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

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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
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 and GE\textsubscript{avg}, 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 $^2\text{H}_2\text{O}$ 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/m\textsuperscript{2}, 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 μg·kg\textsuperscript{-1}·min\textsuperscript{-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 $^2\text{H}_2\text{O}$ (99.9% H; Cambridge Isotope Laboratories, Andover, MA) per kilogram body water. Body water was calculated to be 50% of total body weight in *Cambridge Isotope Laboratories, Andover, MA*.

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

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**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 (Rₐ) and the corresponding plasma glucose concentration. The fractional contribution of gluconeogenesis to glucose release was determined as the ratio of deuterium enrichment on C₅, glucose was isolated by successive ion-exchange chromatography and to facilitate its own disposal, GEᵦₐᵥₑᵦ can also be calculated by subtracting GEᵦₑᵦ from GEₑᵦₑᵦ.

Plasma glucose clearance rate (PCR) and GEᵦₑᵦ are related according to the following equation

\[ PCR(t) = GEᵦₑᵦ + \frac{SA}{AUC[ΔGₜ]} \]

where \( Rₐ \) 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 mmol/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).
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 \((0.27 \pm 0.02\) vs. \(0.16 \pm 0.01\) mU·kg\(^{-1}\)·min\(^{-1}\), \(P < 0.01\)), and the corresponding basal insulin concentrations \((88 \pm 5\) vs. \(65 \pm 3\) 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 \((31 \pm 5\) vs. \(28 \pm 8\) pmol/l, \(P = 0.77\)) and glucagon \((65 \pm 7\) vs. \(66 \pm 8\) pg/ml, \(P = 0.85\)) concentrations did not differ on the two study days (Fig. 6). Basal FFA concentrations \((0.34 \pm 0.04\) vs. \(0.17 \pm 0.02\) 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.
Cortisol excess impairs glucose tolerance.

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 ± 108 vs. 751 ± 72 mmol·l⁻¹·h⁻¹, P < 0.05). Consequently, net glucose effectiveness (4.42 ± 0.52 vs. 6.65 ± 0.83 mmol·kg⁻¹·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 ($GE_b^u$) and to suppress its own production ($GE_b^p$). Because of the infusion of a glucose tracer, the present design also allows a calculation of these hot indexes of glucose effectiveness. Whereas $GE_b^p$ values were higher during saline than during steroid infusion (3.82 ± 0.51 vs. 2.39 ± 0.24 ml·kg⁻¹·min⁻¹, P < 0.05), $GE_b^u$ (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·kg⁻¹·min⁻¹, P = 0.15).

Plasma glucose clearance rate (PCR) is related to $GE_b^u$, $R_d$, and glucose concentration as specified in Eq. 3. $R_d$ was calculated in each subject by substituting in $R_d$ the pretest values of PCR and glucose concentration. In the present experiments, $R_d$ did not differ on the two study days (−4.08 ± 3.53 vs. 1.37 ± 1.14 mmol·kg⁻¹·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·kg⁻¹·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 mmol·kg⁻¹·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 ± 285 vs. 1,211 ± 162 mmol/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 μmol·kg⁻¹·min⁻¹;
The glucose infusion was started at 0 min. [3-3 H]glucose were infused in all subjects. Consequently, the concentrations were higher during steroid than during saline infusion. Therefore, identical amounts of infusions (650,515 μmol/kg·min) during steroid and saline. Identification of an impairment of glucose clearance during hypercortisolemia. During steroid infusion, cortisol concentrations increased threefold from baseline to ~1,200 nmol/l. This concentration is equivalent to the adrenal response seen after severe trauma or surgical stress. 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 hypercortisolism (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 hypercortisolism 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 hypercortisolism 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⁻¹min⁻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 hypercortisolism (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 onward, implying an abnormal suppression of EGR by glucose in the presence of hypercortisolism. Moreover, GE_e 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_e liver, 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_e liver 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_e liver 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 this 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 hypercortisolism affects insulin action and glucose effectiveness.

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