pomc-null mice (13–15). Hochgeschwender et al. (15) replaced glucocorticoid in Pomc-null mice using CORT. However, there was no quantification of plasma levels.
achieved through this strategy. The authors reported that glucocorticoid supplementation alleviated hypoglycemia on insulin challenge, but they did not comment on other aspects of the phenotype such as food intake and body morphology. In studies to date only published in abstract form, Smart and Low (13) used a genetic approach to “rescue” pituitary POMC activity in Pomc−/− mice (14). A strain of mice transgenically overexpressing POMC peptides selectively in pituitary corticotrophs was backcrossed onto Pomc−/− mice. The mice subsequently generated had a functionally restored pituitary adrenal axis with circulating glucocorticoid and became more obese and more hyperphagic than nontransgenic Pomc−/− mice (13). More recently, this group has also reported this transgenic rescue strategy to result in Pomc−/− mice developing hyperglycemia (14). However, the level of circulating corticosterone achieved is uncertain in this model, and detailed data are not yet available.

Normal physiological diurnal variability in circulating plasma corticosterone cannot be satisfactorily achieved by administration of exogenous corticosterone alone. Our strategy aimed, successfully, to give enough corticosterone to Pomc−/− mice to achieve plasma levels comparable with those we have previously documented in unstressed, acclimatized wild-type animals (9). The partial suppression of CRH that was seen in wild-type and Pomc−/− animals would have been expected to occur even if perfect “physiological” replacement was achieved because exogenous CORT would be replacing the need for endogenously produced CORT. The fact that CRH is only partly suppressed can be taken as an indication that grossly supraphysiological levels of CORT were not administered. Having stated that, leptin and insulin levels did rise in the CORT-treated wild-type animals, suggesting that they may have been exposed to somewhat supraphysiological levels of CORT.

Based on plasma corticosterone and CRH mRNA expression, our CORT treatment strategy had a similar effect on wild-type and Pomc−/− mice, yet the impact on the phenotypes exhibited by the two genotypes was markedly different. In particular, only Pomc−/− mice significantly increased their body weight. This increase was entirely due to an increase in fat mass with significant accumulation in all anatomical depots. As expected, this increase in fat mass was accompanied by an increase in plasma leptin levels. Glucocorticoid treatment also caused a significant increase in plasma leptin levels in wild-type animals, despite there being no change in fat mass. This may be a reflection of the previously reported direct effect glucocorticoids have on the expression of leptin (16).
CORT treatment also only affected food intake in Pome⁻/⁻ mice, accentuating the established hyperphagia seen in these mice. Subsequent analysis of orexigenic peptide expression within the hypothalamus gave insights into the potential mechanisms underlying this. The differences in expression levels of AgRP were most striking. Corticosterone-deplete Pome⁻/⁻ mice had less than one-half the expression level seen in wild-type mice yet were still more hyperphagic. Furthermore, although glucocorticoid treatment resulted in absolute expression levels of AgRP that were higher in CORT-treated wild-type, glucocorticoid-treated Pome⁻/⁻ mice had a significantly higher food intake. This may be explained by the fact that Pome⁻/⁻ mice (in contrast to intact wild-type animals) do not produce any anorexigenic melanocortin peptides to act as agonist at melanocortin-4 receptors, leaving unopposed the orexigenic, inverse-agonist effect of AgRP. A further increase in AgRP levels after glucocorticoid treatment, therefore, is manifest as an even greater increase in food intake.

There was no significant difference in the expression patterns of arcuate NPY mRNA expression between wild-type and Pome⁻/⁻ mice, in agreement with our previously published results (8). However, in marked contrast to the changes seen in AgRP expression levels, glucocorticoid supplementation did not effect changes in the expression of NPY in either wild-type or Pome⁻/⁻-null mice. Thus, these two orexigenic peptides that are coexpressed within the same set of hypothalamic arcuate neurons and are exposed to the same peripheral signals of metabolism appear to respond in different ways to glucocorticoid.

Previous studies have examined the effect of lack of glucocorticoids on AgRP and NPY mRNA expression in the hypothalamus (10,17–29). Makimura et al. (10) reported that adrenalectomy in wild-type mice reduced AgRP but not NPY mRNA expression. Similarly, in ob/ob mice, adrenalectomy restored only AgRP but not NPY mRNA to wild-type levels (10). These data are consistent with the expression patterns we found in untreated glucocorticoid-deplete Pome⁻/⁻ mice. However, the levels of NPY mRNA in the hypothalamus have been reported to decrease (18,19,23,29), increase (20), or remain the same (21,22) after adrenalectomy.

There are also data highlighting the permissive effects glucocorticoid have on the actions of AgRP and NPY. Drazen et al. (11) demonstrated that adrenalectomy alters the sensitivity of the central melanocorticergic system, reporting that both leptin and the melanocortin agonist melanotan II have a more potent anorexigenic effect in adrenalectomized animals. Furthermore, the orexigenic effect of intracerebroventricular AgRP was absent in adrenalectomized rats but restored by glucocorticoid replacement (11). Sainsbury et al. (24) have shown that the increased food intake, body weight, and insulin after chronic intracerebroventricular infusion of NPY to intact rats is not seen in adrenalectomized animals. Importantly, Zakrzewska et al. (26) demonstrated giving back glucocorticoid centrally to adrenalectomized animals restored the effects of intracerebroventricular NPY to those seen in intact animals. Thus another potential mechanism underlying the hyperphagia seen in CORT-treated Pome⁻/⁻ mice may be this permissive effect on both AgRP and NPY action.

We have previously reported that MCH expression in the lateral hypothalamus is increased in Pome⁻/⁻ mice (8). In this study, we have again found MCH expression to be increased in Pome-null mice. However, there was no change in expression level after glucocorticoid replacement, indicating that changes in MCH expression do not underlie the increased hyperphagia seen with CORT treatment.

The reduction in resting basal metabolic rate (as measured by oxygen consumption) seen in Pome-null mice remained unaffected by glucocorticoid treatment. Thus the phenotypic feature of a deficit in energy expenditure that we have previously described in Pome⁻/⁻ mice (8) appears to be as a result of melanocortin deficiency rather than secondary to glucocorticoid insufficiency. This is in keeping with the phenotype of Mc4R⁻/⁻ mice, another well-characterized model of melanocortin dysfunction, which are corticosterone replete but still consume 20% less oxygen then wild-type mice (30).

Glucocorticoid treatment to Pome⁻/⁻ mice from weaning resulted in animals becoming profoundly insulin resistant and hyperglycemic with a rapid deterioration in their general condition by 12 weeks of age. This is in contrast to the previous report by Hochgeschwender et al. (15), who stated that continuous supplementation of corticosterone to Pome⁻/⁻ mice for more than 4 months did not lead to hyperglycemia. However, this conclusion was based on fasting blood glucose measurements, in contrast to our study in which all measurements were in the fed state. Another difference may be that the corticosterone levels achieved in both studies are not comparable. Certainly, in our study, the polydipsia resulting from uncontrolled hyperglycemia led to a rapid escalation of corticosterone ingestion and supraphysiological levels of plasma corticosterone. It remains uncertain as to why this was not seen in the previously published study.

Nevertheless, it is striking that wild-type mice with an intact melanocortin system did not become hyperglycemic despite also becoming insulin resistant, and our results are consistent with the growing body of evidence linking the central melanocortin system with glucose homeostasis (31–35). Well-characterized murine models of melanocortin dysfunction such as Mc4r⁻/⁻ mice and yellow agouti A⁺/+ mice develop severe hyperinsulinemia (31,32). Significantly, the hyperinsulinemia in Mc4r⁻/⁻ mice precedes the onset of hyperphagia and obesity (32). Transgenic overexpression of neuronal POMC peptides in leptin-deficient ob/ob mice (36) and yellow agouti A⁺/+ mice (37) can significantly ameliorate hyperglycemia and insulin resistance by enhancing peripheral insulin sensitivity, independent of effects on food intake and body weight. Pharmacological studies have also shown that central administration of α-melanocyte-stimulating hormone can increase both insulin-stimulated glucose disposal and insulin-induced suppression of hepatic gluconeogenesis (33,34). Furthermore, pharmacological blockade of central melanocortin receptors can result in insulin resistance (33). Finally, although humans affected by melanocortin-4 receptor deficiency are euglycemic, they are also particularly insulin resistant with plasma insulin concentrations significantly elevated compared with those in appropriate
obese controls (35). Thus, an intact MC system is required for normal insulin sensitivity in peripheral tissues. That more severe disruption of glucose homeostasis is not more prominent in Pomc−/− mice, we believe, due to their concomitant glucocorticoid insufficiency.

In summary, mice deficient in POMC peptides appear to be hypersensitive to the adverse metabolic consequences of glucocorticoid therapy. At plasma levels of corticosterone that have little impact on the phenotype of POMC-replete wild-type mice, Pomc−/− mice become significantly more obese and hyperphagic. The glucocorticoid deficiency seen in Pomc-null mice may mask the severe adverse metabolic consequences of the lack of melanocortin peptides.

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