Disengaging insulin from corticosterone: roles of each on energy intake and disposition

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Warne JP, Akana SF, Ginsberg AB, Horneman HF, Pecoraro NC, Dallman MF. Disengaging insulin from corticosterone: roles of each on energy intake and disposition. Am J Physiol Regul Integr Comp Physiol 296: R1366–R1375, 2009. First published March 11, 2009; doi:10.1152/ajpregu.91016.2008.—Corticosterone and insulin play complex roles in the amount and composition of calories ingested, and the utilization and deposition of this energy. Understanding the interplay of these two hormones is complicated because increasing concentrations of corticosterone dose-dependently increase circulating insulin levels. We addressed individual contributions of each hormone by controlling, at steady-state levels, corticosterone (by adrenalectomy and exogenous replacement) and insulin (by streptozotocin-induced destruction of pancreatic β-cells and exogenous replacement) across a spectrum of concentrations in rats, creating 8 hormonal combinations. For 5 days after surgery, all rats received chow. At day 5, they were subdivided into those that continued to receive chow and those that had a choice between chow, lard, and 32% sucrose for a further 5 days. During the choice/chow period, total calories ingested were stimulated by corticosterone and choice diet, and subject to a corticosterone-insulin interaction. Sucrose, but not lard, intake was stimulated by insulin. Body weight was increased by insulin, decreased by high corticosterone, and unaffected by diet. White adipose tissue depot weights were stimulated by insulin, corticosterone, and diet. Plasma triglycerides, free fatty acids, total ketone bodies, glucose, and glycerol were all significantly increased by corticosterone and the choice diet but inhibited by insulin. In contrast, plasma leptin was only increased by insulin and diet, plasma glucagon and liver glycogen was only affected by insulin and liver triglycerides, and arcuate nucleus proopiomelanocortin mRNA was only influenced by diet. Collectively, these data show that corticosterone and insulin determine the intake, form, and compartmentalization of energy both independently and interactively.

The ratios of these circulating hormones (39) can change from “normal” over the long term, depending on conditions. For instance, chronic stressors may elevate overall glucocorticoids in the circulation for days or weeks, while, if food intake is reduced appreciably, insulin concentrations are reduced (10). On the other hand, after relief from a chronic stressor, or under conditions of post-traumatic stress disorder, circulating glucocorticoids and responsiveness of the hypothalamic-pituitary-adrenal axis can be decreased from normal, while insulin remains at normal concentrations (14, 19). Thus, it is important to determine not only the metabolic effects of “normal” relationships between glucocorticoids and insulin, but also to examine the effects of small independent increases or decreases in one or the other hormone on energy balance.

It is impossible in vivo to determine the selective role of glucocorticoids on energy balance, because as glucocorticoids increase, insulin concentrations also increase (24, 40), producing dual hormonal effects. Previously, we have approached this problem using implanted corticosterone pellets that provide steady-state peak circadian/stress levels, inhibiting endogenous secretion (1), and streptozotocin (STZ) treatment to destroy pancreatic β-cells, rendering the rat type I diabetic, with steady-state venous insulin delivery (40–42, 44). Those studies, however, did not explore varying steady-state corticosterone levels, the goal of the present studies.

Although both hormones are normally secreted in response to acute stimuli, and do not maintain steady-state concentrations, we have shown that appropriate mean steady-state corticosterone concentrations provided to adrenalectomized rats normalize most variables disrupted by adrenal removal (1). Constant insulin infusions to diabetic rats also normalize many of the aberrations induced by STZ-diabetes (44). Clearly, the constant infusion paradigm does not mimic normal physiology, but it has the virtue of distinguishing between the actions of corticosterone and those of insulin, something impossible to achieve by controlling either hormone alone.

Our goal was to explore whether the metabolic effects of increasing glucocorticoid concentrations are steroid mediated, or are dependent on to increases in circulating insulin levels. Rats underwent ADX and STZ treatment and implanted with corticosterone and insulin pellets to provide steady-state circulating levels that correspond to under-, normal- and over-replacement of both hormones. Sham-operated, nondiabetic rats were included as adrenal- and pancreas-intact reference. Finding all measured variables normalized (i.e., to values in intact rats) by predominantly one pair of corticosterone/insulin doses would validate the utility of this steady-state approach and heighten the significance of normal concentrations of the hormones. Because dietary choices, hence energy balance, are affected by the levels of the two hormones (24, 44), we...
examined two dietary groups: chow only (Chow Groups) and those with ad libitum access to chow, lard, and sucrose (Choice Groups).

MATERIALS AND METHODS

Male rats (Sprague-Dawley, Charles River, Hollister, CA) were housed individually in hanging wire cages in a temperature- and light-controlled room (22°C, lights on 0700–1900). Rats adapted to their new environment with ad libitum access to chow (3.31 kcal/g with 29.85% calories from protein, 16.71% from fat, and 56.44% from carbohydrates; Purina Chow #5008, Purina, St. Louis, MO), and water for 4 days before experimentation. At experimental onset (day 0), rats weighed 253 ± 1 g. The experiments were approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

Experimental timeline. All animal preparation was performed on day 0 (Fig. 1A). Water was replaced with 0.5% NaCl. Rats were allowed 5 days to recover with chow ad libitum. On day 5, cages were installed with a second bottle and cup; these were filled with an ad libitum supply of lard (9 kcal/g) and 32% sucrose solution (4 kcal/g dry weight) for Choice Groups, or remained empty for Chow Groups. On day 10, all rats were killed by decapitation, rectal temperature (°C) taken using a gel-lubricated microthermoprobe (Physiotemp IT-18), and samples were collected.

Surgical procedures and treatment groups. Rats were anesthetized using ketamine (75 mg/kg im) and xylazine (10 mg/kg im). Ketoprofen (10 mg/kg sc) was provided as an analgesic immediately after surgery. Eight hormonal manipulations were performed (Fig. 1B). A ninth, nondiabetic, sham ADX group served as a reference. Surgeries were performed in six cycles, with at least 1 rat from each of the 18 final groups included in each cycle. By the end of the experiment, accounting for rats that died after STZ during the study [up to 10% losses (25)] and excluding those with partial ADX (assessed at autopsy), there were 5 rats/group in most groups. The exceptions were Chow Groups (% corticosterone-units of insulin/day): 0%-0 U (n = 4), 0%-1 U (n = 4), 30%-4 U (n = 4). For the choice groups, the exceptions were 0%-0 U (n = 6), 0%-1 U (n = 4), 30%-4 U (n = 6), and 100%-4 U (n = 6).

Rats were bilaterally adrenalectomized via a dorsal midline skin incision and flank incisions. Corticosterone pellets [100 mg: 0% (wax), 30% (with 70% wt/wt cholesterol)] or 100% corticosterone (Steraloids, Newport, RI) were inserted into a subcutaneous pocket caudal to the posterior edge of the skin incision. Sham operation involved all procedures (wax pellet) except adrenal removal.

Subcutaneous injection of STZ (Sigma Chemicals, St. Louis, MO; 65 mg/kg in citrate buffer pH 4.2) induced insulin-dependent diabetes. Rats in control groups were injected with citrate buffer (2 ml/kg). For each replacement concentration of corticosterone, there were three replacement doses of insulin: 0 U, 2 U, or 4 U/day. ADX rats without corticosterone replacement are extremely sensitive to insulin (11), and received 0 U and 1 U insulin/day. Insulin was provided by inserting zero (0 U/day), half (1 U/day), one (2 U/day) or two (4 U/day) Linplant sustained released insulin implant(s) (LinShin Canada, Ontario, Canada) into a subcutaneous pocket caudal to the posterior edge of the skin incision on the side opposite to the corticosterone pellet. The incision was closed with silk suture.

Tissue collection and assays. Trunk blood was collected into chilled tubes containing 0.1 ml EDTA (65 mg/ml), plasma was separated and stored at −80°C. The whole brain (immersed in Tissue-Tek; Sakura Finetek, Torrance, CA) and liver biopsies (100 mg) were removed, snap frozen, and stored at −80°C. WAT depots [subcutaneous (scWAT), epididymal (eWAT), perirenal (pWAT), and...
Glucocorticoids, insulin, and energy balance

Plasma corticosterone, insulin, glucagon, and leptin concentrations were measured using colorimetric commercial kits (MBI Biomedicals, Orangeburg, NY; Linco Research, St. Charles, MO). Plasma glucose, triglycerides, glycerol, total ketone bodies, and free fatty acids (FFA) were measured using colorimetric methods (Megadiagnostics, Los Angeles, CA; Sigma-Aldrich, St. Louis, MO; Wako Chemicals, Neuss, Germany). Liver glycogen was extracted in 30% potassium hydroxide (30 min, 100°C), precipitated in 95% ethanol (1:1 vol/vol, 30 min, 4°C), quantified by colorimetric plate assay (adapted from Ref. 27). Liver triglycerides were extracted in chloroform:methanol (2:1 vol/vol) (13), measured by colorimetric plate assay (as for plasma) and both glycogen and triglycerides were standardized to grams wet weight. All assays were performed as previously described (44).

ArcN NPY and POMC mRNA expression was detected and semi-quantified by in situ hybridization, as previously described (43). Briefly, the frozen brains were sectioned (14 μm) in the coronal plane, slide mounted, fixed in 4% paraformaldehyde, acetylated, and dehydrated. Specific 35S-labeled antisense cRNA probes (26) were applied overnight at 55°C to each section (1 × 106 cpm/slide). Sections were then rinsed in 2 × SSC, treated with RNase A (20 μg/ml) in 10 mM Tris, 5 mM EDTA, 0.5 M NaCl at 37°C for 1 h), washed in SSC (2 × 0.1 × at room temperature, 0.1 × for 1 h at 68°C, 0.1 × at room temperature), dehydrated in ethanol, and air-dried. Sections were exposed to Hyperfilm MP (Amersham Pharmacia, Buckinghamshire, UK) until a signal was obtained without saturation. The integrated optical density (IOD) from three consecutive sections around −2.8 mm relative to bregma, based on the coordinates of Paxinos and Watson (33), was determined for each rat using National Institutes of Health Image with the IOD corrected for background using a macro designed by Dr. Serge Campeau (University of Colorado).

Statistical analyses. Data are presented as the means ± SE of the mean. Data from the ADX/STZ-treated groups were analyzed by three-way ANOVA to determine the overall individual effects of each experimental manipulation (the factors) and the interactions between these factors. The factors for the three-way ANOVAs were insulin dose (0 U/day, 1−2 U/day, or 4 U/day), corticosterone dose (0%, 30%, or 100%) and diet (chow or choice). The statistical results of the three-way ANOVAs performed are shown for all variables in Supplemental Tables 1 and 2 (see online version of this article). Three-way ANOVAs were performed for data obtained during the first 5 days of the study, despite no differences in insulin provided at this point to verify that there were no significant differences between the Choice and Chow Groups prior provision of lard and sucrose to the former. Three-way ANOVAs were also performed on the lard and sucrose data, despite these not being available to the Chow groups during the last 5 days of the study, so all calorie intake analyses were performed in a similar manner, and expectedly, the factor of diet was highly significant. Three-way ANOVAs were followed by one-way ANOVAs to incorporate the two reference groups and post hoc tests of individual group differences (Tukey test). All statistical analyses were performed using SPSS 16.0 software with significance defined at P < 0.05.

RESULTS

Corticosterone and insulin levels. Both corticosterone and insulin concentrations were only significantly influenced by manipulation of the respective hormone, unaffected by diet with no interactions (P < 0.001, Supplemental Table 1). As expected, increasing the pellet composition (corticosterone, Fig. 1C) or number (insulin, Fig. 1D) produced significant, dose-dependent increases at concentrations predicted by previous studies (1, 24). Plasma values for corticosterone were as follows: 0% pellet 1.2 ± 0.4 μg/dl; 30% pellet 3.9 ± 0.4 μg/dl, 100% pellet 16.2 ± 1.5 μg/dl. Plasma values for insulin were as follows: 0 U 1.2 ± 0.2 ng/ml, 1 U 2.7 ± 0.2 ng/ml, 2 U 4.4 ± 0.9 ng/ml, and 4 U 12.6 ± 2.0 ng/ml. Intact controls showed appropriate corticosterone and insulin levels for time of day at 1.7 ± 0.6 μg/dl and 3.4 ± 0.7 ng/ml, respectively.

Table 1. Comparison of intact versus adrenalectomized and streptozotocin-treated rats with varying corticosterone and insulin replacement concentrations

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Comparison of intact vs. adrenalectomized and streptozotocin-treated rats with varying corticosterone (0%) and insulin replacement (U/day) concentrations, illustrating the steady-state hormonal combinations that most closely mimic that observed in adrenal intact, nondiabetic rats for the variables examined in both the chow and choice dietary groups. ArcN, arcuate nucleus; NPY, neuropeptide Y; POMC, proopiomelanocortin; WAT, white adipose tissue; FFA, free fatty acids. ∗denotes statistically indistinguishable with a highly similar mean; (‡) denotes statistically indistinguishable but mean not similar; X denotes statistically different levels of the variable in question, comparing intact and the specific corticosterone-insulin combination. *No significant differences among any of the corticosterone-insulin combinations. †Body weight gain during the last 5 days of the study. The 30%−2 U corticosterone-insulin steady-state combination mimics very well the caloric intake and disposition of adrenal intact, nondiabetic controls; 30% corticosterone replacement approximates the mean daily plasma corticosterone concentration, and 2 U/day insulin produces AM plasma levels that are indistinguishable from the intact controls.
**First 5-day caloric intake and body weight.** During the 5 days after surgery, body weight was significantly influenced by both corticosterone and insulin ($P < 0.001$, Fig. 1E, and Supplemental Table 1). Insulin stimulated body weight gain. Corticosterone produced more complicated effects: 30% pellets enhanced weight gain, whereas 100% pellets promoted weight loss, compared with those with 0% pellets. ADX/STZ rats with 30% corticosterone and 4 U/day insulin pellets showed body weight gain and caloric intake that approximated that of the intact controls. In contrast, caloric intake was significantly stimulated by corticosterone, but not insulin ($P < 0.001$, Fig. 1F, Supplementary Table 1). There was a significant corticosterone-insulin interaction ($P = 0.017$). Increasing insulin levels stimulated caloric intake at 30% corticosterone and inhibited it at 100% corticosterone.

**Choice/chow caloric intake (second 5 days).** Corticosterone and diet affected chow intake, and there was a significant corticosterone-insulin interaction ($P < 0.01$, Supplemental Table 1). Examination of individual group differences (Fig. 2A) shows corticosterone stimulates chow intake in the chow group at 0 U/day insulin ($P < 0.05$). With choice, sucrose intake was significantly stimulated by insulin (Supplemental Table 1); 2 U/day or higher insulin was significantly greater than 0 U/day at 30% corticosterone, 1 U/day was higher than 0 U/day at 0% corticosterone (Fig. 2B). Lard intake (Fig. 2C) was unaffected by any hormonal manipulation. Consequent to the additional consumption of lard and sucrose, chow intake was reduced significantly (Fig. 2A), although total calories were increased overall ($P < 0.001$, Fig. 2D, Supplemental Table 1), significantly in the 0%-0 U/day, 30%-0 U/day, 30%-2 U/day, and 100%-2 U corticosterone-insulin combinations, as well as in the intact group. When expressed as percent calories, sucrose showed a significant effect of insulin ($P < 0.004$) and diet ($P < 0.001$) and a significant insulin-diet interaction ($P = 0.01$, Supplemental Fig. 1A; Supplemental Table 1). This was notably evident when comparing 0 U and 1 U at 0% corticosterone, 0 U and 2–4 U at 30% corticosterone, and 0 U to 4 U at 100% corticosterone. Percent of lard and chow calories was only predictably affected by diet ($P < 0.01$, Supplemental Fig. 2, Supplemental Table 1).

When examining macronutrient content (Supplemental Fig. 2), both carbohydrate and protein intake are significantly ($P < 0.05$) affected by corticosterone. Provision of the choice diet reduced protein and increased fat intake, evident in most corticosterone-insulin combinations and in the sham-operated rats, compared with the chow-fed rats. The overall percentage of protein, carbohydrate, and fat ingestion in the chow groups was identical since this was the only calorie source. The pattern of protein intake in the choice groups was similar to that of chow intake, since this was the sole source of protein. When expressed as a percentage of calories, the three macronutrients only showed significant effects of diet ($P < 0.001$, Supple-
ment Table 1), with protein intake decreased with the choice diet, fat intake increased and carbohydrate increased under certain hormonal combinations (Supplemental Fig. 1B).

Choice/chow body weight gain. All rats gained body weight during the last 5 days, significantly influenced by both corticosterone and insulin, but not diet (P < 0.05, Fig. 2E, Supplemental Table 1). Any weight lost during the first 5 days (Fig. 1E) was predominantly regained across the entire 10-day period (P < 0.01, Supplemental Table 1, Fig. 2F). Generally, insulin stimulated body weight gain. Corticosterone showed the same pattern as for the first 5 days. Intact reference groups were indistinguishable in body weight gain from the 30%, 2 U/day insulin groups, and weight gain on the choice diet was greater than on chow. Rectal temperature was unaffected by any manipulation (Supplemental Table 2, mean = 36.9 ± 0.1°C).

Stomach content weight was significantly affected by insulin and diet, and there was a corticosterone-insulin interaction (P < 0.05, Supplemental Table 2). Insulin (2 U or 4 U/day) reduced stomach contents at 30% and 100% corticosterone on the chow diet, and also (nonsignificant) at 100% corticosterone on the choice diet (Fig. 2G). In the intact groups, there was a significant increase in stomach content with a choice diet; both groups resembled the 100%-2 U/day corticosterone-insulin combination. Stomach weight was unaffected by any experimental manipulation (Supplemental Table 2, mean = 4.4 ± 0.1 mg/g body wt).

ArcN neuropeptide mRNA and plasma hormones. ArcN POMC mRNA expression was significantly affected by diet, but not insulin or corticosterone (P = 0.037, Fig. 3A, Supplemental Table 2). The corticosterone-insulin combination of 100%-4 U/day had lower expression on the choice diet. Additionally, in both dietary groups at 30% corticosterone, expression was greater at an insulin replacement of 2 U/day than 0 U/day, with 4 U/day insulin intermediate. Intact groups showed no effects of diet. In contrast, there were significant effects of corticosterone and insulin on ArcN NPY mRNA expression (P < 0.05, Fig. 3B, Supplemental Table 2). Corticosterone dose dependently increased and insulin dose dependently decreased expression on the no-choice diet, the latter only evident at 30% and 100% corticosterone replacement doses and when comparing 0 U and 4 U insulin/day. These effects were not significant on a choice diet.

Plasma leptin levels were positively affected by both insulin and diet, and there was an insulin-diet interaction (P < 0.05, Fig. 3C, Supplemental Table 2). Post hoc analyses revealed dose-dependent increases in leptin with increasing insulin (0 < 2 < 4 U/day) at 30% and 100% corticosterone doses. The choice diet produced a significant elevation in plasma leptin greater than the chow diet in the 30%-4 U/day corticosterone-insulin combination. In the intact rats, the choice diet produced an increase in plasma leptin that was similar to the 30%-2 U/day corticosterone-insulin treatment. Plasma glucagon (Fig. 3D) was negatively affected by insulin, but not corticosterone or diet (P = 0.03, Supplemental Table 2). Glucagon levels were lower with 2 and 4 U/day, compared with 0 U/day insulin, in the 100% corticosterone pellet groups on the chow-only diet.

WAT weights. Insulin, corticosterone, and choice diet significantly stimulated all four WAT depots (scWAT, mWAT, eWAT, and pWAT, all P < 0.05, Fig. 4A–D, Supplemental Table 2). Choice diet also stimulated WAT weights in the intact group, which closely resembled the 30% corticosterone-2 U/day insulin groups. For scWAT and eWAT, the choice diet at this corticosterone-insulin combination left-shifted the dose response for 30% corticosterone. The choice diet at 30% corticosterone increased mWAT and eWAT weights above those in the chow group and maintained an insulin dose-response. Importantly, corticosterone requires a threshold level of insulin (between that supplied by the 1 U and 2 U/day pellets) to have a positive effect on WAT weight.

Liver and plasma metabolite measures. Insulin was also the only controlled factor affecting levels of liver glycogen (P < 0.001, Fig. 5A, Supplemental Table 2); 4 U/day insulin produced significantly higher liver glycogen levels than 0 U/day insulin at both the 30% or 100% corticosterone replacement doses. Liver triglyceride content was also significantly affected by diet (P = 0.002, Fig. 5B, Supplemental Table 2),
with significant increases evident with the 0%-0 U/day, 30%-2 U/day and 30%-4 U/day corticosterone-insulin combinations.

Plasma glucose (Fig. 5C) was influenced by corticosterone, insulin, and diet (P < 0.05, Supplemental Table 2). Insulin reduced, and both corticosterone and choice diet increased plasma glucose levels, significantly when comparing (corticosterone dose at insulin dose) 0% to 100% at 0 U/day and 30% to 100% at 4 U/day for the former and 0%-0 U and 100%-2 U for the latter.

Plasma triglycerides (Fig. 5D), glycerol (Fig. 5E), FFA (Fig. 5F), and total ketone bodies (Fig. 5G) were significantly (P < 0.05) elevated by increasing corticosterone and choice diet and dose dependently reduced by increasing insulin concentrations (Supplemental Table 2). Furthermore, there was an insulin-diet interaction for plasma FFA and triglycerides and a significant corticosterone-insulin-diet interaction for plasma ketone bodies (P = 0.04, Supplemental Table 2). Significant insulin-induced reductions in these circulating metabolites were only evident in rats provided with 30% or 100% corticosterone pellets, but not 0% pellets, on chow diet. This was also evident examining plasma triglycerides, FFA and ketones, but not glycerol, in rats fed the choice diet. Diet did not affect these circulating metabolites in the intact, reference rats.

DISCUSSION

By successfully controlling the levels of both corticosterone and insulin, we have demonstrated the independent and important actions of both hormones in the context of diet on calorie intake, adiposity, plasma hormone, and metabolite levels and ArcN neuropeptide mRNA expression. Although the circulating concentrations of both hormones were clamped and not allowed to fluctuate as they do physiologically, it is clear that steady-state values of corticosterone and insulin provided by 30% corticosterone pellets and 2 U/day insulin results in rats that are nearly indistinguishable for the majority of measured variables from intact controls (Table 1). It is, however, the experimentally altered relationships between the two hormones that reveal the key roles of each on energy intake and disposition under the two dietary conditions.

On a chow diet, the hormonal conditions of low corticosterone and low insulin (the 0%, 0 U/day group) are characterized by low circulating levels of triglycerides, FFA, glycerol and ketones, low liver triglycerides and glycogen, low WAT depot weights, and high plasma glucose and glucagon levels. This is also consistent with the low caloric intake and body weight gain. Hence, the calories ingested are kept in the circulation as glucose, with limited capacity of the rodent to store excess energy. Interestingly, the mRNA expression of ArcN POMC and NPY was also low. Previous studies have similarly shown that ADX reduces both orexigenic and anorexigenic gene expression (36, 46), suggesting a more complicated scenario and currently undefined mechanism by which ADX rats exhibit reduced food intake and body weight (23).

Shifting insulin levels from low to high on a low corticosterone background (0%, 0 U/day to 30%, 4 U/day) changes the metabolic landscape. Circulating glucose levels were reduced with energy now stored in the liver as glycogen and in WAT. The shift to high liver glycogen exists after very modest increases in insulin (e.g., 2 U/day), also evident in our other studies (44). Because the liver has a limited capacity to store glycogen (37), the WAT deports expand as the need for further energy storage increases. Collectively, these data are consistent with the established stimulatory actions of insulin on glycogenesis, glycogen storage (9), and glucose uptake and conversion to lipid in WAT (35). Insulin also inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenolysis directly (29) and indirectly by affecting substrate availability (4). The latter is exemplified in this study by further reduced levels of circulating FFA, triglyceride, and glycerol levels. Insulin also inhibits lipolysis in adipocytes via inhibition of hormone-sensitive lipase (2). Furthermore, without fatty acid substrates, ketogenesis is predictably minimal (30). Body weight was highest, in the 30%/2 U/day groups, and not different from control; probably, this is attributable to the high WAT weights and insulin-induced amino acid incorporation into muscle (16).

Caloric intake was greater in the 30%-4 U/day corticosterone-insulin combination, compared with 0%-0 U/day. This
increase in calorie intake could be attributed to the shift from 0% to 30% corticosterone, which can modestly increase food intake (24). However, insulin has been shown to centrally inhibit food intake (7, 20). Thus, increasing insulin replacement from 0 U/day to 4 U/day would be predicted to reduce calorie intake. Although it is possible that the steady-state dosage failed to achieve levels sufficient to exert this effect since our insulin replacement was done peripherally, this does not account for the ability of similar levels of insulin to reduce calorie intake at 100% corticosterone replacement. Rats, like humans, have a pronounced circadian rhythm of glucocorticoid secretion (34), which rises before the active phase (during the dark cycle for rats), coinciding with periods when the rat will eat the majority of their daily calories and is entrained by both light and food availability. Hence, peak circadian levels of corticosterone might be required to facilitate the effects of insulin on food intake, which remains to investigated.

Finally, plasma glucose concentrations with 4 U/day insulin tended to be low at 30% corticosterone. ArcN POMC and plasma leptin levels were also high; however, ArcN NPY was unchanged. This is consistent with a stimulatory role for insulin on both POMC expression (22) and leptin release (18); the latter could also be associated with the larger WAT depots.

Increasing corticosterone levels on a low insulin background (0%-0 U/day to 100%-0 U/day) results in high caloric intake and high circulating levels of glucose, FFA, glycerol, triglycerides, and ketones. WAT depot weight and levels of liver triglycerides and glycogen were low, and body weight was also reduced. This is consistent with the role of glucocorticoids in promoting energy mobilization and mimics concentrations of glucocorticoids and insulin in the fasting state; presumably, glucocorticoid-induced suppression of protein synthesis and enhanced protein breakdown account for the body weight loss (32). FFAs inhibit glucose uptake and glycogen synthase and increase hepatic glucose output (4); thus, the increase in circulating FFA presumably due to glucocorticoid-induced lipolysis and gluconeogenesis resulted in the low liver glycogen and high plasma glucose observed. Any additional acetyl-CoA not shunted to FFA synthesis would likely have been directed to ketogenesis (5), which would account for the elevated plasma ketone bodies observed. Plasma glucagon was also high; during insulin deficiency, high glucagon levels augment ketogenesis when FFA levels are also high (30).

These data demonstrate an absolute requirement for insulin to facilitate energy storage; without this, the energy consumed remains in the circulation. Although our study clearly shows that corticosterone stimulates WAT weight, this action does not occur at very low levels of circulating insulin. In vitro studies have illustrated that glucocorticoids augment insulin signaling and glucose uptake (15) and both promote differentiation of preadipocytes into mature, lipid-storing adipocytes (17).
It is also notable that 30% or 100% corticosterone in combination with the lower plasma insulin levels serve to elevate ArcN NPY and POMC mRNA expression, compared with lower circulating corticosterone concentrations. Corticosterone replacement to adrenalectomized rats increases NPY mRNA levels (36) via a pathway involving adenosine monophosphate-activated protein kinase (38). Counter to our study, ADX has been shown to reduce POMC mRNA expression (36); however, this might have been associated with changes in circulating insulin levels apparent in that study. Insulin reduces POMC mRNA expression (22); our previous studies using high (100% pellet) corticosterone levels have shown inconsistent effects of insulin on negatively regulating POMC mRNA expression (43). Our study uncovers a complex corticosterone-insulin relationship in the regulation of POMC expression that might account for discrepancies between studies.

High levels of both corticosterone and insulin (100% and 4 U/day) are characterized by low plasma glucose, triglycerides, FFAs, ketones and glycerol, and high liver glycogen and triglycerides. Body weight gain is also low; however, at no time was there net body weight loss, illustrating the potent capacity of insulin to protect from the catabolic actions of glucocorticoids. Calorie intake was reduced by the shift from 0 U/day insulin supplementation to 4 U/day at 100% corticosterone, yet intake was still greater when both hormones were low. Thus, although insulin was able to counteract the actions of glucocorticoids for the most part, the greater calorie intake observed compared with when both hormones were low still required storage beyond the capacity of glycogen storage; hence, the liver accumulated triglycerides. Only at high corticosterone concentrations provided by a 100% pellet does the effect of insulin on reducing plasma glucagon levels emerge, as previously reported (40, 41).

Provision of lard and sucrose to eat results in a change in the amount and composition of calories ingested that impacts the metabolic landscape and is influenced by corticosterone and insulin. Total caloric intake was overall increased by the provision of lard and sucrose in addition to chow, as previously reported (24). However, total calorie intake was similar across the various hormonal manipulations. Furthermore, insulin increased sucrose, but not lard, intake counter to observations in previous studies (24, 44). These findings could be attributed to several factors. First, this cohort of rats became comparatively less diabetic with STZ treatment; initial body weight loss was not as great, enabling full body weight recovery; second, the hyperglycemia was not as pronounced (40–42, 44); and third, all rats ate a considerable proportion of their calories as lard. This suggests that a threshold of hyperglycemia is required to suppress lard, but not sucrose, ingestion. Secondly, the rats in the previous studies (40–42, 44) were also subject to intra-abdominal surgery that may have affected the resultant magnitude of the diabetes. Third, our previous studies were performed in adrenal-intact rats provided with 100% corticosterone pellets to suppress endogenous secretion. The intact complement of other important adrenal products (aldosterone, androgens, catecholamines) might have influenced the food intake outcome. Nevertheless, the effects of insulin on sucrose ingestion support the notion that insulin serves to direct composition of the diet toward palatable food sources.

Perspectives and Significance

These studies show both insulin and corticosterone are important in the intake and disposition of energy. In some instances, the two hormones act similarly, possibly synergistically; in other instances, they are clearly antagonistic. For many of the variables, the 30% corticosterone-2 U/day insulin combination (Table 1) most closely resembled the “normal” intact reference groups across both diets. However, high glucocorticoids and low insulin are characteristic of persistent stressors, and low glucocorticoids and high insulin are common to post-traumatic stress disorder. Thus, metabolic effects on either side of “normal” are relevant to other frequently encountered conditions. Hence, determining the ratio of the two hormones is important in understanding the full metabolic outcomes when one hormone is diagnosed as clinically dysregulated.

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