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Impaired basal glucose effectiveness but unaltered fasting glucose release and gluconeogenesis during short-term hypercortisolemia in healthy subjects

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Nielsen, Michael F., Andrea Caumo, Visvanathan Chandramouli, William C. Schumann, Claudio Cobelli, Bernard R. Landau, Hendrik Vilstrup, Robert A. Rizza, and Ole Schmitz.

Impaired basal glucose effectiveness but unaltered fasting glucose release and gluconeogenesis during short-term hypercortisolemia in healthy subjects. *Am J Physiol Endocrinol Metab* 286: E102–E110, 2004. First published September 9, 2003; 10.1152/ajpendo.00566.2002.—Excess cortisol has been demonstrated to impair hepatic and extrahepatic insulin action. To determine whether glucose effectiveness and, in terms of endogenous glucose release (EGR), gluconeogenesis, also are altered by hypercortisolemia, eight healthy subjects were studied after overnight infusion with hydrocortisone or saline. Glucose effectiveness was assessed by a combined somatostatin and insulin infusion protocol to maintain insulin concentration at basal level in the presence of prandial glucose infusions. Despite elevated insulin concentrations ($P < 0.05$), hypercortisolemia resulted in higher glucose ($P < 0.05$) and free fatty acid concentrations ($P < 0.05$). Furthermore, basal insulin concentrations were higher during hydrocortisone than during saline infusion ($P < 0.01$), indicating the presence of steroid-induced insulin resistance. Postabsorptive glucose production ($P = 0.64$) and the fractional contribution of gluconeogenesis to EGR ($P = 0.33$) did not differ on the two study days. During the prandial glucose infusion, the integrated glycemic response above baseline was higher in the presence of hydrocortisone than during saline infusion ($P < 0.05$), implying a decrease in net glucose effectiveness (4.42 ± 0.52 vs. 6.65 ± 0.83 ml·kg⁻¹·min⁻¹; $P < 0.05$). To determine whether this defect is attributable to an impaired ability of glucose to suppress glucose production, to stimulate its own uptake, or both, glucose turnover and “hot” (labeled) indexes of glucose effectiveness (GE) were calculated. Hepatic GE was lower during cortisol than during saline infusion (2.39 ± 0.24 vs. 3.82 ± 0.51 ml·kg⁻¹·min⁻¹; $P < 0.05$), indicating a defect in the ability of glucose to restrain its own production. In addition, in the presence of excess cortisol, glucose disappearance was inappropriate for the prevailing glucose concentration, implying a decrease in glucose clearance ($P < 0.05$). The decrease in glucose clearance was confirmed by the higher increment in [³H]glucose during hydrocortisone than during saline infusion ($P < 0.05$), despite the administration of identical tracer infusion rates. In conclusion, short-term hypercortisolemia in healthy individuals with normal β -cell function decreases insulin action but does not alter rates of EGR and gluconeogenesis. In addition, cortisol impairs the ability of glucose to suppress its own production, which due to accumulation of glucose in the glucose space results in impaired peripheral glucose clearance. These results suggest that cortisol excess

impairs glucose tolerance by decreasing both insulin action and glucose effectiveness.

insulin action

EXCESS CORTISOL is characterized by various metabolic disturbances, including the presence of fasting hyperglycemia and impaired glucose tolerance (21, 23, 34, 37). Furthermore, excess cortisol induces hepatic and extrahepatic insulin resistance. However, the mechanism(s) responsible for cortisol-induced insulin resistance are poorly understood (28, 41).

Under conditions of daily living, after ingestion of a carbohydrate meal, glucose and insulin rise and fall in a tightly coordinated manner. The rise in plasma glucose concentration stimulates insulin secretion, which results in suppression of glucose release and stimulation of glucose uptake (12, 31). However, glucose per se also plays a pivotal role in the regulation of postprandial glucose metabolism. Hyperglycemia suppresses glucose release and stimulates glucose disposal through a mechanism referred to as glucose effectiveness (7). Thus, in addition to pancreatic β -cell function and insulin action, postprandial glucose tolerance depends on the ability of glucose per se to stimulate its own metabolism. Whereas several studies have determined the effects of cortisol on insulin action, the impact of excess cortisol on gluconeogenesis and glucose effectiveness, both of which contribute to hyperglycemia in insulin-resistant states such as diabetes mellitus, has to our knowledge not yet been examined.

Glucose effectiveness is traditionally assessed by use of the minimal model, which is based on the frequently sampled intravenous glucose tolerance (FSIGT) test. However, whereas a close correlation has been established between the glucose clamp-derived estimate of insulin action and the minimal model-derived insulin sensitivity index, the ability of the minimal model to accurately estimate glucose effectiveness is less certain (15, 40). The present study, therefore, sought to determine the effects of cortisol on glucose effectiveness by using a minimal model-independent approach (5). Furthermore, to determine whether altered glucose effectiveness is due to an impaired ability of glucose to suppress glucose production and/or the ability of glucose to stimulate its own uptake, rates

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of glucose production and utilization were determined using the tracer-dependent glucose infusion ("hot-GINF") method. Tracer-dependent indexes of glucose effectiveness, i.e., GE_b^* and GE_{liver} , were calculated by use of a model-independent approach. The effects of cortisol on postabsorptive gluconeogenesis were assessed by combining the isotope dilution method with the 2H_2O technique (25).

MATERIALS AND METHODS

Subjects. After approval from the Ethics Committee, County of Aarhus, eight healthy subjects (5 males and 3 females) with a mean age of 26 yr (21–29 yr), lean body mass of 62.4 ± 4.5 kg, and a body mass index of 24.4 ± 0.8 kg/m², and without any family history of diabetes gave written consent to participate in the study. Lean body weight was measured using bioelectric impedance (Animeter; HTS-Engineering APS, Odense, Denmark). All participants were Caucasian. Female volunteers were studied in the middle of the menstrual cycle. No recruited subjects were taking any medication. At least 3 days before study, subjects were instructed not to engage in vigorous exercise. During the study period, volunteers were encouraged to refrain from changes in eating behavior and exercise activities.

Experimental design. Subjects were admitted to the clinical research center at 2130 on the evening before the study (Fig. 1). Study subjects were instructed to eat their last meal at 1800. On the subject's admission, an 18-gauge cannula was inserted into a forearm vein of each arm. One cannula was used for venous blood sampling and the other for hormone and tracer infusion.

Each subject was studied on two occasions in random order separated by 3 mo. This time interval was chosen to ensure negligible amounts of deuterium-labeled glycogen on the second study day. On one occasion, an infusion of hydrocortisone ($2.0 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Solu-Cortef, Pharmacia & Upjohn) dissolved in saline was started at 2200 and maintained at that rate until 1630 (*time 300*) of the next day. On the second occasion, saline was infused at a rate of 1.8 l/h. At 2200, 2400, and 0200, the subjects drank 1.7 ml of 2H_2O (99.9% H; Cambridge Isotope Laboratories, Andover, MA) per kilogram body water. Body water was calculated to be 50% of total body weight in women and 60% in men. Water ingested ad libitum thereafter was enriched to be 0.5% 2H_2O to maintain isotopic steady state. At 0500 (*time -390*), a primed continuous infusion of [$3\text{-}^3\text{H}$]glucose (17 μCi prime, 0.17 $\mu\text{Ci}/\text{min}$ continuous) was started and maintained throughout the experiment. At 0630, the hand used for blood sampling was placed in a heated Plexiglas box and maintained at a temperature of

$\sim 55^\circ\text{C}$ throughout the study to allow sampling of arterialized venous blood. Postabsorptive glucose release (EGR) and gluconeogenesis were determined from 0700 to 0730.

At 0730 (*time -240*), an intravenous infusion containing somatostatin ($60 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Ferring, Kiel, Germany), glucagon ($0.65 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Novo Nordisk, Bagsværd, Denmark), and growth hormone ($2 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Novo Nordisk) was begun in all subjects. An infusion of insulin (Insulin Actrapid; Novo Nordisk) was also started at rates of $0.15 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ on the saline day and $0.25 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ on the cortisol day. Plasma glucose concentration was measured at 15-min intervals with a glucose oxidase method (Beckman Instruments, Brea, CA), and the insulin infusion rate was adjusted to maintain glucose at ~ 5 mmol/l. The last adjustment of this "basal" insulin infusion was made ≥ 30 min before start of the prandial glucose infusion and was maintained at that rate for the remainder of the study.

At 1130 (*time 0*), a 5-h preprogrammed variable glucose infusion was started with an infusion pump (model PhD 2000; Harvard Apparatus, South Natick, MA) driven by a PC 2/30 265 computer (IBM, Rochester, MN). Glucose was infused in a manner mimicking the systemic rate of appearance of glucose that occurs after ingestion of 50 g of glucose (1). The pattern and amount of glucose infused normalized to lean body mass were equal on the two study days.

In an effort to maintain specific activities constant, the rate of basal [$3\text{-}^3\text{H}$]glucose was adjusted ($-390\text{--}0$ min, 100%; $0\text{--}30$ min, 70%; $30\text{--}60$ min, 46%; $60\text{--}120$ min, 55%; $120\text{--}150$ min, 53%; $150\text{--}300$ min, 61%) according to the anticipated suppression of EGR during the prandial glucose infusion. Furthermore, all glucose infused contained [$3\text{-}^3\text{H}$]glucose. Hence, glucose specific activities remained constant on the two study days.

To avoid the confounding effects of counterregulation during hypoglycemia, the experiments were terminated when plasma glucose concentrations fell below 2.7 mmol/l (50 mg/dl).

Analytical techniques. Arterialized plasma glucose was measured in duplicate immediately after sampling. Samples for hormone analysis were placed on ice, centrifuged at 4°C , separated, and stored at -20°C until assay. Serum insulin was determined by two-site enzyme-linked immunosorbent assay (2). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay. Serum free fatty acids (FFA) were measured enzymatically using a Wako NEFA (nonesterified fatty acid) Test Kit (Wako Chemicals, Neuss, Germany).

The measurement of deuterium enrichment on carbons 2 and 5 in glucose was performed as previously described (25). Briefly, 15 ml of

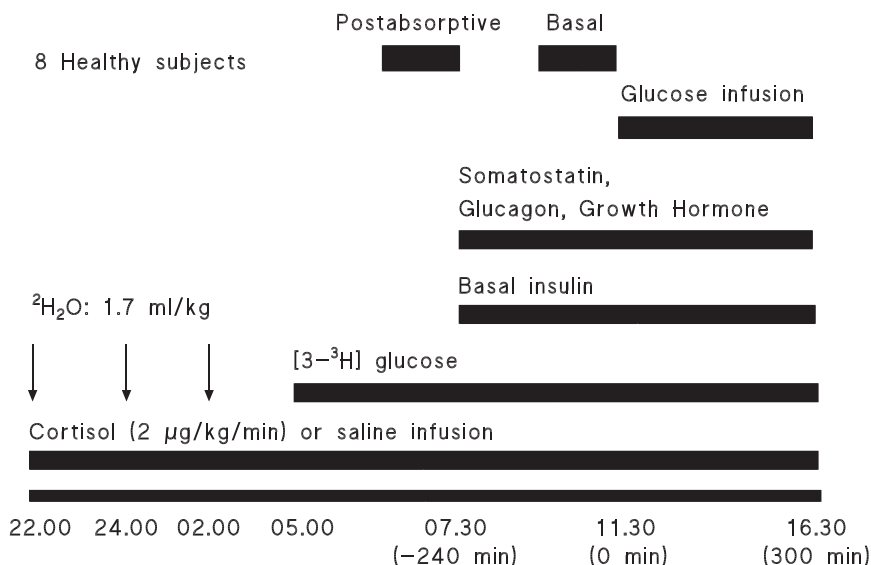


Fig. 1. Experimental design as described in MATERIALS AND METHODS.

blood were diluted with 30 ml of demineralized water and deproteinized using 15 ml of 0.3 N ZnSO₄ and 15 ml of 0.3 N Ba(OH)₂. The samples were centrifuged at 2,000 rpm for 15 min, and the pellet was diluted in 15 ml of demineralized water to wash out the remaining glucose. Glucose was isolated by successive ion-exchange chromatography and high-performance liquid chromatography. For determination of deuterium enrichment on C5, glucose was first converted to xylose, and the C5 of glucose with its hydrogens was cleaved by periodate oxidation to formaldehyde, which was condensed with ammonium hydroxide to form hexamethylenetetramine (HMT). The ²H bound to C2 of glucose was isolated after conversion of glucose to ribitol-5-phosphate and arabitol-5-phosphate and treatment to form HMT. HMT was analyzed on a Hewlett-Packard mass spectrometry system. Standard solutions of glucose of known enrichment were run along with the unknown samples to calibrate for instrument variations.

Calculation of glucose turnover. Glucose turnover, i.e., EGR and glucose utilization, was calculated using Steele's equations for non-steady state (45). The pool correction factor and the glucose volume of distribution were assumed to be 0.65 and 260 ml/kg, respectively. Glucose specific activity, calculated as the ratio between tracer (dpm/ml) and plasma glucose concentration, was smoothed from 0 to 300 min by use of the OOPSEG program of Bradley et al. (8). Glucose production and utilization at *time 0* were calculated using the average specific activity from -30 to 0 min. Glucose clearance was calculated as the ratio between glucose utilization (R_d) and the corresponding plasma glucose concentration. The fractional contribution of gluconeogenesis to glucose release was determined as the ratio of deuterium bound to carbon 5 in glucose to that bound to carbon 2 (25). A quantitative estimate of postabsorptive gluconeogenesis was calculated by multiplying the mean glucose release from 0700 to 0730 by the mean of the fractional contribution of gluconeogenesis to EGR from 0630 to 0730.

Assessment of glucose effectiveness. The calculation of net glucose effectiveness (GE_b, ml·kg⁻¹·min⁻¹), i.e., the ability of glucose to suppress glucose release and stimulate glucose utilization, has been described elsewhere (5, 32). Net glucose effectiveness at basal insulin concentration was calculated as the ratio between the area under the curve (AUC) of the exogenous glucose infusion rate (GIR) and the AUC of the glucose concentration above basal (ΔG)

$$GE_b = \frac{AUC[GIR(t)]}{AUC[\Delta G(t)]} \quad (1)$$

Because equal amounts of glucose were infused in each subject on the two study days, the excursion of glucose concentration above basal is inversely related to net glucose effectiveness. The greater the glycemic excursion, the lower the net glucose effectiveness (GE_b).

To determine the effects of glucose on glucose production and utilization, hot (tracer-determined) indexes of glucose effectiveness, i.e., GE_b^{*} and GE_{liver}, were calculated from glucose and [3-³H]glucose concentrations (5). Whereas GE_b^{*} (ml·kg⁻¹·min⁻¹) determines the ability of glucose at basal insulin to facilitate its own disposal, GE_{liver} (ml·kg⁻¹·min⁻¹) determines the effect of glucose to suppress its own production. These estimates are equivalent to those derived using the glucose clamp technique. The effect of glucose on glucose disposal was calculated as follows

$$GE_b^* = \frac{\{AUC[\Delta GIR^*(t)] - PCR_b G_b AUC[\Delta SA(t)]\}}{\{AUC[\Delta G^*(t)] - G_b AUC[\Delta SA(t)]\}} \quad (2)$$

where ΔGIR* is the tracer infusion rate above basal, G* is the tracer concentration, SA is the tracer specific activity, and PCR_b is the basal plasma glucose clearance rate.

The effect of glucose on glucose production (GE_{liver}, ml·kg⁻¹·min⁻¹) was calculated by dividing the area below basal of EGR by the area above basal of glucose concentration. Since GE_b constitutes the effects of glucose both to suppress its own production

and to facilitate its own disposal, GE_{liver} can also be calculated by subtracting GE_b^{*} from GE_b.

Plasma glucose clearance rate (PCR) and GE_b^{*} are related according to the following equation

$$PCR(t) = GE_b^* + R_{d,0}/G(t) \quad (3)$$

where R_{d,0} equals the hypothetical rate of glucose uptake at zero glucose concentration.

Assessment of basal insulin sensitivity. Insulin action was determined from postabsorptive glucose and insulin concentrations with the homeostatic model assessment analysis for insulin sensitivity (HOMA), as proposed by Turner and colleagues [Matthews et al. (26) and Turner et al. (46)]. To account for differences in FFA concentrations, insulin sensitivity was also calculated on the basis of the recently revised QUICKI method, as suggested by Perseghin et al. (38).

Statistical analysis. Data in the text and Figs. 1–9 are expressed as means ± SE. All rates are expressed per kilogram of lean body weight. Integrated responses and responses above basal were calculated using the trapezoidal rule. Integrated response was defined as total area above zero during saline or steroid infusion. Postabsorptive measures were obtained from 0700 to 0730 (-270 to -240 min) (Fig. 1). Basal measures, i.e., those obtained in the presence of basal insulin concentrations, were defined as the means of values from 1100 to 1130 (-30–0 min). A paired Student's *t*-test was used to test for differences during saline or steroid infusion. The Wilcoxon rank sum test was used for data not fulfilling the criteria for normal distribution (27). A *P* value <0.05 was considered statistically significant.

RESULTS

Postabsorptive glucose, FFA, and hormone concentrations. Postabsorptive cortisol (1,243 ± 133 vs. 386 ± 50 nmol/l, *P* < 0.01), glucose (6.72 ± 0.20 vs. 4.91 ± 0.13 mmol/l, *P* < 0.01), insulin (52 ± 5 vs. 33 ± 2 pmol/l, *P* < 0.05), and C-peptide concentrations (594 ± 55 vs. 476 ± 34 pmol/l, *P* < 0.05) were greater after overnight cortisol infusion than during saline infusion (Fig. 2). During the night, circulating FFA concentrations increased on both study days (Fig. 3). However, FFA concentrations were higher during cortisol than during saline infusion (0.71 ± 0.07 vs. 0.47 ± 0.06 mmol/l, *P* < 0.01). Postabsorptive plasma glucagon concentrations did not differ on the two study days (51 ± 8 vs. 48 ± 9 pg/ml, *P* = 0.82).

Postabsorptive EGR, gluconeogenesis, and insulin sensitivity. Despite higher glucose and insulin concentrations during cortisol than during saline infusion, postabsorptive glucose production (12.4 ± 0.5 vs. 11.9 ± 0.7 μmol·kg⁻¹·min⁻¹, *P* = 0.64) and gluconeogenesis (8.1 ± 0.6 vs. 7.1 ± 0.5 μmol·kg⁻¹·min⁻¹, *P* = 0.33) did not differ on the two study days (Fig. 4). Gluconeogenesis accounted for 62.9 ± 4.6 and 59.8 ± 3.9% of total glucose release during cortisol and saline infusion, respectively. Insulin sensitivity, determined by both the HOMA analysis (2.58 ± 0.30 vs. 1.19 ± 0.14; *P* < 0.01) and the revised QUICKI method (0.35 ± 0.01 vs. 0.43 ± 0.01; *P* < 0.01), was markedly lower during cortisol than during saline infusion, implying steroid-induced insulin resistance.

Although postabsorptive glucose concentrations were higher during steroid infusion, glucose uptake did not differ significantly on the two study days (12.4 ± 0.5 vs. 11.9 ± 0.7 μmol·kg⁻¹·min⁻¹; *P* = 0.64). Consequently, glucose clearance was lower during steroid than during saline infusion (1.89 ± 0.06 vs. 2.42 ± 0.18 ml·kg⁻¹·min⁻¹; *P* < 0.05).

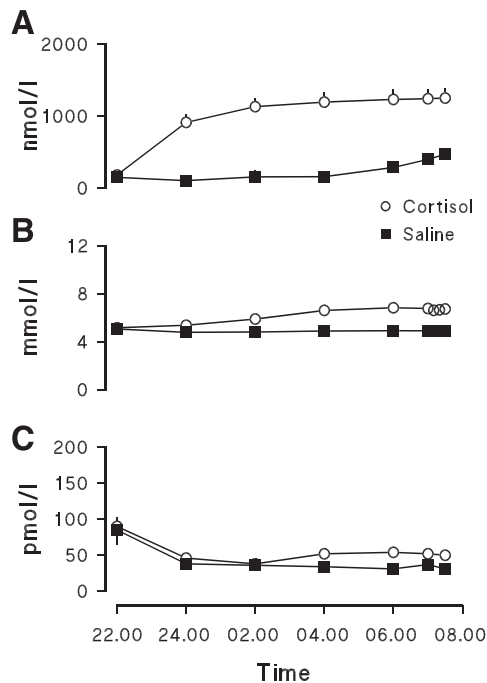


Fig. 2. Nocturnal plasma cortisol (A), glucose (B), and insulin (C) concentrations during cortisol (○) or saline (■) infusion.

Substrate and hormone concentrations during prandial glucose infusion. “Basal” insulin infusion rate, defined as the infusion rate required to maintain glucose concentration at ~ 5 mM (0.27 ± 0.02 vs. 0.16 ± 0.01 mU \cdot kg $^{-1}\cdot$ min $^{-1}$, $P < 0.01$), and the corresponding basal insulin concentrations (88 ± 5 vs. 65 ± 3 pmol/l, $P < 0.05$) were higher during cortisol than during saline infusion, indicating the presence of steroid-induced insulin resistance (Fig. 5). Basal C-peptide (31 ± 5 vs.

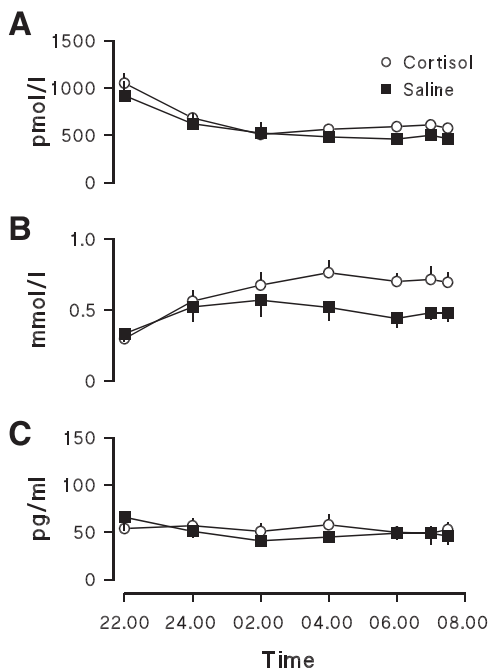


Fig. 3. Nocturnal C-peptide (A), free fatty acid (FFA, B), and glucagon (C) concentrations during cortisol (○) or saline (■) infusion.



Fig. 4. Postabsorptive rates of endogenous glucose release and gluconeogenesis during nocturnal cortisol or saline infusion. Gluconeogenesis was calculated by multiplying the mean ratio of deuterium enrichment at carbon 5 to that at carbon 2 in glucose by the mean rate of fasting endogenous glucose release from -30 to 0 min.

28 ± 8 pmol/l, $P = 0.77$) and glucagon (65 ± 7 vs. 66 ± 8 pg/ml, $P = 0.85$) concentrations did not differ on the two study days (Fig. 6). Basal FFA concentrations (0.34 ± 0.04 vs. 0.17 ± 0.02 mmol/l, $P < 0.01$) were higher during cortisol than during saline infusion.

In the presence of prandial glucose infusion, basal insulin concentrations remained constant and higher during cortisol than during saline infusion. Glucose infusion did not alter FFA or glucagon concentrations. During somatostatin infusion, C-peptide concentrations were completely suppressed on both study days, implying suppressed endogenous insulin secretion during the prandial glucose infusion.

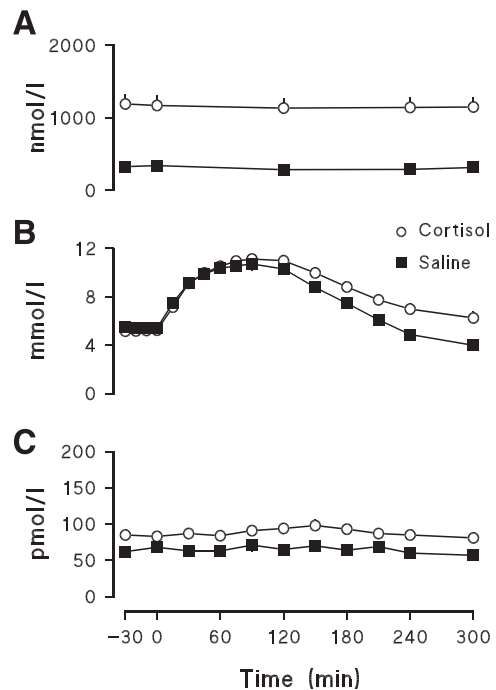


Fig. 5. Plasma cortisol (A), glucose (B), and serum insulin (C) concentrations during prandial glucose infusion in the presence of cortisol (○) or saline (■) infusion. The glucose infusion was started at 0 min.

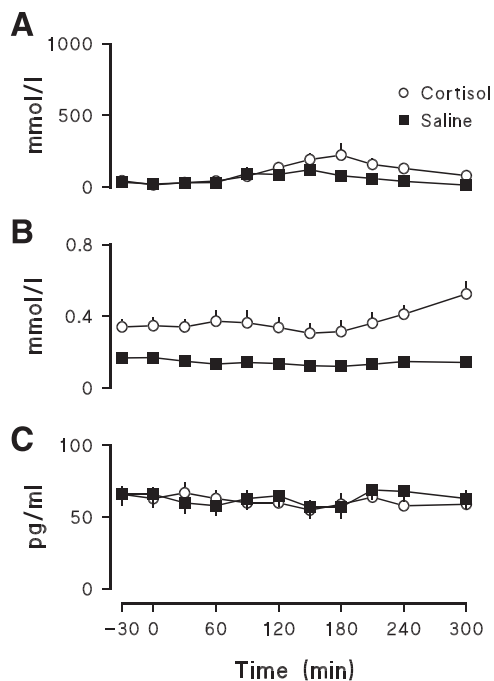


Fig. 6. C-peptide (A), FFA (B), and plasma glucagon (C) concentrations during prandial glucose infusion in the presence of cortisol (○) or saline (■) infusion. The glucose infusion was started at 0 min.

Estimate of glucose effectiveness during the prandial glucose infusion. Before the prandial glucose infusion, glucose concentrations (5.20 ± 0.12 vs. 5.44 ± 0.13 mmol/l, $P = 0.27$) were comparable and constant on both study days. In the presence of constant basal insulin concentrations, the glycemic excursion during glucose infusion was greater during cortisol than during saline infusion ($1,082 \pm 108$ vs. 751 ± 72 mmol \cdot l $^{-1}\cdot$ 5 h $^{-1}$, $P < 0.05$). Consequently, net glucose effectiveness (4.42 ± 0.52 vs. 6.65 ± 0.83 ml \cdot kg $^{-1}\cdot$ min $^{-1}$, $P < 0.05$) was impaired during cortisol infusion (Fig. 7).

Net glucose effectiveness, GE_b , is a composite measure of the ability of glucose to promote its own uptake (GE_b^*) and to suppress its own production (GE_{liver}). Because of the infusion of a glucose tracer, the present design also allows a calculation of these hot indexes of glucose effectiveness. Whereas GE_{liver}

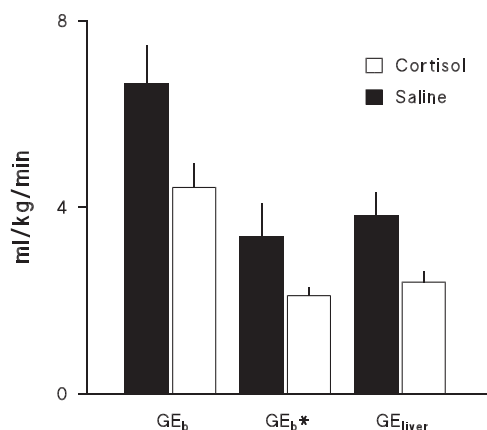


Fig. 7. Estimates of glucose effectiveness during basal insulin concentrations. Net glucose effectiveness (GE_b) constitutes the combined effects of glucose to stimulate its own uptake (GE_b^*) and suppress its own production (GE_{liver}).

values were higher during saline than during steroid infusion (3.82 ± 0.51 vs. 2.39 ± 0.24 ml \cdot kg $^{-1}\cdot$ min $^{-1}$, $P < 0.05$), GE_b^* (although higher during saline than during steroid infusion in 6 of 8 subjects) did not differ significantly on the two study days (3.37 ± 0.72 vs. 2.10 ± 0.19 ml \cdot kg $^{-1}\cdot$ min $^{-1}$, $P = 0.15$).

Plasma glucose clearance rate (PCR) is related to GE_b^* , $R_{d,0}$, and glucose concentration as specified in Eq. 3. $R_{d,0}$ was calculated in each subject by substituting in Eq. 3 the pretest values of PCR and glucose concentration. In the present experiments, $R_{d,0}$ did not differ on the two study days (-4.08 ± 3.53 vs. 1.37 ± 1.14 μ mol \cdot kg $^{-1}\cdot$ min $^{-1}$; $P = 0.24$). During the prandial glucose infusion, PCR changed as a consequence of the change in glucose concentration. Mean rates of PCR during glucose infusion were lower during cortisol than during saline infusion (2.23 ± 0.08 vs. 2.77 ± 0.20 ml \cdot kg $^{-1}\cdot$ min $^{-1}$; $P < 0.05$).

Glucose turnover during prandial glucose infusion. To determine the effects of glucose on glucose kinetics, rates of EGR and glucose disappearance were measured during the prandial glucose infusion (Figs. 8 and 9). In the presence of basal insulin concentrations, postabsorptive EGR values (14.2 ± 0.6 vs. 12.3 ± 0.4 μ mol \cdot kg $^{-1}\cdot$ min $^{-1}$; $P < 0.05$) were higher during saline than during cortisol infusion. During the prandial glucose infusion, EGR rates were suppressed promptly and equally on the two study days ($1,570 \pm 285$ vs. $1,211 \pm 162$ μ mol/kg; $P = 0.26$). Because equal rates of glucose production were obtained despite higher glucose concentrations during steroid infusion, these results imply a defect in the ability of glucose to suppress EGR during excess hydrocortisone concentrations.

In the presence of basal insulin concentrations, basal glucose rates of disappearance were higher during saline than during cortisol infusion (14.3 ± 0.6 vs. 12.3 ± 0.4 μ mol \cdot kg $^{-1}\cdot$ min $^{-1}$;

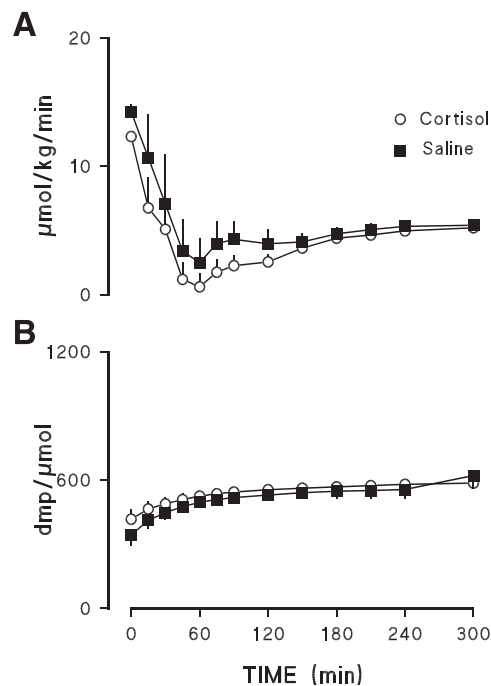


Fig. 8. Endogenous glucose release (A) and [3 H]glucose specific activity (B) during prandial glucose infusion in the presence of cortisol (○) or saline (■) infusion. The glucose infusion was started at 0 min.

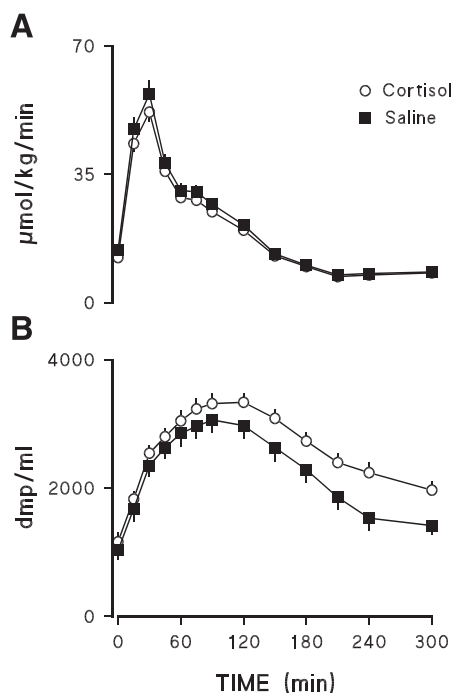


Fig. 9. Rate of disappearance (A) and $[3\text{-}^3\text{H}]$ glucose concentrations (B) during prandial glucose infusion in the presence of cortisol (○) or saline (■) infusion. The glucose infusion was started at 0 min.

$P < 0.05$). During the prandial glucose infusion, glucose disappearance did not differ on the two study days ($5,869 \pm 281$ vs. $5,532 \pm 171$ mmol/kg; $P = 0.31$). Consequently, in the presence of excess cortisol, rates of disappearance were inappropriate for the higher glucose concentrations, indicating impaired plasma glucose clearance (2.77 ± 0.20 vs. 2.23 ± 0.08 ml·kg $^{-1}$ ·min $^{-1}$; $P < 0.05$).

The same tracer infusion profile was applied during saline and steroid infusions. Therefore, identical amounts of $[3\text{-}^3\text{H}]$ glucose were infused in all subjects. Consequently, the excursion of $[3\text{-}^3\text{H}]$ glucose concentration during the prandial glucose infusion yields a model-independent assessment of glucose uptake. In the present experiments, $[3\text{-}^3\text{H}]$ glucose concentrations were higher during steroid than during saline infusion ($650,515 \pm 62,403$ vs. $791,385 \pm 38,411$ dpm·ml $^{-1}$ ·5 h $^{-1}$; $P < 0.05$). This difference in tracer concentration implies a lower rate of glucose disappearance and confirms the presence of an impairment of glucose clearance during hypercortisolemia.

DISCUSSION

Previous studies have demonstrated that excess cortisol induces hepatic and extrahepatic insulin resistance (3, 18, 20, 28, 41, 42, 48). Metabolic effects may be induced by the normal circadian variation in cortisol secretion (10, 11). Besides coincident disturbances in protein and lipid metabolism, hypercortisolemia is also characterized by hyperinsulinemia and hyperglycemia (13, 21, 23, 34, 37, 43). The mechanism(s) by which glucocorticoids induce alterations in glucose metabolism are, however, sparsely elucidated. Glucocorticoids are reported to decrease glucose transport (36), and conflicting results are reported on the effects of glucocorticoids on insulin binding (6, 14, 22).

Under conditions of daily living, glucose and insulin concentrations rise and fall in a tightly coordinated manner. An increase in either glucose or insulin will suppress glucose production and stimulate glucose uptake. The concurrent rise in concentration of both glucose and insulin will exert a greater response than a rise in either alone (31). Previous studies have established that excess cortisol, measured either during clamp conditions or in the presence of physiological changes in insulin concentrations, alters insulin action. However, the effects of glucocorticoids on glucose effectiveness, i.e., the ability of glucose to regulate its own metabolism, have to our knowledge never been examined. The present study was designed to determine the effects of hypercortisolemia on post-absorptive and postprandial glucose metabolism. Hydrocortisone was infused at a rate known to induce hepatic and extrahepatic insulin resistance (41). During steroid infusion, cortisol concentrations increased threefold from baseline to $\sim 1,200$ nmol/l. This concentration is equivalent to the adrenal response seen after severe trauma or surgical stress (9, 30, 35, 47).

Despite higher fasting insulin concentrations, both glucose and FFA concentrations were higher during steroid than during saline infusion. Although insulin action was not directly measured, both HOMA analysis and the revised QUICKI method suggest that insulin action was impaired, implying that the presence of insulin resistance was partially compensated by an increase in insulin secretion. Fasting EGR, gluconeogenesis, and, by implication, fasting glycogenolysis were unaltered during hydrocortisone infusion; that is, net glycogenolysis to the extent that there is glycogen cycling, with perhaps more cycling on hydrocortisone administration (24). Because glucose and insulin concentrations were both higher during hydrocortisone than during saline infusion, the present data suggest an impaired ability of insulin and glucose to suppress postabsorptive glucose production during excess cortisol concentrations.

The effects of glucocorticoids on hepatic glucose metabolism have been studied in both in vivo and in vitro experiments. Gluconeogenic conversion of alanine to glucose has been demonstrated to be increased during acute rises in plasma cortisol (19). Furthermore, phosphoenolpyruvate carboxykinase (PEPCK) activity is increased by glucocorticoids, consistent with an increase in gluconeogenesis during hypercortisolemia (16). As pointed out by Sasaki et al. (44), dexamethasone-induced increase of PEPCK gene transcription is either blunted or totally inhibited by elevated insulin, suggesting that insulin is a dominant hormone in the regulation of gluconeogenesis (44). Therefore, in the present experiments, the observation of unaltered rates of gluconeogenesis and glucose production during hypercortisolemia may be attributed to the inhibition of insulin on PEP kinase transcription. This conclusion is supported by studies in overnight-fasted dogs in which a fivefold elevation in plasma cortisol resulted in minimally elevated rates of gluconeogenesis despite increased plasma insulin and glucose concentrations (20).

Although several studies have determined the effects of hydrocortisone on insulin action (3, 28, 41, 48), the effects of glucocorticoids on glucose effectiveness are unknown. The classic minimal model analysis based on the FSIGT was originally designed to estimate insulin action. The model also allows an estimate of glucose effectiveness, but the accuracy of

that estimate has been challenged. It has been demonstrated that the cold minimal model in its original form provides an overestimate of glucose effectiveness when assessed in the presence of changing glucose concentrations (15). For that reason, we chose in the present experiments to assess glucose effectiveness by use of a minimal model-independent approach in which insulin was maintained at basal concentrations and the glycemic excursion was measured during a standardized glucose infusion (5).

We have previously demonstrated that the estimate of glucose effectiveness is sensitive to even subtle changes in insulin concentrations (32). This is the case because EGR is inhibited and glucose uptake is stimulated by insulin. Because cortisol induces hepatic and extrahepatic insulin resistance to obtain the same biological effect, higher insulin concentrations were required during hypercortisolemia than during saline infusion. To ensure that the response to exogenous glucose infusion was assessed in the presence of biologically equivalent amounts of insulin, the appropriate basal insulin concentration was individually determined on each occasion in each study subject. We defined basal as the insulin concentration necessary to maintain the subject's glucose concentration constant at 5.5 mmol/l. Inspection of Fig. 5 indicates that this goal was clearly achieved during both saline and steroid infusions.

As depicted in Fig. 5, the basal insulin infusion rate (0.27 vs. 0.16 mU·kg TBW⁻¹·min⁻¹; $P < 0.01$) and the corresponding insulin concentrations (88 ± 5 vs. 65 ± 3 pmol/l; $P < 0.05$) were higher during cortisol than during saline infusion, indicating the presence of steroid-induced insulin resistance. Moreover, basal insulin concentrations were maintained constant from -30 until the end of the prandial glucose infusion. Because each subject received the same glucose infusion profile, the higher glycemic excursion, measured either in terms of peak glucose concentrations or as integrated area above baseline, indicates a decrease in net glucose effectiveness during hypercortisolemia (Fig. 5).

The higher glucose concentrations during steroid infusion could have been due to inappropriately high rates of EGR, inappropriately low rates of glucose utilization, or a combination of both. Thus, to gain insight into the mechanism by which glucose effectiveness is impaired, glucose production and rates of glucose utilization were determined using the hot-GINF method. Moreover, model-independent estimates of hot indexes of glucose effectiveness were calculated on the basis of glucose and [3-³H]glucose concentrations.

During the prandial glucose infusion, EGR decreased promptly and equally on the two study days. However, these rates of glucose production were obtained in the presence of higher glucose concentrations during steroid infusion. Inspection of Fig. 8 suggests that, whereas suppression of EGR appeared unaltered during the early part of the prandial glucose infusion, the rate of suppression was inadequate from 150 min and onward, implying an abnormal suppression of EGR by glucose in the presence of hypercortisolemia. Moreover, GE_{liver}, calculated as the ratio between the area below basal of EGR and the area above basal of glucose, was lower during steroid than during saline infusion, demonstrating that the ability of glucose to suppress glucose production is reduced in the presence of excess cortisol concentrations.

Rates of glucose disappearance also did not differ in the presence or absence of excess hydrocortisone (Fig. 9A). Equal

rates of glucose disappearance were obtained in the presence of higher glucose concentrations, which suggests that glucose clearance was impaired. Other evidence of an impairment of glucose clearance during hydrocortisone infusion comes from the inspection of the glucose tracer data (Fig. 9B). In the present experiments, equal rates of [3-³H]glucose were infused on the two study days. Therefore, the greater increment in the concentration of [3-³H]glucose during steroid than during saline infusion suggests that the tracer disappearance rate was reduced during the hydrocortisone infusion. Altered rates of glucose clearance, however, do not necessarily indicate that peripheral glucose effectiveness, GE_b^{*}, is also different. In fact, glucose clearance and glucose effectiveness are related but measure slightly different aspects of glucose metabolism. According to Eq. 3, glucose clearance is the sum of GE_b^{*} and a variable component determined by the ratio between R_{d,0} (i.e., the hypothetical intercept with the R_d axis of the R_d vs. glucose relationship) and the time-varying glucose concentration. We found GE_b^{*} to be reduced in six of eight subjects in the presence of hydrocortisone infusion; however, this reduction was not statistically significant. Also, R_{d,0} did not differ on the two study days. Nevertheless, because of the marked difference in the time courses of plasma glucose concentration, mean PCR was lower during hydrocortisone than during saline infusion.

All in all, the present experiments provide evidence that impaired glucose effectiveness during steroid infusion is attributable to a defect in the ability of glucose to suppress its own production. Moreover, this defect produces an elevation of the glucose level that results in impaired peripheral glucose clearance.

The finding of impaired basal glucose effectiveness during excess cortisol is in contrast to experiments by Baron et al. (4), who studied the effects of a 12-h cortisol infusion on non-insulin-mediated glucose uptake (NIMGU) (4). In these experiments, no effects of cortisol on NIMGU was demonstrated. Because glucose effectiveness in the present experiments was determined during basal insulin concentrations, and NIMGU was measured in the presence of near-zero insulin conditions, these conditions suggest that cortisol has different effects on glucose metabolism in the presence or absence of insulin.

The present experiments suggest that impaired glucose effectiveness is a contributing mechanism to glucose intolerance during conditions of excess cortisol. Various mechanisms may account for that finding. At basal insulin, glucose-mediated glucose uptake may be induced by a mass action effect facilitated by GLUT transport proteins. However, mass action is not the only mechanism. Hyperglycemia has been demonstrated to recruit insulin-independent glucose transporters (GLUT1 and GLUT2) to the cell surface via a Ca²⁺-dependent mechanism, which is mechanistically different from the insulin-dependent mechanism mediated via phosphatidylinositol 3-kinase (33). Furthermore, hyperglycemia has been demonstrated to induce translocation of GLUT4 transporters to the plasma membrane in muscle (17). In addition, experiments by Petersen et al. (39) have suggested that hyperglycemia inhibits hepatic glycogenolysis primarily through inhibition of glycogen phosphorylase, whereas insulin inhibits glycogenolysis primarily through stimulation of glycogen synthase. It is currently unknown how glucocorticoids interfere with these mechanisms in a

way that ultimately leads to impaired glucose effectiveness. It is well established that glucocorticoids affect gene expression, but these hormones may also exert their effects through modulation of posttranscriptional events. Further experiments are required to determine through which pathways hypercortisolemia effects insulin action and glucose effectiveness.

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