Co-administration of 5α-reductase inhibitors worsens the adverse metabolic effects of prescribed glucocorticoids

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Abstract

Context. Glucocorticoids (GC) are commonly prescribed, but their use is associated with adverse metabolic effects. 5α-reductase inhibitors (5αRI) are also frequently prescribed, mainly to inhibit testosterone conversion to dihydrotestosterone. However, they also prevent the inactivation of GCs.

Objective. We hypothesised that 5αRIs may worsen the adverse effects of GCs.

Design. Prospective, randomised study.

Patients. 19 healthy male volunteers (age; 45±2 years, BMI; 27.1±0.7kg/m^2).

Interventions. Participants underwent metabolic assessments; 2-step hyperinsulinemic, euglycemic clamp incorporating stable-isotopes, adipose tissue microdialysis and biopsy. Participants were then randomised to either prednisolone (10mg daily) or prednisolone (10mg daily) plus a 5αRI (finasteride 5mg daily or dutasteride 0.5mg daily) for 7 days; metabolic assessments were then repeated.

Main Outcome Measures. Ra glucose, glucose utilization (M-value), glucose oxidation, non-esterified fatty acids (NEFA) levels.

Results. Co-administration of prednisolone with a 5αRI increased circulating prednisolone levels (482±96 *vs*. 761±57nmol/L, p=0.029). Prednisolone alone did not alter Ra glucose (2.55±0.34 *vs* 2.62±0.19mg/kg/min, p=0.86), M-value (3.2±0.5 *vs* 2.7±0.7mg/kg/min, p=0.37), or glucose oxidation (0.042±0.007 *vs* 0.040±0.004mmol/hr/kg/min, p=0.79). However, co-administration with a 5αRI increased Ra glucose (2.67±0.16 *vs.* 3.05±0.18mg/kg/min, p<0.05) and decreased M-value (4.0±0.5 *vs.* 2.6±0.4mg/kg/min, p<0.05), and oxidation (0.043±0.003 *vs.* 0.036±0.002mmol/hr/kg, p<0.01). Similarly, prednisolone did not impair insulin-mediated suppression of circulating NEFA (43.1±28.9 *vs.* 36.8±14.3μmol/L, p=0.81), unless co-administered with a 5αRI (49.8±8.6 *vs.* 88.5±13.5μmol/L, p<0.01).

Conclusions. We have demonstrated that 5αRIs exacerbate the adverse effects of prednisolone. This study has significant translational implications, including the need to consider GC dose adjustments, but also the necessity for increased vigilance for the development of adverse effects.

Precis

A prospective randomized study in 19 healthy male volunteers showed that co-administration of 5α-reductase inhibitors exacerbates the adverse metabolic effects of prednisolone.

Introduction

2-3% of the population of the United Kingdom and United States are currently prescribed glucocorticoid (GC) therapy (1). GC use, both acute and chronic, is known to be associated with a number of significant adverse effects. Recurrent short-course administration is also associated with increased morbidity and mortality (2). Adverse metabolic features include obesity, skeletal muscle myopathy, hypertension, insulin resistance and diabetes mellitus and are collectively termed '*Iatrogenic Cushing's syndrome'*.

5α-reductases (5αR) have a crucial role in the metabolism of testosterone and glucocorticoids (3). They metabolise testosterone to the more potent androgen, 5α-dihydrotestosterone, and inactivate cortisol to 5α-dihydrocortisol, which is then in turn metabolised to tetrahydrocortisol by 3α-hydroxysteroid dehydrogenase. There are two isoforms of 5αR; 5αR type 1 (5αR1) is found in the liver, non-genital skin, muscle, adipose tissue and brain whilst 5αR type 2 (5αR2) is mainly expressed in the male reproductive tissues such as prostate, epididymis and seminal vesicles but also in the liver (3). 5αR therefore simultaneously enhances androgen and limits active GC availability and represent a potent pre-receptor regulatory step in steroid hormone action.

The 5αR have an established role in the regulation of metabolic phenotype. 5αR1 knock-out male mice are glucose intolerant, and have a higher incidence of hepatosteatosis and liver fibrosis (4,5). Cross-sectional studies in humans have shown that 5αR activity correlates positively with BMI (6-8) and tracks longitudinally over time with both weight and insulin resistance (6). Conversely, weight loss is associated with reduced 5αR activity (6-8). 5αR inhibitors (5αRI) such as dutasteride and finasteride are prescribed widely for their anti-androgenic effects in conditions such as benign prostate hyperplasia (BPH), prostate cancer, alopecia, as well as in some patients with polycystic ovary syndrome (PCOS).

Dutasteride is a non-selective 5αRI inhibiting both 5αR1 and 2, whilst finasteride is a selective 5αR2 inhibitor. The ability of these drugs to regulate metabolic phenotype has only been examined in a very small number of studies. Dutasteride (and not finasteride) treatment worsened skeletal muscle and hepatic insulin sensitivity and increased hepatic triglyceride accumulation (9,10). Most recently, analysis of data from primary care prescriptions as suggested a significant association between 5αRI prescriptions and the incidence of type 2 diabetes (T2D) (11).

Patients who are prescribed GCs often have other comorbidities necessitating treatment with other medications and there are numerous examples of drug interactions altering GC exposure, leading to clinical signs and symptoms of GC excess. Such medications include protease inhibitors, anti-fungals, antibiotics immunosuppressive medications and combined oral contraceptives (12-14). Despite the well-recognised role of 5αR in GC metabolism (including synthetic GCs (3)), their ability to negatively impact upon the adverse effect profile associated with prescribed GC has not been explored.

We have therefore undertaken a detailed, proof-of-concept experimental medicine study in healthy volunteers, to test the hypothesis that 5αRIs can worsen the metabolic impact of prescribed prednisolone, putatively, through decreased metabolism and generation of inactive metabolites, and / or increased prednisolone clearance (15,16).

Materials and Methods

Clinical protocol

The clinical protocol received full ethical approval from the Wales 7 Research Ethics Committee (reference 15/WA/0071) (https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/find-it-2/). Nineteen healthy male volunteers were recruited from local advertisement, the Oxford Biobank, (reference 08/H0606/107+5), NHS hospitals and GP surgeries. All were aged 18-65 years, had a BMI 20-35kg/m^2, did not have T1D or T2D, were normotensive, had not used GC therapy within the last 6 months and were not on any medications known to impact upon GC metabolism. The participants attended the Clinical Research Unit, Churchill Hospital (Oxford, UK) at 8:00 AM and underwent subcutaneous abdominal adipose biopsy after an overnight fast (from 24:00h). Aspiration of adipocytes (approximately 1g of tissue) was achieved using a needle and syringe and liposuction following administration of local anaesthetic. At 9:00 AM an adipose microdialysis catheter (CMA Microdialysis, Solna, Sweden) was inserted under local anaesthetic into the subcutaneous abdominal adipose tissue 5cm to one side of the umbilicus. Using the microdialysis pump, a microdialysate solution (0.9% sterile saline solution) was introduced into the catheter (perfusion rate = 0.3μl/minute). Samples were collected every 30 minutes until the completion of the hyperinsulinemic clamp (see below).

2-step hyperinsulinemic euglycemic clamp

On commencement of the two-step hyperinsulinemic euglycemic clamp, a bolus of [U-^{13}C]-glucose (Cambridge Isotope Laboratories, Andover, USA) was administered (2mg/kg over 1 minute followed by a continuous infusion in 0.9% saline (20μg/kg/min)). At the same time a [2,2-$^{2}H_2$]-palmitate (Cambridge Isotope Laboratories, Andover, USA) in human serum albumin infusion was started (0.03μmol/kg/min). Blood glucose was monitored at 15-minute intervals during the initial 120min (t=0-120min) basal phase. At t=120min, an insulin infusion (Actrapid; Novo Nordisk) was infused at 20mU/m^2/min (low-dose) alongside an infusion of

20% dextrose supplemented with [U-^{13}C]-glucose enriched to 4%; blood glucose levels were monitored at 5-minute intervals (t=120-360min). At t=240min, the insulin rate was increased to 100mU/m^2/min (high-dose) and continued for another 120 minutes (t=240-360min). In addition to blood glucose sampling, blood samples were taken and at 3 time points in the last 30 minutes of each phase (basal, low- and high-insulin) for steady state measurements of insulin, whole body glucose turnover (Ra glucose, Gd glucose), endogenous glucose production rate (EGP) and lipolysis (Ra palmitate). Glucose and palmitate disposal rates were calculated using a modified version of the Steele equations (17,18). Exhaled breath samples were collected from the participants at 60-minute intervals throughout the study to allow for analysis of glucose oxidation ($^{13}CO_2$ production).

Participants were then randomised to one of three drug regimen arms; prednisolone 10mg once daily (OD), prednisolone 10mg and finasteride 5mg OD or prednisolone 10mg and dutasteride 0.5mg OD. The medications were taken for 7 days and the participants returned to the research facility on the last day of administration and all investigations were repeated.

Biochemical and stable isotope analysis

Cholesterol, liver biochemistry and plasma glucose were measured using standard laboratory methods (Roche Modular system, Roche Ltd, Lewes, UK). Insulin was measured using a commercially available colorimetric ELISA (Mercodia, Uppsala, Sweden) with an in-house coefficient of variation of less than 15%. NEFA, beta-hydroxybutyrate (OHB) and glycerol serum concentrations were measured using the 600/650 iLAB clinical chemistry analyser (Instrumentation Laboratory, Milano, Italy). Microdialysis samples were analysed using a mobile photometric, enzyme-kinetic analyser (CMA ISCUS Flex, Solna, Sweden) for glycerol, pyruvate, glucose and lactate.

Prednisolone, prednisone, cortisol and cortisone were extracted from participants' serum, calibration standards and quality control by liquid:liquid extraction using diethyl ether. The

upper solvent layer containing the steroids was separated from the bottom aqueous layer using an ice-bath (Thermofisher) filled with ethanol (VWR). A rotary evaporator was used to dry down extracts and they were then reconstituted in 50:50 methanol water before analysis on the mass spectrometer.

Analysis was performed by LC-MS/MS, specifically a Shimadzu HPLC system coupled to an API 5000 tandem mass spectrometer (Sciex, Warrington, UK). The ion source used was atmospheric pressure ionisation (APCI) and the software was Analyst version 1.7.

Plasma enrichment of [U-^{13}C]-glucose was measured using gas chromatography-mass spectrometry (model 5973; Agilent Technologies, Cheshire, UK). The plasma lipids were extracted according to the method of Folch (19) and the NEFA fraction isolated by solid phase extraction (Bond Elut NH2- Aminopropyl columns). Following methylation of the NEFA fraction, the samples were run on GC (model 5890; Agilent Technologies, Cheshire, UK) to determine the relative amount of individual fatty acids. Whole body lipolysis (Ra palmitate) was calculated using extracted total circulating lipid and by measuring deuterium ([2,2-$^{2}H_2$]-palmitate) enrichment in the NEFA fraction. [2,2-$^{2}H_2$]-palmitate enrichments were determined by gas chromatography-mass spectrometry using a 5890 GC coupled to a 5973N MSD (Agilent Technologies; CA, USA). Ions with mass-to-charge ratios (m/z) of 270 (M+0) and 272 (M+2) were determined by selected ion monitoring (20).

RNA extraction and RNA-sequencing

Total RNA from adipose tissue biopsies was extracted using the Tri-Reagent system (Sigma-Aldrich, Dorset, UK). Assessment of RNA quality was performed using a Nanodrop spectrophotometer (Thermo Scientific, Hemel Hempstead, UK). Total RNA was enriched for polyA-tailed mRNA using oligo (dT) beads. The Illumina TruSeq Stranded mRNA HT Sample Prep Kit was used to prepare cDNA libraries for sequencing. In-house 8bp indexes (21) were used to multiplex samples (10-plex), which were then sequenced over 1 lane of an

Illumina HiSeq4000 machine using HiSeq 3000/4000 PE Cluster Kit and SBS Kit. Paired-end sequencing (75bp) was performed at a depth of ~25 million read pairs per sample.

Reads were mapped with STAR 2.5.1b (22) on default settings with GENCODE version 19 (23) as transcriptome and GRCh37 as genome reference. Gene level reads counts for all protein-coding and long intergenic non-coding RNA (lincRNA) transcripts present in GENCODE version 19 were quantified in a strand-specific manner with featureCounts (24) from the Subread package v1.5.0-p2. For plotting purposes, we also normalized the gene counts to transcripts per million (TPM).

Statistical approach

Data are presented as mean ± SE unless otherwise stated. In the first instance, data was analysed as three groups; prednisolone only, prednisolone + finasteride and prednisolone + dutasteride. Delta changes for the variables were calculated as the difference: follow-up (7 days post treatment) minus baseline. Shapiro-Wilk test was run to check for normality of distribution of the data. For normally distributed variables, one-way ANOVA was conducted to determine if there were differences between the three groups both for baseline measures and delta changes. For non-normally distributed variables, Kruskal-Wallis test was done instead. There were no outliers, as assessed by boxplot, and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances.

In cases where the differences between the three groups was not significant, further stratification was done by merging the two groups that received both prednisolone and a 5αRI, thus dividing the participants into two groups: prednisolone only and prednisolone + 5αRI. Paired t-tests were then used to compare individual variables before and after intervention within each participants' group where the data was normally distributed and in cases the data was not normally distributed the Wilcoxon test was used instead. Absolute

change between the two groups, as well as post-treatment comparisons, were calculated using unpaired t-test where the data was normally distributed or using the Mann-Whitney test where the data was not normally distributed.

Statistical analyses were performed using SPSS, version (IBM, Chicago, IL) and GraphPad Prism 8 software package (GraphPad Software, La Jolla, CA) for MacOS. Area under the curve (AUC) analysis was performed using the trapezoidal method.

For RNA-sequencing data, differential expression analysis was performed using edgeR (25) in R 3.2.2 on normalised gene counts for all autosomal protein-coding and lincRNA genes that were expressed at > 2 count per million (CPM) in all samples. A paired model was fitted to the data, and significance was determined by empirical Bayes moderated t-statistics implemented in edgeR. Differentially regulated genes were defined by a false discovery rate (Benjamini-Hochberg method) adjusted p-value <5%.

Results

Clinical characteristics

Nineteen healthy male volunteers were recruited (mean age 45±2years, BMI 27.1±0.7kg/m^2). There were no significant differences when comparing participants randomised to prednisolone treatment alone (n=6), prednisolone + finasteride (n=7) or prednisolone + dutasteride (n=6). Subsequently, the two arms that received prednisolone + 5αRI were merged and post-hoc analysis was undertaken as two groups; prednisolone alone (n=6) and prednisolone + 5αRI (n=13). Detailed demographic and biochemical data are presented in Table 1.

Circulating prednisolone levels were significantly high in participants taking 5αRIs compared to those taking prednisolone alone (482±96 vs. 761±57nmol/L, p=0.029). There was no difference in circulating prednisone levels (95±16 vs. 105±9nmol/L, p=0.54) (Figure 1a, Table1).

There was no impact of either prednisolone alone or co-administration of prednisolone + 5αRI on fasting glucose or insulin levels, or on circulating lipids and liver chemistry. In addition, the changes observed in the composition of the fatty acid pool where similar in both groups (Table1).

5αRI co-administration with prednisolone impairs hepatic insulin sensitivity

Basal Ra glucose was not altered by treatment with prednisolone alone. However, when combined with 5αRIs, Ra glucose increased significantly; the absolute change between the two groups was not significant (p=0.30) (Table 2, Figure 1b and c). EGP rate was unchanged by prednisolone treatment, but following 5αRI co-administration EGP significantly increased, consistent with worsening hepatic insulin sensitivity (Table 2, Figure

1d and e). The absolute change between the two groups was not statistically significant (p=0.15).

5αRIs increase GC-induced peripheral insulin resistance and glucose oxidation

Prednisolone alone had no impact on the M-value during the low- or high-dose insulin infusion. However, when combined with a 5αRI, under low-dose insulin infusion it decreased significantly (Table 2, Figure 1f and g). Gd was decreased by prednisolone alone. Although the magnitude in reduction of Gd with prednisolone + 5αRI was similar to prednisolone alone, this did not reach statistical significance (p=0.080) (Table 2). There were no differences in the absolute changes between the groups (Table 2).

$^{13}CO_2$ production from the infused [U-^{13}C]-glucose was used as a marker of glucose uptake and subsequent oxidation. Co-administration of prednisolone + 5αRI (but not prednisolone alone), decreased glucose oxidation across the 2-step clamp (both low- and high-dose insulin phases) (Table 2). The absolute change between the two groups, under both low- and high-dose insulin phases, was not different (Table 2).

5αRI co-administration with prednisolone impairs adipose tissue insulin sensitivity

Prednisolone alone had no impact on insulin-mediated suppression of circulating NEFA levels during the low- or high-dose insulin infusion. However, when combined with a 5αRI, there was a significant reduction in the ability of low-dose insulin (but not high-dose insulin) to suppress circulating NEFA levels (Table 2, Figure 2a, b and c).

Prednisolone alone, as well as prednisolone + 5αRI were equally effective in impairing the ability of insulin to supress circulating glycerol under low-, but not high-dose insulin infusions. There was no significant difference in the absolute change between the two groups across the clamp (Table 2, Figure 2d, e and f).

Adipose tissue lipolysis (as measured using [2,2-$^{2}H_2$]-palmitate) was decreased by insulin (both low- and high-dose), but there was no impact of prednisolone or prednisolone + 5αRI treatment (Table 2). Insulin decreased fatty acid oxidation as measured by circulating levels of beta-hydroxybutyrate. Prednisolone had no impact on the ability of insulin to suppress beta-hydroxybutyrate levels, but when co-administered with a 5αRI, insulin-mediated suppression of beta-hydroxybutyrate was impaired (Table 2, Figure 2g and h). The absolute change between the two groups was different but did not reach statistical significance (p=0.17) (Table 2, Figure 2i). There were no differences following high dose insulin infusion (Table 2).

Adipose tissue microdialysis was used to specifically examine the impact of treatment upon subcutaneous adipose tissue. Insulin decreased adipose tissue interstitial glycerol levels in a dose-dependent manner (Table 2). However, neither prednisolone alone, nor prednisolone + 5αRI had any impact on subcutaneous adipose interstitial fluid levels of glycerol, glucose, pyruvate, and lactate or their response to low- and high-dose insulin infusion (Table 2).

Subcutaneous adipose tissue gene expression

RNA sequencing analysis identifed only 11 genes (PLA2G2A, ETNK2, MALL, EDN1, SOX7, FAM166B, LINC00844, ARNTL, KRT1, NFKBIA, GADD45B) that were regulated by prednisolone treatment, several of which are recognised GC-targets (including endothelin 1, aryl hydrocarbon receptor nuclear translocator like, NFKB inhibitor alpha and growth arrest and DNA damage inducible beta) (26-29). The expression of only a single gene changed following prednisolone + 5αRI treatment (ribosomal protein L41 pseudogene 1).

Finasteride and dutasteride have a similar action to augment the metabolic impact of prednisolone.

A *post-hoc* subgroup analysis was used to compare the administration of either finasteride or dutasteride. There were no differences in the fasting basal metabolic parameters between the subgroups (data not shown). Prednisolone levels were similar in those individuals treated with either prednisolone + finasteride or prednisolone + dutasteride (806±66 *vs.* 709±99nmol/L, p=0.89). In both cases, levels were higher than in participants treated with prednisolone alone (482±96 *vs.* 806±66 *vs.* 709±99nmol/L, p=0.046).

The low-dose insulin mediated suppression of NEFA was similar in individuals treated with finasteride + prednisolone ($p<0.05$) and dutasteride + prednisolone ($p=0.058$); there was no significant difference between the groups (Figure 3a, b and c). Changes in EGP were similar in individuals treated with either finasteride or dutasteride and there was no significant difference between the groups (Figure 3d and e).

Interestingly, the changes in glycerol, OHB, M-value and Gd under high-dose insulin were more marked in individuals treated with finasteride (Table 3). Changes in other metabolic variables were not different between the two groups and are summarized in Table 3.

Discussion

In this proof-of-concept, experimental medicine study, we have shown that the metabolic impact of prednisolone (10mg daily) for 7 days is relatively modest. However, when co-administered with a 5αRI, circulating prednisolone levels are increased and the adverse metabolic effects of prednisolone on peripheral, hepatic and adipose tissue insulin sensitivity are augmented. Whilst in the acute phases of immune and inflammatory conditions, dose of prednisolone higher than 10mg are often used, longer-term maintenance doses are often lower and therefore co-administration of drugs that can impact on prednisolone metabolism may well have a clinical impact. Given that combined administration is not infrequent either in men with BPH or in women with PCOS, these data have broad clinical implications.

Previous studies have examined the metabolic effects of short-term isolated GC treatment, including both prednisolone and hydrocortisone. High doses (30-75mg) of prednisolone for 1-15 days' duration causes pancreatic beta-cell dysfunction and reduced glucose tolerance (30,31). In healthy male volunteers, treatment with low (7.5mg) and high (30mg) dose prednisolone for 2 weeks decreased the ability of insulin to suppress EGP and lipolysis, and high- (but not low-) dose treatment decreased glucose disposal and increased fasting insulin levels (32,33). In patients with inflammatory rheumatologic disease, a 7-10-day course of prednisolone (6mg) increased basal EGP, reduced glucose disposal and increased peripheral insulin resistance (34,35). These data are consistent with the current study, although the magnitude of effect that we observed with prednisolone treatment alone was less than in the published studies and this is likely to reflect the fact that this was a shorter treatment duration (1-week). Both dose, and duration are important and we have shown that acute administration of high-dose of intravenous hydrocortisone (0.2mg/kg•hr) increases EGP, limits glucose disposal and induces systemic insulin resistance (36).

The role of 5αR in the regulation of metabolic phenotype is still not entirely understood. Cross-sectional observation studies provided the first evidence of dysregulation of 5αR activity, demonstrating increased activity with weight gain and insulin resistance and reduced activity with weight loss (6-8). In rodent models, 5αR1 KO male mice are more prone to the development of glucose intolerance as well as hepatosteatosis and liver fibrosis (4,5). It is important to note however, that mice (contrasting with humans) do not express 5αR2 in the liver and therefore direct extrapolation to clinical studies cannot be made.

Very few translational, interventional clinical studies have been performed. A retrospective clinical analysis has suggested that long-tern dutasteride treatment is associated with hyperglycaemia and adverse circuiting lipid profiles (37). In smaller mechanistic studies, isolated treatment with dutasteride alone have demonstrated increased skeletal muscle and hepatic insulin resistance and increased hepatic triglyceride content (9,10). Finasteride was without effect, suggestive of a specific role for 5αR1. More recently, data have been published suggesting an increased risk of incident T2D associated with both dutasteride and finasteride (11) although the analysis did not examine the impact of co-prescription of these medications with GCs.

Building on the established role of the 5αRs in glucocorticoid metabolism (38), the aim of the current study was to test the impact of co-administration of prednisolone and 5αRIs. We have previously shown that both finasteride (5mg daily) and dutasteride (0.5mg daily) (in the absence of exogenous glucocorticoid, for a 3-week duration) have no impact on fasting glucose, fasting insulin, M-value across a 2-step hyperinsulinemic euglycemic clamp, Ra glucose, M-value, glucose disposal (Gd), circulating NEFA or Ra glycerol (10). We have therefore concluded that the changes that we observed in the prednisolone + 5αRI arm of this study are due to the combination of treatment rather than the 5αRI treatment alone. This

study therefore provides the first evidence to suggest that co-administration of GC and 5αRI can precipitate the development of adverse metabolic consequences.

There are very few differences in GC metabolites when comparing individuals taking finasteride or dutasteride (10). The limited additional impact of combined 5αR1 and 2 inhibition suggests that 5αR2 may be most critical for glucocorticoid metabolism. Our data would endorse this observation; our subgroup analysis showed the impact of finasteride was similar (or indeed slightly more marked) than that of dutasteride.

There are many examples of co-prescriptions of medications altering GC half-life and availability with resultant adverse metabolic effects. In particular, ritonavir, itraconazole, erythromycin, cyclosporin and oral contraceptives have been shown to increase circulating GC levels (12,13,39). In contrast, drugs such as carbamazepine, phenytoin, phenobarbital, rifampicin decrease GC levels due to increased P-450 activity (12,13,39). In cases were these medications are co-administered with GCs dose adjustments and vigilance as to the development of adverse effects need to be considered.

There are a number of limitations to this study; the sample size is modest although does reflect the complex and sensitive nature of the investigations that were performed. The study was powered to detect a 15% change in EGP, and sample size estimates suggested that 8 participants taking both prednisolone and 5αRIs would be needed (additional volunteers were recruited to account for potential drop-outs or failed sample analysis). We did not include a dedicated arm of participants treated with 5αRI alone as we have already reported findings from participants treated in this way (10). Whilst it is established that 5αRs are able to metabolise prednisolone and prednisone (40), there are very limited data with respect to other synthetic GCs and therefore it may not be possible to extrapolate our findings to all prescribed steroids across all routes of administration.

In conclusion, we have demonstrated for the first time that co-administration of prednisolone with a 5αRI worsens metabolic phenotype. These data not only demonstrate the potent ability of the pre-receptor 5αR system to regulate exogenous GC action, but raise important clinical questions with respect to vigilance and surveillance for adverse effects as well as the potential need to consider dose adjustments.

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Figure legends

Figure 1. The effect of prednisolone and co-administration with a 5αRI on circulating prednisolone and prednisone levels (A), glucose production (Ra Glucose) (B and C), endogenous glucose production (EGP) rate (D and E) and glucose utilization (M-value) (F and G) during a two-step hyperinsulinemic euglycemic clamp. Absolute change refers to the difference between pre- and post-treatment values (C, E and G). Data shown are from low-dose insulin infusion (B - G). Data are medians and error bars are IQR. Datapoints represent individual patients. White circles represent pre-treatment levels and black squares post-treatment levels (7 days), light grey circles are prednisolone alone and dark grey squares, prednisolone + 5αRI.

Figure 2. The effect of prednisolone and co-administration with a 5αRI on circulating non-esterified fatty acids (NEFA) (A, B and C), glycerol (D, E and F) and β-hydroxybutyrate (OHB) (G, H and I) levels across a 2-step hyperinsulinemic euglycemic clamp. Absolute change refers to the difference between pre- and post-treatment values (C, F and I). Data are medians and error bars are IQR. Datapoints represent individual patients. White circles represent pre-treatment levels and black squares post-treatment levels (7 days), light grey circles are prednisolone alone and dark grey squares, prednisolone + 5αRI.

Figure 3. The effect of co-administration of prednisolone with finasteride or dutasteride on circulating non-esterified fatty acids (NEFA) (A, B and C) levels across a 2-step hyperinsulinemic euglycemic clamp and endogenous glucose production (EGP) (D and E) during the low-dose insulin phase of a 2-step hyperinsulinemic euglycemic clamp. Absolute change refers to the difference between pre- and post-treatment values. Data are medians and error bars are IQR. Datapoints represent individual patients. White circles represent pre-

treatment levels, light grey squares are post-treatment (7 days) finasteride + prednisolone and dark grey squares, post-treatment (7 days) with dustasteride + prednisolone.

Tables

Table 1. Clinical characteristics and changes in fasting biochemistry before and after randomization to 7 days of treatment with either prednisolone (10mg once daily) or prednisolone (10mg once daily) and a 5αRI (finasteride (5mg once daily) or dutasteride (0.5mg once daily)).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; DHT, dihydrotestosterone; HDL, high-density lipoprotein; SBP, systolic blood pressure. Local reference ranges are included in parentheses.

[a]P <0.05, before *vs.* after treatment.

[b]P <0.01, before *vs.* after treatment.

[c]P <0.05, before treatment between the two groups.

*P <0.05, after *vs.* after treatment.

Clinical Variable	**Prednisolone**		**Prednisolone + 5αRI**	
	Before	*After*	*Before*	*After*
Age, y	46.7 ± 3.9		44.5 ± 2.5	
Weight, kg	89.4 ± 2.8		86.2 ± 3.9	
BMI, kg/m^2	27.8 ± 1.2		26.7 ± 0.9	
SBP, mm Hg	140.8 ± 3.1		141.8 ± 3.0	
DBP, mm Hg	86.7 ± 2.5		82.0 ± 2.6	
HbA1c, mmol/mol (20-42)	34.3 ± 1.2		32.1 ± 1.0	
Fasting glucose, mmol/L	4.8 ± 0.2	5.1 ± 0.3	4.5 ± 0.1	4.7 ± 0.1
Fasting insulin, pmol/L	20.4 ± 6.2	33.2 ± 9.9	33.4 ± 6.9	29.4 ± 4.2
HDL cholesterol, mmol/L	1.4 ± 0.1[a]	1.5 ± 0.1[a]	1.2 ± 0.1	1.3 ± 0.1

Total cholesterol, mmol/L	5.1 ± 0.5	5.0 ± 0.4	4.8 ± 0.3	4.9 ± 0.4
AST, IU/L (15-42)	21.7 ± 1.9	18.2 ± 1.7	19.0 ± 1.3	19.5 ± 2.6
Bilirubin, mmol/L (<21)	19.2 ± 4.0	14.0 ± 2.5	12.9 ± 1.8	11.2 ± 1.4
ALT, IU/L (10-45)	22.7 ± 3.4	24.8 ± 4.1	22.1 ± 3.4	20.9 ± 3.5
ALP, IU/L (30-130)	58.7 ± 6.3	47.0 ± 11.2	62.0 ± 6.6[a]	55.8 ± 5.3[a]
Albumin, g/L (32-50)	37.5 ± 1.1	37.5 ± 0.7	37.6 ± 0.6	36.2 ± 0.6
Palmitic acid 16:00, %	32.0 ± 3.3[c]	28.0 ± 1.2	27.5 ± 1.3[c]	26.5 ± 1.0
Stearic acid 18:00, %	22.9 ± 1.9[a]	19.2 ± 1.9[a]	21.6 ± 1.4[a]	18.5 ± 0.9[a]
Oleic acid 18: 1n-9, %	21.6 ± 4.1[a]	28.3 ± 1.7[a]	26.9 ± 2.5[b]	31.6 ± 2.0[b]
Linoleic acid 18: 2n-6, %	13.1 ± 1.2	16.0 ± 2.9	15.0 ± 1.8	13.6 ± 1.2
Fasting triglycerides, µmol/L	659 ± 140	568 ± 138	823 ± 116	786 ± 91
Serum prednisolone, nmol/L		482 ± 96*		761 ± 57*
Serum prednisone, nmol/L		95 ± 16		105 ± 9

Table 2. The effect of prednisolone and co-administration of prednisolone and a 5αRI on glucose and lipid metabolism during a two-step hyperinsulinemic euglycemic clamp.

Abbreviations: EGP, endogenous glucose production; Gd, glucose disposal; NEFA, non-esterified fatty acids. Data shown are before, after and absolute change following randomization to 7 days of treatment with either prednisolone (10mg once daily) or prednisolone (10mg once daily) and a 5aRI (finasteride (5mg once daily) or dutasteride (0.5mg once daily)).

[a]P <0.05, before *vs*. after treatment.

[b]P <0.01, before *vs*. after treatment.

[c]P <0.001, before *vs*. after treatment.

*P <0.05, after vs. after treatment.

	Prednisolone			**Prednisolone + 5αRI**		
Metabolic Variable	*Before*	*After*	*Absolute change*	*Before*	*After*	*Absolute change*
NEFAs, µmol/L						
Basal	490.3 ± 53.0	393.5 ± 67.3	-96.8 ± 63.9	405.8 ± 30.3	465.9 ± 39.2	60.1 ± 45.2
Low insulin	43.1 ± 28.9	36.8 ± 14.3*	-6.3 ± 16.5[a]	49.8 ± 8.6[b]	88.5 ± 13.5[b,*]	38.7 ± 10.9[a]
High insulin	13.8 ± 4.8	16.3 ± 4.0	2.5 ± 3.3	22.6 ± 4.3	20.4 ± 3.0	-2.2 ± 3.6
Glycerol, µmol/L						
Basal	33.0 ± 3.1	29.9 ± 4.2	-3.2 ± 4.6	27.2 ± 1.9	29.2 ± 2.6	2.0 ± 3.7
Low insulin	8.2 ± 1.5[b]	9.9 ± 1.5[b]	1.8 ± 0.3	7.1 ± 0.9[b]	10.8 ± 1.1[b]	3.7 ± 1.2
High insulin	6.9 ± 1.4	7.1 ± 0.9	0.3 ± 0.8	6.0 ± 0.6	7.4 ± 1.0	1.4 ± 1.0
β-hydroxybutyrate, µmol/L						
Basal	135.2 ± 35.5	93.5 ± 39.0	-41.7 ± 43.8	69.0 ± 14.1	87.8 ± 19.3	18.7 ± 21.1
Low insulin	15.9 ± 2.7	16.7 ± 1.8*	0.8 ± 2.0	19.9 ± 2.1[a]	25.6 ± 2.2[a,*]	5.7 ± 2.1
High insulin	9.0 ± 1.2	10.0 ± 1.8	1.0 ± 2.3	13.6 ± 1.0	13.6 ± 0.9	0.0 ± 0.8
M-value, mg/kg ・min						

Low insulin	3.2 ± 0.5	2.7 ± 0.7	-0.5 ± 0.5	4.0 ± 0.5[a]	2.6 ± 0.4[a]	-1.4 ± 0.5
High insulin	11.5 ± 1.2	12.0 ± 1.9	-1.2 ± 2.0	10.9 ± 1.1	9.8 ± 0.9	-1.1 ± 0.9
M/I-value, mg/kg ・min ・pmol ・L						
Low insulin	0.026 ± 0.005[a]	0.015 ± 0.003[a]	-0.010 ± 0.003	0.026 ± 0.004[a]	0.017 ± 0.002[a]	-0.010 ± 0.003
High insulin	0.027 ± 0.006	0.045 ± 0.015	0.015 ± 0.009	0.027 ± 0.004	0.026 ± 0.003	-0.001 ± 0.002
Ra glucose, mg/kg ・min						
Basal	2.55 ± 0.34	2.62 ± 0.19	0.07 ± 0.38	2.67 ± 0.16[a]	3.05 ± 0.18[a]	0.38 ± 0.13
EGP, mg/kg ・min						
Low insulin	1.40 ± 0.26	1.29 ± 0.29	-0.11 ± 0.36	1.11 ± 0.16[a]	2.05 ± 0.30[a]	0.93 ± 0.40
Gd, mg/kg ・min						
Low insulin	2.5 ± 0.6[a]	1.8 ± 0.6[a]	-0.8 ± 0.2	2.9 ± 0.5	2.0 ± 0.3	-0.8 ± 0.4
High insulin	11.0 ± 2.2	10.4 ± 2.4	-0.6 ± 1.5	8.8 ± 1.1	8.4 ± 1.1	-0.4 ± 1.2
Ra palmitate, mg/kg ・min						
Basal	1.98 ± 0.26	1.73 ± 0.17	-0.26 ± 0.32	1.60 ± 0.09	1.85 ± 0.21	0.25 ± 0.20
Low insulin	0.66 ± 0.11	0.63 ± 0.09	-0.03 ± 0.08	0.60 ± 0.05	0.96 ± 0.30	0.35 ± 0.26
High insulin	0.51 ± 0.06	0.46 ± 0.05	-0.05 ± 0.04	0.49 ± 0.04	0.53 ± 0.08	0.04 ± 0.06
Breath 13-CO_2 AUC, mmol/h/kg						
Low insulin	0.042 ± 0.007	0.040 ± 0.004	-0.002 ± 0.005	0.043 ± 0.003[b]	0.036 ± 0.002[b]	-0.007 ± 0.002
High insulin	0.073 ± 0.003	0.056 ± 0.007	-0.018 ± 0.009	0.070 ± 0.003[c]	0.060 ± 0.002[c]	-0.010 ± 0.002
Adipose microdialysis						
Glycerol AUC, µmol/L ・h						
Basal	227.7 ± 37.1	242.1 ± 50.8	12.0 ± 23.5	336.4 ± 80.8	235.7 ± 23.2	-77.4 ± 53.4
Low insulin	124.0 ± 38.0	151.7 ± 34.9	23.1 ± 17.5	199.9 ± 32.9	199.4 ± 39.1	-0.4 ± 26.2
High insulin	68.0 ± 6.7	98.8 ± 24.9	25.7 ± 21.8	140.4 ± 34.0	136.8 ± 39.1	-3.0 ± 22.6
Pyruvate AUC, µmol/L ・h						
Basal	127.3 ± 11.4	132.2 ± 47.7	4.1 ± 35.9	141.8 ± 18.9	133.0 ±17.2	-6.8 ± 15.5
Low insulin	116.2 ± 17.3	136.0 ± 19.9	16.5 ± 9.6	144.7 ± 16.9	117.3 ± 21.4	-23.2 ± 22.8

High insulin	110.6 ± 15.5	106.1 ± 22.6	-3.8 ± 24.2	127.9 ± 22.3	123.2 ± 29.4	-4.0 ± 18.0
Lactate AUC, μmol/L・h						
Basal	1.7 ± 0.5	1.9 ± 0.7	0.2 ± 0.7	1.8 ± 0.4	1.5 ± 0.2	-0.2 ± 0.3
Low insulin	3.0 ± 1.5	2.4 ± 0.6	-0.5 ± 1.2	2.4 ± 0.5	1.7 ± 0.3	-0.5 ± 0.6
High insulin	3.3 ± 1.4	1.9 ± 0.4	-1.2 ± 0.9	2.4 ± 0.5	1.7 ± 0.4	-0.5 ± 0.5
Glucose AUC, μmol/L・h						
Basal	3.9 ± 0.5	3.7 ± 0.5	-0.2 ± 0.6	4.2 ± 0.3	4.3 ± 0.3	0.1 ± 0.3
Low insulin	3.5 ± 0.7	3.9 ± 0.5	0.4 ± 0.9	3.3 ± 0.3	3.1 ± 0.4	-0.2 ± 0.4
High insulin	3.5 ± 0.7	3.5 ± 0.7	0.0 ± 0.9	3.2 ± 0.4	2.6 ± 0.3	-0.5 ± 0.4

Table 3. The impact of specific 5αRI (finasteride 5mg once daily or dutasteride 0.5mg once daily) co-administered with prednisolone (10mg once daily) on glucose and lipid metabolism during a two-step hyperinsulinemic euglycemic clamp.

Abbreviations: EGP, endogenous glucose production; Gd, glucose disposal; NEFA, non-esterified fatty acids. Data shown are before, after and absolute change following randomization to 7 days of treatment with either prednisolone (10mg once daily), prednisolone (10mg once daily) and finasteride (5mg once daily) or prednisolone (10mg once daily) and dutasteride (0.5mg once daily).

[a]P <0.05, before vs after treatment.

[b]P <0.01, before vs after treatment.

*P <0.05, after vs after treatment.

	Prednisolone + Finasteride			**Prednisolone + Dutasteride**		
Metabolic Variable	*Before*	*After*	*Absolute change*	*Before*	*After*	*Absolute change*
NEFA, µmol/L						
Basal	427.0 ± 49.6	500.2 ± 61.9	73.2 ± 76.9	381.1 ± 33.3	425.9 ± 44.9	44.8 ± 47.3
Low insulin	33.1 ± 5.4[a]	81.8 ± 19.0[a]	48.7 ± 17.8	69.3 ± 14.2	96.3 ± 20.6	27.0 ± 11
High insulin	18.2 ± 2.7	18.2 ± 3.4	0.0 ± 1.1	27.8 ± 8.6	23.0 ± 5.2	-4.8 ± 8.0
Glycerol, µmol/L						
Basal	29.0 ± 3.1	31.5 ± 4.2	2.6 ± 6.3	25.2 ± 2.0	26.6 ± 2.6	1.3 ± 3.8
Low insulin	6.7 ± 1.0	11.3 ± 2.0	4.7 ± 1.9	7.5 ± 1.5	10.0 ± 1.0	2.5 ± 1.3
High insulin	5.7 ± 0.7	8.1 ± 1.8	2.4 ± 1.7	6.3 ± 1.1	6.6 ± 0.6	0.2 ± 0.7
β-hydroxybutyrate, µmol/L						
Basal	59.9 ± 8.5	88.3 ± 29.4	28.5 ± 28.4	78.2 ± 27.7	87.2 ± 27.9	9.0 ± 33.4
Low insulin	18.5 ± 1.9[a]	26.9 ± 3.6[a]	8.4 ± 3.0	21.3 ± 3.8	24.3 ± 2.6	3.0 ± 2.8
High insulin	14.9 ± 1.1	14.1 ± 1.5	-0.8 ± 1.5	12.2 ± 1.5	13.1 ± 1.2	0.9 ± 0.8
M-value, mg/kg・min						

Low insulin	3.7 ± 0.4	2.5 ± 0.6	-1.1 ± 0.6	4.3 ± 1.1	2.7 ± 0.5	-1.6 ± 0.9
High insulin	12.9 ± 0.8[a]	9.9 ± 1.3[a]	-2.9 ± 1.2[a]	8.7 ± 1.8	9.6 ± 1.3	1.0 ± 0.9[a]
M/I-value, mg/kg ・min ・pmol ・L						
Low insulin	0.024 ± 0.003	0.018 ± 0.004	-0.006 ± 0.004	0.029 ± 0.008	0.015 ± 0.003	-0.014 ± 0.006
High insulin	0.034 ± 0.004	0.028 ± 0.005	-0.005 ± 0.003	0.019 ± 0.005	0.023 ± 0.003	0.004 ± 0.003
Ra glucose, mg/kg ・min						
Basal	2.81 ± 0.20	3.07 ± 0.18	0.26 ± 0.12	2.52 ± 0.27	3.04 ± 0.36	0.52 ± 0.24
EGP, mg/kg ・min						
Low insulin	1.30 ± 0.23	1.90 ± 0.20	0.60 ± 0.32	0.89 ± 0.21	2.22 ± 0.63	1.32 ± 0.80
Gd, mg/kg ・min						
Low insulin	2.7 ± 0.4	1.9 ± 0.5	-0.8 ± 0.6	3.1 ± 1.0	2.2 ± 0.5	-0.9 ± 0.7
High insulin	10.8 ± 1.3	8.3 ± 1.2	-2.5 ± 1.4[a]	6.5 ± 1.4	8.7 ± 1.9	2.2 ± 1.7[a]
Ra palmitate, mg/kg ・min						
Basal	1.70 ± 0.11	2.11 ± 0.36	0.41 ± 0.34	1.48 ± 0.14	1.54 ± 0.10	-0.05 ± 0.15
Low insulin	0.62 ± 0.08	1.24 ± 0.54	0.63 ± 0.47	0.58 ± 0.07	0.62 ± 0.05	0.04 ± 0.06
High insulin	0.58 ± 0.06	0.63 ± 0.14	0.05 ± 0.12	0.39 ± 0.04	0.41 ± 0.03	0.02 ± 0.03
Breath 13-CO_2 AUC, mmol/hr/kg						
Low insulin	0.039 ± 0.003	0.033 ± 0.002*	-0.005 ± 0.002	0.048 ± 0.006	0.040 ± 0.002*	-0.008 ± 0.005
High insulin	0.065 ± 0.004[a]	0.056 ± 0.003[a]	-0.009 ± 0.003	0.076 ± 0.005[b]	0.064 ± 0.004[b]	-0.010 ± 0.003

Figure 1

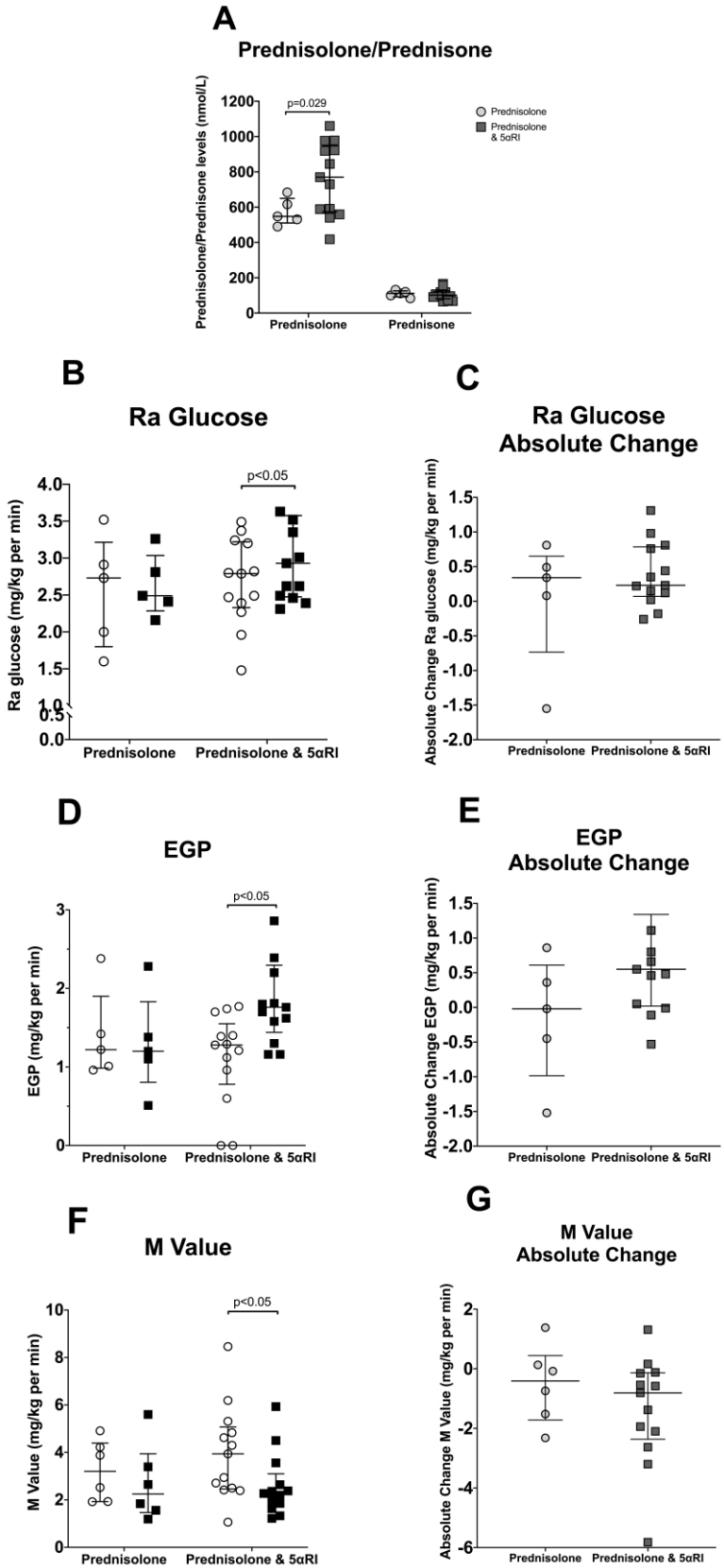

Figure 2

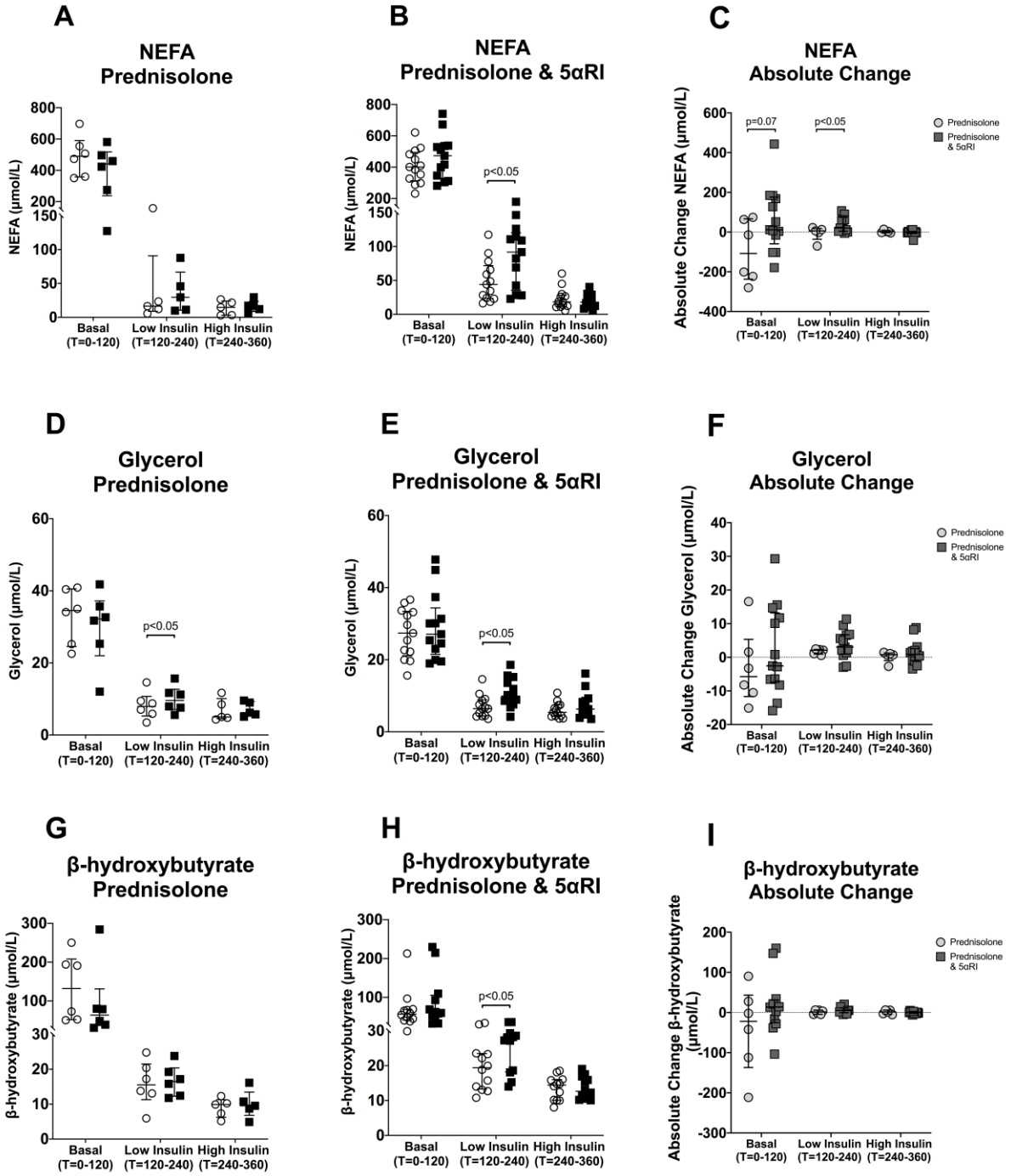

Figure 3