

# Effects of Low-Dose Prednisolone on Hepatic and Peripheral Insulin Sensitivity, Insulin Secretion, and Abdominal Adiposity in Patients With Inflammatory Rheumatologic Disease

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**OBJECTIVE**—The metabolic effects of low-dose prednisolone and optimal management of glucocorticoid-induced diabetes are poorly characterized. The aims were to investigate the acute effects of low-dose prednisolone on carbohydrate metabolism and whether long-term low-dose prednisolone administration increases visceral adiposity, amplifying metabolic perturbations.

**RESEARCH DESIGN AND METHODS**—Subjects with inflammatory rheumatologic disease without diabetes mellitus were recruited. Nine subjects (age,  $59 \pm 11$  years) not using oral glucocorticoids were studied before and after a 7- to 10-day course of oral prednisolone 6 mg daily. Baseline data were compared with 12 subjects (age,  $61 \pm 8$  years) using continuous long-term prednisolone ( $6.3 \pm 2.2$  mg/day). Basal endogenous glucose production (EGP) was estimated by  $6,6\text{-}^2\text{H}_2$  glucose infusion, insulin sensitivity was estimated by two-step hyperinsulinemic-euglycemic clamp, insulin secretion was estimated by intravenous glucose tolerance test, and adipose tissue areas were estimated by computed tomography.

**RESULTS**—Prednisolone acutely increased basal EGP ( $2.44 \pm 0.46$  to  $2.65 \pm 0.35$  mg/min/kg;  $P = 0.05$ ) and reduced insulin suppression of EGP ( $79 \pm 7$  to  $67 \pm 14\%$ ;  $P = 0.03$ ), peripheral glucose disposal ( $8.2 \pm 2.4$  to  $7.0 \pm 1.6$  mg/kg/min;  $P = 0.01$ ), and first-phase ( $5.9 \pm 2.0$  to  $3.9 \pm 1.6$  mU/mmol;  $P = 0.01$ ) and second-phase ( $4.6 \pm 1.7$  to  $3.6 \pm 1.4$  mU/mmol;  $P = 0.02$ ) insulin secretion. Long-term prednisolone users had attenuated insulin suppression of EGP ( $66 \pm 14$  vs.  $79 \pm 7\%$ ;  $P = 0.03$ ) and nonoxidative glucose disposal ( $44 \pm 24$  vs.  $62 \pm 8\%$ ;  $P = 0.02$ ) compared with nonglucocorticoid users, whereas basal EGP, insulin secretion, and adipose tissue areas were not significantly different.

**CONCLUSIONS**—Low-dose prednisolone acutely perturbs all aspects of carbohydrate metabolism. Long-term low-dose prednisolone induces hepatic insulin resistance and reduces peripheral nonoxidative glucose disposal. We conclude that hepatic and peripheral insulin sensitivity should be targeted by glucose-lowering therapy for glucocorticoid-induced diabetes.

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Glucocorticoids (GCs) are potent anti-inflammatory agents that are commonly used to treat a broad range of inflammatory and autoimmune conditions. They are prescribed long-term to ~0.75% of the general population, most frequently to patients with inflammatory rheumatologic disease (1). Despite the advent of disease-modifying antirheumatologic drugs and biologic therapies, long-term GC prescription rates are still increasing (1). Long-term prescription of GCs is most prevalent in the elderly, at prednisolone-equivalent doses of <10 mg daily (1,2).

The acute effects of high-dose GCs on carbohydrate metabolism have been extensively investigated. High-dose GCs cause peripheral tissue insulin resistance (3), because they reduce both oxidative and nonoxidative glucose disposal (4). The effects of high-dose GCs on endogenous glucose production (EGP) and hepatic insulin sensitivity are less clear, but a number of studies report a deleterious effect (3,5,6). High-dose GCs also acutely reduce insulin secretion (7,8), which will contribute to their hyperglycemic effect.

There are fewer data regarding the metabolic consequences of typical long-term (lower) GC doses, which are generally considered to be modest (9–11). Furthermore, which components of carbohydrate metabolism are perturbed by low-dose GCs is not clear. We recently reported that older patients with inflammatory rheumatologic disease using long-term low-dose prednisolone had a higher postglucose load plasma glucose concentration but a slightly lower fasting plasma glucose concentration than matched controls not using prednisolone (12). Our finding of a lower fasting plasma glucose concentration suggests that basal EGP was not increased in subjects using low-dose prednisolone. This is consistent with a previous study by van Raalte et al. (13) reporting that prednisolone 7.5 mg/day for 14 days did not significantly change basal EGP. However,

van Raalte et al. (13) did report a reduction in hepatic insulin sensitivity, suggesting that carbohydrate metabolism in the liver may be adversely affected by low-dose prednisolone.

The effects of GCs on carbohydrate metabolism predominantly have been studied after short courses in healthy young adults. Findings in younger patients may not be translatable to older patients in whom increased visceral adiposity is likely to increase susceptibility to the diabetogenic effects of GCs (14). In addition, long-term GC therapy may further increase visceral adiposity and enhance the effects of GCs on carbohydrate metabolism (15,16).

The aim of this study was to investigate the acute effects of low-dose prednisolone on carbohydrate metabolism in an older population typical of patients for whom prednisolone is most frequently prescribed. An additional aim was to assess whether there is an increase in visceral adiposity that further amplifies metabolic perturbations during long-term low-dose prednisolone administration. This information will provide a foundation for future studies targeting therapy for GC-induced hyperglycemia at the major metabolic abnormalities induced by low-dose prednisolone.

## RESEARCH DESIGN AND METHODS

### Subjects and study design

Subjects aged 40 years or older with inflammatory rheumatologic disease were recruited from the outpatient clinic of our institution. We studied 9 subjects who had not been administered any oral GC for at least 6 months (non-GC users) and 12 subjects using a stable continuous oral prednisolone dose of 4–10 mg/day for at least 6 months (GC users). The two groups were matched for sex, age, BMI, inflammatory disease activity (assessed by C-reactive protein) and physical activity (assessed by modified Baecke physical activity score) (17). Subjects were excluded from the study if they had known diabetes mellitus, significant hepatic disease (liver transaminases more than three-times the upper limit of normal, known cirrhosis, or chronic hepatitis), severe renal disease (serum creatinine >200  $\mu\text{mol/L}$ ), severe congestive cardiac failure (New York Heart Association class IV), or were using medications known to significantly affect carbohydrate metabolism. Undiagnosed diabetes mellitus was

excluded at a screening visit with an oral glucose tolerance test. One subject in the GC users group was omitted from the EGP and rate of glucose disposal (Rd) analysis because of a technical problem with the basal  $6,6\text{-}^2\text{H}_2$  glucose infusion.

To determine the acute effects of prednisolone, non-GC users were studied before and after a 7- to 10-day course of oral prednisolone 6 mg daily. To assess the long-term effects of prednisolone, baseline data from non-GC users were compared with baseline data from the matched GC users. The study was approved by the Southern Adelaide Clinical Human Research Ethics Committee, Flinders Medical Centre, and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

### Study protocol

Carbohydrate metabolism and body composition were assessed using a standardized 2-day protocol. At each visit, subjects presented to the Endocrine Research Unit of the Repatriation General Hospital at 0800 h after an overnight fast. The day 1 study protocol involved assessment of basal EGP, followed by a two-step hyperinsulinemic-euglycemic clamp study. On day 2, subjects underwent a frequently sampled intravenous glucose tolerance test, dual-energy X-ray absorptiometry scan, and abdominal computed tomography (CT) scan. Subjects were asked to refrain from alcohol and exercise for 2 days before the study visits, and no subject smoked during study visits. Subjects consumed all regular medications with water in the morning before the study visits, including their prescribed prednisolone dose.

### Basal endogenous glucose production and two-step hyperinsulinemic-euglycemic clamp

An intravenous cannula was inserted into the antecubital fossa of one arm for administration of infusions. A distally sited cannula was inserted into the contralateral arm for blood sampling and was heated for the duration of the study to achieve arterialization of venous blood. After baseline blood samples were drawn, subjects were administered a primed (5 mg/kg) continuous (3 mg/kg/h) infusion of  $6,6\text{-}^2\text{H}_2$  glucose (Cambridge Isotopes Laboratories, Andover, MA) for 120 min to estimate basal EGP. Steady state was defined as 90 to 120 min after completion of the priming bolus.

A two-step hyperinsulinemic-euglycemic clamp study followed immediately. In the

first step, the low-dose clamp study, human neutral insulin (Actrapid; Novo Nordisk Pharmaceuticals, New South Wales, Australia) was infused for 120 min at 15  $\text{mU/m}^2/\text{min}$ . The basal  $6,6\text{-}^2\text{H}_2$  glucose infusion was continued at 50% of the initial rate (1.5 mg/kg/h) during this step. In the second step, the high-dose clamp study, the basal  $6,6\text{-}^2\text{H}_2$  glucose infusion was ceased and subjects were administered a primed (320  $\text{mU/m}^2/\text{min}$  for 2 min followed by 160  $\text{mU/m}^2/\text{min}$  for 2 min) human neutral insulin infusion at 80  $\text{mU/m}^2/\text{min}$  for 120 min. During both the low-dose and high-dose clamp studies, subjects were administered a variable infusion of 25% glucose (Baxter Healthcare, New South Wales, Australia) enriched to 2.6% with  $6,6\text{-}^2\text{H}_2$  glucose to maintain euglycemia with a target glucose concentration of 5 mmol/L. Blood samples were drawn every 5 to 10 min to titrate the variable glucose infusion and steady state was defined as the last 30 min of each step of the clamp study.

EGP was calculated using the Steele equation (18) as modified by Finegood et al. (19) assuming a pool fraction of 0.65 and a volume of distribution of 20% of body weight. The calculations accounted for the background natural abundance of  $^{13}\text{C}$  and other isomers with the same gas chromatography–mass spectrometry retention time and mass as  $6,6\text{-}^2\text{H}_2$  glucose. Negative EGP values during the high-dose clamp were assigned to zero as previously described (19,20). The percentage of EGP suppression during the low-dose clamp study was considered a marker of hepatic insulin sensitivity. The Rd was calculated using nonsteady-state calculations as previously described (18,19). The mean glucose infusion rates during steady state corrected for fat-free mass (FFM) and for serum insulin concentration (I) during the high-dose clamp study were used as a measure of peripheral tissue insulin sensitivity.

### Indirect calorimetry

Indirect calorimetry was performed using a ventilated hood technique (ParvoMedics TrueOne 2400 Metabolic Measurement System; ParvoMedics, Sandy, UT) during the final 30 min of assessment of basal EGP and during each step of the two-step hyperinsulinemic-euglycemic clamp study. After an equilibrium period of 10 min, oxidative glucose disposal was calculated using data from the final 20 min of indirect calorimetry recordings and the equations of Frayn (21).

### Frequently sampled intravenous glucose tolerance test

After placement of an intravenous cannula in each arm and collection of baseline blood samples to measure glucose and insulin concentration, a 25% glucose (Baxter HealthCare) bolus (300 mg/kg, maximum 25 g) was administered over 60 s. Further blood samples were then drawn from the contralateral arm at minutes 1, 2, 3, 4, 6, 8, 10, and then every 10 min until 60 min after the glucose bolus. The areas under the curve for glucose and insulin were calculated using the trapezoidal method (22). Insulin secretion was calculated from the ratio of area under the curve for insulin to area under the curve for glucose, with first-phase insulin secretion from minutes 0 to 10 after the glucose bolus and second-phase insulin secretion from minutes 10 to 60 after the glucose bolus.

### Body composition

CT was used to assess hepatic fat content and subcutaneous and visceral adipose tissue volumes. All CT images were 1 cm thick and taken on a GE Lightspeed Pro 16 (GE Healthcare, General Electric Company, Pewaukee, WI). Scans were analyzed by a single operator who was blinded to patient visit and group. Hepatic fat content was quantified from a CT slice centered at the T12/L1 disc space, with the average Hounsfield units determined from three regions of interest manually placed in the liver, avoiding major vessels. Subcutaneous and visceral adipose tissue areas were obtained from a CT slice centered at the L4/L5 disc space. Separation lines were manually plotted at the outside margin of the skin and the abdominal wall musculature in continuity with fascia of the paraspinal muscles. Adipose tissue was defined as tissue with a density between and including  $-50$  to  $-150$  Hounsfield units. Total fat mass and FFM were measured by dual energy X-ray absorptiometry on a GE Medical Systems Lunar Prodigy (GE Healthcare, General Electric Company).

### Laboratory analysis

Glucose samples were analyzed immediately at the bedside on a glucose analyzer (Yellow Springs Instrumentation, Yellow Springs, OH). Glycosylated hemoglobin was measured using boronate affinity chromatography on a Primus PDQ (Immuno, Sydney, Australia) with a between-run coefficient of variation (CV)  $<3\%$ . C-reactive protein was measured using a Tina-quant

immunoturbidimetric assay (Roche Diagnostics GmbH, Mannheim, Germany) on a Roche/Hitachi Modular Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) with a CV  $<4\%$ . Urine urea was analyzed by kinetic ultraviolet spectrophotometric assay (Roche Diagnostics GmbH) on a Roche/Hitachi Modular Analyzer (Hitachi High-Technologies Corporation). The between-run CV for urine urea is 2.9% at 1,456 mmol/L and 3.1% at 279 mmol/L. Serum insulin was analyzed by radioimmunoassay (Linco Research, St Charles, MO) with a between-run CV of 9.1% at 12.5 mU/L, 2.8% at 50 mU/L, and 4.9% at 200 mU/L. Nonesterified fatty acids were measured by enzymatic colorimetric assay (Wako, Osaka, Japan). The between-run nonesterified fatty acids CVs were 4.5% at 0.0625 mmol/L and 1.7% at 0.25 mmol/L.

To perform isotope ratio glucose analysis, serum 6,6- $^2\text{H}_2$  glucose samples underwent protein precipitation using acetone, followed by a two-step derivatization with hydroxylamine hydrochloride and then acetic anhydride (23,24). The glucose and 6,6- $^2\text{H}_2$  glucose derivatives were assayed by gas chromatography–mass spectrometry (a 6890 gas chromatograph was interfaced to an Agilent 5973 Mass Selective Detector; Agilent Technologies, New South Wales, Australia). Single ion monitoring was used to maximize sensitivity, with acquisition of ions  $m/z$  328 (glucose) and  $m/z$  330 (6,6- $^2\text{H}_2$  glucose). Baseline 6,6- $^2\text{H}_2$  glucose samples were performed in duplicate with a mean CV of 0.4%. The between-run CV of unenriched 25% glucose was 0.8%.

### Statistical analysis

Statistical analysis was performed using IBM SPSS version 19 for Windows (IBM, New York, NY). Data are presented as mean  $\pm$  SD for normally distributed data and as median (interquartile range) if the distribution was not normal.  $P < 0.05$  was considered statistically significant. The effects of acute prednisolone administration in non-GC users were analyzed with a Wilcoxon signed-rank test, whereas the effects of long-term prednisolone therapy in GC users compared with non-GC users were analyzed using the Mann-Whitney  $U$  test. A sample size of 12 per group had 78% power to detect a change of 3.6 mg/kg FFM per minute difference in FFM value between GC users and non-GC users at the 0.05 significance level.

## RESULTS

### Subject characteristics

GC users were using a mean prednisolone dose of  $6.3 \pm 2.2$  mg/day, with the mean duration of continuous prednisolone therapy being  $81 \pm 62$  months. All non-GC users and 11 of the 12 GC users had a diagnosis of either rheumatoid arthritis or seronegative arthritis (Table 1). There were no significant differences in sex, age, BMI, waist circumference, family history of diabetes, physical activity score, or C-reactive protein between non-GC users and GC users (Table 1). Whereas fasting plasma glucose concentration was significantly higher in the GC users during the screening oral glucose tolerance test, there was no significant difference in 2-h postglucose load plasma glucose concentration (Table 1). Glycosylated hemoglobin was not significantly different between non-GC users and GC users (Table 1).

### Body composition

In non-GC users, acute prednisolone administration did not significantly alter hepatic ( $60 \pm 4$  vs.  $60 \pm 5$  Hounsfield units;  $P = 0.86$ ), subcutaneous ( $257 \pm 130$  vs.  $261 \pm 129$   $\text{cm}^2$ ;  $P = 0.31$ ), or visceral ( $108 \pm 82$  vs.  $111 \pm 80$   $\text{cm}^2$ ;  $P = 0.31$ ) adipose tissue. There were no significant differences in total fat mass, hepatic adiposity, subcutaneous adipose tissue area, or visceral adipose tissue area between non-GC users and GC users (Table 1).

### Endogenous glucose production and hepatic insulin sensitivity

In non-GC users, basal EGP was increased by 8% after acute administration of prednisolone ( $P = 0.050$ ; Fig. 1). Basal EGP was 14% higher in GC users compared with non-GC users, although this did not reach statistical significance ( $P = 0.16$ ; Fig. 1). Acute prednisolone administration in non-GC users significantly attenuated insulin suppression of EGP during the low-dose clamp from  $79 \pm 7$  to  $67 \pm 14\%$  ( $P = 0.03$ ; Fig. 1). Insulin suppression of EGP during the low-dose clamp was lower in GC users than in non-GC users ( $66 \pm 14$  vs.  $79 \pm 7\%$ ;  $P = 0.03$ , Fig. 1). EGP was not significantly different from zero during the high-dose clamp study in all groups. Glucose and insulin concentrations and 6,6- $^2\text{H}_2$  glucose enrichment during the clamp studies are reported in the Supplementary Materials (Supplementary Figs. A–C and Supplementary Table 2).

Table 1—Subject baseline characteristics and baseline body composition data

	Non-GC users	GC users	P
n	9	12	
RA/SNA/other, n	3/6/0	8/3/1	0.14
Female, n (%)	4 (44)	6 (50)	0.80
Age, years	59 ± 11	61 ± 8	0.29
BMI, kg/m <sup>2</sup>	27.5 ± 5.8	27.4 ± 3.3	0.92
Waist circumference, cm	95 ± 18	95 ± 11	0.97
Family history of diabetes, n (%)	1 (11)	3 (25)	0.60
Physical activity score	19 ± 11	17 ± 7	0.94
C-reactive protein, mg/L*	3.9 (1.2–7.3)	4.2 (1.8–14.8)	0.41
Fasting plasma glucose, mmol/L	4.7 ± 0.3	5.1 ± 0.6	0.01
2-h plasma glucose, mmol/L	6.7 ± 1.1	7.2 ± 1.5	0.39
Glycosylated hemoglobin, % (mmol/mol)	5.6 ± 0.2 (38 ± 2)	5.6 ± 0.3 (38 ± 3)	0.72
Total fat mass, kg	26 ± 12	28 ± 10	0.78
Liver attenuation, Hounsfield units*	59 (56–64)	60 (52–64)	0.83
Visceral adipose tissue volume, cm <sup>2</sup>	108 ± 82	97 ± 38	1.00
Subcutaneous adipose tissue volume, cm <sup>2</sup> *	218 (173–381)	257 (157–357)	0.94

Data are mean ± SD unless otherwise noted. \*Median (interquartile range). RA, rheumatoid arthritis; SNA, seronegative arthritis.

### Peripheral tissue insulin sensitivity

In non-GC users, mean glucose infusion rates during steady state corrected for FFM and for serum insulin concentration was reduced by 14% after acute administration of prednisolone ( $P = 0.04$ ; Fig. 2A). Although mean glucose infusion rates during steady state corrected for FFM and for serum insulin concentration was 14% lower in GC users compared with non-GC users, this difference was

not statistically significant ( $P = 0.32$ ; Fig. 2A). In non-GC users, the Rd was reduced by 15% after acute prednisolone administration ( $P = 0.01$ ; Fig. 2B). The Rd was 17% lower in GC users than in non-GC users, although this difference did not reach statistical significance ( $P = 0.27$ ; Fig. 2B). In non-GC users, there was no significant change in the percentage of nonoxidative glucose disposal after acute prednisolone administration

( $62 \pm 8$  vs.  $59 \pm 15\%$ ;  $P = 0.44$ ; Fig. 2B). However, nonoxidative glucose disposal was reduced in GC users compared with non-GC users ( $44 \pm 24$  vs.  $62 \pm 8\%$ ;  $P = 0.02$ ; Fig. 2B).

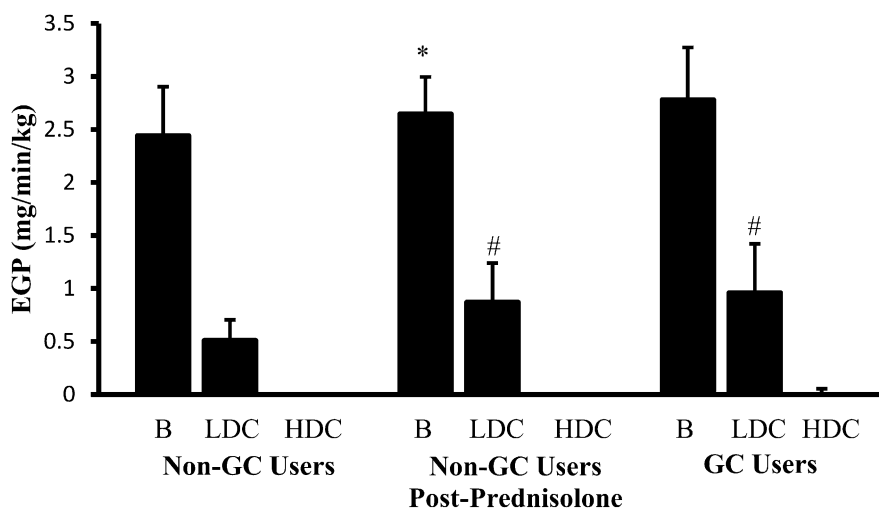
Neither acute nor long-term prednisolone administration significantly changed basal nonesterified fatty acids concentration or suppression of lipolysis in the low-dose or high-dose clamp study (Supplementary Table 1).

### Insulin secretion

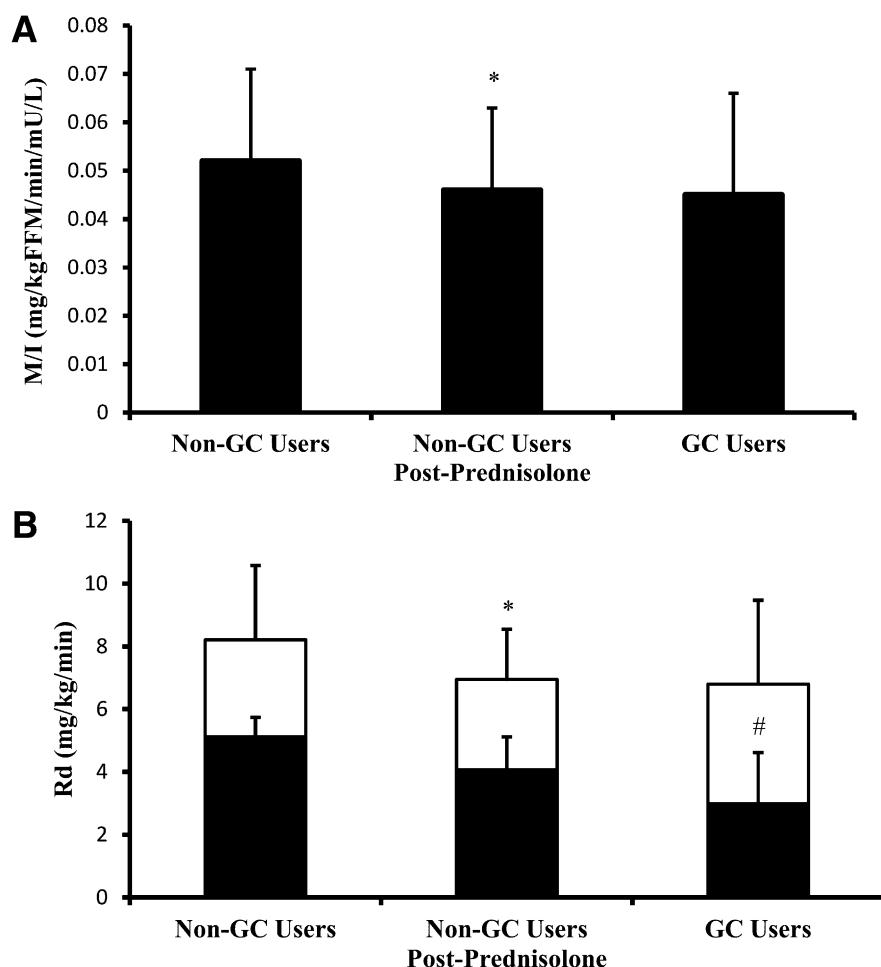
In non-GC users, after acute prednisolone administration, first-phase insulin secretion ( $5.9 \pm 2.0$  vs.  $3.9 \pm 1.6$  mU/mmol;  $P = 0.01$ ; Fig. 3A) and second-phase insulin secretion ( $4.6 \pm 1.7$  vs.  $3.6 \pm 1.4$  mU/mmol;  $P = 0.02$ ; Fig. 3B) were reduced. In contrast, there was no difference in first-phase insulin secretion ( $5.7 \pm 3.5$  vs.  $5.9 \pm 2.0$  mU/mmol;  $P = 0.57$ ; Fig. 3A) or second phase insulin secretion ( $5.1 \pm 3.1$  vs.  $4.6 \pm 1.7$  mU/mmol;  $P = 0.94$ ; Fig. 3B) in GC users and in non-GC users.

**CONCLUSIONS**—This study represents a systematic assessment in the older patient of the effect of typical long-term therapeutic prednisolone doses on carbohydrate metabolism using gold standard metabolic techniques. We have demonstrated that acute low-dose prednisolone significantly reduces hepatic insulin sensitivity and appears to increase basal EGP. A reduction in hepatic insulin sensitivity also was present in patients using similar doses of prednisolone long-term. Low-dose prednisolone acutely reduces peripheral insulin sensitivity and the percentage of glucose undergoing nonoxidative glucose disposal is lower in patients using long-term low-dose prednisolone. Insulin secretion is reduced after acute, but not long-term low-dose prednisolone administration. Visceral adiposity was not increased in subjects using short-term or long-term prednisolone, suggesting that GCs did not alter body composition and thus did not amplify perturbations of carbohydrate metabolism. These findings provide insight into the temporal effects of low-dose prednisolone on components of carbohydrate metabolism and the potential metabolic perturbations that could be targeted to reverse these effects.

There is emerging support for targeting diabetes therapy at its specific underlying pathophysiology (25). The paradigm for this is monogenic diabetes, in which an understanding of the



**Figure 1**—Endogenous glucose production in non-GC users before and after administration of low-dose prednisolone for 7–10 days and in long-term GC users. Data are mean ± SD. B, basal EGP; LDC, low-dose clamp; HDC, high-dose clamp. \* $P = 0.05$  compared with basal EGP in non-GC users at baseline. # $P < 0.05$  compared with percentage of insulin suppression of EGP in non-GC users at baseline.



**Figure 2**—A: Mean glucose infusion rate at steady state during high-dose clamp study corrected for FFM and for serum insulin concentration (M/I) in non-GC users before and after administration of low-dose prednisolone for 7–10 days and in long-term GC users. Data are mean  $\pm$  SD. \* $P < 0.05$  compared with non-GC users at baseline. B: Rate of oxidative (white bars) and non-oxidative (black bars) Rd at steady state during high-dose clamp study in non-GC users before and after administration of low-dose prednisolone for 7–10 days and in long-term GC users. Data are mean  $\pm$  SD. \* $P < 0.05$  compared with total Rd in non-GC users at baseline. # $P < 0.05$  compared with percentage of nonoxidative glucose disposal in non-GC users at baseline.

derangements of carbohydrate metabolism has led to the use of directed therapies and improved glycemic control (26). It is hoped that targeting therapy at the metabolic perturbations underlying hyperglycemia in patients with type 2 diabetes will have a similar benefit (27). Although low-dose GCs are associated with an increased odds ratio of development of new-onset diabetes, there remains a dearth of evidence regarding optimal treatment for GC-induced hyperglycemia (28). We have thus sought to define the changes in carbohydrate metabolism induced by low-dose prednisolone in older patients to enable a specific therapeutic approach in this setting.

In this study, basal EGP was 8% higher after short-term low-dose prednisolone administration ( $P = 0.050$ ).

Previous reports of GC-mediated effects on basal EGP have been inconsistent, with van Raalte et al. (13) finding no change in basal EGP after low-dose prednisolone; however, withdrawal of a low GC dose in patients with Addison's disease markedly reduced basal EGP (29). It is likely that differing patient characteristics alter their susceptibility to the metabolic effects of low-dose GCs and underlie the variability in the literature. In subjects using long-term low-dose prednisolone, basal EGP was 14% higher, although this result did not reach statistical significance. The lack of statistical significance may represent the variability inherent in a cross-sectional study. Confirmation of our findings with a larger sample size should be performed in the patient

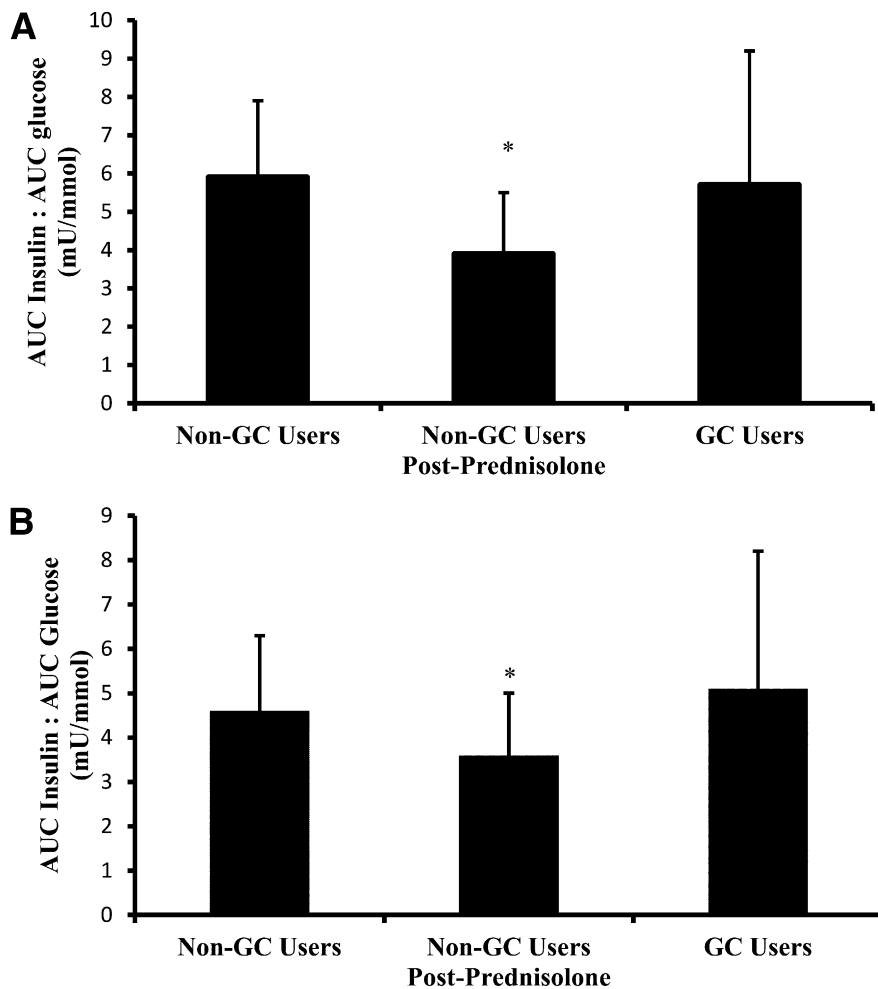
population for whom low-dose GCs are usually prescribed.

We have demonstrated that both acute and long-term low-dose prednisolone robustly induce hepatic insulin resistance, consistent with a previous report (13). Hepatic insulin resistance was not secondary to increased hepatic adiposity, because we did not find a change in hepatic fat content. Our results suggest that adverse changes in hepatic carbohydrate metabolism induced by low-dose prednisolone will increase both fasting and postprandial glucose concentration. As such, metformin, a drug that primarily targets hepatic glucose regulation (30), should be a cornerstone of glucose-lowering therapy to reverse these pathophysiologic changes.

In our study, low-dose prednisolone acutely reduced peripheral insulin sensitivity. Although it is well-established that high-dose GCs acutely induce peripheral insulin resistance (3,4,6,31), few studies have characterized the effect of typical lower GC doses. van Raalte et al. (13) reported that a 2-week course of prednisolone 7.5 mg daily did not significantly reduce peripheral insulin sensitivity in healthy young men. Our contrasting findings suggest that older subjects with an underlying long-term inflammatory disease comprise a group of patients who have increased vulnerability to the adverse metabolic effects of low-dose GCs.

The degree of peripheral insulin resistance in subjects using long-term low-dose prednisolone therapy was similar to that seen after acute low-dose prednisolone. However, this reduction in insulin sensitivity did not reach statistical significance. We do report that long-term low-dose prednisolone significantly reduces the percentage of nonoxidative glucose disposal. Our results suggest that increasing peripheral insulin sensitivity is likely to be important if the effects of low-dose prednisolone are to be reversed. However, current pharmacologic options to improve peripheral tissue insulin sensitivity in GC users are limited because thiazolidinediones have a similar adverse effect profile as GCs, including increasing fat mass (32) and reduction of bone density (33). Although exercise may improve peripheral insulin sensitivity, the underlying disease state could limit its therapeutic applicability.

In this study, neither acute nor long-term low-dose prednisolone influenced basal or insulin-suppressed lipolysis. It has been previously demonstrated that a cortisol infusion yielding a higher GC



**Figure 3**—A: First-phase insulin secretion (area under the curve [AUC] insulin:AUC glucose) in non-GC users before and after administration of low-dose prednisolone for 7–10 days and in long-term GC users. Data are mean  $\pm$  SD. \* $P < 0.05$  compared with non-GC users at baseline. B: Second-phase insulin secretion (AUC insulin:AUC glucose) in non-GC users before and after administration of low-dose prednisolone for 7–10 days and in long-term GC users. Data are mean  $\pm$  SD. \* $P < 0.05$  compared with non-GC users at baseline.

dose than that used in this study increased lipolysis (34). However, lower GC doses did not increase lipolysis (13), and Kauh et al. (35) reported a dose-dependent effect of prednisolone on lipolysis. We hypothesize that the lack of effect on lipolysis in our study reflects the low GC dose studied.

We found a disparate effect of acute and long-term low-dose prednisolone on insulin secretion. Acute low-dose prednisolone administration reduced first-phase and second-phase insulin secretion, consistent with previous reports of acute  $\beta$ -cell dysfunction after exposure to medium-dose and high-dose GCs (8,36,37). However, insulin secretion was not reduced in patients using long-term prednisolone. Whereas this may represent a type II error, first-phase and second-phase insulin

secretion results are very similar in the GC users and non-GC users before prednisolone. Furthermore, van Raalte et al. (8) raised the possibility that the acute reduction in insulin secretion is not sustained with longer administration of GCs, although the prednisolone doses used were different in their short-term and medium-term studies. Our study does not provide insight into the possible mechanisms or the exact timing underlying the apparent recovery of insulin secretion. There are potentially multiple mechanisms involved, including a compensatory  $\beta$ -cell response to GC-induced insulin resistance. Nevertheless, the clinical implications of our results are that if insulin secretion is only transiently reduced by low-dose prednisolone, then insulin secretagogues are not required

long-term to reverse this pathophysiologic change.

We hypothesized that visceral adiposity would be increased in patients using long-term prednisolone and that this would further exacerbate its effects on carbohydrate metabolism. However, there was not an increase in visceral adiposity in this patient group and perturbations of carbohydrate metabolism were not amplified. We chose to match our subjects for BMI based on a previous report that patients using long-term GC therapy have increased abdominal fat without a significant increase in BMI (15). A prospective study would be required to fully quantify the effect of body compositional change. However, it would be extremely challenging to recruit patients requiring initiation of GCs to achieve inflammatory disease control for these complex metabolic investigations.

Low-dose GCs are prescribed as treatment for a variety of inflammatory and autoimmune conditions, and our results are likely to apply to other patient groups. However, van Raalte et al. (13) reported that low-dose prednisolone induced less metabolic effects in healthy young adults than we have demonstrated in an older patient population with chronic inflammatory disease. Metabolic studies in younger subjects with chronic inflammatory diseases treated with similar GC doses would be useful to confirm the widespread applicability of our findings.

This study's strengths lie in the use of gold standard metabolic investigations and the study of patients with an underlying chronic disease rather than healthy volunteers. However, we acknowledge that the study is subject to limitations. Complex metabolic studies such as this are often limited by a small sample size, which reduced our power to detect significant changes across all comparators. In particular, this patient group was frail and difficult to recruit. Insulin secretion was quantified by measuring insulin during the intravenous glucose tolerance test and, unlike C-peptide, insulin concentrations can be affected by hepatic clearance. However, the consistent insulin concentrations across all groups during the euglycemic clamp studies suggest that insulin clearance is not altered by low-dose GC administration. Furthermore, an intravenous glucose tolerance test does not assess the incretin effect on insulin secretion, which has been shown previously to be reduced by acute GC administration (38). Finally, we did not

assess whether changes in glucagon concentration mediated changes in EGP. However, the effect of glucagon predominates at low glucose concentrations, not those found during a hyperinsulinemic-euglycemic clamp study (39), and previous studies have reported that low-dose prednisolone does not alter serum glucagon concentration (6,40),

In summary, we have demonstrated that acute low-dose prednisolone impairs multiple components of carbohydrate metabolism. In patients using long-term low-dose prednisolone, visceral adiposity was not increased and perturbations of carbohydrate metabolism were not amplified. Patients using long-term low-dose prednisolone have hepatic insulin resistance and reduced peripheral nonoxidative glucose disposal, but no change in insulin secretion. Our findings demonstrate that low-dose prednisolone exerts a major deleterious effect on carbohydrate metabolism. When prescribing GCs, careful consideration should be given to these potential adverse metabolic effects, and alternative anti-inflammatory agents should be considered. Treatment for GC-induced diabetes should increase hepatic insulin sensitivity and peripheral non-oxidative glucose disposal to reverse the pathophysiologic changes induced by low-dose prednisolone.

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C.J.P. was responsible for study design, subject recruitment, data acquisition, laboratory analysis, data analysis, and manuscript preparation. B.L.M. was responsible for data acquisition. A.B.J. was responsible for the  $6,6\text{-}^2\text{H}_2$  glucose data analysis and manuscript revision. A.P. was responsible for laboratory analysis and manuscript revision. M.D.S. was responsible for subject recruitment and manuscript revision. J.R.G. was responsible for study design, data analysis, and manuscript revision. C.H.T. was responsible for study design, supervision, and manuscript revision. M.G.B. was responsible for obtaining funding, study design, data analysis, supervision, and manuscript revision. C.J.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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