0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(9):3553–3559

doi: 10.1210/jc.2007-0445

Effects of Cortisol on Carbohydrate, Lipid, and Protein Metabolism: Studies of Acute Cortisol Withdrawal in Adrenocortical Failure

Jens Juel Christiansen, Christian B. Djurhuus, Claus H. Gravholt, Per Iversen, Jens Sandahl Christiansen, Ole Schmitz, Jørgen Weeke, Jens Otto Lunde Jørgensen, and Niels Møller

Medical Department M, Aarhus Sygehus and Medical Research Laboratories, Clinical Institute, Aarhus University Hospital, DK-8000 C Århus, Denmark

Context: Cortisol is an important catabolic hormone, but little is known about the metabolic effects of acute cortisol deficiency.

Objective: The objective of the study was to test whether clinical symptoms of weight loss, fatigue, and hypoglycemia could be explained by altered energy expenditure, protein metabolism, and insulin sensitivity during cortisol withdrawal in adrenocortical failure.

Design, Participants, and Intervention: We studied seven women after 24-h cortisol withdrawal and during replacement control during a 3-h basal period and a 3-h glucose clamp.

Results: Cortisol withdrawal generated cortisol levels close to zero, a 10% decrease in basal energy expenditure, increased TSH and T_3 levels, and increased glucose oxidation. Whole-body glucose and phenylalanine turnover were unaltered, but forearm phenylalanine turnover was increased. During the clamp glucose, infusion rates rose by 70%, glucose oxidation rates increased, and endogenous glucose production decreased. Urinary urea excretion decreased by 40% over the 6-h study period.

Conclusions: Cortisol withdrawal increased insulin sensitivity in terms of increased glucose oxidation and decreased endogenous glucose production; this may induce hypoglycemia in adrenocortical failure. Energy expenditure and urea loss decreased, indicating that weight and muscle loss in Addison's disease is caused by other mechanisms, such as decreased appetite. Increased muscle protein breakdown may amplify the loss of muscle protein. **(*J Clin Endocrinol Metab* 92: 3553–3559, 2007)**

CORTISOL AND OTHER glucocorticoids are prominent metabolic hormones (1). Cortisol increases availability of all fuel substrates by mobilization of glucose (2, 3), free fatty acids (4), and amino acids from endogenous stores (5, 6). Thus, when in excess, cortisol is an overall catabolic hormone, which decreases lean body mass and muscle mass and may increase energy expenditure (7). At the same time, glucocorticoids increase appetite and food intake (7) and may increase fat mass (8, 9). Cortisol also induces insulin resistance (2, 3), and it has been proposed that glucocorticoids could contribute to the metabolic syndrome and cardiovascular disease (10, 11). Physiologically these actions are important during stress, such as fasting and exercise and clinically during glucocorticoid excess (Cushing's syndrome), adrenonocortical failure, hypoglycemia, and inflammatory disease (1, 7, 10, 12). Cortisol exerts its effects after uptake of free hormone from the circulation and binding to intracellular corticoid receptors, being members of the steroid receptor hormone superfamily of nuclear transcription factors (1).

First Published Online July 3, 2007

Abbreviations: CTR, Control conditions; CW, cortisol withdrawal; EE, energy expenditure; FFA, free fatty acid; IGFBP, IGF binding protein; I_{pt}, rate of phenylalanine conversion to tyrosine; M-value, amount of exogenous glucose necessary to maintain euglycemia; PheBal, phenylalanine balance; Q_p, phenylalanine flux; RaPhe, phenylalanine rate of appearance; RER, respiratory exchange ratio.

Knowledge concerning the metabolic effects of glucocorticoids is primarily based on studies in which varying amounts of cortisol or synthetic glucocorticoids have been given or on studies of patients with endogenous hypersecretion, whereas much less is known about the metabolic effects of glucocorticoid deficiency. In general, patients with Addison's disease present clinically with weight loss, muscular weakness, and fatigue and a tendency to hypoglycemia (13), but there is a lack of controlled studies in the field and the nature of the weight loss and alterations in metabolism remains uncertain in these patients. One study aimed at identifying the role of cortisol in hypoglycemic counterregulation has suggested that patients with adrenocortical failure have normal endogenous glucose production and glucose use when receiving glucocorticoid replacement (14).

Based on controlled observations during glucocorticoid excess together with empirical clinical observations in patients with Addison's disease, we hypothesized that glucocorticoid deficiency primarily leads to increased insulin sensitivity, decreased energy expenditure and decreased protein loss. If so, increased insulin sensitivity could explain the tendency to hypoglycemia, whereas weight loss and muscle weakness and fatigue by inference would involve other mechanisms, such as decreased appetite and lethargy.

The present study was therefore designed to define the metabolic effects of acute cortisol withdrawal on glucose, lipid, and amino acid metabolism in patients with adrenocortical failure. The primary aim was to test whether these patients would exhibit increased hepatic and peripheral in-

sulin sensitivity (leading to propensity to hypoglycemia). For this purpose we studied seven patients with adrenocortical failure with and without acute cortisol withdrawal and used indirect calorimetry, glucose and amino acid tracer dilution, microdialysis, and forearm arteriovenous and glucose clamp techniques to identify metabolic abnormalities.

Subjects and Methods

Study population

The study population comprised seven females with adrenocortical failure [Addison's disease (n = 6), monotropic ACTH failure (n = 1)] and a mean age of 39 yr (range 22–54). All participants were on stable hydrocortisone (15–30 mg/d) and fluorocortisone (0–0.20 mg/d) treatment. Three of six naturally menstruating patients used combined oral contraceptives. One patient was postmenopausal. Other concomitant medication included thyroid hormones (two patients), calcium and vitamin D supplementation (three patients), bisphosphonate (one patient), and hydroxocobalamin (one patient). None of the participants had diabetes. Participants were studied during the early follicular phase (d 5–10) of their regular cycle or in the corresponding phase of a tablet cycle. The protocol was approved by the Aarhus County Ethical Scientific committee (no. 1998/4375). All participants gave informed oral and written consent.

Study protocol (Fig. 1, A and B)

The patients were examined twice in a controlled, randomized fashion: 1) under normal control conditions (CTR) with cortisol replacement and 2) after 24 h cortisol withdrawal (CW) (Fig. 1B). On each occasion the patients were admitted to the hospital 24 h before the actual study day. Each study day was separated by at least 1 month. The experiment consisted of a fasting 180-min (basal) period followed by a 180-min hyperinsulinemic, euglycemic clamp. Blood samples were taken in triplicate after 150 and 330 min (Fig. 1A).

Hydrocortisone substitution (Solucortef; Pharmacia, Stockholm, Sweden) or saline was administrated as a continuous infusion in a hand vein. The infusion rate was modified according to Hangaard *et al.* (15) (1400–0200 h: 0.014 mg/kg·h, 0200–1400 h: 0.026 mg/kg·h) and maintained during examination. The mean infusion rates of hydrocortisone were 0.87 ± 0.05 mg/h (1400–0200 h) and 1.6 ± 0.10 (0200–1400 h). During cortisol withdrawal condition, patients took the normal early morning dose the day before evaluation and then paused until the end of evaluation. The day before examination, three standardized meals [total 2000 kcal (15% protein, 50% carbohydrate, 35% fat)] were served (0830, 1200. and 1800 h), and thereafter patients were fasting. Intermeal snacks and caloric beverages were avoided. Starting at 0800 h the following day, fuel metabolism was estimated during fasting (basal period) and meal simulation by means of insulin and amino acid stimulation (clamp period). After insertion of microdialysis catheters in femoral and abdominal adipose tissue, one iv catheter was placed retrogradely in a deep antecubital vein and one catheter was inserted retrogradely in a heated dorsal hand vein of the contralateral arm, allowing collection of venous blood draining the forearm muscle and oxygenated blood from digital capillary shunting, respectively. Criteria for correct positioning were oxygen saturations less than 70 and above 91%, respectively. A third catheter was placed anterogradely in a cubital vein of the heated hand for infusions. Urine was collected from 0800 to 1400 h for measurements of urea excretion.

The project was conducted and monitored according to the International Conference on Harmonisation-Good Clinical Practice guidelines (The Good Clinical Practice unit, Aarhus University Hospital).

Tracers and clamp

At 800 h priming doses of [3-^{3}H] glucose (NEN Life Science Products, Boston, MA; 20 μCi), L-[^{15}N]phenylalanine (0.7 mg/kg), L-[$^2H_4$]tyrosine (0.5 mg/kg), and L-[^{15}N]tyrosine (0.3 mg/kg; Cambridge Isotope Laboratories, Inc., Woburn, MA) were given to accomplish an early plateau. A continuous infusion of [3-^{3}H]glucose (20 μCi/h), L-[^{15}N]phenylalanine (0.7 mg/kg·h), and L-[$^2H_4$]tyrosine (0.5 mg/kg·h) was started and

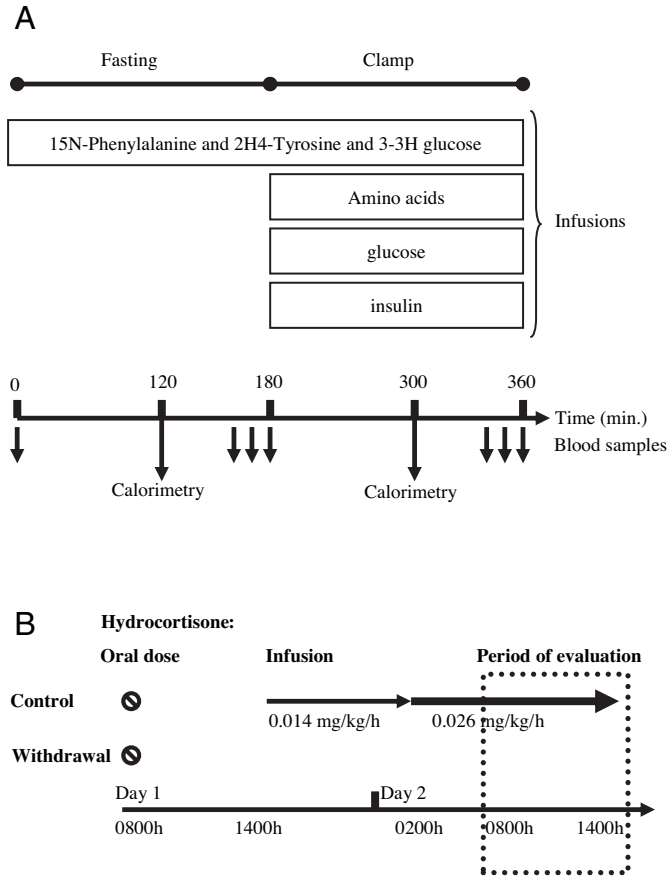

FIG. 1. A, Determination of phenylalanine, tyrosine, and glucose kinetics. The experiment consisted of a fasting 180-min (basal) period followed by a 180-min hyperinsulinemic, euglycemic clamp. Blood samples were taken in triplicate after 150 and 330 min to ensure steady state. Indirect calorimetry was performed after 120 and 300 min. B, Hydrocortisone substitution scheme in the hydrocortisone substituted control condition and during hydrocortisone withdrawal. On the day before the study day, participants received their usual hydrocortisone tablet in the morning (⃠) but did not receive their afternoon or evening dose. On the day of continuous cortisol replacement, participants started infusion of cortisol at 1400 h, whereas on the day of cortisol withdrawal, participants received a saline infusion.

maintained for 6 h. Tracer solutions were prepared according to good manufacturing practice guidelines by the hospital pharmacy (Århus University Hospital).

After 3 h a constant amount (0.6 mU/kg·min) of insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused; based on measurements every 10 min, plasma glucose was clamped at 5.0 mmol/liter by infusion of variable rates of a 20% glucose solution. To minimize rapid dilution of the labeled glucose pool with unlabeled glucose, 3-^{3}H glucose was added to the glucose infused during the clamp (100 μCi per 500 ml 20% glucose). During the clamp, hepatic glucose production was calculated by subtracting the amount of exogenous glucose necessary to maintain euglycemia (M-value) from the isotopically determined overall appearance rate for glucose. During the clamp period, a mixed amino acid infusion was given (Vamin 14 g N/liter, 0.0176 ml/kg·min) to avoid hypoaminoacidemia.

Blood flow measurements

The sc adipose tissue blood flow in the abdominal and femoral region in which dialysis was performed was measured by the local 133Xe washout method. In short, 3.7 MBq (0.1 ml) 133Xe were injected sc abdominal and femoral. Disappearance of ^{133}Xe was continuously measured starting 30 min after injection using a NaI detector (Mediscint;

Oakfield Instruments Ltd., Oxfordshire, UK) as previously described (16). Forearm blood flow was determined by means of venous occlusion plethysmography (17).

Indirect calorimetry

Indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed for 30 min in the basal state and during the clamp. The initial 5 min were used for acclimatization, and calculations of respiratory exchange ratio (RER) and resting energy expenditure (EE) were based on mean values of 25 measurements of 1 min each. Likewise, rates of protein lipid and glucose oxidation were estimated corrected for urinary excretion of urea (18). Net nonoxidative glucose disposal was calculated by subtraction of glucose oxidation from the isotopically assayed total glucose disposal.

Protein and glucose kinetics

Whole-body phenylalanine kinetics were estimated from the equations of Thompson *et al.* (17). Regional phenylalanine kinetics were estimated as previously described (18).

Phenylalanine flux (Q_p) and tyrosine flux were calculated as follows:

$$Q_{flux}=i[(E_i/E_p)-1]$$

in which i is the rate of tracer infusion (micromoles per kilogram per minute), and E_i and E_p are enrichment of the tracer infused and plasma enrichment of the tracer at isotopic plateau, respectively. The rate of phenylalanine conversion to tyrosine (I_{pt}) was calculated as follows:

$$I_{pt}=\text{tyrosineflux}\times([^{15}N]Tyr_{ei}/[^{15}N]Phe_{ei})\times[Q_p/(I_p+Q_p)]$$

where $[^{15}N]Tyr_{ei}$ and $[^{15}N]Phe_{ei}$ are the isotopic enrichments of the respective tracers in plasma and I_p is the infusion rate of [^{15}N]phenylalanine (micromoles per kilogram per hour). Protein synthesis is calculated by subtraction I_{pt} for Q_p because phenylalanine is irreversibly lost by either conversion into tyrosine or incorporation into protein. In the forearm study, phenylalanine balance (PheBal) was calculated as follows:

$$\text{PheBal}=(\text{PheA-PheV})\times F$$

in which Phe_A and Phe_V are phenylalanine concentrations in arteries and veins and F is blood flow.

Regional protein breakdown represented by phenylalanine rate of appearance (RaPhe) was calculated as follows (19):

$$\text{RaPhe}=\text{PheA}[(Phe_{EA}/Phe_{EV})-1]\times F$$

in which Phe_{EA} and Phe_{EV} represent phenylalanine isotopic enrichment in arteries and veins.

Local rate of disappearance was calculated as:

$$\text{RdPhe}=\text{PheBal}+\text{RaPhe}$$

The specific activity of tritiated glucose was measured as previously described (20). Rates of appearance and disappearance of glucose were calculated using Steele's equation for nonsteady state, and a pool fraction of 0.65 was used. Endogenous glucose production during the clamp was calculated by subtracting the rate of glucose infusion (M-value) from glucose rates of appearance, as determined isotopically. L-[^{15}N]phenylalanine, L-[$^{2}H_4$]tyrosine and L-[^{15}N]tyrosine were measured as their *t*-butyldimethylsilyl ether derivatives under electron ionization conditions (18).

Plasma concentrations of amino acids were determined by an HPLC system (HP 1090 series 2 HPLC, 1046 fluorescence detector, and cooling system) with precolumn *O*-phthalaldehyde derivatization. In addition, concentrations of phenylalanine and tyrosine were measured by mass spectrometry using d8 β-methylphenylalanine and C6 α-methyltyrosine as internal standards, respectively (18). Plasma glucose levels were measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments, Palo Alto, CA).

TABLE 1. Circulating concentrations of hormones and metabolites in the basal state under cortisol-treated CTR and during CW in seven female subjects with adrenocortical failure

	CTR	CW	*P* value
Cortisol (nmol/liter)	272.3 ± 43.1	29.4 ± 10.2	0.001
Glucagon (nmol/liter)	34 ± 7.4	36 ± 5.8	0.6
Insulin (pmol/liter)	70.6 ± 27.4	56.1 ± 14.3	0.5
Leptin (μg/liter)	11 ± 1.5	12 ± 2.5	0.4
GHBP (nmol/liter)	2.0 ± 0.28	2.7 ± 0.45	0.2
Free IGF-I (μg/liter)	0.64 ± 0.13	0.41 ± 0.05	0.2
IGF-I (μg/liter)	179 ± 11	164 ± 21	0.2
IGF-II (μg/liter)	815 ± 39	840 ± 59	0.5
IGFBP-1 (μg/liter)	5 ± 0.5	5 ± 1.6	0.7
IGFBP-3 (μg/liter)	5216 ± 232	5295 ± 247	0.7
C-peptide (pmol/liter)	803 ± 67	642 ± 39	0.3
TT3 (nmol/liter)	1.14 ± 0.09	1.49 ± 0.16	0.01
TT4 (nmol/liter)	82.5 ± 6.4	75.8 ± 12.3	0.5
TSH (mU/liter)	1.75 ± 0.42	3.48 ± 0.55	0.04
Glucose (mmol/liter)	4.8 ± 0.1	4.6 ± 0.1	0.05
FFAs (μmol/liter)	0.61 ± 0.06	0.57 ± 0.14	0.7
3-Hydroxybutyrate (μmol/liter)	162 ± 22	101 ± 7	0.05
Glycerol (μmol/liter)	61 ± 4	69 ± 6	0.1
Urea (μmol/liter)	4.74 ± 0.31	4.14 ± 0.42	0.1

GHBP, GH binding protein; TT3, total T_3; TT4, total T_4.

Microdialysis and calculations

Microdialysis fibers (CMA 60 microdialysis catheter; CMA, Stockholm, Sweden) were placed in abdominal and femoral sc adipose tissue. Immediately after placement, fibers were perfused at a rate of 1 μl/min (CMA-107 perfusion pump; CMA). To the perfusion fluid [Ringer chlo-

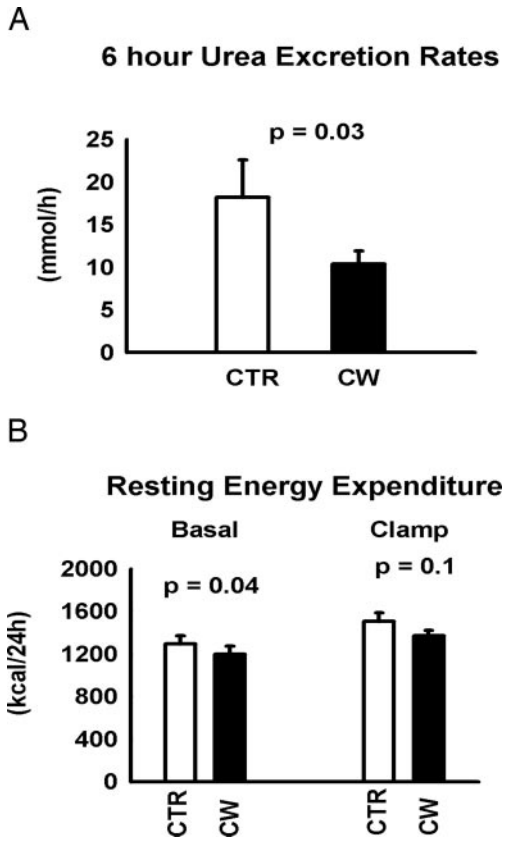

FIG. 2. A, Six-hour urea excretion rate. B, Resting EE in the basal state and during a euglycemic clamp in seven female subjects with adrenocortical failure after 24 h CW and during cortisol-treated CTR.

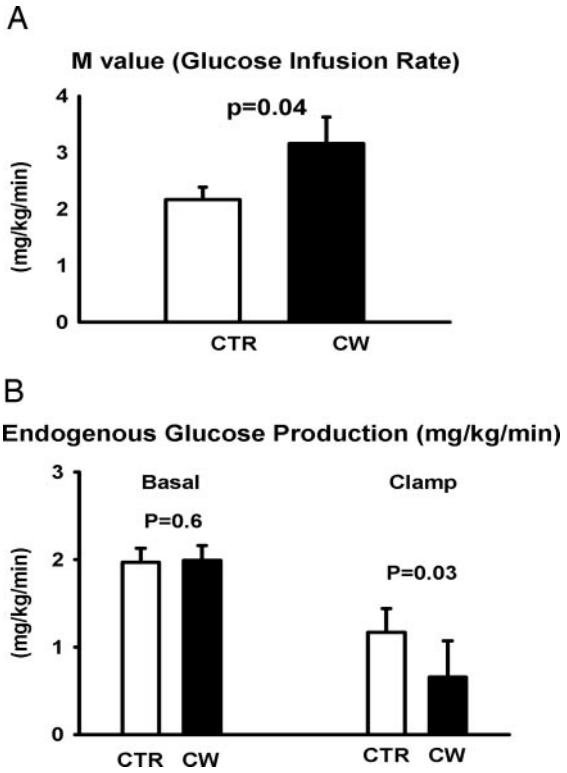

FIG. 3. A, Glucose infusion rates during the euglycemic clamp. B, Endogenous glucose production during basal state and euglycemic clamp in seven female subjects with adrenocortical failure after 24-h CW and during cortisol-treated CTR.

ride, T1; CMA; Na^+, 147 mmol/liter; K^+, 1.4 mmol/liter; Ca^{2+}, 2.3 mmol/liter; Cl^-, 156 mmol/liter (pH, 6); and osmolality, 290 mosmol/kg] was added a small amount of [^{3}H]glycerol to measure the relative recovery by the internal reference method (21).

Samples were collected every hour from 2100 to 1400 h. An automated spectrophotometric kinetic enzymatic analyzer (CMA 600; CMA, Solna, Sweden) was used for duplicate measurements of glycerol, glucose, urea, and lactate in the microdialysate. Changes in interstitial glycerol concentration was used as an index of lipolysis (22).

Assays

GH was measured with a double monoclonal immunofluorometric assay (DELFIA, Wallac, Finland). Plasma glucagon and serum C-peptide were measured by RIAs (Immunoclear, Stillwater, MN). Serum IGF-I was measured by in-house, noncompetitive, time-resolved immunofluorometric assays (23). Serum IGF binding protein (IGFBP)-3 was measured by an immunoradiometric assay (Diagnostic System Laboratories Inc., Webster, TX) and GHBP with an in-house. time-resolved, immunofluorometric assay (24). Insulin was determined by a commercial ELISA (Dako, Glostrup, Denmark). Blood levels of alanine, glycerol, 3-hydroxybutyrate, and lactate were assayed with an automated fluorometric method (25).

Urea excretion in urine was determined by an indophenol method and serum urea by a commercial kit (COBASINTEGRA; Roche, Hvidovre, Denmark). Cortisol was measured by an automated chemiluminescence system (Chiron Diagnostics, Fernwald, Germany). Free fatty acids (FFAs) were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany). Total concentrations of T_4 and T_3 were measured in serum as previously described (26), whereas TSH was measured in a solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite; Diagnostic Products Corp., Los Angeles, CA).

Statistics

Statistic calculations were done with SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL). As appropriate for parametric and nonparametric data, measurements are expressed as mean ± SEM or median and range, and comparisons were done with Student's paired *t* test or Wilcoxon signed rank test. Significance levels less than 5% were considered significant. Based on a power calculation using expected change in M-value as the primary end point, we originally planned to recruit eight patients for the study. Based on previous studies, we assumed an expected difference in M-values of 0.25 mg/kg·min and with a SD of 0.2 mg/kg·min.

Results

Circulating hormones and metabolites (Table 1)

During cortisol withdrawal, basal circulating cortisol concentrations fell from normal physiological levels of 272 ± 43 to very low levels of 29 ± 10 nmol/liter ($P < 0.001$). In addition TSH and total T_3 rose significantly. All other measured hormones, including insulin and C-peptide, were comparable. During the glucose clamp, comparable insulin (cortisol withdrawal *vs.* CTR: 373 ± 63 *vs.* 316 ± 38 pmol/liter, $P = 0.2$) and C-peptide values (data not shown) were recorded. In the basal state, glucose concentrations were slightly decreased during cortisol withdrawal, as were 3-hydroxybutyrate concentrations, whereas circulating FFAs and glycerol were comparable. During the glucose clamp, comparable glucose concentrations were recorded.

Indirect calorimetry and glucose turnover (Figs. 2 and 3 and Tables 4 and 5)

In the basal state, cortisol withdrawal decreased resting EE (Fig. 2B) and increased RER. During the clamp, EE also tended to decrease with cortisol withdrawal and RER increased. Cortisol withdrawal induced increased glucose oxidation under fasting and clamp conditions and diminished protein oxidation under clamp conditions only and a trend toward lower lipid oxidation.

Cortisol withdrawal did not alter total glucose fluxes or nonoxidative glucose disposal but stimulated oxidative glucose disposal. During the clamp, glucose infusion rates rose (Fig. 3A), and suppression of endogenous glucose production was augmented during cortisol withdrawal (Fig. 3B).

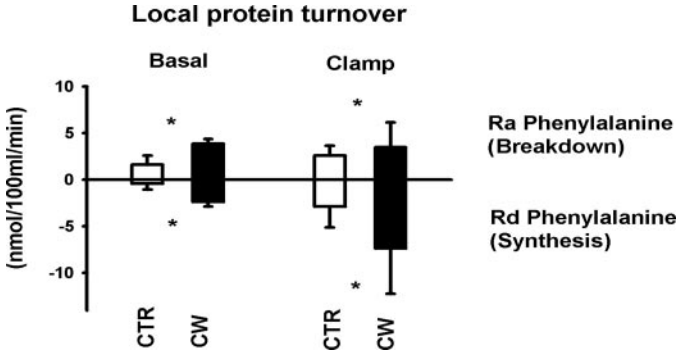

FIG. 4. Local protein turnover (forearm) in basal state and during the euglycemic clamp under cortisol-treated CTR and CW in seven female subjects with adrenocortical failure. *, $P < 0.05$.

TABLE 2. Circulating concentrations of amino acids in the basal state under cortisol-treated CTR and during CW in seven female subjects with adrenocortical failure

Amino acid	Basal			Clamp		
	CTR	CW	*P*	CTR	CW	*P*
Asp						
Gln+His	295 ± 27	286 ± 17	0.6	342 ± 33	312 ± 13	0.6
Ser	298 ± 18	281 ± 16	0.3	378 ± 14	354 ± 12	0.1
Gln+His	970 ± 71	895 ± 16	0.1	1121 ± 100	1007 ± 61	0.1
Gly	658 ± 70	750 ± 49	0.2	1080 ± 108	1172 ± 29	0.9
Thr	305 ± 22	341 ± 27	0.1	621 ± 31	632 ± 51	0.5
Citr	95 ± 10	90 ± 12	0.8		108 ± 7	
Arg	181 ± 11	167 ± 6	0.2	405 ± 19	405 ± 35	0.8
Ala	516 ± 30	599 ± 36	0.02	1029 ± 79	1030 ± 52	0.9
Tau	282 ± 16	293 ± 18	0.4	305 ± 21	285 ± 25	0.5
Tyr	124 ± 7	166 ± 10	0.005	180 ± 16	250 ± 8	0.01
Val+Met	312 ± 12	316 ± 15	0.8	552 ± 22	555 ± 17	0.6
Phe	122 ± 3	124 ± 5	0.7	235 ± 7	228 ± 7	0.3
Isoleu	109 ± 6	99 ± 7	0.07	217 ± 11	202 ± 12	0.3
Leu	196 ± 6	196 ± 12	0.9	313 ± 10	300 ± 11	0.3
Orn	174 ± 15	159 ± 9	0.7	189 ± 31	195 ± 20	0.2
Lys	346 ± 17	356 ± 17	0.6	624 ± 34	545 ± 1	0.07

Values are expressed as nmol/liter.

Urea excretion, whole-body protein metabolism, and forearm protein metabolism (Figs. 2 and 4 and Tables 2 and 3)

Urea excretion rates measured over the entire 6-h study period decreased in the absence of cortisol (Fig. 2A). CW did not affect whole-body phenylalanine or tyrosine fluxes, neither in the basal state nor during the clamp (Table 3). In the basal state, both forearm rates of appearance (a measure of local muscle protein breakdown) and disappearance (a measure of muscle protein synthesis) for phenylalanine more than doubled (Fig. 4). Circulating levels of all amino acids generally remained constant (Table 2). Forearm blood flow was comparable in the two situations.

Microdialysis

Basal sc glycerol concentrations in the thigh (344 ± 75 *vs.* 239 ± 38 μmol/liter, $P = 0.09$) and abdomen (572 ± 188 *vs.* 365 ± 73 μmol/liter, $P = 0.14$) tended to be elevated during cortisol withdrawal. Subcutaneous lactate was not affected and glucose concentrations were decreased ($P = 0.05$ and 0.09). Adipose tissue blood flow did not differ between the two treatments (data not shown).

Discussion

Our study was designed to define the metabolic effects of acute cortisol withdrawal in patients with adrenocortical failure. We accomplished cortisol concentrations close to zero *vs.* normal physiological concentrations of 270 nmol/liter and observed in line with our hypotheses decreased resting energy expenditure and decreased urinary urea excretion together with increased glucose oxidation and increased central and peripheral sensitivity to insulin. We also found unaltered whole-body amino acid turnover despite increased forearm muscle protein fluxes. Basal glucose turnover was also unaltered, as were circulating levels of FFAs, lipid oxidation rates, and interstitial glycerol levels. Apart from increased T_3, levels of all major metabolic hormones were comparable, so these findings in all likelihood are due to lack of cortisol *per se*.

Our data clearly show that acute cortisol withdrawal dramatically increases insulin sensitivity; during the clamp M-values were increased by close to 70%, endogenous glucose production was halved and glucose oxidation was increased by more than 50%. These findings are in line with previous studies reporting insulin resistance during short-term cortisol excess (2, 3, 27) and that cortisol promotes lipolysis (28), rendering it possible that increased levels of FFAs in the circulation may contribute to the observed insulin resistance. In our study we did not observe any changes in circulating FFA levels or lipid oxidation rates, suggesting that the observed acute increase in insulin sensitivity is directly related to lack of cortisol. It should be noted that we primarily saw an increase in glucose oxidation and that both endogenous

TABLE 3. Whole-body phenylalanine and tyrosine fluxes in the basal state and during the euglycemic clamp under cortisol-treated CTR and during CW in seven female subjects with adrenocortical failure

	Basal			Clamp		
	CTR	CW	*P* value	CTR	CW	*P* value
Total Qphe	37.2 ± 2.6	35.2 ± 2.8	0.2	62.3 ± 2.0	59.4 ± 3.6	0.8
Endogenous Qphe				24.3 ± 2.0	21.5 ± 3.6	0.8
Total Qtyr	32.8 ± 1.8	34.7 ± 3.1	0.06	45 ± 2.0	47 ± 4.7	0.9
Endogenous Qtyr				31.7 ± 2.0	33.2 ± 4.7	0.9
Qpt	4.3 ± 0.2	4.8 ± 0.4	0.2	8.9 ± 0.7	10.7 ± 1.4	0.6
Protein synthesis	32.9 ± 2.6	30.4 ± 2.5	0.1	53.3 ± 2.4	48.8 ± 2.8	0.6

Values are expressed as μmol/kg·h. Qphe, flux of phenylalanine; Qtyr, flux of tyrosine; Qpt, flux of phenylalanine to tyrosine conversion.

TABLE 4. RER and EE and substrate oxidation rates estimated by indirect calorimetry in basal state and during the euglycemic clamp under cortisol-treated CTR and during CW in seven female subjects with adrenocortical failure

	CTR	CW	*P* value
Basal			
RER	0.83 ± 0.01	0.88 ± 0.01	0.01
EE (kcal per 24 h)	1295 ± 76	1197 ± 76	0.04
Protein oxidation (kcal per 24 h)	455 ± 196	268 ± 63	0.2
Glc oxidation (kcal per 24 h)	357 ± 79	577 ± 52	0.003
Lipid oxidation (kcal per 24 h)	483 ± 114	352 ± 51	0.3
Clamp			
RER	0.87 ± 0.02	0.93 ± 0.01	0.04
EE (kcal per 24 h)	1507 ± 79	1372 ± 51	0.1
Protein oxidation (kcal per 24 h)	466 ± 66	248 ± 28	0.03
Glc oxidation (kcal per 24 h)	598 ± 61	922 ± 89	0.03
Lipid oxidation (kcal per 24 h)	443 ± 94	201 ± 63	0.1

Glc, Glucose.

glucose production and glucose disposal/oxidation was affected. The molecular mechanisms underlying these events appear to be complex; animal studies have suggested that glucocorticoids may both stimulate and inhibit insulin receptor substrate-1 and phosphatidylinositol 3-kinase signaling (29, 30), and cell culture studies suggest that glucocorticoids inhibit GLUT 4 translocation in adipocytes (31).

It is well described that long-term glucocorticoid excess in Cushing's syndrome decreases lean body mass (8, 9) and that short-term excess increases whole-body protein breakdown (5, 6). One recent study specifically targeted at muscle failed to observe any effects of short-term prednisone treatment on muscle protein metabolism, possibly because of coexisting elevations of insulin and IGF-I levels (32). In the present study, we found that cortisol withdrawal induced a substantial decrease in urea excretion of more than 40% in the presence of unaltered circulating amino acid concentrations and whole amino acid fluxes. This suggests a direct action on hepatic ureagenesis, and studies in humans have indeed shown that 4 d of synthetic glucocorticoid treatment increases hepatic synthesis of urea by 25% (33). In forearm muscle we observed that cortisol withdrawal increased both phenylalanine appearance and disposal rates, suggesting that both protein breakdown and synthesis were stimulated. Our study involved infusion of amino acids during the glucose clamp to avoid a decrease in circulating amino acids, and it has been reported that increased amino acid concentrations may increase insulin-stimulated muscle protein synthesis (34). One report has shown that insulin stimulated muscle protein synthesis is increased after adrenalectomy in rats (35). Other studies have suggested that short-term, high-dose dexamethasone treatment may increase net amino acid loss across the forearm (36), so it is possible that glucocorticoids have a diphasic action on muscle protein metabolism, *i.e.* increase muscle protein breakdown at both very high and very low concentrations.

TABLE 5. Glucose turnover expressed as glucose rate of disappearance, nonoxidative disposal, and oxidative disposal in basal state and during the euglycemic clamp under cortisol-treated CTR and during CW in seven female subjects with adrenocortical failure

Glucose (mg/kg·min)	CTR	CW	*P* value
Basal			
Rate of disappearance	1.99 ± 0.17	2.02 ± 0.14	0.6
Nonoxidative disposal	0.959 ± 0.264	0.361 ± 0.164	0.1
Oxidative disposal	1.08 ± 0.24	1.79 ± 0.21	0.003
Clamp			
Rate of disappearance	3.19 ± 0.30	4.35 ± 0.42	0.1
Nonoxidative disposal	1.614 ± 0.117	1.396 ± 0.252	0.3
Oxidative disposal	1.99 ± 0.12	2.89 ± 0.26	0.03

In our study we observed a 10% reduction in energy expenditure after cortisol withdrawal. Although many studies have failed to observe any effect of glucocorticoids on energy expenditure (28, 32, 37), a carefully conducted study by Tataranni *et al.* (7) reported that synthetic glucocorticoid treatment for 4 d, independently of thermic effects of food, induces increments of around 5–10% in 24-h energy expenditure together with a quite impressive increase in appetite and *ad libitum* food intake. Thus, these observations in concert with our own observations of decreased energy expenditure are fully compatible with the notion that weight loss in adrenocortical failure is caused by decreased appetite and food intake, rather than increased metabolic rate. The mechanisms behind the reduction in metabolic rate after cortisol withdrawal are unclear, but there may be direct hypothalamic effects of cortisol responsible for the reduction (38).

There is evidence that steroid hormones regulate mitochondrial function (39, 40), and a recent study in humans has shown that mitochondrial ATP production in the presence of palmitoyl-carnitine increases after prednisone treatment, compatible with the notion that lack of glucocorticoids selectively inhibits FFA oxidation (32). In our study we found a statistically insignificant decrease in fat oxidation of around 25%. It is noteworthy that the decrease in EE occurred despite increased TSH and T_3 levels. It is well described that cortisol inhibits TSH secretion (41), a phenomenon that may explain circadian TSH patterns and the decrease in T_3 often encountered in nonthyroidal illness. The finding of increased T_3 and unaltered/decreased T_4 levels suggest that lack of cortisol may stimulate peripheral T_4 to T_3 conversion.

The present study has some limitations. We performed extensive metabolic examination of a limited number of subjects 24 h after cortisol withdrawal. Isolated adrenocortical failure is a rare disorder, and it was possible to recruit only seven subjects for the study; the occurrence of type 2 errors therefore cannot be excluded. In addition, it is possible that some of the effects of glucocorticoids are time and dose dependent and that the use of a different model, *e.g.* low-dose hydrocortisone treatment, for a longer period would have yielded a different outcome. It should also be noted that more prolonged alterations of glucocorticoid status may change insulin levels and body composition and thus interfere with metabolism.

Our results, which to our knowledge are novel, show that in patients with Addison's disease, hypoglycemia may be caused by increased overall insulin sensitivity, involving both glucose production and glucose disposal. We also found decreased energy expenditure and decreased nitrogen loss, compatible with the notion that loss of weight and lean body mass in adrenocortical failure is caused by central mechanisms, such as reduced appetite and food intake.

Acknowledgments

Elsebeth Hornemann, Hanne Pedersen, and Lone Svendsen are thanked for expert technical help.

Received March 5, 2007. Accepted June 26, 2007.

Address all correspondence and requests for reprints to: Jens Juel Christiansen, Medical Department M, Aarhus Sygehus NBG, Århus University Hospital, DK-8000 C Århus, Denmark. E-mail: juel@dadlnet.dk.

This work was supported by grants from The FOOD Study Group/Ministry of Food, Agriculture, and Fisheries and the Ministry of Family and Consumer Affairs and a grant from the Danish Health Research Council, Grant 9600822 from the Århus University-Novo Nordisk Center for Research in Growth and Regeneration. J.J.C. is supported with a Ph.D. research fellowship from the University of Aarhus. The microdialysis catheters and other utensils were generously supplied by Roche Diagnostics.

Disclosure Summary: J.J.C., C.B.D., O.S., J.W., and N.M. have nothing to declare. C.H.G. received lecture fees from Novo Nordisk. P.I., J.S.C., and J.O.L.J. received consulting fees and lecture fees from Novo Nordisk and Pfizer.

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