Short-term prednisone use antagonizes insulin’s anabolic effect on muscle protein and glucose metabolism in young healthy people

Kevin R. Short, Maureen L. Bigelow, and K. Sreekumaran Nair

Endocrinology Research Unit, Mayo Clinic College of Medicine, Rochester, Minnesota

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Glucocorticoids are regularly used in clinical practice to treat conditions like inflammation and asthma. Among the undesirable effects of glucocorticoids are hyperglycemia and hyperinsulinemia (14, 16, 25, 29, 35), as well as muscle wasting and weakness with prolonged use (9, 10, 12). Chronic excess of glucocorticoids, such as that seen with Cushing’s syndrome, is well known to be associated with a reduction in lean mass and increased abdominal fat mass (4, 33). The mechanism accounting for glucocorticoid effects on muscle is not well understood but likely involve changes in the balance between protein synthesis and breakdown leading to net protein loss. There are several studies on regulation of muscle protein synthesis and catabolism in rodents, but most used higher doses of glucocorticoids than those typically given to humans (19, 20, 28, 34). Human studies in this area are so far inconclusive. In women using prednisone (Pred) for ~9 yr to treat rheumatoid arthritis, muscle protein fractional synthesis rate was lower compared with arthritic patients not using steroid treatment, but the affected women were also undergoing knee surgery, which may have contributed to the observed differences (7). When healthy people were given Pred for 5 days (0.8 mg·kg⁻¹·day⁻¹) appearance of urinary 3-methylhistidine, a marker of muscle protein breakdown, was increased, although this metabolite can also appear from non-muscle tissues such as the gut (1). Although these data suggest that muscle protein synthesis may be reduced or breakdown increased by glucocorticoid use, the evidence is indirect.

A more direct approach, the arteriovenous sampling method, has been used in three prior studies to measure muscle anabolism and catabolism across the forearm or leg of healthy participants. When oral dexamethasone (8 mg/day) was used for 3 or 4 days, neither phenylalanine uptake (a marker of protein synthesis) nor appearance (from protein breakdown) across the forearm was significantly altered during basal, postabsorptive conditions (14, 16). Phenylalanine net balance, however, was reduced, indicative of net catabolism, in response to the 3-day, but not the 4-day protocol (14, 16). We previously reported that 6 days of Pred use (0.5 mg·kg⁻¹·day⁻¹) produced no change in either whole body or leg protein kinetics in healthy young participants (29). Although Pred use resulted in clear changes in hormones and metabolites (e.g., increased insulin, IGF-I, and glucose) and reduced leg blood flow, we found no evidence of changes in amino acid balance across the leg, changes in muscle protein synthesis and breakdown, or muscle protein fractional synthesis rate.

The lack of measurable effects of glucocorticoids on muscle protein metabolism in our prior investigation (29) was unexpected given the clinical observation of lean tissue loss in conditions of glucocorticoid excess. In prior studies in which Pred use was shown to increase whole body protein catabolism (1, 2, 8, 24), oral daily doses of 0.5–0.8 mg·kg⁻¹·day⁻¹ were given for 6–7 days. Since the dose of Pred used in our prior study (0.5 mg·kg⁻¹·day⁻¹ for 6 days) was at the lower end of the range used in those earlier studies, we considered the possibility that the effects of Pred on muscle protein metabolism would become evident at higher dosages. Additionally, measurements in our previous study were conducted in the postabsorptive state following an overnight fast. However, the impact of glucocorticoids may be more evident when insulin or amino acids are elevated. For example, in the fed state, whole body leucine oxidation and net protein balance typically increase, but Pred use was shown to blunt these responses (1, 8). Likewise, dexamethasone use prevented the acute increase in forearm phenylalanine net balance during hyperinsulinemia or amino acid infusion (14, 16). Those results suggest that a primary means for glucocorticoids to cause protein wasting may
be through interference with the anabolic effects of insulin or amino acids.

Thus, the purpose of the present study was to investigate the effect of glucocorticoids on whole body and leg muscle protein metabolism during the fasting postabsorptive state and during hyperinsulinemia. We studied young healthy volunteers in double-blind manner following a higher dose of oral Pred (0.8 mg·kg⁻¹·day⁻¹ for 6 days vs. placebo control) than in our prior study. An arteriovenous three-pool model with muscle biopsies was used with multiple labeled amino acid tracers.

**MATERIALS AND METHODS**

**Materials.** L-[¹³C,¹⁵N]leucine (Leu, 97.2 atom percent excess, APE), L-[¹⁵N]phenylalanine (Phe, 97.6 APE), L-[¹⁵N]tyrosine (Tyr, 96.8 APE), L-[¹³C,H]Tyr (97.5 APE), and [6,6-²H₂]glucose (94.1 APE) were purchased from Cambridge Isotope Laboratories, (Woburn, MA). Isotope solutions were tested before use for their isotopic and chemical purity, were prepared under sterile conditions, and were determined to be bacteria and pyrogen free. The study protocol was approved by the Institutional Review Board of the Mayo Foundation. All procedures were clearly explained to the study volunteers before informal oral and written consent was obtained.

**Participants.** Seven young healthy people (4 women, 3 men), volunteers, were recruited from the local area (Rochester, MN). Average characteristics (means ± SE) of the group were: age 33 ± 3 yr, height 174 ± 3 cm, weight 75.0 ± 4.8 kg, body mass index 24.6 ± 1.0 kg/m², body-fat-free mass 48.3 ± 3.7 kg, and body fat 29.8 ± 2.8%. Body composition was measured using dual-energy X-ray absorptiometry. Health status was assessed by medical history, physical examination, blood chemistry (including liver enzymes, creatinine, electrolytes, and glucose), complete blood count, urinalysis, and electrocardiogram. Exclusion criteria included tobacco use, β-blockers, or any medications that could affect metabolism or blood coagulation, diabetes or other endocrine disorders, and debilitating chronic illness. Participants were not taking medications or engaged in a regular exercise program at the time of the study. Each participant completed two similar trials, receiving either oral Pred or placebo in a randomized, double-blind manner for 6 days. Regular lifestyle patterns were maintained throughout the study period, a diet estimated to meet energy requirements and 15% of energy from carbohydrate, 30% and 55% of energy from fat and protein, respectively, was provided on days 3–5. Strenuous physical activity was avoided on those same days. On the morning of day 5, the last dose of Pred or placebo was taken at 0830. By 0930, a polyethylene venous catheter was placed in an antecubital arm vein for infusion of isotopically labeled tracers. Primed, continuous infusions of [¹³C,¹⁵N]Leu (10.4 μmol·kg⁻¹·h⁻¹; thereafter), [¹⁵N]Phe (4.1 μmol·kg⁻¹·h⁻¹; thereafter), [¹³C,H]Tyr (3.0 μmol·kg⁻¹·h⁻¹; thereafter), [¹⁵N]Tyr (1.6 μmol·kg⁻¹·h⁻¹; thereafter), and [6,6-²H₂]glucose (13.9 μmol·kg⁻¹·h⁻¹; thereafter) were started (time 00 min) and continued for 9 h. Participants were transported a short distance to the Vascular Radiology Laboratory for placement of leg catheters for infusion and sample collection. French sheaths were inserted into the femoral artery and vein of the right leg on the first trial and in the left leg on the second trial. A femoral artery catheter was inserted through the sheath with the catheter tip in the common iliac artery. This catheter was used for arterial blood sampling, and the sheath was used to infuse indocyanine green. The distal tip of the venous sheath was placed in the external iliac vein a few centimeters above the inguinal ligament. Normal saline was infused to maintain patency of the arterial and venous catheters.

Each study day was divided into two phases. The basal phase comprised the postabsorptive period up to 240 min, when saline was infused in the forearm catheter. This was followed by a euglycemic hyperinsulinemic clamp (insulin phase). Insulin was infused systemically through the forearm catheter at 1.5 mIU·kg⁻¹·min⁻¹ (9 pmol·kg⁻¹·min⁻¹). Dextrose infusion was adjusted to maintain plasma glucose concentration, measured every 10 min, at 5 mmol/l.

**Concentration and enrichment of tracer amino acids was measured during the insulin phase using indirect calorimetry (DeltaTrac; SensorMedics, Yorba Linda, CA) from 360 to 405 min. Upon completion of the study, leg catheters were removed and participants remained overnight for observation before being discharged.

**Hormone and metabolite assays.** Glucose was measured with a Beckman Glucose Analyzer (Beckman Instruments, Porterville, CA). Nonesterified free fatty acids were measured using an enzymatic colorimetric assay (NEFA C; Wako Chemicals, Richmond, VA). Plasma amino acid concentrations were measured with a high-performance liquid chromatography (HP 1090, 1046 fluorescence detector and cooling system) using precolumn O-phthalaldehyde derivatization (11). Urinary nitrogen content was measured using a Beckman GM7 Analox Microstat (Beckman Instruments, Fullerton, CA).

Insulin and human growth hormone were measured with two-site immunoenzymatic assays (Access System; Beckman Instruments, Chaska, MN). Glucagon and C-peptide were measured by direct radioimmunoassays (Linco Research, St. Louis, MO). After separation from their binding proteins with a simple organic solvent, total insulin-like growth factor I (IGF-I) and IGF-II were measured with two-site immunoradiometric assays (Diagnostic Systems Laboratories, Webster, TX). IGF-binding protein (BP)-1 and -3 were also measured with two-site immunoradiometric assays, while IGFBP-2 was measured by a double antibody radioimmunoassay (Diagnostic Systems Laboratories).

**Tracer analyses.** Amino acids in arterial and venous plasma were prepared by acidification with addition of acetic acid and separation on a cation exchange column (AG-50-X8; Bio-Rad, Hercules, CA). Concentration and enrichment of tracer amino acids was measured under electron ionization conditions using a gas chromatograph-mass spectrometer (GC-MS, HP5973 MSD; Hewlett-Packard Instruments, Avondale, CA). After formation of tert-butyldimethylsilyl ester derivatives, amino acid enrichment was measured by fragment ion monitoring at m/z 200/201/302/303/304 for [¹³C,¹⁵N]Leu and [¹⁵C]Leu.
337/336 for [13C]Phe, 467/466 for [15N]Tyr, and 470/466 for [3H]Tyr, using appropriate standard curves for each analyte. Concentration measurements were performed in a separate run on the same instrument by monitoring m/z 302/308 for Leu, 336/342 for Phe, and 466/472 for Tyr and comparison of samples to standard curves for each amino acid. The plasma enrichment of [13C]ketocisocaproate (KIC), the deamination product of Leu, was measured after formation of its quinaxalinol-TMS derivative using electron ionization in the same GC-MS and monitoring of m/z 233/232. KIC concentration was measured simultaneously by comparison with ketoisovalerate, which was added as an internal standard. All plasma samples were analyzed in duplicate.

Muscle samples were used to separate the amino-acyl tRNA as previously described (15). After derivatization with methyl heptfluorobutylate, the enrichments of [13C,15N]Leu and [15N]Phe were measured in duplicate on the HP5973 GC-MS under positive chemical ionization conditions, monitoring the fragment ions at m/z 321/319 using a GC-MS (TSQ; Thermo Finnigan, Sommerset, NJ). Samples were run in duplicate and compared with a standard curve.

Calculations. Whole body amino acid kinetics were calculated from the mean values of isotopic enrichment at steady state from 220 to 240 min (basal phase) and from 510 to 540 min (insulin phase). Flux rates of Leu, Phe, and Tyr were determined by tracer dilution (18, 21). Leu carbon flux was calculated using arterial [13C]KIC as a surrogate for intracellular Leu enrichment (17, 26), while arterial [13C,15N]Leu was used to calculate Leu nitrogen flux (18, 21). Tyrf carbon flux was determined from [3H]Tyr enrichment. Rates of Phe conversion to Tyrf, Phe incorporation into protein (protein synthesis), Leu transamination to KIC, and KIC reamination to Leu were calculated as previously described (18, 21).

Amino acid kinetics across the leg were calculated using both two- and three-pool models (18, 21, 32). The two-pool calculation uses arterial and venous concentration and enrichment and the rate of leg blood flow to provide an estimate of net balance and rates of appearance (Ra) and disappearance (Rd) of a given amino acid. The three-pool model uses those same values plus intracellular tracer enrichment to determine rates of amino acid transport among the arterial, venous, and tissue, as well as protein synthesis and breakdown (32). We used muscle amino-acyl tRNA enrichment for the intracellular free pool, since it should be the immediate precursor supply for protein synthesis and because this approach has been previously validated (32).

Glucose uptake across the leg was calculated using the same arterial-venous balance equations used for amino acids. Whole body glucose Ra was determined from tracer dilution using Steele’s equation for steady state (30).

Muscle strength tests. Three tests of upper body strength were conducted on the morning of day 5 of each study phase. Isometric handgrip strength was measured as the best of 6 maximal efforts. Chest press and arm (biceps) curl strength were determined as the one-repetition maximum weight lifted during a progressive series of attempts. Two familiarization sessions were completed 1 and 2 wk prior to commencing the study to ensure reliable maximal efforts with a minimal number of attempts. No lower body exercises were performed to minimize the chance that muscle activation would affect leg protein metabolism the following day.

Statistical analysis. Summarized values are reported as means ± SE. Two-way analysis of variance for repeated measures was used to test comparisons between placebo and Pred trials and the basal and insulin phases within trial. Paired t-tests were used for pairwise differences. Alpha level was set to 5% to define statistical significance.

RESULTS

Plasma hormones, NEFA, and amino acids. Compared with the placebo trial, Pred resulted in increased levels of circulating insulin (70%), C-peptide (45%), glucagon (28%), and IGF-I (19%) and lower IGFBP-3 (7%) in the basal phase of the study (Table 1). During insulin infusion, insulin concentration increased by design, but the final level was 13% lower in Pred compared with placebo. Also during the insulin phase, C-peptide levels were suppressed compared with basal values and became 50% lower in Pred than with placebo. Glucagon increased during the insulin clamp only during the Pred trial, to a level that was 103% higher than that of placebo. IGF-I concentration was 19% higher during the Pred trial vs. placebo and did not change from basal to insulin phase during either study. IGF-II concentration declined 10% during the insulin clamp in the placebo trial only, but otherwise did not differ among treatments. In both the Pred and placebo trials, IGFBP-1 decreased from basal during the insulin clamp, falling 71 and 78%. Also during insulin infusion, IGFBP-2 concentration increased 15%, whereas IGFBP-3 decreased 10%, but these changes occurred in the placebo trial only and did not result in significant differences compared with Pred.

NEFA concentration in the basal phase was not different in placebo (0.652 ± 0.093 mmol/l) vs. Pred (0.649 ± 0.087 mmol/l, P = 0.955). NEFA levels were suppressed (P <

### Table 1. Plasma hormones during basal and hyperinsulminemic phases of each study

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Prednisone</th>
<th>Placebo</th>
<th>Prednisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, µU/ml</td>
<td>4.2±0.7</td>
<td>7.1±0.8*</td>
<td>44.2±3.0†</td>
<td>38.2±2.8†</td>
</tr>
<tr>
<td>pmol/l</td>
<td>(25.0±4.1)</td>
<td>(42.8±4.7)</td>
<td>(265.1±18.2)</td>
<td>(229.4±16.8)</td>
</tr>
<tr>
<td>C-peptide, ng/ml</td>
<td>1.01±011</td>
<td>1.46±0.11*</td>
<td>0.45±0.08†</td>
<td>0.07±0.01†</td>
</tr>
<tr>
<td>nmol/l</td>
<td>(0.33±0.04)</td>
<td>(0.48±0.06)</td>
<td>(0.15±0.03)</td>
<td>(0.02±0.00)</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>83±6</td>
<td>107±8*</td>
<td>94±5</td>
<td>190±31*</td>
</tr>
<tr>
<td>ng/l</td>
<td>(83±6)</td>
<td>(107±8)</td>
<td>(94±5)</td>
<td>(190±31)</td>
</tr>
<tr>
<td>Growth hormone, ng/ml</td>
<td>3.78±2.78</td>
<td>2.30±0.58</td>
<td>5.16±3.64</td>
<td>6.99±2.40</td>
</tr>
<tr>
<td>µg/l</td>
<td>(3.78±2.78)</td>
<td>(2.30±0.58)</td>
<td>(5.16±3.64)</td>
<td>(6.99±2.40)</td>
</tr>
<tr>
<td>IGF-I (total), ng/ml</td>
<td>280±36</td>
<td>332±32*</td>
<td>275±35</td>
<td>328±29*</td>
</tr>
<tr>
<td>IGF-II, ng/ml</td>
<td>576±33</td>
<td>551±39</td>
<td>519±27†</td>
<td>522±30</td>
</tr>
<tr>
<td>IGFBP-1, ng/ml</td>
<td>20.7±4.7</td>
<td>16.7±5.0</td>
<td>4.7±1.9†</td>
<td>4.8±1.3†</td>
</tr>
<tr>
<td>IGFBP-2, ng/ml</td>
<td>541±158</td>
<td>525±161</td>
<td>623±169</td>
<td>495±143</td>
</tr>
<tr>
<td>IGFBP-3, ng/ml</td>
<td>338±250</td>
<td>3140±238*</td>
<td>3020±242†</td>
<td>3146±196</td>
</tr>
</tbody>
</table>

Values are given as means ± SE. *Pred vs. Placebo, P < 0.05; †insulin vs. basal within trial, P < 0.05.
0.001) during insulin infusion compared with basal, but the decline was less in Pred (0.181 ± 0.011 mmol/l, 135% higher than placebo, \( P < 0.001 \)) than placebo (0.077 ± 0.007 mmol/l).

Circulating concentrations of amino acids were, on average, 24% higher in Pred vs. placebo in the basal phase of the study [Supplemental Table S1 (supplemental materials are found in the online version of this article)]. Several individual amino acids were increased with Pred, with the highest difference observed for Ala (124% higher compared with placebo). Phe was 20% higher (\( P < 0.05 \)), with similar trends for Leu (19% higher, \( P < 0.08 \)), and Tyr (15% higher, \( P < 0.06 \)). During the insulin infusion, the confusion of the mixed amino acid solution achieved the goal of preventing a decline in the concentrations of Leu and the total amino acids measured in both placebo and Pred trials. The total amino acid concentration increased 22% from basal in the placebo trial but was no longer a difference between trials during the insulin phase. Only Gly and Ser differed between trials in the insulin phase. The concentration of several of individual amino acids was, however, changed from basal to the insulin phase in both trials, as noted in Supplemental Table S1.

**Leg blood flow.** Leg blood flow in the basal phase while on Pred (266 ± 33 ml/min) was 18% lower (\( P = 0.030 \)) than during the placebo trial (218 ± 28 ml/min). During the insulin phase, leg blood flow increased 55% above the basal rate in both trials (\( P < 0.007 \)), but there was no longer a statistically significant difference between Pred (346 ± 46 ml/min) and placebo (407 ± 22 ml/min, \( P = 0.215 \)).

**Amino acid kinetics.** There were no differences between trials for enrichment of amino acid tracers or KIC in arterial plasma or muscle amino-acetyl tRNA, with the exception of \([^{15}\text{N}]\text{Phe} \) tRNA, which was lower in the Pred trial during the basal phase (Supplemental Table S2). During the insulin phase, enrichment of several compounds differed significantly from basal, though during both phases the enrichment levels were at steady state during the measurement period.

Whole body kinetics of Phe, Tyr, and Leu did not differ between the Pred and placebo trials in either the basal or insulin phases of the study (Fig. 1). However, in both trials, insulin infusion resulted in reduced fluxes of Phe and Tyr, hydroxylation of Phe to Tyr, Phe incorporation into protein, and Leu carbon flux, with no change in Leu nitrogen flux (deamination to KIC). The reamination of KIC to Leu was unaffected by insulin in the placebo trial but was increased 19% over basal by insulin in the Pred trial. This resulted in a trend (\( P = 0.079 \)) for the rate of KIC to Leu reamination to be higher (27%) in the Pred trial than with placebo during the insulin infusion phase of the study.

There were no differences between trials for basal leg \( R_a \) or \( R_d \) or net balance of Leu, Phe, or Tyr calculated from the two-pool model (Fig. 2). During insulin infusion, \( R_a \) of Leu carbon (Pred trial only) and Tyr (both trials) declined from baseline, whereas \( R_d \) of Leu carbon and Phe increased only in the placebo trial, the latter change resulting in values that were significantly greater than the corresponding value for the Pred trial. As a result of these and smaller, nonsignificant changes in \( R_a \) and \( R_d \), net balances of Leu, Phe, and Tyr were all increased during insulin infusion compared with the basal phase (Fig. S1). However, for each of these amino acids the net balance value during insulin infusion was significantly lower during the Pred trial compared with placebo. There were no significant differences between trials for basal KIC reamination to Leu (placebo, 1.80 ± 0.18 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg leg FFM}^{-1} \), Pred.

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**Fig. 1.** Whole body amino acid kinetics. A: Phe and Tyr kinetics did not differ between placebo (Plac) or prednisone (Pred) treatments in either basal or insulin clamp phases of the study but declined from basal during the insulin clamp. B: Leu carbon (C) flux was not altered by Pred but declined with insulin infusion. Leu nitrogen (N) flux (deamination) was not changed by Pred or insulin. KIC-to-Leu (reamination) was increased by insulin only in the Pred trial. *Insulin phase vs. basal phase, \( P < 0.05 \).
1.88 ± 0.27 μmol·min⁻¹·kg leg FFM⁻¹) or during insulin infusion (placebo, 1.87 ± 0.25 μmol·min⁻¹·kg leg FFM⁻¹, Pred, 1.83 ± 0.27 μmol·min⁻¹·kg leg FFM⁻¹), and there were no significant differences in reamination between basal and insulin phases.

The leg three-pool model calculations confirmed that during the basal phase of the study there were no significant differences between trials in the amino acid kinetics among artery, vein, and tissue or in the intracellular rates of protein synthesis and breakdown (Fig. 3, A and B). During the insulin infusion phase, both Leu and Phe delivery (Fin) to the leg increased, and several of the other intercompartmental rates also increased. Most, but not all, of the insulin-induced changes were found to be not significantly different between trials. An exception, however, was the change in protein synthesis measured using the Phe tracer. Although there was not a significant change in protein synthesis rate with insulin within either trial, the delta over baseline was higher for the placebo trial vs. Pred.

Basal net balance of protein synthesis and breakdown derived from the three-pool model was negative for Leu and Phe in both trials (Fig. 4). During insulin infusion, these net balance values increased above basal in both Pred and placebo trials, achieving positive balance for all but the Phe tracer in the Pred trial. However, the net balance of Leu and Phe achieved during the insulin phase were both significantly lower during the Pred trial than with placebo.

**Glucose metabolism.** Basal glucose concentration was 16% higher in the Pred trial than with placebo. By design, glucose levels were held at similar concentrations during insulin infusion, so there was no longer a difference between trials (Table 2). The amount of glucose required to maintain euglycemia during the insulin phase was 72% lower during the Pred trial than with placebo. The tracer enrichment was at steady state during the sample collection times in both phases of both trials, although arterial glucose enrichment tended to increase during insulin infusion compared with basal in both trials. Endogenous rate of glucose appearance did not differ between trials in either the basal or insulin phases and tended to decline from basal rates during insulin infusion in both the placebo (21%, P = 0.070) and Pred (11%, P = 0.095) trials. Leg glucose uptake did not differ between trials in the basal phase. Insulin infusion resulted in increased leg glucose uptake in both trials but was 65% lower in Pred than with placebo.
Indirect calorimetry was performed during the insulin infusion phase of the study. During the Pred trial, resting oxygen consumption, \( \bar{V}O_2 \), was 6% higher (268 ± 110 ml/min) than during the placebo trial (253 ± 17 ml/min, \( P = 0.039 \)), whereas carbon dioxide production, \( \bar{V}CO_2 \), was not different between trials (221 ± 13 vs. 224 ± 18 ml/min for placebo and Pred, respectively). As a result, in the Pred trial there were trends for 5% lower respiratory exchange ratio (Pred 0.83 ± 0.01, placebo 0.87 ± 0.01, \( P = 0.095 \)), 35% higher fat oxidation (Pred 4.65 ± 0.35 g/h, placebo 3.46 ± 0.68, \( P = 0.095 \)), and 5% higher energy expenditure (Pred 1.30 ± 0.12 kcal/min, placebo 1.23 ± 0.09 kcal/min, \( P = 0.081 \)). Urinary nitrogen excretion was not significantly different during the Pred trial (47 ± 9 mmol/h) vs. placebo (36 ± 5 mmol/h, \( P = 0.16 \)).

Muscle function. There were no statistically significant differences (\( P < 0.30 \)) in muscle strength between trials. The peak strength values for placebo and Pred conditions, respectively, were 52.8 ± 9.8 and 55.7 ± 10.8 kg for chest press, 39.2 ± 8.7 and 41.5 ± 10.4 kg for arm curl, and 45.3 ± 4.7 and 45.3 ± 4.6 kg for isometric handgrip.

**DISCUSSION**

Since glucocorticoid excess is associated with loss of lean tissue, the aim of this investigation was to determine whether short-term glucocorticoid administration adversely affects protein dynamics across the leg. Pred use for 6 days in healthy participants had little impact on leg muscle or whole body protein metabolism in the basal state, but during hyperinsulinemia while maintaining glucose and amino acid levels, the normal increase in leg muscle anabolism was significantly blunted. This finding was confirmed using two different methodological approaches (2- and 3-pool modeling) and with two different tracers for amino acid kinetics.
Glucose metabolism parameters down across the leg in healthy participants (23). In the present study, hyperinsulinemia and a modest increase in amino acids caused a significant increase in leg protein net balance, which was less robust in the Pred trial. Therefore, with maintained or slightly elevated concentrations of most amino acids and a \(-50\%\) increase in leg blood flow, amino acid delivery to the muscle was increased during the hyperinsulinemic phase in both trials. Both inward transport from artery to tissue and net uptake (inward minus outward transport; not shown) were increased above basal values during hyperinsulinemia in both the placebo and Pred conditions, although leg protein synthesis and breakdown rates did not significantly change. Both the two-pool and three-pool models support the finding that leg amino acid net balance \((R_d - R_a)\) in the 2-pool model, protein synthesis \(-\) protein breakdown in the 3-pool model) was increased during the insulin phase and that Pred attenuated this effect. Thus, the net anabolic effect of insulin in the postprandial range was blunted by glucocorticoid use and could potentially lead to muscle loss if continued unabated over several weeks. The effect of glucocorticoids to interfere with the anabolic response appears to be through the intracellular signaling pathways that are regulated by insulin and amino acids. It was reported that high-dose dexamethasone administration inhibits the insulin- or amino acid-mediated phosphorylation of p70 S6 kinase in rats (6, 27, 28) and the amino acid effect in signaling pathways that are regulated by insulin and amino acids. Available to explore these signaling pathways in the present investigation, so further work is needed to determine whether impaired signaling accounts for the effect of Pred to inhibit insulin-mediated muscle anabolism in humans and whether this effect can be reversed through intervention.

There was no effect of Pred use on whole body protein kinetics in the basal, postabsorptive period, in agreement with our previous study (29) and another study in which dexamethasone was used for 3 days (14). In contrast, increased postabsorptive whole body protein breakdown was reported in young healthy participants who were given similar doses glucocorticoids for 6–7 days (1, 2, 8, 24). There is no clear explanation for the differences among these studies, since similar conditions and participants were used. A potential limitation of the current study and our prior study is the small sample size of six to seven participants, which could prevent detection of small differences in whole body protein metabolism resulting from Pred use. However, the current results demonstrate that effects of insulin infusion were consistently evident under both treatment conditions, which supports the idea that any effects of Pred on whole body protein metabolism in this population are so small that they may not be physiologically relevant and would require impractically large sample size. The current

separate labeled amino acid tracers (Leu and Phe). Additionally, insulin-mediated leg glucose uptake was significantly reduced by Pred. Thus, Pred use resulted in muscle insulin resistance to both carbohydrate and protein metabolism, which could contribute to hyperglycemia and muscle wasting with continued use.

Consistent with our previous study (29), Pred had no effect on whole body or whole muscle metabolism in normal healthy participants during the basal, postabsorptive period. Basal leg blood flow was 18% lower on the Pred trial than with placebo, in agreement with the 25% reduction in our prior study (29). Amino acid delivery to the leg during the basal period was relatively unchanged by Pred; however, because concentrations of several amino acids tended to increase (15–20% for Leu, Phe, and Tyr). Thus, under postabsorptive conditions there was no evidence that glucocorticoids directly affected leg whole muscle amino acid uptake or protein synthesis or breakdown. It was previously shown that insulin plus amino acid infusion increased protein synthesis and reduced protein breakdown across the leg in healthy participants (23). In the present

### Table 2. Glucose metabolism parameters

<table>
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<tr>
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<th>Basal</th>
<th>Prednisone</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Pred</td>
<td>Placebo</td>
</tr>
<tr>
<td>Arterial glucose, mg/dl</td>
<td>91±2</td>
<td>106±1*</td>
<td>88±2</td>
</tr>
<tr>
<td>mmol/l</td>
<td>(5.0±0.1)</td>
<td>(5.9±0.1)</td>
<td>(4.9±0.1)</td>
</tr>
<tr>
<td>Glucose infusion rate, μmol·min(^{-1})·kg FFM(^{-1})</td>
<td>NA</td>
<td>NA</td>
<td>6.14±0.47</td>
</tr>
<tr>
<td>Arterial [H(_2)O]glucose, mC</td>
<td>1.63±0.04</td>
<td>1.56±0.08</td>
<td>2.05±0.18†</td>
</tr>
<tr>
<td>Endogenous Ra, μmol·min(^{-1})·kg FFM(^{-1})</td>
<td>19.0±1.1</td>
<td>20.0±1.2</td>
<td>14.9±2.4</td>
</tr>
<tr>
<td>Leg uptake, μmol·min(^{-1})·kg FFM(^{-1})</td>
<td>1.8±0.5</td>
<td>1.9±0.6</td>
<td>33.5±2.2†</td>
</tr>
</tbody>
</table>

Values are given as means ± SE. R\(_{an}\), rate of appearance; NA, not applicable. *Pred vs. Placebo, P < 0.05; †insulin vs. basal within trial, P < 0.05.
study highlights the importance of protein dynamic studies at the regional level, because whole body studies showed no effect of prednisone. Both whole body and regional studies measure only the average protein turnover of many proteins and do not provide any information on the potential impact of prednisone on individual protein synthesis and breakdown.

During hyperinsulinemia, whole body protein breakdown and Phe conversion to Tyr declined as expected (18), but Pred use did not alter this response. Thus, Pred promotes insulin resistance to protein metabolism in skeletal muscle, but this effect is obscured when measured in the larger whole body pool, which represents contributions from many other tissues. It should be noted that, while our study measured primarily the effect of hyperinsulinemia, amino acids exert a stronger effect than insulin on protein anabolism (23). Thus, during hyperinsulinaemia, such as after a meal, an inhibitory effect of Pred on whole body protein metabolism may be more evident. In two earlier investigations, Pred use for 6 days by young healthy participants was shown to blunt the rise in whole body Leu oxidation and net balance during an enterally infused meal solution (1, 8).

In addition to effects on muscle protein metabolism, Pred induced insulin resistance in glucose and lipid metabolism, as shown by impaired insulin-mediated glucose uptake in the leg (65% lower on the Pred trial) and blunted suppression of circulating free fatty acids. Pred did not affect endogenous glucose production in either the postabsorptive or hyperinsulinemic phases, in agreement with prior work (2, 3, 13), implicating peripheral insulin resistance as the main target of Pred. Additionally, we found evidence of Pred effects on pancreatic hormones during the insulin infusion phase. During insulin infusion, C-peptide concentration declined more in the Pred trial than with placebo, suggesting greater reduction in endogenous insulin secretion. It is therefore possible that the resulting lower circulating insulin in the Pred trial may account for some of the blunted responses to hyperinsulinemia. Additionally, glucagon concentration doubled from the basal to the insulin phase in the Pred trial but was unchanged in the placebo trial. The reason for this unexpected, apparently novel result is not known, but glucagon promotes protein catabolism and may therefore have played a role in the present findings (5). These effects could be mitigated in future studies with the use of somatostatin to inhibit endogenous pancreatic hormone secretion with controlled replacement of selected hormones and metabolites. To examine whether other hormonal changes might explain the responses in protein or glucose metabolism, we measured components of the of the IGF system. IGF-I was increased by Pred during basal and insulin phases, consistent with our prior report (29), but neither this finding nor the other minor changes in IGFs or IGFBP5 were associated with the protein or glucose outcomes.

In summary, prednisone administration for 6 days does not significantly alter leg muscle or whole body protein metabolism in young healthy participants. During hyperinsulinemia, however, the effect of prednisone to blunt muscle anabolism and leg glucose uptake is evident. These findings demonstrate that the effect of glucocorticoids to promote muscle loss is most likely to occur through an inhibition of anabolic responses to insulin and amino acids, as would occur following a meal.

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