Glucocorticoids Fail to Cause Insulin Resistance in Human Subcutaneous Adipose Tissue In Vivo

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Context: It is widely believed that glucocorticoids cause insulin resistance in all tissues. We have previously demonstrated that glucocorticoids cause insulin sensitization in human adipose tissue in vitro and induce insulin resistance in skeletal muscle.

Objective: Our aim was to determine whether glucocorticoids have tissue-specific effects on insulin sensitivity in vivo.

Design: Fifteen healthy volunteers were recruited into a double-blind, randomized, placebo-controlled, crossover study, receiving both an overnight hydrocortisone and saline infusion. The tissue-specific actions of insulin were determined using paired 2-step hyperinsulinemic euglycemic clamps incorporating stable isotopes with concomitant adipose tissue microdialysis.

Setting: The study was performed in the Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Main Outcome Measures: The sensitivity of sc adipose tissue to insulin action was measured.

Results: Hydrocortisone induced systemic insulin resistance but failed to cause sc adipose tissue insulin resistance as measured by suppression of adipose tissue lipolysis and enhanced insulin-stimulated pyruvate generation. In primary cultures of human hepatocytes, glucocorticoids increased insulin-stimulated p-ser473akt/protein kinase B. Similarly, glucocorticoids enhanced insulin-stimulated p-ser473akt/protein kinase B and increased Insulin receptor substrate 2 mRNA expression in sc, but not omental, intact human adipocytes, suggesting a depot-specificity of action.

Conclusions: This study represents the first description of sc adipose insulin sensitization by glucocorticoids in vivo and demonstrates tissue-specific actions of glucocorticoids to modify insulin action. It defines an important advance in our understanding of the actions of both endogenous and exogenous glucocorticoids and may have implications for the development and targeting of future glucocorticoid therapies. **(*J Clin Endocrinol Metab* 98: 1631–1640, 2013)**

The clinical indications for the use of glucocorticoids (GCs), both topical and systemic, are many and varied. Although their therapeutic efficacy is not in doubt, their side effect profile to induce Cushing's syndrome, characterized by central obesity, insulin resistance, and in some cases overt type 2 diabetes mellitus, creates a significant additional health burden that can limit their use. This has a high clinical relevance because approximately 1%–2% of the populations of the United States and the United Kingdom use GC therapy (1, 2). In addition, endogenous GCs and

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Abbreviations: akt/PKB, protein kinase B; BMI, body mass index; ct value, calculated threshold line; DNL, de novo lipogenesis; GC, glucocorticoid; HFD, high-fat diet; INS-½-max NEFA, insulin concentration causing half-maximal suppression of NEFA; INS-½-max GLY, insulin concentration causing half-maximal suppression of glycerol; IRS, insulin receptor substrate; NEFA, nonesterified fatty acid; Ra glucose, rates of glucose production.

their metabolism may have a fundamental role in the pathogenesis of obesity, insulin resistance, and type 2 diabetes mellitus. Modification of GC action has evolved as a potential treatment target (3). There is therefore an urgent need to determine the tissue-specific actions of GCs to understand the mechanisms underpinning their side effect profile that might lead to the development of newer classes of agent with improved adverse effect profiles.

In human preadipocytes, we and others have demonstrated that synthetic and endogenous GCs cause insulin sensitization in a dose- and time-dependent manner (4–6); furthermore, these effects persist with prolonged (7 days) administration (7). Insulin signals through a tyrosine kinase, membrane bound receptor that upon insulin binding, autophosphorylates and activates a signaling cascade with the phosphorylation of a number of insulin receptor substrates (IRSs). This in turn results in phosphatidylinositol 3-kinase activation and the phosphorylation and activation of akt/protein kinase B (PKB) ultimately leading to the translocation of glucose transporter type 4-containing vesicles to the cell membrane to permit glucose uptake. The effects of GCs to enhance insulin action in adipose tissue are mediated through the increased tyrosine phosphorylation of IRS1 and increased mRNA and protein expression of IRS2 (4). We have proposed that GC-mediated enhanced insulin action will not only fuel adipocyte differentiation that is critically dependent on both insulin and GCs (8) but will also drive lipid accumulation within the adipocyte (5); both of these mechanisms will contribute to increased fat mass after GC treatment. These observations contrast with those in skeletal muscle (primary cultures and cell lines) in which GCs decrease insulin-stimulated glucose uptake through the increased inactivating serine phosphorylation of IRS1 (9) that targets it for degradation and limits its ability to interact with the insulin receptor (10).

However, several important questions remain unanswered. First, it is crucial to determine whether these observations translate into the clinical setting. Although there is little doubt that GCs cause whole-body insulin resistance when given to both animals and humans (11, 12), we have begun to challenge the widely held belief that GCs cause global pan-tissue insulin resistance. Second, the studies published to date have almost exclusively focused on the sc adipose tissue depot; however, GC administration causes a more dramatic (although not exclusive) increase in intraabdominal adipose tissue (13, 14). Although there has been a suggestion that the omental depot may respond differently to GC treatment (15), detailed studies have not been performed. Finally, it is widely believed that GCs cause hepatic insulin resistance, yet in rodent hepatocytes, GCs and insulin work synergistically to promote lipid accumulation (16). Studies in human models have not been performed.

Materials and Methods

Clinical protocol

The clinical protocol received full ethical approval from the South Birmingham Local Research Ethics Committee (reference 10/H1207/15). Fifteen healthy volunteers [mean age 33 ± 2 years, body mass index (BMI) 26.6 ± 1.0 kg/m^2, 9 male, 6 female] were recruited from local advertisement, and all were nondiabetic, were on no regular medication, were normotensive, and had not used GCs within the last 6 months. Female volunteers had pregnancy excluded and were not taking any hormonal contraception. At 5:00 PM, volunteers were admitted to the research facility, and total body water was estimated using bioimpedance (model BC418MA; Tanita, Amsterdam, The Netherlands). To determine the rates of de novo lipogenesis (DNL), volunteers were given oral ^2H_2O (3 g/kg total body water in 2 divided doses) at 6:00 and 10:00 PM followed by drinking water enriched to 0.4%. An adipose tissue microdialysis catheter (CMA Microdialysis, Solna, Sweden) was inserted under a local anesthetic 10 cm lateral to the umbilicus and microdialysis samples taken (0.3 μL/min) hourly until 12:00 AM, 2-hourly overnight and then every 30 minutes during the hyperinsulinemic clamp. A standardized meal was provided at 6:00 PM (carbohydrate 45 g, protein 23 g, and fat 20 g) and after the meal, the volunteer remained fasted until the end of the clamp. Volunteers were randomized to receive iv hydrocortisone (0.2 mg/kg·h) or saline in a double-blind protocol, and the infusion commenced at 6:00 PM until the end of the clamp the next day.

At 08:00 AM the next morning, the volunteers underwent a 2-step hyperinsulinemic euglycemic clamp after an overnight fast. A bolus of U-^{13}C-glucose (CK Gas Ltd, Hook, United Kingdom) was administered (2 mg/kg) over 1 minute followed by a constant infusion rate (0.02 mg/kg/min) for 2 hours prior to starting the glucose and insulin infusions. Basal steady-state samples were taken at 3 time points during the final 30 minutes of the 2 hours before insulin and glucose infusions. At 10:00 AM, insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused at 20 mU/m^2 · min with concomitant 20% glucose enriched with U-^{13}C-glucose to 4%. Arterialized blood samples were taken at 5-minute intervals and the glucose infusion rate changed to maintain fasting glycemic levels. Steady-state samples were taken at 3 time points in the final 30 minutes, 2 hours after starting the insulin infusion. The insulin infusion rate was then increased to 100 mU/m^2 · min for 2 hours with sampling as described above. Rates of glucose production (Ra glucose) and glucose disposal were calculated by using modified versions of the Steele equations (17, 18).

Volunteers then underwent a washout period of at least 2 weeks before undergoing an identical protocol but receiving the alternative infusion. All volunteers therefore received both infusions in a random order, separated by at least 2 weeks, with investigators and volunteers blinded to the nature of each infusion. A schematic diagram detailing the protocol is included in the Supplemental Data (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Biochemical and stable isotope analysis

Blood counts and biochemistry including nonesterified fatty acids (NEFAs), cortisol, and insulin were measured using standard laboratory methods or commercially available ELISAs, and details are provided in the Supplemental Data. Microdialysate samples were collected in microvials and analyzed using a mobile photometric, enzyme-kinetic analyzer (CMA Iscus Flex, Solna, Sweden) for glucose, pyruvate, lactate, and glycerol. The enrichment of U-^{13}C-glucose in plasma was determined by gas chromatography-mass spectrometry (model 5973; Agilent Technologies, Cheshire, United Kingdom).

Deuterium enrichment of the body water pool was measured using the Gasbench II (http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_27060.pdf) coupled online to a ThermoFinnigan Deltaplus XP isotope ratio mass spectrometer (ThermoFinnigan MAT GmbH, Bremen, Germany). Deuterium enrichment in the palmitate fraction of total plasma triglycerides was measured on an automated GC/TC/isotope ratio mass spectrometer system (ThermoFinnigan Delta Pus XP; http://www.thermo.com/eThermo/CMA/PDFs/Product/productPDF_27059.pdf). Full methods are described in the Supplemental Data.

Calculation of the contribution of DNL to total palmitate synthesis

The fraction of endogenous palmitate synthesis produced by DNL and was calculated from the incorporation of $^{2}H_2O$ in the palmitate present in the plasma total triglyceride pool and was calculated using the following formula: fraction = (Δ $^{2}H/^{1}H$ ratio in palmitate methylester/Δ $^{2}H/^{1}H$ ratio in waterpool)(34/22), where 34 is the total number of H atoms in palmitate methylester and 22 is the number of water molecules incorporated into palmitate via DNL (19–21).

Primary human adipocytes

Paired primary human sc and omental intact adipocytes were isolated from adipose tissue of healthy, nondiabetic donors aged 43–81 years (10 males, 13 females, median 66 years), BMI 21–38.8 kg/m^2 (median 27.7 kg/m^2) undergoing elective surgery for nonmalignant, noninflammatory conditions as described previously (22). None were taking GC therapy or any drugs known to impact on insulin sensitivity. After isolation, cells were incubated in serum-free media for 24 hours prior to treatment with GCs (for specific conditions, see Results). In all cell culture experiments investigating insulin signaling cascade protein phosphorylation, the media were spiked with human insulin (0.1 μg/mL; Sigma, Poole, United Kingdom) for the final 15 minutes of the treatment period. All treatments and reagents were supplied by Sigma unless otherwise stated.

Primary human hepatocytes

Primary human hepatocytes were purchased from Celsis In Vitro Technologies (Baltimore, Maryland). All donors were healthy, male, and nondiabetic and were on no regular medications with negative viral hepatitis serology, and none consumed alcohol above the recommended limits (n = 4, aged 54 ± 14 years, BMI 28.4 ± 3.3 kg/m^2). Cells were cultured overnight in Williams' Medium E without any supplements before being treated with GCs (cortisol dose range 100, 250, and 1000 nM, 24 hours). For insulin signaling studies, media were spiked with insulin 15 minutes prior to cell harvest as described above. Lipogenesis was measured by the uptake of 1-[^{14}C]acetate into the lipid component as described previously (5) and expressed as disintegrations per minute per well.

RNA extraction, reverse transcription, and real-time PCR

Total RNA was extracted using the Tri-Reagent system. RNA integrity, reverse transcription, and real-time PCR were performed as described previously (4). All probes and primers were supplied by an Assay on Demand (Life Technologies, Paisley, United Kingdom). All reactions were normalized against the housekeeping gene 18S rRNA. Data were expressed as the cycle number at which logarithmic PCR plots cross a calculated threshold line (ct values) and used to determine Δct values[(Δct = (ct of the target gene) − (ct of the housekeeping gene)]. Fold changes were calculated using the transformation [fold increase = 2−difference in ΔCT].

Protein extraction and immunoblotting

Protein extraction and immunoblotting were performed as described previously (5). Primary [anti-PKB/akt; Biosource, Nivelles, Belgium; and antiphospho-PKB/akt (serine 473), R&D Systems, Abingdon, United Kingdom] and secondary antibodies (Dako, Glostrop, Denmark) were used at a dilution of 1:1000. Membranes were reprobed for β-actin. Primary and secondary antibodies were used at a dilution of 1:5000 (Abcam PLC, Cambridge, United Kingdom). Bands were quantified with Genesnap by Syngene (Cambridge, United Kingdom).

Statistical approach

Data are presented as mean ± SE unless otherwise stated. Area under the curve analysis was performed using the trapezoidal method. For comparison of single variables, paired *t* tests have been used (or nonparametric equivalents in which data were not normally distributed). Where repeated samples were taken (either during an individual investigation or for comparison of the same investigation between the 2 different infusions), repeated-measures ANOVA on ranks was used, incorporating Dunn's test as a post hoc analysis. All analyses were performed using the SigmaStat 3.1 software package (Systat Software, Inc, Point Richmond, California).

Results

Overnight hydrocortisone infusion significantly increased circulating cortisol levels (1139 ± 60 vs 405 ± 42 nmol/L, $P < .00001$ vs saline). There was no impact on blood pressure, renal function, or electrolytes (data not shown). Total cholesterol levels were unchanged; however, high-density lipoprotein cholesterol and circulating NEFA concentrations increased, whereas triglyceride levels decreased. Osteocalcin has recently been implicated in GC-mediated changes in metabolic phenotype (23), and circulating osteocalcin levels were significantly lower after overnight hydrocortisone infusion (1.9 ± 0.6 vs 11.8 ± 1.5 ng/mL, $P < .005$ vs saline). Absolute data are presented in Supplemental Table 1.

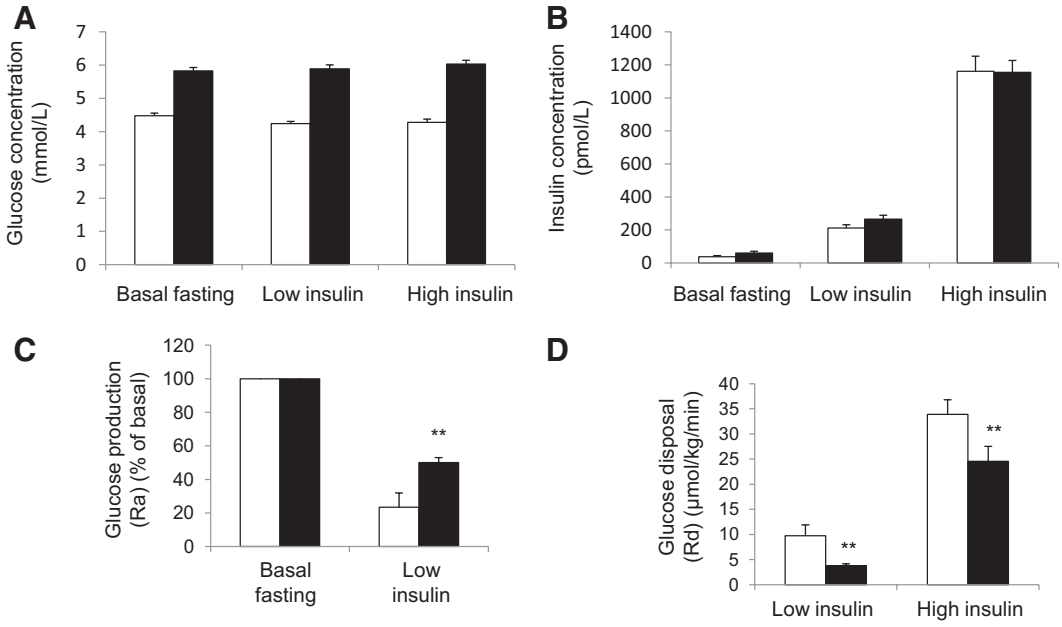


Figure 1. Circulating glucose (A) and insulin (B) concentrations during a 2-step hyperinsulinemic euglycemic clamp. Overnight hydrocortisone infusion (0.2 mg/kg · h) causes insulin resistance as measured by the suppression of glucose production rate (C) and glucose disposal (D). Open bars, saline; black bars, hydrocortisone. $^{**}P < .01$ vs saline.

GCs cause systemic insulin resistance

Fasting insulin levels were unchanged (59.6 ± 11.3 vs 38.0 ± 6.5 pmol/L, $P = .11$), but glucose concentrations increased (5.8 ± 0.1 vs 4.5 ± 0.1 mmol/L, $P < .0001$) (Figure 1, A and B) after hydrocortisone infusion, representing a failure of insulin secretion in the face of systemic insulin resistance. During the 2-step hyperinsulinemic clamp, glucose infusion rates in response to both low and high insulin concentrations decreased in 13 of 15 volunteers after hydrocortisone treatment, consistent with insulin resistance (low insulin: 1.6 ± 0.1 vs 3.0 ± 0.4 mg/kg · min, $P < .01$; high insulin: 7.3 ± 0.7 vs 9.1 ± 0.5 mg/kg · min, $P < .005$). Endorsing this observation, body weight-adjusted rate of glucose disposal rates flowing both low- and high-dose insulin infusion were lower after hydrocortisone in comparison with saline (low insulin: 3.8 ± 0.6 vs 9.8 ± 2.2 μmol/kg · min, $P < .01$; high insulin: 24.6 ± 3.0 vs 33.0 ± 2.9 μmol/kg · min, $P < .005$) (Figure 1A), indicative of systemic insulin resistance, largely reflecting the actions of GC upon skeletal muscle.

Hepatic insulin sensitivity

Despite the increase in fasting blood glucose concentrations, endogenous Ra glucose production rates were not different after hydrocortisone or saline infusions (12.9 ± 0.6 vs 11.9 ± 1.1 μmol/kg · min, $P = .2$) (Figure 1). Glucose production rates were lower after low-dose insulin infusion in the saline arm (6.5 ± 0.6 vs 3.2 ± 1.1 μmol/kg · min, $P < .005$), However, the suppression of glucose production rate by insulin was lower in the hydrocortisone arm in comparison with saline, indicative of hepatic insulin resistance (Ra glucose percentage of basal: 50.0 ± 3.0 vs 23.4 ± 8.5%, $P < .01$) (Figure 1C).

To understand the mechanisms that might contribute to these observations, additional experiments were performed in primary cultures of human hepatocytes. Insulin stimulated akt/PKB serine 473 phosphorylation (p-ser473akt/PKB) (data not shown), and although treatment with cortisol had no significant impact on total akt/PKB protein levels, insulin-stimulated p-ser473akt/PKB increased, consistent with GC-induced insulin sensitization, contrasting with our in vivo observations (Supplemental Figure 2).

De novo lipogenesis

The percentage contribution of DNL to palmitate synthesis decreased in 12 of 15 volunteers after hydrocortisone infusion, but this failed to reach statistical significance (4.1 ± 1.0 vs 5.2 ± 1.3%, $P = .22$) (Figure 2A). Due to the combinations of stable isotopes used within the clinical protocol, it was not possible to measure rates of DNL under hyperinsulinemic conditions.

Extending our clinical observations, cortisol decreased DNL in the primary cultures of human hepatocytes [data are expressed as a percentage change from control (100%); 85.6 ± 6.6% (100 nM), 73.5 ± 7.9% (250 nM), 55.0 ± 5.6% (1000 nM), $P < .05$] (Figure 2B). Insulin alone (5 nM, 24 hours) had a modest impact on acetate incorporation into lipid (129.1 ± 13.0%); however, co-incubation with cortisol (1000 nM, 24 hours) significantly enhanced insulin-stimulated lipogenesis (148.8 ± 10.4%, $P < .05$ vs insulin treatment alone) (Figure 2C), indicating

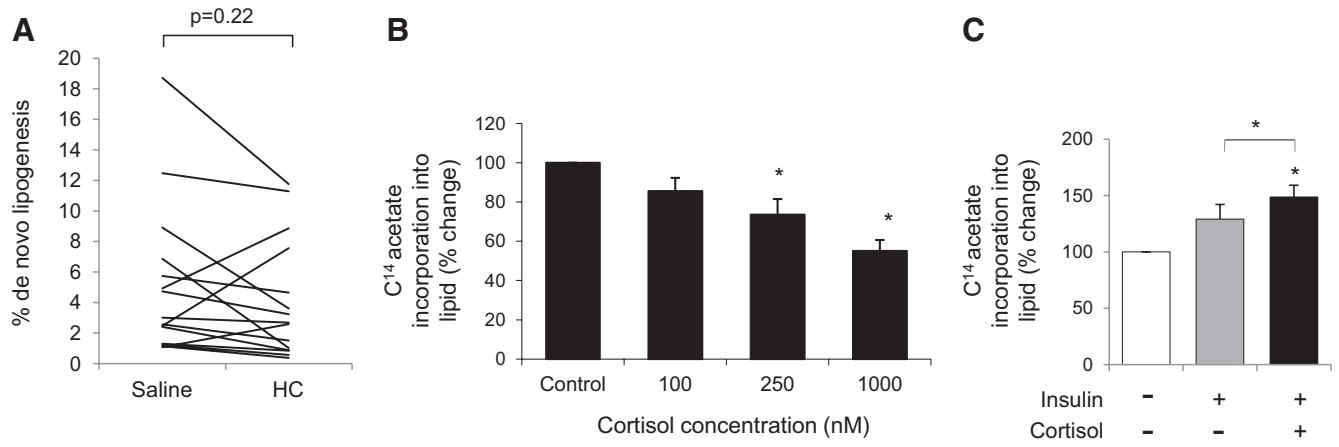


Figure 2. The contribution of de novo lipogenesis to palmitate synthesis does not differ after overnight hydrocortisone or saline infusion (A). However, in primary cultures of human hepatocytes, cortisol causes a dose-dependent decrease in C^{14}-acetate incorporation into lipid (n = 4). *$P < .05$ vs control (B). Although insulin has a modest effect to stimulate lipogenesis, this is augmented in the presence of cortisol (1000 nM, 24 hours) (n = 4), *$P < .05$ (C).

that GCs and insulin are acting synergistically to enhance lipid accumulation within hepatocytes.

Depot-specific, adipose tissue insulin sensitization by GCs

Hydrocortisone increased circulating NEFA levels, and these decreased after both low- and high-dose insulin infusion (Figure 3A). The magnitude of suppression of circulating NEFAs by insulin (both low and high dose) was significantly greater after hydrocortisone infusion (low insulin: 564 ± 40 vs 403 ± 38, $P < .01$ μmol/L · h; high insulin: 712 ± 35 vs 443 ± 38 μmol/L · h, $P < .005$) (Figure 3B). Adipose tissue interstitial fluid glycerol release increased after overnight hydrocortisone (395 ± 49 vs 286 ± 40 μmol/L · h, $P < .05$) and was suppressed by insulin (low insulin: 143 ± 18 μmol/L · h; high insulin: 66 ± 14 μmol/L · h, both $P < .05$ vs basal) (Figure 3, C and D). The magnitude of the suppression of lipolysis by insulin (both high and low dose) was not different after hydrocortisone or saline, providing no evidence for adipose tissue insulin resistance (Figure 3E).

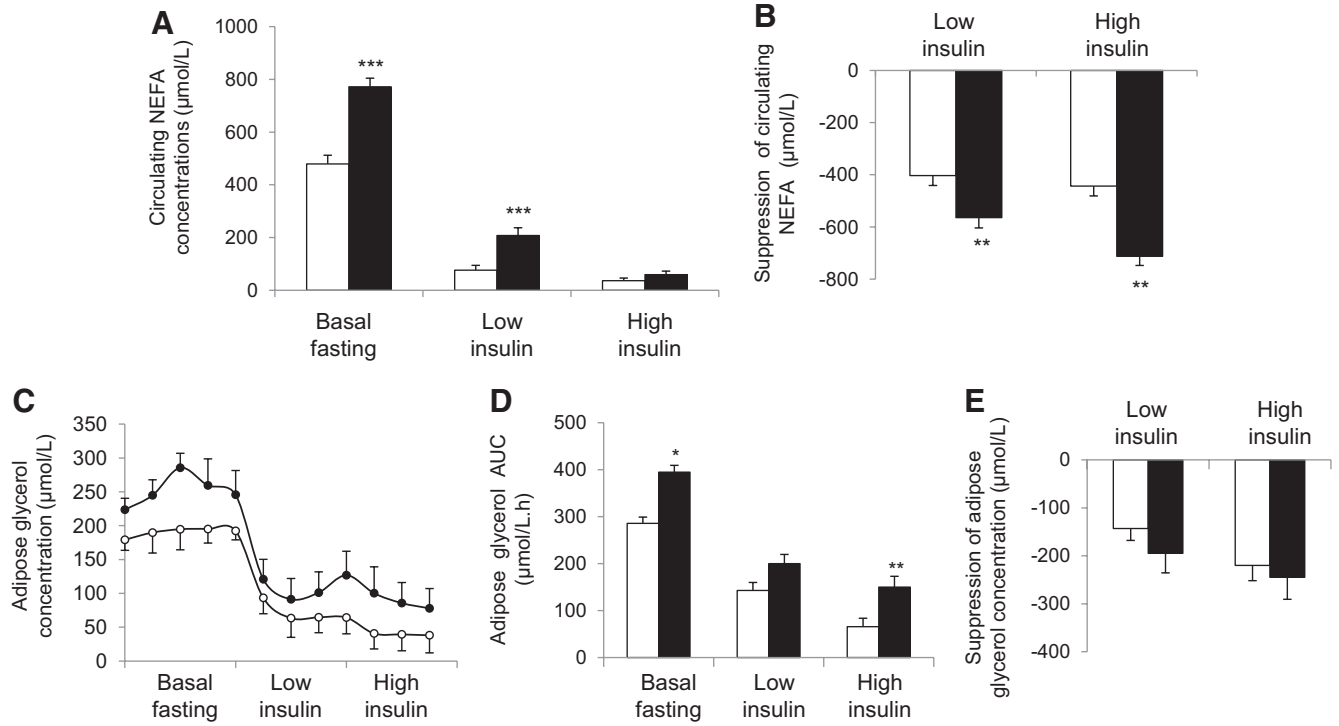
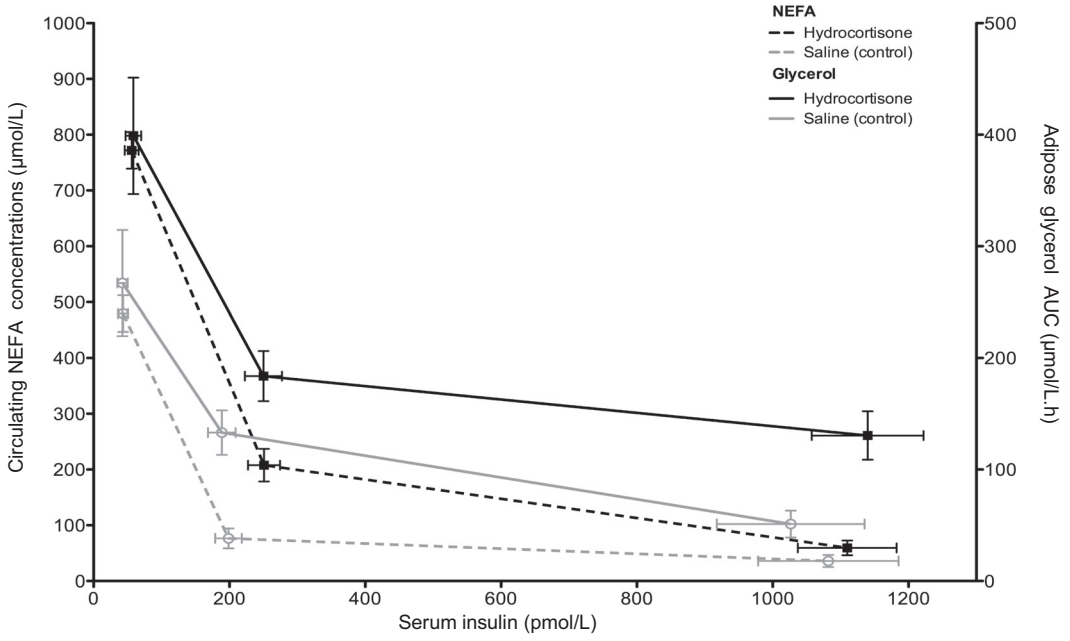


Figure 3. Overnight hydrocortisone infusion increases circulating NEFA levels, which are suppressed after low- and high-dose insulin infusions as part of the hyperinsulinemic euglycemic clamp (A). The magnitude of the suppression of circulating NEFAs by insulin is greater after overnight hydrocortisone infusion in comparison with saline (B). Subcutaneous adipose tissue interstitial fluid glycerol concentrations are increased by hydrocortisone and are suppressed by insulin (C and D). The magnitude of the suppression of adipose interstitial fluid glycerol by low- and high-dose insulin is not different after saline or hydrocortisone infusion (E). Open bars/circles, saline; black bars/circles, hydrocortisone. *$P < .05$, **$P < .01$, ***$P < .005$ vs saline.



Insulin conc. (pmol/L) ½-maximal suppression of **circulating NEFA**		
Saline	Hydrocortisone	p
65 14	110 16	0.001

Insulin conc. (pmol/L) ½-maximal suppression of **sc adipose glycerol**		
Saline	Hydrocortisone	p
115 12	140 19	0.1

Figure 4. Insulin suppresses circulating NEFAs and subcutaneous adipose tissue glycerol after both hydrocortisone and saline infusions. The insulin concentration causing half-maximal suppression of circulating NEFAs was greater after hydrocortisone. However, the insulin concentration needed to cause half-maximal suppression of glycerol release, specifically within subcutaneous adipose tissue, was not different.

Based on the assumption that high-dose insulin causes maximal suppression of circulating NEFAs as well as sc adipose glycerol generation, the insulin concentrations causing half-maximal suppression of NEFA (INS-½-max NEFA) and glycerol (INS-½-max GLY) were calculated for each subject using regression analysis. INS½-max NEFA was higher after hydrocortisone (65 ± 14 vs 110 ± 16 pmol/L, $P < .005$). However, INS-½-max GLY was not different between saline and hydrocortisone (115 ± 12 vs 140 ± 19 pmol/L, $P = .1$) (Figure 4), suggesting depot specificity of action.

Hydrocortisone alone had no effect on pyruvate generation from adipose tissue in vivo (99 ± 13 vs 88 ± 15 μmol/L · h, $P = .6$). As expected, insulin increased interstitial fluid pyruvate generation (low insulin: 134 ± 17 μmol/L · h; high insulin: 151 ± 18 μmol/L · h, both $P < .005$ vs basal) (Figure 5, A and B), and this was dramatically enhanced after hydrocortisone, consistent with insulin sensitization (high insulin: 284 ± 23 μmol/L · h, $P < .005$ vs saline) (Figure 5C).

Hydrocortisone suppressed lactate levels in adipose interstitial fluid (2.3 ± 0.3 vs 4.3 ± 0.7 μmol/L · h, $P < .05$) (Figure 5, D and E). Insulin infusion had no effect on lactate generation; however, in combination with hydrocortisone lactate, concentrations increased after high-dose insulin infusion, potentially as a consequence of increased pyruvate (high insulin: 3.5 ± 0.4 μmol/L · h, $P < .01$ vs basal) (Figure 5F).

Cortisol (1000 nM, 24 hours) had no impact on insulin receptor expression or phosphorylation in paired isolated intact sc and omental adipocytes (data not shown). However, insulin-stimulated p-ser473akt/PKB increased in sc adipocytes pretreated with cortisol as did IRS2 mRNA expression (Figure 6, A and B, and Supplemental Table 2). In contrast, parallel experiments performed in paired isolated intact omental adipocytes failed to show regulation of insulin-stimulated p-ser473akt/PKB or IRS2 expression (Figure 6, C and D, and Supplemental Table 2). In both the sc and omental depots, cortisol increased hormone-sensitive lipase expression, consistent with its known lipolytic effect (Figure 6, E and F, and Supplemental Table 2). The response to GC treatment did not differ in isolated intact adipocytes from lean or obese individuals.

Discussion

Combining in vitro cell biology with clinical data, we have established tissue-specific actions of GCs upon insulin action. We have demonstrated that short-term iv GC administration causes skeletal muscle insulin resistance but, in contrast, in sc adipose tissue, causes insulin sensitization

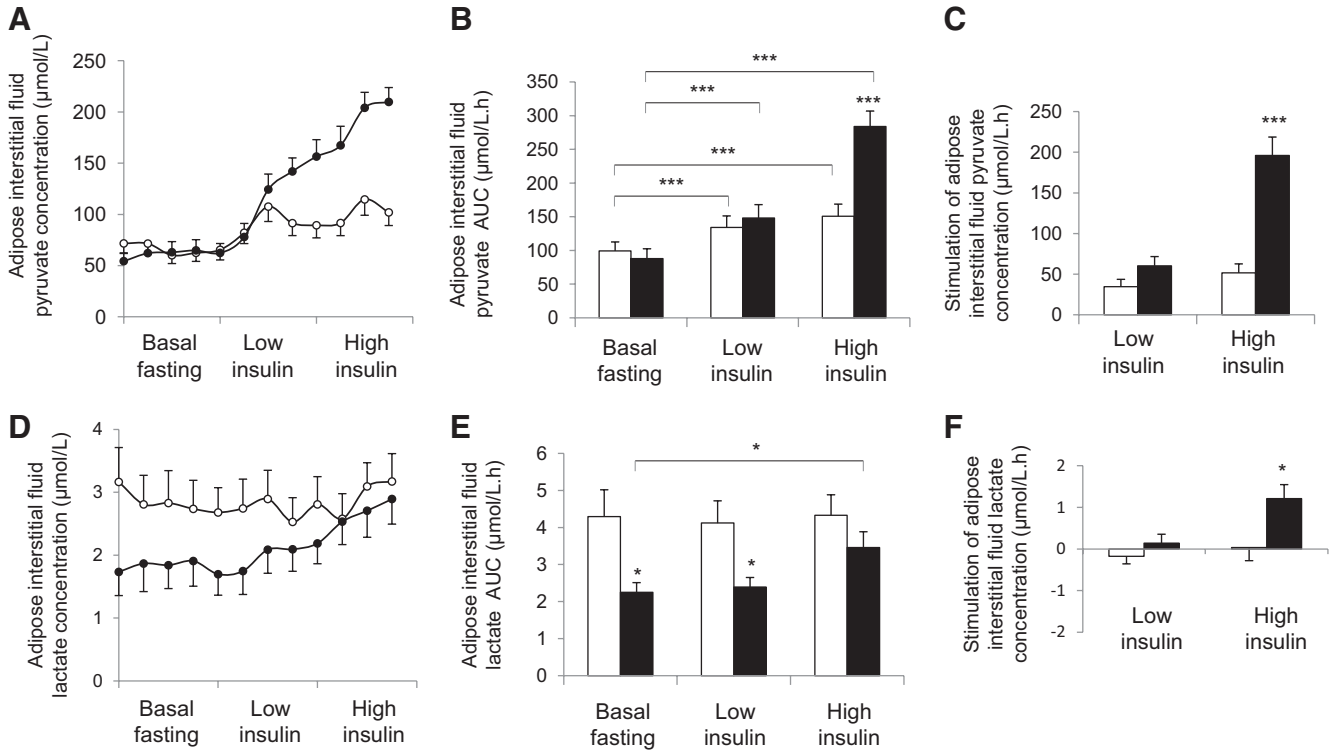


Figure 5. Hydrocortisone infusion does not impact on basal rates of pyruvate generation within sc adipose tissue. Insulin increases adipose interstitial fluid pyruvate levels, which is augmented in the presence of hydrocortisone (A–C). Hydrocortisone infusion decreases lactate concentrations within sc adipose tissue. Although insulin alone does not affect lactate levels, in the presence of hydrocortisone, lactate levels increase in adipose tissue interstitial fluid (D–F). Open bars/circles, saline; black bars/circles, hydrocortisone. $*P < .05$, $***P < .005$ vs saline or basal.

(increased insulin stimulated pyruvate generation) in vivo, which was endorsed by experiments in isolated intact adipocytes as well as previously published observations in differentiated preadipocytes (4, 5, 7). Similarly, GCs cause insulin sensitization in primary human hepatocyte cultures and augment the lipogenic action of insulin but appeared to cause hepatic insulin resistance in vivo as evidenced by decreased insulin mediated suppression of endogenous glucose production.

Postprandial glucose uptake is largely driven by the action of insulin on skeletal muscle. There is little doubt that GCs cause whole-body insulin resistance (11), and we would argue that this reflects their action upon skeletal muscle. We have shown decreased glucose disposal rates after hydrocortisone compared with saline under both low and high insulin concentrations. The molecular mechanisms that underpin GC-mediated insulin resistance in skeletal muscle are not fully understood. We have previously shown that GCs increase serine phosphorylation of IRS1 at residue 307, and this impairs the ability if IRS1 to interact with the insulin receptor and in addition targets it for proteasomal degradation (9). Interestingly, insulin resistance was not observed in 2 volunteers, and this did not appear to be related to basal insulin sensitivity, BMI, or sex. This may well represent variability in the susceptibility to the adverse effects of GCs that could be underpinned by differences in GC receptor expression or prereceptor GC metabolism and/or clearance.

The interaction between GCs and insulin action in human liver has not previously been investigated in detail. Insulin was less able to suppress glucose production after hydrocortisone compared to saline infusion which would be consistent with GC-induced hepatic insulin resistance. However, our primary hepatocyte culture experiments demonstrated increased insulin stimulated p-ser473akt/PKB after GC treatment. The differences between our in vivo and in vitro observations are likely to reflect the multiple factors in vivo that are able to regulate hepatic insulin sensitivity, contrasting with relatively simplistic cell culture models and emphasize the importance of translating laboratory-based findings into the clinical setting. Although accepting that there may be species specificity of the response to GCs, rodent studies have provided conflicting evidence; high-dose prednisolone (10 mg/kg · d) for 7 days given to mice fed a normal chow diet caused hepatic insulin resistance (24). However, when fed a high-fat diet (HFD) for 6 weeks with an identical dose of prednisolone given for the final 7 days of the HFD, there was no evidence of worsening hepatic insulin resistance during hyperinsulinemic euglycemic or hyperglycemic clamps (25).

The interaction between GCs and insulin has been examined previously in rodent models (26, 27), and in these

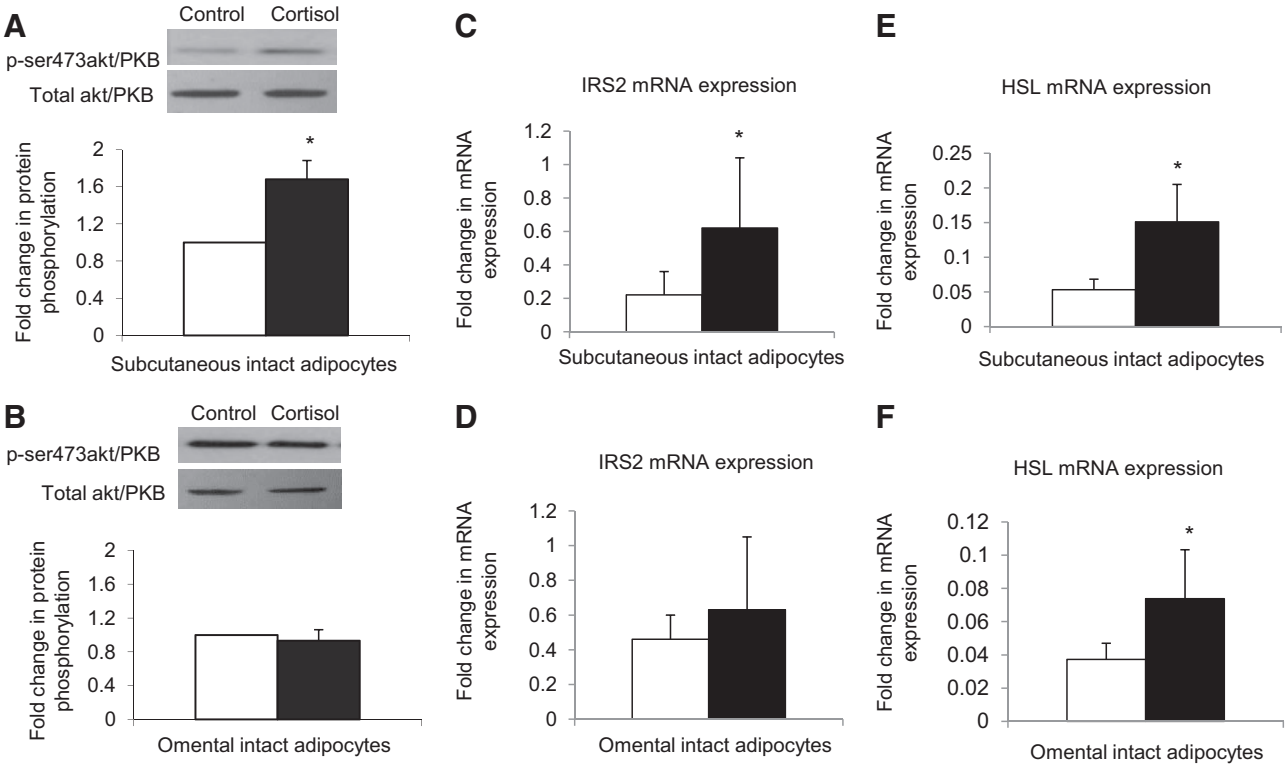


Figure 6. Cortisol (1000 nM, 24 hours) increases insulin-stimulated p-ser473akt/PKB in isolated intact sc (A) but not omental adipocytes (B). IRS2 mRNA expression is increased by cortisol in sc adipocytes only (C and D). Hormone-sensitive lipase mRNA expression is increased by cortisol in both sc and omental adipocytes (E and F) (n = 3–6 experiments in triplicate). Open bars, control; black bars, cortisol. *$P < .05$ vs untreated control.

systems there is evidence to suggest that GCs together with insulin may enhance lipogenesis (16). In primary cultures of human hepatocytes, GC-suppressed lipogenesis in the absence of insulin. However, in contrast, although insulin stimulates lipogenesis, GCs and insulin worked synergistically to further enhance lipid synthesis as has been shown in primary cultures of human adipocytes (5). In our clinical study, hydrocortisone infusion decreased lipogenesis in 12 of 15 subjects in the fasting state (80%). If our in vitro observations in the presence of insulin translate to the clinical setting, then it is likely that in the postprandial state, GCs and insulin working together will drive triglyceride synthesis within the liver. Rodent models have provided some support for this hypothesis; the combination of HFD and GC treatment significantly worsens metabolic phenotype and exacerbates the development of hepatic steatosis (28). Importantly, our in vitro data were generated using hepatocytes exclusively from male donors, but there were no differences in response in the clinical study between male and female volunteers. It therefore seems unlikely that there is a sexual dimorphism in the interaction between GC and insulin. There is an emerging interest in the interaction between bone and metabolic phenotype. Until very recently, this had not been explored in the context of GC administration. However, hepatic overexpression of osteocalcin is able to ameliorate the effects of exogenous GCs upon hepatic insulin sensitivity (23), and in this clinical study, we have been able to show that short-term administration of GCs (albeit at pharmacological doses) dramatically suppressed osteocalcin levels. Although this is only observational data, it remains plausible that this may be a crucial mechanism regulating the adverse metabolic effects of GCs.

GCs are potent regulators of lipid metabolism in adipose tissue (29). We and others have shown that GCs cause insulin sensitization in human adipose tissue and that this is functionally important leading to increased glucose uptake and lipogenesis (4, 6). This contrasts with observations in rodents in which GCs appear to cause adipocyte insulin resistance and reflects the fact that rodents do not develop a classical Cushing's phenotype when treated with GCs, highlighting the importance of conducting studies using human models (30, 31). Fasting NEFA levels increased in the hydrocortisone group, largely reflecting adipose tissue lipolysis driven by hormone-sensitive lipase and adipose triglyceride lipase (32, 33). Insulin suppresses lipolysis and it was able to do this in both the hydrocortisone and saline arm. Although the magnitude of the suppression of circulating NEFA levels by insulin was greater in the hydrocortisone arm INS-½-max NEFA was also increased. Using adipose tissue microdialysis, sc interstitial fluid glycerol concentrations paralleled circulating NEFA levels. Insulin (both low and high dose) was able to suppress glycerol release to a similar degree in both hy-

drocortisone and saline arms, and there was no difference in INS-½-max GLY. The differences between the effects of hydrocortisone on circulating NEFAs vs interstitial fluid glycerol generation may well reflect adipose depot-specific actions. Insulin stimulated pyruvate generation in sc adipose tissue interstitial fluid, and although hydrocortisone alone was without effect, it dramatically augmented the effect of insulin consistent with insulin sensitization. Our in vitro observations have suggested a depot specificity of effect of pharmacological doses of GCs; in sc adipocytes, cortisol caused insulin sensitization with increased insulin-stimulated p-ser473akt/PKB and IRS2 mRNA expression, and it is likely that these 2 mechanisms both contribute independently to insulin sensitization. However, these responses were absent in the paired adipocytes isolated from the omental depot and are consistent with depot-specific observations reported previously (15). Performing clinical studies to assess the dynamic response to GCs is challenging and fraught with technically difficulty, principally due to lack of access to the intraabdominal adipose tissue depot.

There are few published data that have examined the tissue-specific interaction between GCs and insulin in vivo. Prednisolone 7.5 mg daily for 2 weeks had few adverse metabolic effects in contrast to 30 mg daily, which increased hepatic glucose production rate, decreased suppression of hepatic glucose production by insulin, and interestingly decreased lipolysis (34). Prednisone 10 mg (that requires activation by 11β-hydroxysteroid dehydrogenase type 1) given to healthy male volunteers daily for 1 week had a minimal impact upon glucose disposal, whereas higher doses of prednisone (25 mg daily) decreased glucose disposal (35). The discrepancies between these observations and our own may perhaps reflect the duration of treatment, the route of administration and/or differences between prednisolone/prednisone and hydrocortisone.

Importantly, there are limitations in the interpretation of the data from this study. Due to the challenges in obtaining primary cultures for in vitro experiments, the metabolic characteristics of the donors of the cells may not be as closely matched to those in the clinical study as would have been desired, although important discrepancies have been avoided (coexistent diabetes and other pathology, medication including glucocorticoids). As part of the clamp studies, we have not infused somatostatin and this may result in differences in insulin concentration between the peripheral and portal circulations. Furthermore, the lower dose of insulin chosen (20 mU/m^2 · min) may, in some individuals, fully suppress endogenous glucose production, limiting the interpretation of the data. However, this was not the case in our study (Figure 1A). Finally, we have not measured adipose tissue blood flow as part of the microdialysis protocol; however, we are reassured by data from previous studies that has shown that hydrocortisone has no effect on adipose tissue blood flow (36).

In conclusion, this study has enhanced our understanding of the impact of both pharmacological GC treatment as well as endogenous GCs upon insulin action and metabolic phenotype. We have challenged the concept that GCs cause insulin resistance in all tissues and clearly demonstrated a tissue-specific interaction between GCs and insulin in vivo. There is little doubt that GCs cause skeletal muscle insulin resistance. In the liver, in the fed state when insulin is high, they may potentiate the action of insulin to drive lipogenesis, and this may explain the development of hepatic steatosis in Cushing's syndrome (37). In adipose tissue, GCs drive adipocyte differentiation (8) and specifically within the sc depot, enhancing insulin action will decrease lipid turnover through decreased lipolysis and increased lipogenesis. In the omental depot, this positive interaction between GCs and insulin does not occur, perhaps resulting in sustained NEFA release and increased lipid turnover that may fuel the global GC-associated metabolic phenotype with NEFA delivery to liver and skeletal muscle. Understanding this interaction has significant clinical implications. The timing and patterns of GC administration in the fed or fasted state are likely to be important. Prolonged exposure to GCs in the absence of insulin in the fasting state may lead to adverse fuel mobilization rather than safe storage. Furthermore, synthetic GCs with differing and often prolonged pharmacokinetic profiles in comparison with cortisol are likely to have different metabolic effects in vivo, reflecting the critical interaction with insulin.

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