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Original Article

ORIGINAL ARTICLE

GLUCOCORTICOIDS, BONE, AND INSULIN RESISTANCE

PARKER ET AL.

Glucocorticoid-Induced Insulin Resistance in Men Is Associated With Suppressed Undercarboxylated Osteocalcin¹

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ABSTRACT

In mice, glucocorticoid-induced insulin resistance occurs largely through impaired osteoblast function and decreased circulating undercarboxylated osteocalcin (ucOC). Whether these mechanisms contribute to glucocorticoid-induced insulin resistance in humans has yet to be established. In addition, the effects of glucocorticoids on the exercise-induced increase in circulating ucOC and insulin sensitivity are also unknown. We hypothesized that acute glucocorticoid treatment would lead to basal and postexercise insulin resistance in part through decreased circulating ucOC and ucOC-mediated skeletal muscle protein signaling. Nine healthy men completed two separate cycling sessions 12 hours after ingesting either glucocorticoid (20 mg prednisolone) or placebo (20 mg Avicel). The homeostatic model assessment was used to assess basal insulin sensitivity and a 2-hour euglycemic–hyperinsulinemic clamp was commenced 3 hours after exercise to assess postexercise insulin sensitivity. Serum ucOC and skeletal muscle protein signaling were measured. Single-dose glucocorticoid ingestion increased fasting glucose (27%, $p < 0.01$) and insulin (83%, $p < 0.01$), and decreased basal insulin sensitivity (-47%, $p < 0.01$). Glucocorticoids reduced insulin sensitivity after cycling exercise (-34%, $p < 0.01$), reduced muscle GPRC6A protein content (16%, $p < 0.05$), and attenuated protein phosphorylation of mTOR^{Ser2481}, Akt^{Ser374}, and AS160^{Thr642} (59%, 61%, and 50%, respectively; all $ps < 0.05$). Serum ucOC decreased (-24%, $p < 0.01$) which correlated with lower basal insulin sensitivity ($r = 0.54$, $p = 0.02$), lower insulin sensitivity after exercise ($r = 0.72$, $p < 0.05$), and attenuated muscle protein signaling ($r = 0.48$ - 0.71 , $p < 0.05$). Glucocorticoid-induced basal and postexercise insulin resistance in humans is associated with the suppression of circulating ucOC and ucOC-linked protein signaling in skeletal muscle. Whether ucOC treatment can offset glucocorticoid-induced insulin resistance in human subjects requires further investigation. © 2018 American Society for Bone and Mineral Research

KEY WORDS: GLUCOCORTICOID METABOLISM; ANTI-INFLAMMATION; GLYCEMIC CONTROL; HIGH-INTENSITY INTERVAL EXERCISE; INSULIN SIGNALING

Introduction

Osteoblasts synthesize and secrete noncollagenous proteins including osteocalcin (tOC). The undercarboxylated form of osteocalcin (ucOC) is reported to regulate insulin secretion and sensitivity in mice.^(1,2) For example, ucOC-deficient mice are insulin resistant, whereas treatment of obese mice with ucOC restores insulin secretion and sensitivity and enhances

insulin-stimulated glucose uptake following ex vivo muscle contraction.^(1,3,4) In humans, ucOC may also regulate glycemic control and postexercise insulin sensitivity.⁽⁵⁻⁹⁾ However, evidence for this relies on cross-sectional and observational studies.⁽¹⁰⁾ The daily control of glucose and insulin is important for the prevention and management of type 2 diabetes and other cardiometabolic conditions.⁽¹¹⁾ As such, further exploration of potential novel targets that mediate glycemic control, including the mechanisms behind the postexercise enhancement of insulin sensitivity, is warranted.

Insulin resistance associated with acute glucocorticoid (GC) treatment is reported to occur largely through the suppression of osteocalcin secretion rather than canonical GC signaling in the liver or skeletal muscle.^(12,13) In humans, acute GC ingestion decreases both insulin sensitivity⁽¹⁴⁾ and circulating ucOC,⁽¹⁵⁾ but whether there is a causal relationship between these traits has yet to be established. In addition, acute exercise increases circulating ucOC, which is associated with improved postexercise insulin sensitivity in humans⁽⁷⁾ and is reported to play an important role in skeletal muscle function and adaptation to exercise training in mice.^(16,17)

GC treatment and exercise are also associated with changes in circulating bone remodeling markers including β -isomerized C-terminal telopeptides (β -CTX) and procollagen 1 N-terminal propeptide (P1NP),^(14,16) which may also influence insulin sensitivity. However, this remains largely unexplored in humans.

In rodents, the ucOC-mediated glucose uptake signaling pathway in skeletal muscle includes the G protein-coupled receptor family C group 6-member A (GPRC6A) as the receptor with downstream activation of protein signaling pathways involving AMP-activated protein kinase (AMPK), protein kinase B (Akt), mechanistic target of rapamycin (mTOR), extracellular signal-regulated kinase (ERK), Akt substrate 160 (AS160), and/or increased circulation of interleukin-6 (IL-6).^(10,16,18,19) The role of these proteins in GC-induced insulin resistance and/or ucOC-mediated glycemic control in humans remains unclear. Furthermore, whether ucOC mediates postexercise enhancement of insulin sensitivity via these protein signaling

pathways is unknown. We hypothesized that acute GC administration would suppress circulating ucOC, leading to a decrease in insulin sensitivity at rest and after exercise by compromising ucOC-associated muscle protein signaling.

Materials and Methods

Screening

The study protocol has been published.⁽²⁰⁾ A schematic overview of the project protocol and the consort flow chart are provided in Fig. 1 and Supplemental Fig. 1, respectively. Men were specifically recruited to avoid the potential confounding effects of sex on glucose and bone metabolism. Seventeen men were initially screened for eligibility. Exclusion criteria for participation included men with osteoporosis, metabolic or cardiovascular disease, and/or those taking medication known to affect bone metabolism, insulin secretion, or insulin sensitivity. Men with musculoskeletal and/or orthopedic conditions (such as severe osteoarthritis) that prevented normal daily function, or were undergoing medication or supplementation that could affect research outcomes such as glucocorticoids, warfarin therapy, or vitamin K, were also excluded from participation. Two participants did not meet the inclusion criteria and 4 declined to participate. Two participants voluntarily withdrew from the study prior to completion. Nine young men that were recruited from the general public completed the randomized, double-blinded, crossover study.

Participants were asked to refrain from physical activity (48 hours), and alcohol and caffeine ingestion (24 hours) prior to all testing sessions. Eligible participants provided a fasting blood sample that was analyzed at Austin Health Pathology (Melbourne, Australia) for glucose, HbA1c, and insulin. Body composition was analyzed via DXA (Software version 9.1; GE Lunar Prodigy, Madison, WI, USA) to assess body fat, lean body mass, fat mass in the abdominal region, and BMD to exclude osteoporosis.

On a separate day, participants completed a graded exercise test (GXT) on a cycle ergometer (Lode Excalibur Sport; Lode Medical Technology, Groningen, The Netherlands) to measure peak cycling aerobic capacity (VO_{2peak}), maximal power output during the VO_{2peak} test

($W_{\max}$), and heart rate peak (HR_{peak}). The GXT protocol consisted of 1-min cycling stages starting at 20 W, which increased by 20 W every min until participants were unable to maintain a cycling cadence of 60 rpm or greater. Inspired and expired gas was analyzed via indirect calorimetry (Quark CPET; Cosmed, Rome, Italy). The $W_{\max}$ and HR_{peak} obtained during the GXT were used to calculate the workload for the main exercise sessions.

Experimental phase

After screening, participants underwent two identical sessions, with the exception of either prior placebo or GC ingestion, which included a single session of exercise, 3 hours of rested recovery, concluding with a 2-hour euglycemic–hyperinsulinemic clamp.

Twenty-four hours prior to their first session, participants completed a food diary that was replicated prior to subsequent sessions. Participants arrived in the laboratory in the morning after an overnight fast. A resting muscle biopsy and blood sample was taken; then participants performed a single session of high-intensity interval exercise (HIIE) on a cycle ergometer. The HIIE included a 6-min warm-up at 50% to 60% HR_{peak} , followed by 4×4 -min cycling intervals at 90% to 95% HR_{peak} , interspersed with 2-min active recovery periods at 50% to 60% HR_{peak} . The workloads (W) for both exercise sessions were adjusted accordingly to achieve the participants targeted HR, and thus maintain the same relative exercise intensity between sessions. The target HR for the exercise sessions was determined by the Karvonen heart rate reserve method: exercise target HR = (% of desired exercise intensity $\times$ ($HR_{\text{peak}} - HR_{\text{rest}}$)) + resting HR_{rest} . After completing the exercise, participants recovered for 3 hours on a bed, after which a 2-hour euglycemic–hyperinsulinemic clamp (clamp) was performed. Muscle biopsies were also taken prior to the clamp (3 hours postexercise) and after 2 hours of the clamp. Blood samples were taken immediately; 30 min; 1, 2, and 3 hours postexercise; and 1.5 and 2 hours during the clamp.

The procedures for the two experimental sessions were identical, with the exception that participants orally ingested a capsule of placebo (Avicel-microcrystalline cellulose NF PH105) or glucocorticoid (20 mg of prednisolone), 12 hours prior to the commencement of

exercise. The timing of ingestion was based on previously published data on the suppressive effect of acute GC treatment on osteocalcin in humans, which was confirmed during pilot testing in our own laboratory (data not shown).⁽¹⁵⁾ The order of GC or placebo was randomly allocated in a double-blind fashion, using a block randomization model and sealed opaque envelopes. The randomization was completed by a university staff member independent of the research and research team. All researchers remained blinded until data analysis was complete. Both the GC and placebo were compounded by Thompsons Pharmacy (VIC, Australia). The 2 experimental days (GC and placebo) were performed 1 to 3 weeks apart to ensure that the effects of acute GC ingestion and high-intensity exercise were “washed out.”^(21,22)

Euglycemic–hyperinsulinemic clamp

The clamp was performed as previously reported.⁽⁷⁾ A single slow-release tablet of potassium chloride (600 mg) was taken prior to the clamp to minimize the risk of potassium depletion. Insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at 40 mU/m²/min for 120 min generating an elevated, stable insulin concentration in the last 30 min of the clamp with no difference between trials (placebo: 498 ± 24 pmol/L; GC: 507 ± 15 pmol/L, $p < 0.01$ compared with baseline levels, $p = 0.56$ between trials). Insulin sensitivity was determined by the average glucose infusion rate (GIR; mg/kg⁻¹/min⁻¹) during the last 30 min of the insulin-stimulated period (M value) and the GIR expressed relative to per unit of insulin (M/I value).⁽²³⁾ Exogenous glucose was variably infused throughout the clamp to achieve a target blood glucose of ~ 5 mmol/L, which was assessed every 5 min from arterialized blood.

Skeletal muscle and blood sampling

Muscle samples were obtained from the vastus lateralis under local anesthesia (xylocaine 1%; AstraZeneca, Macquarie Park NSW 2113, Australia) utilizing a Bergström needle with suction.⁽²⁴⁾ The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Venous blood was collected from an antecubital vein via an intravenous cannula, with collection tubes containing ethylenediaminetetraacetic acid (EDTA) or clot activator and

serum gel separator. Blood samples were then separated into plasma/serum by centrifugation (10 min at 3500 rpm, 4°C) and immediately aliquoted and stored at -80°C until analyzed.

Biochemical analysis

Whole blood was analyzed immediately for blood glucose and lactate using an automated analysis system (YSI 2300 STAT Plus Glucose & Lactate Analyzer; YSI, Inc., Yellow Springs, OH, USA). Blood was analyzed at Austin Health Pathology using the standard protocols for HbA1c and insulin. Serum tOC was measured using an automated immunoassay (Elecsys 170; Roche Diagnostics, Mannheim, Germany). Serum ucOC was measured by the same immunoassay after adsorption of carboxylated OC on 5 mg/mL hydroxyl-apatite slurry, following the method described by Gundberg and colleagues.⁽²⁵⁾ Serum β -isomerized C-terminal telopeptides (β -CTX, a bone resorption marker) and procollagen 1 N-terminal propeptide (P1NP) were analyzed at Austin Health Pathology using a Roche Hitachi Cobas e602 immunoassay analyzer, according to the manufacturer's guidelines. IL-6 concentrations in serum were analyzed using a commercially available ELISA kit (Product #ab46042, Abcam, Cambridge, UK) as per the manufacturer's instructions. Serum IL-6 data for one participant were found to be a statistically significant outlier ($p < 0.05$; extreme studentized deviate test) and thus was excluded from IL-6 statistical analysis and correlations ($n = 8$). Importantly, exclusion of these data does not alter the main outcomes or conclusions of this study. Insulin resistance was estimated using the homeostatic model assessment (version 2) for insulin resistance (HOMA2-IR) using the Oxford Diabetes Trials Unit calculator (<https://www.dtu.ox.ac.uk/homacalculator>; University of Oxford, UK).

Skeletal muscle protein analysis

Phosphorylation and abundance of specific proteins in whole muscle lysate were determined with all constituents present (ie, no centrifugation), to avoid the potential loss of total cellular protein that can occur with centrifugation.^(26,27) Whole muscle lysate was analyzed as previously reported.⁽²⁸⁾ In brief, 30 cryosections of skeletal muscle (20 μ m) were

homogenized using a TissueLyser II (QIAGEN, Hilden, Germany) in RIPA lysis and extraction buffer [Cell Signaling Technology #9806 (Cell Signaling Technology [CST], Beverly, MA, USA): 20mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM Na₂EDTA, 1mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5mM sodium pyrophosphate, 1mM beta-glycerophosphate, 1mM Na₃VO₄, 1 µg/mL leupeptin] with 100mM dithiothreitol and 0.1% v/v protease and phosphatase inhibitor cocktail added (Sigma-Aldrich #P8340 and #P5726; Sigma-Aldrich, St. Louis, MO, USA). Homogenization was followed by gentle rotation at 4°C for 1 hour. Total protein content of muscle lysate was determined using the commercially available Bio-Rad protein assay and Bradford method as per the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). Twelve µg of protein was prepared in Laemmli sample buffer (Bio-Rad), heated for 5 min at 95°C and separated by electrophoresis in 10% Criterion TGX Stain-Free Pre-Cast Gels (Bio-Rad). Separated proteins were transferred to a polyvinylidene difluoride membrane using a Trans-Blot Turbo Transfer System (Bio-Rad) and subsequently blocked with Tris-buffered saline-tween (TBST) and 5% skim milk for 1 hour at room temperature. Membranes were washed (3 × 5 min) with TBST and incubated at 4°C overnight with the following primary antibodies: phospho-ERK (Thr202/Tyr204; CST #9101), ERK (CST #9102), phospho-mTOR (Ser2481; CST #2974), mTOR (CST #2972), phospho-AMPK α (Thr172; CST #2531), AMPK α (CST #2532), phospho-IRS-1 (Ser307 in human; CST #2384), phospho-AS160 (Ser588, Ser318, Thr642; CST #8730, CST #8619, CST #4288, respectively), AS160 (CST #2447), phospho-Akt (Ser473; CST #9271), Akt (#9272), IRS-1 (Millipore #06-248; Millipore, Billerica, MA, USA), and GPRC6A provided by AVIVA (San Diego, CA, USA). After incubation, membranes were washed with TBST and incubated for 1 hour at room temperature with appropriate dilutions of horseradish peroxidase conjugated secondary antibody in 5% skim milk. Membranes were rewashed and incubated in SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, Waltham, MA, USA) for 5 min prior to imaging with a ChemiDoc Imaging System (Bio-Rad). All densitometry values are expressed

relative to a pooled internal standard and normalized to the total protein content (densitometry) of each sample lane obtained from the stain-free image. There were no significant main or interaction effects for total protein content of IRS1, AS160, Akt, mTOR, and ERK (all $p > 0.1$; data not shown). As such, phosphorylated proteins are expressed relative to antibody-specific total protein content.

Statistical analysis

Data were checked for normality and analyzed using Predictive Analytics Software (PASW v20; SPSS Inc., Chicago, IL, USA). Paired t tests were conducted to compare measures of insulin sensitivity and the percent increase in insulin-stimulated protein phosphorylation between placebo and GC ingestion trials. Comparisons of multiple means were examined using a 2-factor (capsule ingestion $\times$ time point) repeated measures analysis of variance. For all significant interaction and main effects, a priori comparisons of means (baseline versus all postexercise time points; placebo versus glucocorticoid for all time points) were conducted using Fisher's least significant difference test ($p < 0.05$). Spearman's rank correlation coefficients were determined to evaluate correlations between measures of glycemic control, bone remodeling markers, and skeletal muscle protein signaling. Data are reported as mean $\pm$ SEM; statistical analyses were conducted at the 95% level of significance ($p \leq 0.05$).

Study approval

This study was approved by, and conducted in accordance with, the Victoria University Human Research Ethics Committee (HRE14-099) and was registered with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au; ACTRN12615000755538). Verbal and written explanations about the study were provided prior to obtaining written informed consent.

Results

Glucocorticoids decrease tOC, ucOC, and insulin sensitivity

Participant characteristics are reported in Table 1. Compared with placebo, GC decreased basal and postexercise insulin sensitivity, and decreased serum tOC and ucOC (Table 2; Fig. 2A, B). The degree of suppression of tOC and ucOC correlated with the deterioration in basal and postexercise insulin sensitivity (Table 3). GC suppressed serum P1NP and increased serum β -CTx during the clamp (Fig. 2C, D, respectively); changes that were not associated with insulin sensitivity (all $ps > 0.1$; data not shown).

Serum IL-6 significantly increased after exercise and remained elevated after the insulin clamp with both GC and placebo (Fig. 2E). Compared with placebo, GC decreased serum IL-6 at baseline and immediately after exercise, but not 3 hours postexercise or after the insulin clamp. After the insulin clamp, higher tOC and ucOC correlated with lower serum IL-6 (Table 3).

Glucocorticoids decrease muscle insulin protein signaling

GC decreased GPRC6A protein abundance at all time points (Fig. 3A), and attenuated downstream signaling targets including insulin-stimulated p-Akt^{Ser473} and p-mTOR^{Ser2481}, but not p-ERK or p-AMPK, and increased baseline p-Akt^{Ser473} (Fig. 3B–E). GC increased baseline p-IRS1^{Ser307}, p-AS160^{Thr642}, and p-AS160^{Ser318}, which attenuated the percent increase in phosphorylation induced by exercise and the insulin clamp (Fig. 4A–D). Impairment of p-AS160, p-mTOR, and p-Akt signaling correlated with decreased insulin sensitivity and suppressed tOC and ucOC (Table 3). Postexercise insulin-stimulated p-AS160^{Ser318, Thr642}, p-mTOR^{Ser2481}, and p-Akt^{Ser473} were all significantly correlated (Supplementary Table 1). Serum IL-6 after the insulin clamp negatively correlated with insulin-stimulated p-AS160^{Thr642} ($r = -0.57$, $p = 0.02$).

Representative blots for Western blot analysis are provided in Supplementary Fig. 2 and graphical representations of linear regression analyses are provided in Supplementary Figs. 3 and 4.

Discussion

We report that in healthy young men, a single dose of GC induced basal insulin resistance, evident by increased fasting glucose, fasting insulin, and HOMA2-IR, and also decreased postexercise insulin sensitivity: features that were all associated with suppressed ucOC and tOC. Suppressed ucOC and insulin sensitivity were associated with decreased skeletal muscle mTOR–Akt–AS160 axis protein signaling, highlighting a novel signaling pathway for GC-induced insulin resistance in humans.

Osteocalcin, bone remodeling markers, and insulin resistance/sensitivity at rest and following exercise

Chronic GC treatment impairs insulin sensitivity in the liver, and in muscle and adipose tissues. However, short-term GC treatment decreases insulin sensitivity largely by reducing glucose disposal by muscle.^(29,30) This loss of insulin sensitivity appears to be driven mainly by suppressed tOC and ucOC synthesis by osteoblasts, at least in murine models.⁽¹²⁾ Transgenic mice overexpressing the glucocorticoid-inactivating enzyme 11 β -hydroxysteroid dehydrogenase type 2, which blocks glucocorticoid signaling in osteoblasts, maintain osteocalcin and ucOC levels and are protected from GC-induced insulin resistance.⁽¹²⁾

We report that suppression of ucOC and tOC with GC was associated with the development of insulin resistance in healthy young men. Furthermore, the suppression of insulin sensitivity following exercise with GC was associated with lower ucOC and tOC. Previous work in animals supports ucOC as a positive regulator of glycemic control.^(1,3,4,31) In humans, we reported that the increase in ucOC following acute exercise correlates with improved glycemic control.^(7,8) However, previous studies have only investigated whether the exercise-induced increase in osteocalcin secretion correlates with postexercise insulin sensitivity. We extend previous findings by providing evidence that suppressing osteocalcin in humans before and after exercise is associated with decreased insulin sensitivity, supporting ucOC, and perhaps tOC, as a regulator of basal and postexercise human glycemic control.

GC treatment and exercise affect other markers of bone remodeling, including P1NP and β -CTx.^(14,16) We report that acute high-intensity cycling exercise in men decreases serum β -CTx

for up to 3 hours after exercise and remains suppressed during 2 hours of glucose and insulin infusion. P1NP was largely unaffected by acute exercise, but decreased during insulin and glucose infusion. Furthermore, GC significantly decreased P1NP at all time points and attenuated the suppression of serum P1NP and β -CTx during insulin and glucose infusion. In contrast to osteocalcin (tOC and ucOC), there were no correlations between serum P1NP, β -CTx, and measures of glycemic control. These findings support that tOC and ucOC, at least in humans, likely play a larger role than P1NP or β -CTx in whole-body glucose metabolism.

Research in rodents has identified a potential feed-forward loop that exists between exercise-induced IL-6 and osteocalcin that favors the many adaptations of regular exercise including whole-body energy metabolism.⁽¹⁶⁾ However, despite a reduction in IL-6 with GC at baseline and immediately after exercise, we report similar serum IL-6 levels 3 hours after exercise and after insulin stimulation with the clamp. Furthermore, IL-6 after the clamp was negatively correlated with tOC and ucOC, which, if anything, suggests IL-6 as a potential negative regulator in humans of circulating osteocalcin during hyperinsulinemia clamp conditions. Further research in humans is required to confirm this.

The suppression of bone remodeling markers, including tOC, ucOC, P1NP, and β -CTx, has previously been reported following nutrient intake such as an oral glucose tolerance test or a mixed meal or glucose infusion during an euglycemic-hyperinsulinemic clamp.^(32–35) In support of previous findings, we report that tOC, ucOC, P1NP, and β -CTx are suppressed in healthy men after insulin and glucose infusion, supporting the bidirectional feed-forward loop between osteocalcin and glucose. Interestingly, the suppression of bone remodeling markers following insulin/glucose infusion occurred to a lesser extent with GC ingestion, possibly because of already suppressed levels. Further research is required to explore the complex interaction between GC, feeding, and bone remodeling markers.

Glucocorticoid suppression of osteocalcin-associated skeletal muscle protein signaling

GPRC6A is an amino acid-sensing G protein-coupled receptor expressed in tissues responsive to ucOC including skeletal muscle.^(16,19,36) Mice lacking GPRC6A develop insulin

resistance, high circulating insulin, and fat accumulation.^(1,37–39) In addition, hind-limb immobilization in rats suppresses ucOC and GPRC6A expression.⁽⁴⁰⁾ We report decreased GPRC6A protein expression in human skeletal muscle with GC; however, this suppression was not correlated with ucOC or tOC. It is possible that GPRC6A protein expression in human skeletal muscle does not adequately reflect ucOC-mediated activation of the receptor. Although GPRC6A appears to be the predominant osteocalcin receptor in skeletal muscle of mice,⁽¹⁶⁾ whether this is true in humans is unclear. Further mechanistic studies exploring ucOC and skeletal muscle GPRC6A signaling in humans are required.

Intermittent ucOC treatment restores insulin sensitivity in high-fat-diet-fed mice likely through restoration of the IRS, mTOR, and Akt signaling cascade.^(18,41) Furthermore, in mice, ucOC increases insulin-stimulated AS160 phosphorylation and glucose uptake following ex vivo muscle contraction.⁽⁴⁾ We report that activation of insulin protein signaling is associated with ucOC in humans. We observed impairment of p-mTOR^{Ser2481}, p-Akt^{Ser473}, and p-AS160^{Thr642, Ser318} with GC following exercise and insulin stimulation, which correlated with suppressed tOC and ucOC and insulin sensitivity. Interestingly, GC increased baseline p-Akt^{Ser473}, p-IRS1^{Ser307}, and p-AS160^{Thr642, Ser318}, possibly compensating for increased insulin resistance and insulin secretion at baseline. Furthermore, phosphorylation of ERK was not affected by GC ingestion, supporting previous work suggesting a lesser role of ERK in GC-induced insulin resistance.⁽⁴²⁾ Our findings also suggest that AMPK, which was not altered by insulin stimulation or GC, may play a lesser role in ucOC-mediated glucose uptake in humans; we have previously reported a similar finding in mice.⁽³¹⁾ However, it is also possible that the AMPK α (Thr172) phosphorylation site detected by our antibody may not adequately reflect AMPK activity.⁽⁴³⁾ As such, future studies would benefit by directly measuring AMPK activity.

Limitations

Some of the effects of GC on insulin sensitivity may be because of the effects of GC on the liver, adipose tissue, or other signaling pathways in muscle. Nevertheless, short-term (5 days)

GC therapy has minimal effects on muscle protein synthesis and breakdown, mitochondrial function, strength, and resting energy expenditure in men, despite increased insulin resistance.⁽⁴⁴⁾ Similarly, perturbed whole-body energy metabolism in rodents precedes changes to canonical GC receptor signaling in skeletal muscle and liver,⁽¹²⁾ whereas the effect of GC on osteoblast function and osteocalcin synthesis in humans occurs within hours.^(14,15) A limitation of the current study is the use of the HOMA2-IR, which is not the gold standard for measuring glucose and insulin dynamics including hepatic insulin sensitivity. However, the primary aim of this study was to investigate the effects of GC ingestion on postexercise insulin sensitivity—for which we used the gold standard euglycemic–hyperinsulinemic clamp. These findings are limited to young healthy adult men and may not be generalizable to other populations.

Conclusion

We provide evidence that GC-induced basal and postexercise insulin resistance in humans is associated with the suppression of circulating ucOC and ucOC-linked protein signaling in skeletal muscle. Whether ucOC treatment can offset GC-induced insulin resistance in humans requires further investigation.

Disclosure

The authors report no conflicts of interest in this work.

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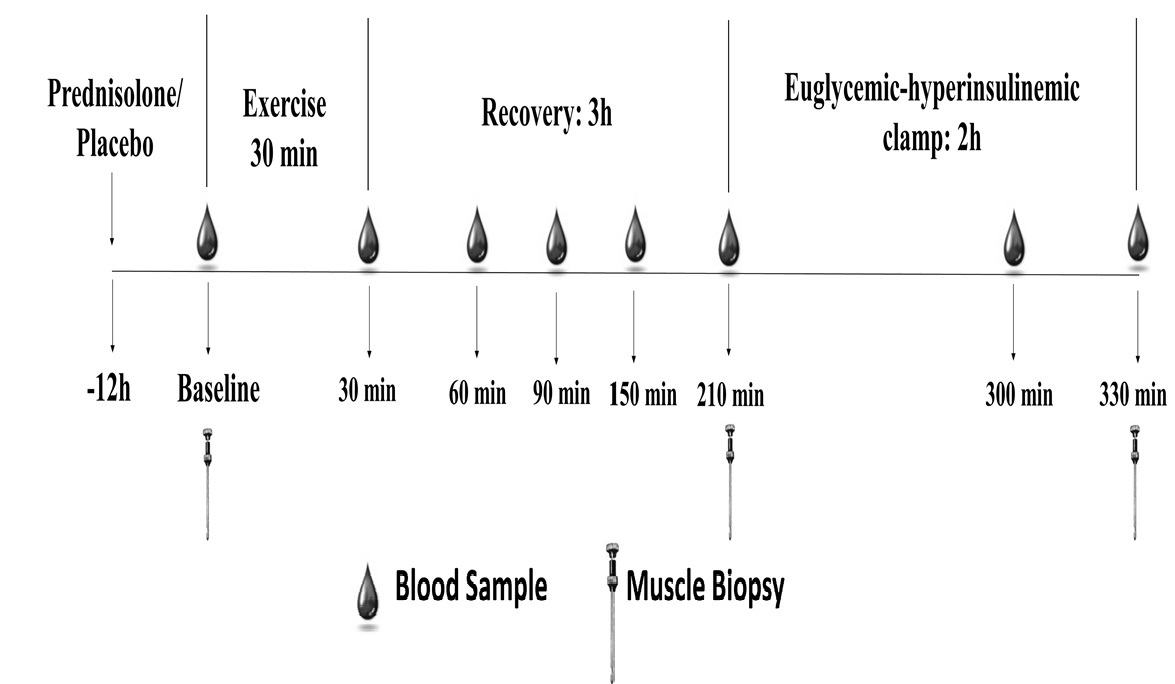
Fig. 1. Schematic depiction of the experimental design adapted from Levinger and colleagues.⁽²⁰⁾

Participants performed the indicated protocol twice, with either prior ingestion of GC (prednisolone) or placebo, orally in a double-blind, randomized, crossover design. The 2 experimental days were separated by a minimum of 1 week.

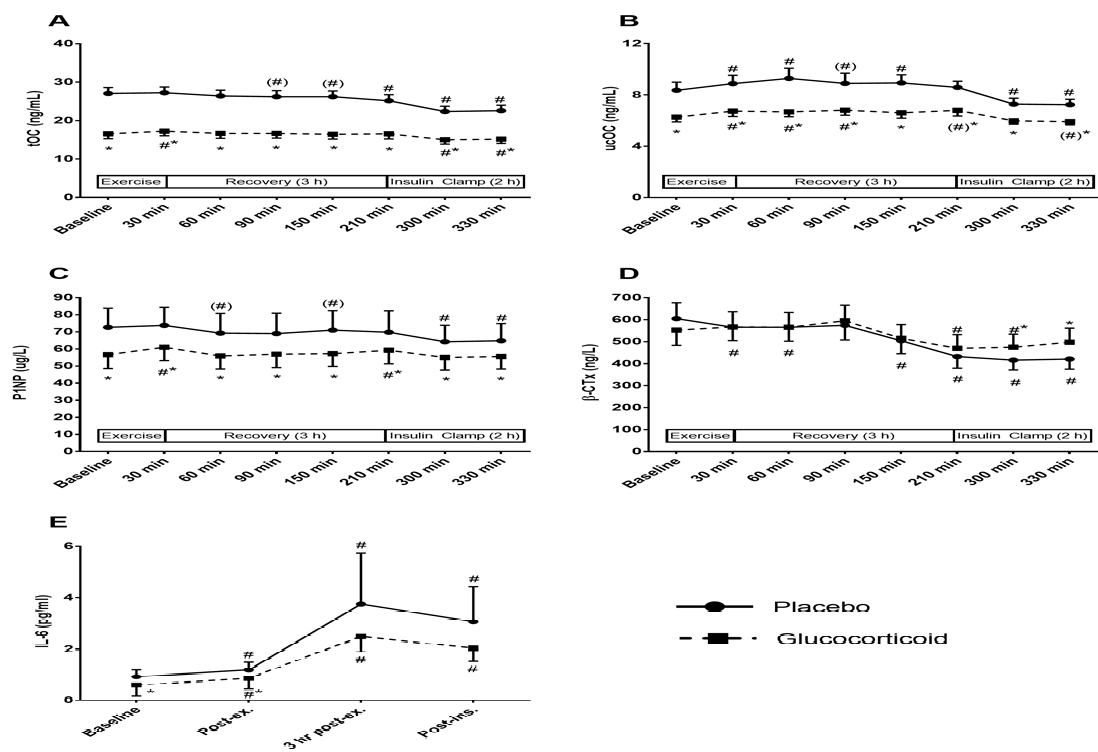
Fig. 2 The effect of glucocorticoid (prednisolone) on serum osteocalcin at baseline, postexercise, and postclamp (after insulin and glucose infusion). (A) Total osteocalcin (tOC), (B) undercarboxylated osteocalcin (ucOC), (C) procollagen type 1 N-terminal propeptide (P1NP), (D) beta C-terminal telopeptide of type 1 collagen (β -CTX), and (E) interleukin-6 (IL-6). Comparisons of multiple means were examined using a 2-factor (capsule ingestion $\times$ time point) repeated measures analysis of variance. A priori comparisons (baseline versus all postexercise time points; placebo versus GC for all time points) were conducted for interaction and main effects ($p \leq 0.1$) using Fisher's least significant difference. * = Significant difference ($p < 0.05$) compared with placebo trial; # = significant difference ($p < 0.05$) compared with baseline. Symbols in parenthesis are $p < 0.1$; $N = 8$ for IL-6 analysis; $N = 9$ for all other analyses. All data are presented as mean $\pm$ SEM.

Fig. 3. The effect of glucocorticoid (prednisolone) on baseline, postexercise, and postclamp (after insulin and glucose infusion) skeletal muscle protein signaling. (A) GPRC6A protein abundance, (B) p-mTOR^{Ser2481}, (C) p-Akt^{Ser473}, (D) p-Erk^{Thr202/Tyr204}, and (E) p-AMPK^{Thr172}. The postclamp percent increase was calculated by expressing the postclamp value of each participant relative to their baseline sample. Comparisons of multiple means were examined using a 2-factor (capsule ingestion $\times$ time point) repeated measures analysis of variance using Fisher's least significant difference where appropriate. Two-tailed paired *t* tests were conducted on the percent change in insulin-stimulated phosphorylation between placebo and glucocorticoid. * = Significant difference ($p < 0.05$) compared with placebo trial; # = significant difference ($p < 0.05$) compared with baseline. Symbols in parenthesis are $p < 0.1$, $N = 9$. All data are presented as mean $\pm$ SEM.

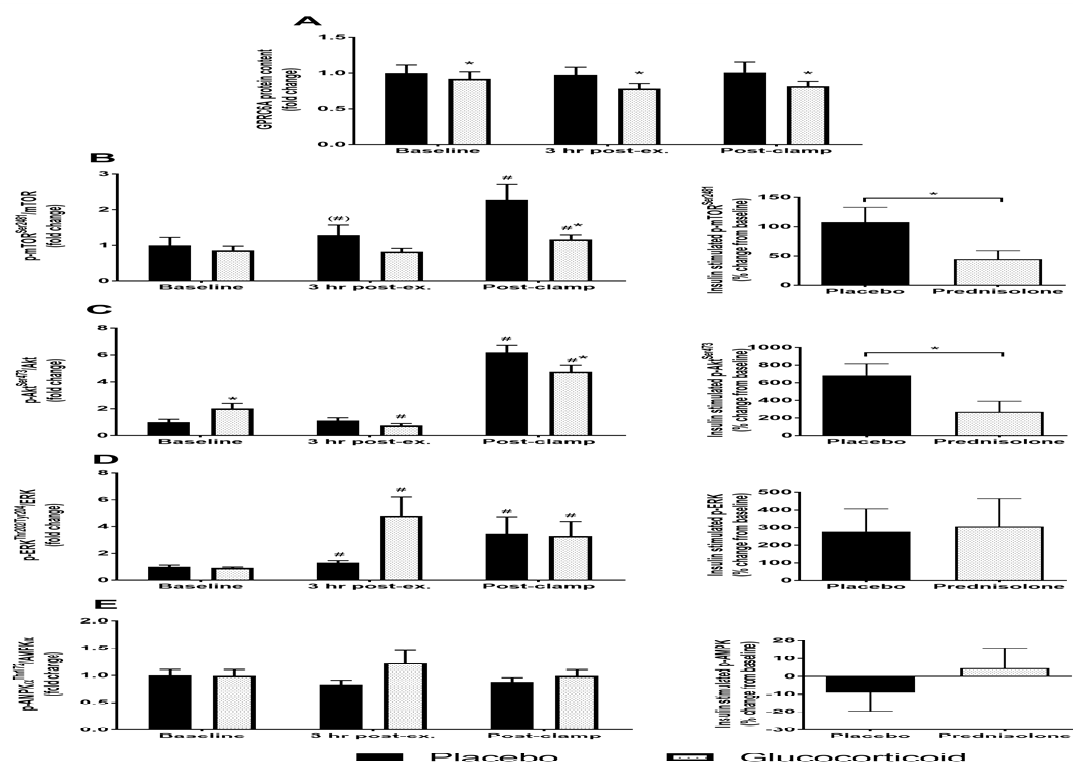
Fig. 4. The effect of glucocorticoid (prednisolone) on baseline, postexercise, and postclamp (after insulin and glucose infusion) skeletal muscle protein signaling. (A) p-IRS-1^{Ser302}, (B) p-AS160^{Thr642}, (C) p-AS160^{Ser318}, and (D) p-AS160^{Ser588}. The postclamp percent increase was calculated by expressing the postclamp value of each participant relative to their baseline sample. Comparisons of multiple means were examined using a 2-factor (capsule ingestion $\times$ time point) repeated measures analysis of variance using Fisher's least significant difference where appropriate. Two-tailed paired *t* tests were conducted on the percent change in insulin-stimulated phosphorylation between placebo and glucocorticoid. * = Significant difference ($p < 0.05$) compared with placebo trial; # = significant difference ($p < 0.05$) compared with baseline. Symbols in parenthesis are $p < 0.1$, $N = 9$. All data are presented as mean $\pm$ SEM.



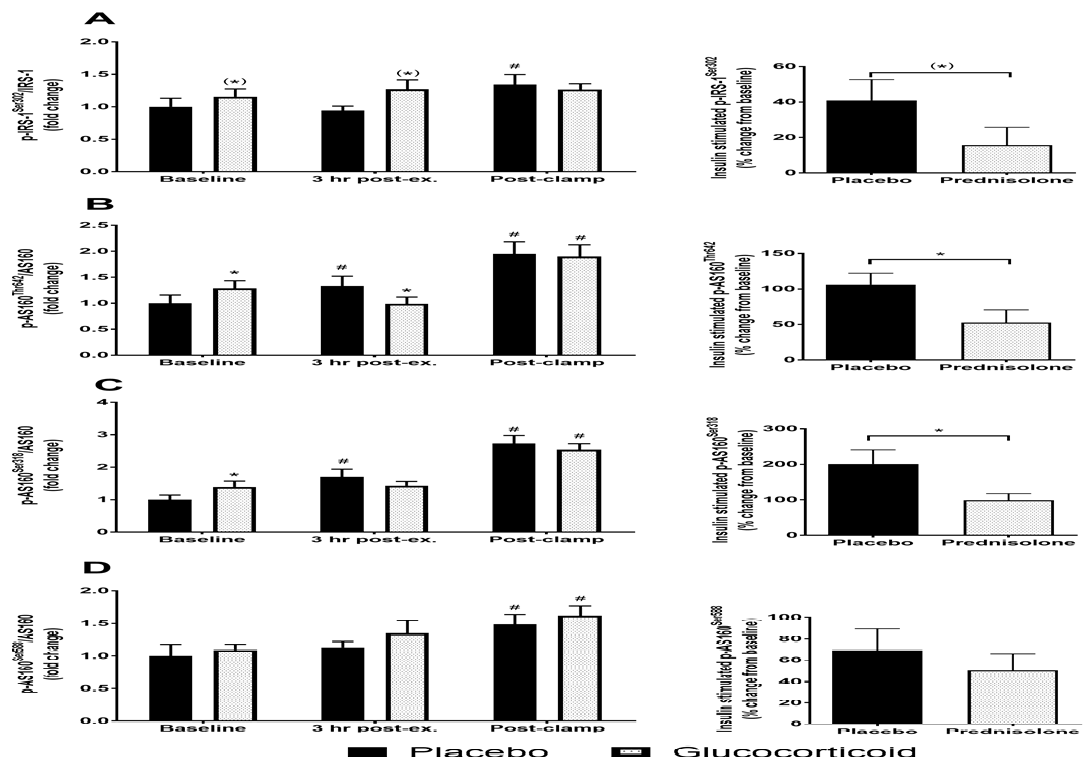
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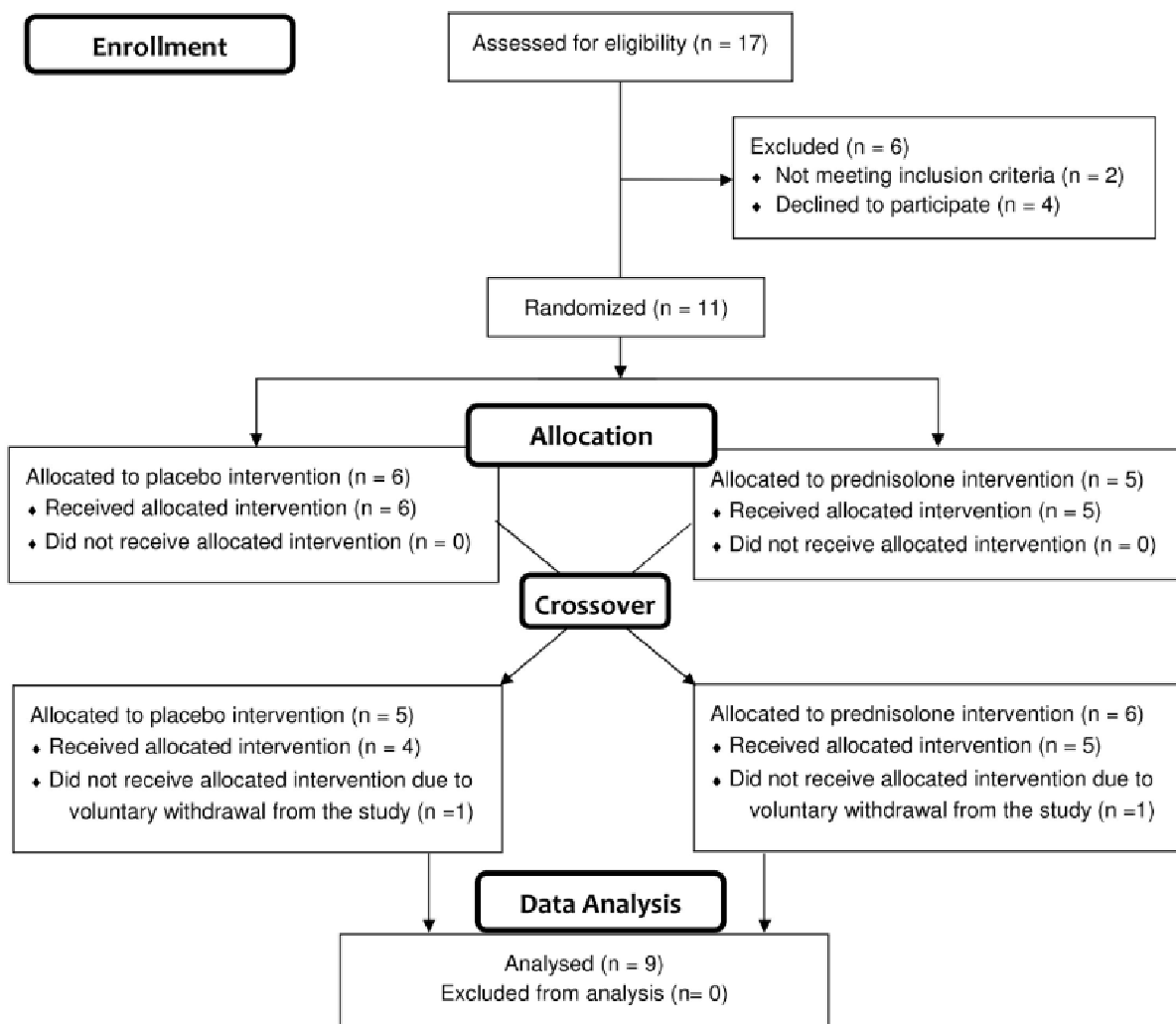
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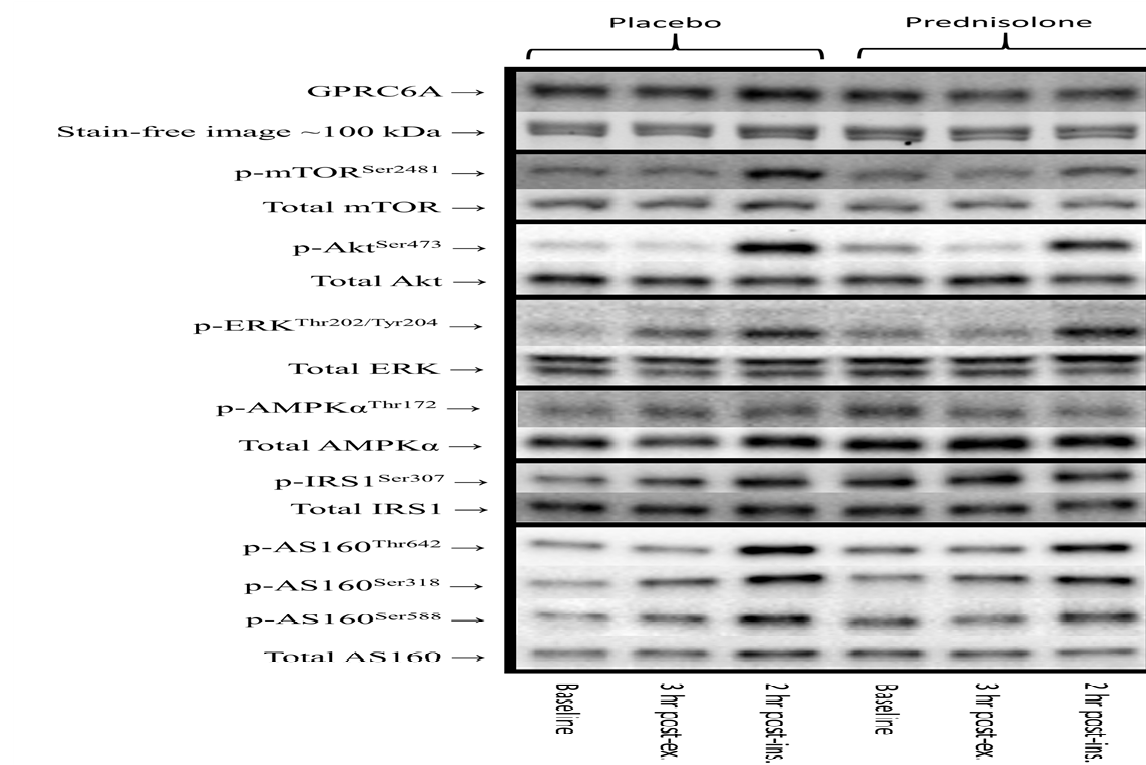
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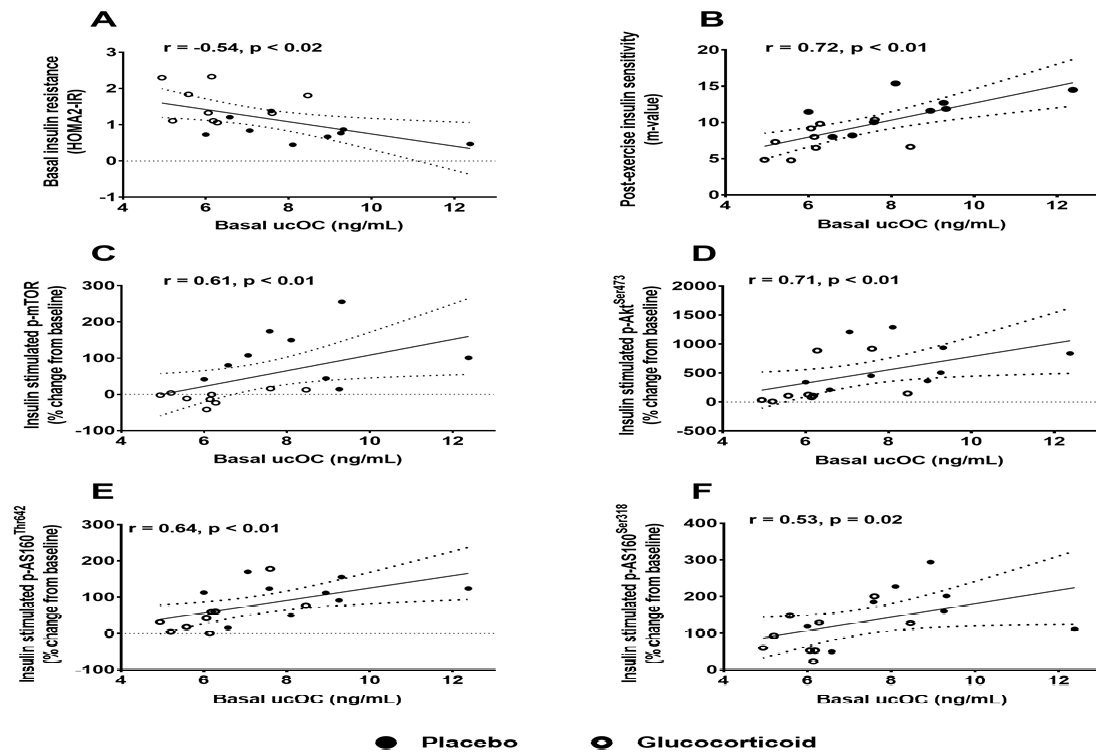
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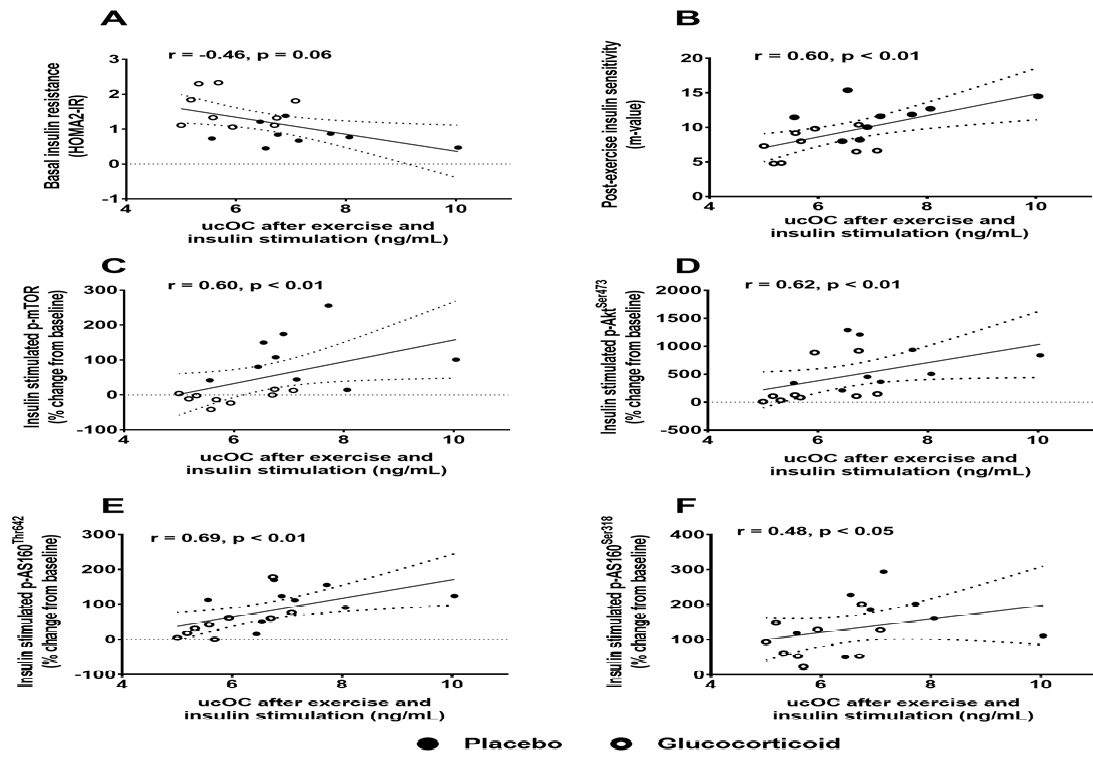
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