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ORIGINAL ARTICLE

Effects of prednisolone on energy and fat metabolism in patients with rheumatoid arthritis: tissue-specific insulin resistance with commonly used prednisolone doses

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Summary

Objective Glucocorticoids can cause postprandial hyperglycaemia, but the effects on postprandial energy and fat metabolism are uncertain. We investigated the effects of acute and chronic low-dose prednisolone on fasting and postprandial energy expenditure and substrate metabolism.

Design An open interventional and cross-sectional study was undertaken.

Patients and measurements Eighteen patients who had not taken oral glucocorticoids for ≥6 months were studied before and after 7 days prednisolone (6 mg/day) to assess the acute effects of prednisolone. Baseline data from patients, not on glucocorticoids, were compared with 18 patients on long-term prednisolone (6·5 ± 1·8 mg/day for >6 months) to assess the chronic effects. Energy expenditure and substrate oxidation were measured using indirect calorimetry before and after a mixed meal. Adipocyte insulin resistance index and insulin-mediated suppression of NEFA were calculated from fasting and postprandial insulin and NEFA concentrations.

Results There were no significant differences in resting energy expenditure or diet-induced thermogenesis with prednisolone. Acute (−2·1 ± 6·2 *vs* −16·3 ± 4·8 mg/min, $P = 0{\cdot}01$) and chronic (−1·4 ± 2·8 *vs* −16·3 ± 4·8 mg/min, $P = 0{\cdot}01$) prednisolone attenuated postprandial suppression of fat oxidation. Chronic (31·6 ± 3·8 *vs* 17·0 ± 3·3, $P = 0{\cdot}007$), but not acute, prednisolone increased adipocyte insulin resistance index. However, insulin-mediated suppression of NEFA was not significantly different after acute or chronic prednisolone.

Conclusions Prednisolone does not alter energy expenditure. However, even at low doses, prednisolone exerts adverse effects on fat metabolism, which could exacerbate insulin resistance and increase cardiovascular risk. Attenuated postprandial suppression of fat oxidation, but not lipolysis, suggests that prednisolone causes greater insulin resistance in skeletal muscle than in adipocytes.

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Introduction

Endogenous glucocorticoid excess (Cushing's syndrome) causes a distinctive change in body composition with reduced lean body mass and increased fat mass, predominantly in the truncal region.[1] Consequently, visceral adiposity is increased, which is associated with increased rates of cardiovascular disease and diabetes. While Cushing's syndrome is rare, exogenous glucocorticoid therapy is prescribed long term to nearly 1% of the adult population to treat inflammatory disease or attenuate disease progression.[2] High-dose glucocorticoid therapy (prednisolone >20 mg/day) also causes central adiposity[3] and is associated with a threefold increased risk of cardiovascular event and stroke.[4] However, most patients on chronic glucocorticoid therapy are prescribed prednisolone equivalent doses of <10 mg/day.[2] The effect of typical therapeutic (lower) glucocorticoid doses on fat mass is less clear, with some studies reporting an increase in central fat mass and others no significant difference.[5,6]

Studies of body composition are valuable but interpretation can be limited by between-subject heterogeneity and relatively small sample size. Defining the perturbations of energy and fat metabolism associated with typical therapeutic glucocorticoid doses will aid an understanding of their contribution to adiposity. While infusion of high doses of glucocorticoids paradoxically increases resting energy expenditure,[7] during longer term administration of exogenous glucocorticoids[8] and in Cushing's

syndrome,[1] resting energy expenditure is not significantly different to controls. Stimulation of appetite, and not reduced energy utilization, is considered to be the major contributor to glucocorticoid-induced adiposity.[9]

Glucocorticoids predominantly increase glucose concentration in the postprandial period.[10] However, the effect of glucocorticoids on diet-induced thermogenesis, the increase in energy expenditure after a meal, has not been studied. A reduction in diet-induced thermogenesis, which comprises obligatory (heat generated by digestion and absorption of food) and facultative (regulated heat production to dissipate food energy) components, may contribute to adiposity and the metabolic syndrome.[11] As glucocorticoids reduce sympathetic nervous system activity and insulin sensitivity, even at typical therapeutic doses,[12] this could reduce diet-induced thermogenesis and contribute to adiposity in patients taking glucocorticoids.

Variable effects of glucocorticoids on fat oxidation have been reported.[13,14] Fasting fat oxidation was increased after a 4–5 h infusion of hydrocortisone.[15] However, there were no significant changes in fasting fat oxidation in patients with Cushing's syndrome[1] and taking chronic glucocorticoid therapy.[5] The effects of glucocorticoids on postprandial lipid metabolism have not been well characterized.

The aim of this study was to assess the acute and chronic effects of commonly used therapeutic prednisolone doses on fasting and postprandial energy and fat metabolism. We hypothesized that, similar to its effects on postprandial glycaemia, glucocorticoid-induced changes in energy and substrate metabolism would predominantly manifest in the postprandial period.

Material and methods

The study was approved by the Southern Adelaide Clinical Human Research Ethics Committee, Flinders Medical Centre, and all patients provided written informed consent in accordance with the Declaration of Helsinki. The effects of prednisolone on whole body insulin sensitivity and vascular function in this cohort have previously been reported.[12]

Patients

Patients aged 50 years or older with rheumatoid arthritis were recruited from the rheumatology outpatient clinic at Repatriation General Hospital, Adelaide, Australia. We studied 18 patients who had not been administered any oral glucocorticoids for at least 6 months (non-GC users) and 18 patients taking a stable continuous oral prednisolone dose of 4–10 mg/day for at least 6 months (GC users). The two groups were matched for age and sex. Patients with active synovitis and those prescribed oral hypoglycaemic agents and /or insulin were excluded from the study.

Study design

Non-GC users were studied before and after a 7-day course of oral prednisolone 6 mg daily to determine the acute effects of prednisolone. Baseline data from non-GC users were compared with data from chronic GC users to determine the chronic effects of prednisolone.

Study protocol

Patients attended the Endocrine Research Unit at Repatriation General Hospital at 0830 h after a 12-h overnight fast. All patients took their regular medications in the morning prior to arrival, including prednisolone. Basic anthropometric measures were recorded. At each visit, after resting supine for a 20-min acclimatization period, energy and substrate metabolism were assessed using indirect calorimetry. Blood samples were collected for estimation of glucose, insulin, lipid profile, nonesterified fatty acids (NEFA) and C-reactive protein (CRP). A mixed meal was then administered over 15 min (10 kcal/kg body weight, 45% carbohydrate, 40% fat and 15% protein), and indirect calorimetry repeated 2 h after the mixed meal. Further blood samples for glucose, insulin and NEFA were taken at 30-min intervals for 120 min. Patients then underwent a dual energy X-ray absorptiometry scan to measure whole body composition.

Indirect calorimetry

Indirect calorimetry was performed using a ventilated hood technique (Parvo Medics True One 2400 Metabolic Measurement System, Parvo Medics, Sandy, UT). After an equilibrium period of 10 min, resting energy expenditure and substrate oxidation rates were calculated from the next 20 min of indirect calorimetry recordings using the equations of Ferrannini.[16] Diet-induced thermogenesis was calculated as the percentage increase in energy expenditure after the mixed meal.[17]

Body composition

Fat mass and lean body mass (LBM) were measured by dual energy X-ray absorptiometry on a GE Medical Systems Lunar Prodigy (GE Healthcare General Electric Company), which also quantified central abdominal fat. Central abdominal fat is the fat within a manually traced region bordered by the upper margin of second and the lower margin of the fourth lumbar vertebral bodies and the outer margin of the ribs.[5]

Whole body insulin sensitivity

As previously described,[12] whole blood glucose was measured at the bedside immediately after venesection on an in-house glucose analyser (YSI 2300 STAT Plus, Yellow Springs Instrumentation, Ohio, USA) by an immobilized glucose oxidase method. Insulin was measured by radioimmunoassay (EMD Millipore, Toronto, Ontario, Canada). Whole body insulin sensitivity was estimated by the composite insulin sensitivity index (Matsuda index).[18]

Lipids and adipocyte insulin sensitivity

Fasting lipid profiles were measured by enzymatic colorimetry (Roche Modular P Unit; Roche Diagnostics GmbH). Serum free

fatty acid concentrations were measured by enzyme colorimetry using a Beckman Synchron CX5 analyser (WAKO NEFA C kit, Denver, CO). Adipocyte insulin resistance index was calculated as the product of fasting plasma insulin and fasting NEFA concentration.[19] Insulin-mediated suppression of NEFA was calculated as the percentage decrease in fasting NEFA concentration divided by the mean plasma insulin concentration during the mixed meal test.[20]

Physical activity

Physical activity was assessed using the Modified Baecke Questionnaire, which is a composite score based on household activity, sports and exercise and other leisure activities. A higher score reflects higher levels of physical activity.[21]

Other laboratory analysis

CRP was measured using a Tinaquant immunoturbidimetric assay (Roche Diagnostics GMBH, Mannheim, Germany) on a Roche Modular Analyser (Hitachi High-Technologies Corporation, Tokyo, Japan). The limit of detection was 0·3 mg/l. The between-run CV was 3·6% at a CRP of 3·9 mg/l and 2·3% at a CRP of 49·5 mg/l. Urinary noradrenaline excretion was measured during the 6-h study period by liquid chromatography/mass spectrometry to estimate sympathetic nervous system activity. The coefficient of variation (CV) for urinary noradrenaline measurement is 5·1% at 300 nmol/l.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 20 for Windows (IBM, New York, USA). A P-value of $<0{\cdot}05$ was considered statistically significant. Patient characteristics are presented as mean ± standard deviation if the distribution was normal and median (interquartile range) if the distribution was not normal. All other data are presented as mean ± standard error of mean. Changes in variables in non-GC users after 7 days prednisolone were analysed using paired t-tests, which are hereafter described in the manuscript as the acute effects of prednisolone. GC users were compared with baseline data from non-GC users using unpaired t-tests if normally distributed or Mann−Whitney U-tests if the distribution was not normal. These results are reported in the manuscript as the chronic effects of prednisolone. As resting energy expenditure is dependent on LBM, it was adjusted for this variable using analysis of covariance.

The primary end-point is the change/difference in diet-induced thermogenesis. In the acute study, a sample size of 17 had 80% power to detect a 3% change in diet-induced thermogenesis at the 0·05 significance level, assuming a standard deviation of 4%. In the chronic study, a sample size of 16 per group had 80% power to detect a 4% difference in diet-induced thermogenesis at the 0·05 significance level, assuming a standard deviation of 4%.

Results

Patient characteristics, body composition and noradrenaline excretion

GC users were taking a mean prednisolone dose of 6·5 ± 1·8 mg/day, with a mean duration of continuous prednisolone therapy of 62 ± 62 months. None of the non-GC users reported receiving long-term prednisolone therapy in the past. There were no significant differences in sex distribution, age, body mass index, C-reactive protein, physical activity score or use of disease-modifying antirheumatic drugs between GC users and non-GC users (Table 1). There were no significant differences in total fat mass, truncal fat mass, central abdominal fat or lean body mass between GC and non-GC users (Table 1). There were no significant differences in serum lipid profile between GC users and non-GC users (Table 1). Urinary noradrenaline excretion was reduced by acute prednisolone (54 ± 8 *vs* 93 ± 23 nmol/6 h, $P = 0{\cdot}02$), but was not significantly different in patients on chronic prednisolone (68 ± 17 *vs* 93 ± 23 nmol/6 h, $P = 0{\cdot}31$). No patient in either group had a diagnosis of diabetes.

Indirect calorimetry

There were no significant differences in resting energy expenditure (1391 ± 52 *vs* 1383 ± 52 Kcal/day, $P = 0{\cdot}67$) or diet-induced thermogenesis (Fig 1) after acute prednisolone. There was no significant difference in resting energy expenditure (1374 ± 27 *vs* 1408 ± 27 Kcal/day, $P = 0{\cdot}38$) or diet-induced thermogenesis (Fig 1) between GC users and non-GC users.

Table 1. Patient characteristics, body composition and lipid profile

	Non-GC users ($n = 18$)	GC users ($n = 18$)	P-value
Female (n, (%))	12 (67)	12 (67)	1·00
Age (years)	64 ± 7	66 ± 7	0·33
BMI (Kg/m^2)	28·1 ± 5·2	27·9 ± 6·1	0·95
C-reactive protein (mg/l)*	2·4 (1·1–4·5)	1·6 (0·5–7·6)	0·44
Physical activity score	12·8 ± 5·7	10·5 ± 5·5	0·22
DMARDs (n)	11	9	0·50
Total fat mass (Kg)	29·6 ± 12·7	29·5 ± 12·9	0·98
Central abdominal fat (Kg)	2·3 ± 1·3	2·2 ± 1·2	0·65
Truncal fat (Kg)	16·2 ± 6·8	15·2 ± 7·0	0·68
Lean body mass (Kg)	43·3 ± 8·4	45·6 ± 8·1	0·41
Total cholesterol (mmol/l)	5·3 ± 0·8	5·3 ± 1·1	0·87
Triglyceride (mmol/l)	1·3 ± 0·6	1·2 ± 0·8	0·74
LDL cholesterol (mmol/l)	3·0 ± 0·9	3·0 ± 0·9	0·77
HDL cholesterol (mmol/l)	1·7 ± 0·6	1·7 ± 0·5	0·82

Data are mean ± SD unless otherwise stated; *median (interquartile range); n, number of patients with specified variable; GC, glucocorticoid; BMI, body mass index; DMARDs, disease-modifying antirheumatic drugs.

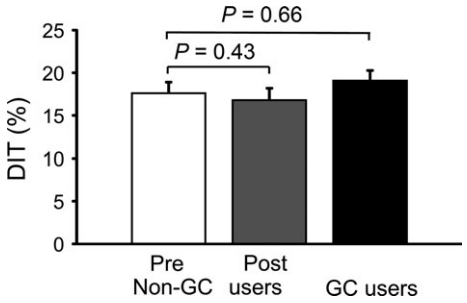

Fig. 1 Diet-induced thermogenesis (DIT) in 18 patients with rheumatoid arthritis who do not take oral glucocorticoids (non-GC users) before (white bars) and after (grey bars) 7 days prednisolone and 18 patients with rheumatoid arthritis on long-term prednisolone (GC users, black bars). Data are mean ± SEM.

There were no significant differences in fasting fat (29·1 ± 6·0 *vs* 37·4 ± 3·9 mg/min, $P = 0{\cdot}14$) or carbohydrate oxidation (88·6 ± 12·4 *vs* 64·0 ± 8·6 mg/min, $P = 0{\cdot}09$) after acute prednisolone. There were no significant differences in fasting fat (34·0 ± 4·0 *vs* 37·4 ± 3·9, $P = 0{\cdot}56$) or carbohydrate (73·4 ± 9·4 *vs* 64·0 ± 8·6 mg/min, $P = 0{\cdot}47$) oxidation between GC users and non-GC users. However, acute and chronic prednisolone were both associated with an impaired ability to suppress fat oxidation (Fig 2a) and increase carbohydrate oxidation (Fig 2b) in response to the meal.

Whole body insulin sensitivity

The time course of changes in glucose and insulin concentrations are shown in Fig 3a and b respectively. As previously reported[12], fasting glucose (5·3 ± 0·3 *vs* 4·6 ± 0·1 mmol/l, $P = 0{\cdot}02$) and glucose AUC over 2 h (844 ± 58 *vs* 733 ± 27 mmol/l*min, $P = 0{\cdot}03$) were higher after acute prednisolone. No significant differences in fasting (4·9 ± 0·2 *vs* 4·6 ± 0·1 mmol/l, $P = 0{\cdot}20$) or glucose AUC over 2 h (822 ± 40 *vs* 733 ± 27 mmol/l*min, $P = 0{\cdot}07$) were observed with chronic prednisolone, although the higher glucose AUC with chronic prednisolone approached statistical significance. There were no significant differences in fasting insulin (28·3 ± 2·0 *vs* 24·2 ± 3·7 μU/ml, $P = 0{\cdot}28$) or insulin AUC (14·3 ± 1·8 *vs* 12·5 ± 1·6 mU/ml*min, $P = 0{\cdot}28$) with acute prednisolone. Fasting insulin (32·2 ± 2·9 *vs* 24·2 ± 3·7 μU/ml, $P = 0{\cdot}10$) and insulin AUC (17·3 ± 2·2 *vs* 12·5 ± 1·6 mU/ml*min, $P = 0{\cdot}09$) were increased by more than 30% in patients on chronic prednisolone, but these differences did not reach statistical significance. The Matsuda index was significantly lower after both acute (2·0 ± 1·0 *vs* 3·6 ± 1·1, $P = 0{\cdot}01$) and chronic (1·9 ± 1·0 *vs* 3·6 ± 1·1, $P = 0{\cdot}04$) prednisolone.

Adipocyte insulin sensitivity

There was no significant difference in fasting NEFA after acute prednisolone (0·7 ± 0·1 *vs* 0·6 ± 0·1 mmol/l, $P = 0{\cdot}24$). GC users had higher fasting NEFA (1·0 ± 0·1 mmol/l *vs* 0·6 ± 0·1 mmol/l, $P < 0{\cdot}001$) than non-GC users. Adipocyte insulin resistance index was higher after chronic, but not acute prednisolone (Fig 4a). However, percentage suppression of NEFA postmeal was not significantly different after acute (85·4 ± 1·9 *vs* 88·5 ± 1·8%, $P = 0{\cdot}09$) or chronic prednisolone. (89·5 ± 1·2 *vs* 88·5 ± 1·8%, $P = 0{\cdot}63$) (Fig 4b). Consequently, insulin-mediated suppression of NEFA was not significantly different after acute or chronic prednisolone (Fig 4c).

Discussion

This study investigated the effects of low-dose prednisolone on fasting and postprandial energy and substrate metabolism in elderly patients with rheumatoid arthritis. Neither acute nor chronic prednisolone was associated with significant changes in resting energy expenditure or diet-induced thermogenesis. In contrast, low-dose prednisolone reduced whole body insulin sensitivity, which was associated with attenuated postprandial suppression of fat oxidation. Chronic, but not acute, prednisolone was associated with higher fasting NEFA and adipocyte insulin resistance index. However, acute and chronic prednisolone did not affect postprandial insulin-mediated suppression of NEFA. These data provide insight into tissue-specific differences in glucocorticoid-induced insulin resistance and suggest potential therapeutic targets to reduce the metabolic effects of glucocorticoids.

The effects of glucocorticoids on diet-induced thermogenesis have not been studied. Diet-induced thermogenesis contributes 10–15% of average daily energy expenditure. Diet-induced thermogenesis is regulated by the sympathetic nervous system[22] and is lower in patients with insulin resistance.[23] Previous studies have reported that resting energy expenditure is not reduced in patients with Cushing's syndrome[1] and taking exogenous glucocorticoids.[8,9] Our study was concordant with these findings, but extended them to demonstrate that there is no significant change in postprandial energy expenditure with acute or chronic low-dose prednisolone. In our cohort of patients, urinary noradrenaline excretion was reduced by 42% with acute prednisolone

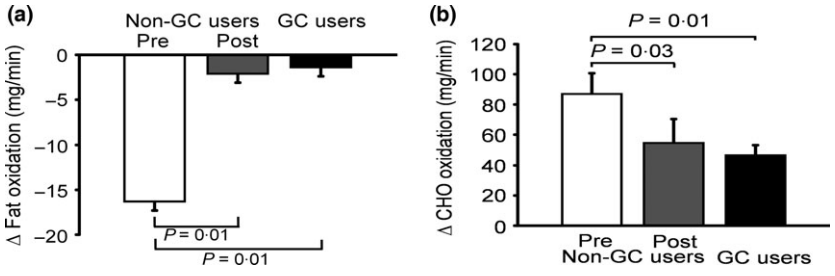

Fig. 2 Postmeal change in fat oxidation (a) and carbohydrate oxidation (CHO oxidation) (b) in 18 patients with rheumatoid arthritis who do not take oral glucocorticoids (non-GC users) before (white bars) and after (grey bars) 7 days prednisolone and 18 patients with rheumatoid arthritis on long-term prednisolone (GC users, black bars). Data are mean ± SEM.

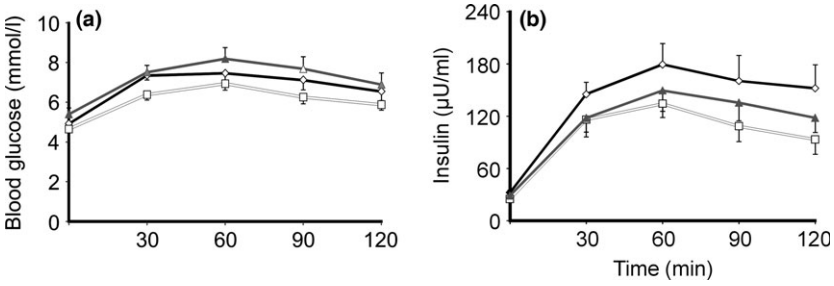

Fig. 3 Time course for glucose (a) and insulin (b) after a mixed meal in 18 patients with rheumatoid arthritis who do not take oral glucocorticoids (non-GC users) before (white bars) and after (grey bars) 7 days prednisolone and 18 patients with rheumatoid arthritis on long-term prednisolone (GC users, black bars). Data are mean ± SEM.

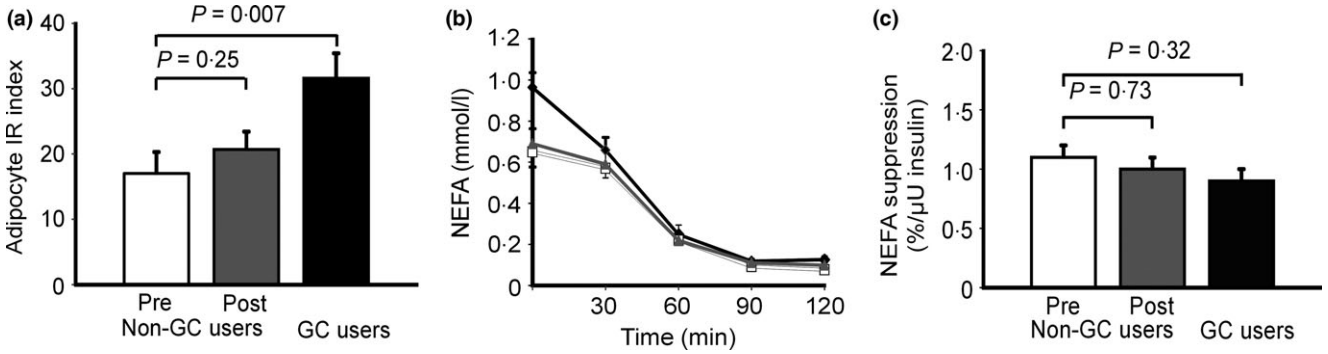

Fig. 4 Adipocyte insulin resistance index (Adipocyte IR index) (a), time course of plasma nonesterified fatty acids (NEFA) (b) and insulin-mediated suppression of NEFA (c) in 18 patients with rheumatoid arthritis who do not take oral glucocorticoids (non-GC users) before (white bars) and after (grey bars) 7 days prednisolone and 18 patients with rheumatoid arthritis on long-term prednisolone (GC users, black bars). Data are mean ± SEM.

and there was a reduction in Matsuda index with acute and chronic prednisolone.[12] However, these changes did not translate into a reduction in diet-induced thermogenesis. It is possible that the effects of glucocorticoids on postprandial energy metabolism manifest only with higher glucocorticoid doses. However, our study is clinically relevant as we studied commonly used therapeutic doses of prednisolone in typical (older) patients at ambient temperature.

In this study, we have systematically assessed the effects of prednisolone on substrate oxidation in the fasting and postprandial state. Previous studies have demonstrated no significant changes in fasting fat oxidation in patients with Cushing's syndrome[1] and taking exogenous glucocorticoids.[24] Concordant with these studies, there were no significant changes in fasting fat oxidation in this study. However, we have demonstrated for the first time that low-dose prednisolone alters postprandial substrate oxidation, with attenuation of suppression of fat oxidation and the concomitant increase in carbohydrate oxidation (Fig 2a and b). There is a parallel between the timing of these postprandial changes in substrate metabolism, termed metabolic inflexibility, and prednisolone-induced changes in blood glucose concentration, which also mainly occurs after a meal.[10] Postprandial glucose uptake and substrate oxidation predominantly occur in skeletal muscle. Consequently, postprandial hyperglycaemia and metabolic inflexibility are consistent with acute and chronic prednisolone causing insulin resistance in skeletal muscle.

In contrast to changes in fat oxidation, elevations of NEFA manifested in the fasting state and only during chronic glucocorticoid excess. Elevated serum NEFA concentrations are indirectly indicative of resistance to insulin-induced suppression of lipolysis in adipocytes. Consistent with previous studies,[25] circulating NEFA and adipocyte insulin resistance index were not significantly different after 7 days of low-dose prednisolone, suggesting insulin sensitivity in adipocytes was not affected by acute prednisolone. However, adipocyte insulin resistance index was increased in patients on long-term prednisolone, with mean fasting NEFA higher than typical NEFA concentrations after an overnight fast.[26] These findings are similar to those observed in patients with Cushing's syndrome, in whom fasting NEFA and subcutaneous adipose tissue lipolysis are increased,[27] but extend them by demonstrating that mild degrees of chronic glucocorticoid excess attenuate insulin-induced suppression of lipolysis. They do, however, need to be verified using stable isotopic assessment of lipid metabolism.

Previous *in vitro, ex vivo and in vivo* studies have reported that glucocorticoids do not cause insulin resistance in adipocytes.[28–30] In contrast, the elevated fasting NEFA in our study suggest that prednisolone does induce insulin resistance in adipocytes as the relatively low insulin concentrations during fasting do not suppress lipolysis. However, in the postprandial period, during which mean insulin concentrations were more than 100 μU/ml in all groups, insulin-mediated suppression of lipolysis was not significantly affected by prednisolone. As these insulin concentrations did not suppress fat oxidation, this suggests that the degree of glucocorticoid-induced insulin resistance is tissue-specific, with lipolysis in adipose tissue less resistant than fat oxidation in skeletal muscle.

These changes in lipid metabolism induced by glucocorticoids may have clinical consequences. It is debated as to whether metabolic inflexibility is a cause or consequence of insulin

resistance, and its clinical significance in humans remains to be clarified. However, in an animal model, inhibition of fat oxidation prevented glucocorticoid-induced insulin resistance in muscle.[31] Elevated fasting NEFA are associated with fat accumulation in liver and skeletal muscle, a major contributor to insulin resistance.[32] Furthermore, patients with higher fasting NEFA have increased rates of hypertension, type 2 diabetes and increased mortality.[33] Inhibition of lipolysis with acipimox was reported to reduce dexamethasone-induced insulin resistance.[34] Consequently, reversing prednisolone-induced changes in lipolysis and fat oxidation could potentially ameliorate the adverse metabolic effects of glucocorticoids and this should be the subject of future studies.

Assessing the metabolic effects of glucocorticoids *in vivo* is challenging. In addition to potential direct effects of glucocorticoids, changes in insulin concentration and sensitivity and catecholamine secretion influence their effects on energy and substrate metabolism. These variables can differ in the fasting and postprandial state. Strengths of this study include that we have systematically assessed the effects of prednisolone on fasting and postprandial energy metabolism and also simultaneously quantified insulin sensitivity and catecholamine secretion in a well-matched cohort of patients with rheumatoid arthritis.

However, we acknowledge our study has limitations. Firstly, as our study has a relatively small sample size, this could have resulted in a type 2 error when analysing some measurements e.g. fasting substrate metabolism. Secondly, we have not used an euglycaemic-hyperinsulinaemic clamp to assess the effect of glucocorticoids on insulin sensitivity, a technique considered to be the gold standard. However, calculation of Matsuda index from a mixed meal produces insulin sensitivity results similar to that from euglycaemic-hyperinsulinaemic clamp studies.[35] Thirdly, our findings may not be applicable to patients on higher glucocorticoid doses (>10 mg/day). Finally, inherent in any cross-sectional study is the possibility that an unmeasured variable affected results. However, the groups were well-matched for a number of key variables.

In summary, we have assessed the effects of commonly used therapeutic prednisolone doses on energy and fat metabolism in a typical (older) patient group with rheumatoid arthritis. Neither acute nor chronic prednisolone was associated with changes in fasting or postprandial energy expenditure. However, prednisolone attenuated postprandial suppression of fat oxidation. Chronic prednisolone also increased fasting NEFA and adipocyte insulin resistance, but did not affect postprandial insulin-mediated suppression of lipolysis. Our study highlights the adverse changes in carbohydrate and fat metabolism associated with mild glucocorticoid excess. The fasting and postprandial changes in fat metabolism demonstrated in our study suggest that glucocorticoids cause greater insulin resistance in skeletal muscle than in adipocytes. Future studies should confirm our findings using more sophisticated metabolic techniques and explore whether therapies targeting these changes in fat metabolism could reduce insulin resistance and the associated metabolic consequences of glucocorticoid therapy.

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Disclosure

The authors have no disclosures to declare.

References

1 Burt, M.G., Gibney, J. & Ho, K.K. (2006) Characterization of the metabolic phenotypes of Cushing's syndrome and growth hormone deficiency: a study of body composition and energy metabolism. *Clinical endocrinology*, **64**, 436–443.
2 Fardet, L., Petersen, I. & Nazareth, I. (2011) Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. *Rheumatology (Oxford)*, **50**, 1982–1990.
3 Fardet, L., Cabane, J., Lebbe, C., *et al.* (2007) Incidence and risk factors for corticosteroid-induced lipodystrophy: a prospective study. *Journal of the American Academy of Dermatology*, **57**, 604–609.
4 Fardet, L., Petersen, I. & Nazareth, I. (2012) Risk of cardiovascular events in people prescribed glucocorticoids with iatrogenic Cushing's syndrome: cohort study. *BMJ*, **345**, e4928.
5 Burt, M.G., Johannsson, G., Umpleby, A.M., *et al.* (2007) Impact of acute and chronic low-dose glucocorticoids on protein metabolism. *Journal of Clinical Endocrinology and Metabolism*, **92**, 3923–3929.
6 Nordborg, E., Schaufelberger, C. & Bosaeus, I. (1998) The effect of glucocorticoids on fat and lean tissue masses in giant cell arteritis. *Scandinavian Journal of Rheumatology*, **27**, 106–111.
7 Djurhuus, C.B., Gravholt, C.H., Nielsen, S., *et al.* (2002) Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. *American Journal of Physiology. Endocrinology and Metabolism*, **283**, E172–E177.
8 Chong, P.K., Jung, R.T., Scrimgeour, C.M., *et al.* (1994) The effect of pharmacological dosages of glucocorticoids on free living total energy expenditure in man. *Clinical endocrinology*, **40**, 577–581.

9 Tataranni, P.A., Larson, D.E., Snitker, S., *et al.* (1996) Effects of glucocorticoids on energy metabolism and food intake in humans. *American Journal of Physiology*, **271**, E317–E325.

10 Burt, M.G., Willenberg, V.M., Petersons, C.J., *et al.* (2012) Screening for diabetes in patients with inflammatory rheumatological disease administered long-term prednisolone: a cross-sectional study. *Rheumatology (Oxford)*, **51**, 1112–1119.

11 de Jonge, L. & Bray, G.A. (1997) The thermic effect of food and obesity: a critical review. *Obesity Research*, **5**, 622–631.

12 Radhakutty, A., Mangelsdorf, B.L., Drake, S.M., *et al.* (2016) Effect of acute and chronic glucocorticoid therapy on insulin sensitivity and postprandial vascular function. *Clinical Endocrinology (Oxford)*, **84**, 501–508.

13 Macfarlane, D.P., Forbes, S. & Walker, B.R. (2008) Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. *Journal of Endocrinology*, **197**, 189–204.

14 Peckett, A.J., Wright, D.C. & Riddell, M.C. (2011) The effects of glucocorticoids on adipose tissue lipid metabolism. *Metabolism*, **60**, 1500–1510.

15 Divertie, G.D., Jensen, M.D. & Miles, J.M. (1991) Stimulation of lipolysis in humans by physiological hypercortisolemia. *Diabetes*, **40**, 1228–1232.

16 Ferrannini, E. (1988) The theoretical bases of indirect calorimetry: a review. *Metabolism*, **37**, 287–301.

17 Vosselman, M.J., Brans, B., van der Lans, A.A., *et al.* (2013) Brown adipose tissue activity after a high-calorie meal in humans. *American Journal of Clinical Nutrition*, **98**, 57–64.

18 Matsuda, M. & DeFronzo, R.A. (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*, **22**, 1462–1470.

19 Malin, S.K., Kashyap, S.R., Hammel, J., *et al.* (2014) Adjusting glucose-stimulated insulin secretion for adipose insulin resistance: an index of beta-cell function in obese adults. *Diabetes Care*, **37**, 2940–2946.

20 Abdul-Ghani, M.A., Molina-Carrion, M., Jani, R., *et al.* (2008) Adipocytes in subjects with impaired fasting glucose and impaired glucose tolerance are resistant to the anti-lipolytic effect of insulin. *Acta Diabetologica*, **45**, 147–150.

21 Voorrips, L.E., Ravelli, A.C., Dongelmans, P.C., *et al.* (1991) A physical activity questionnaire for the elderly. *Medicine and Science in Sports and Exercise*, **23**, 974–979.

22 Valensi, P., Lormeau, B., Dabbech, M., *et al.* (1998) Glucose-induced thermogenesis, inhibition of lipid oxidation rate and autonomic dysfunction in non-diabetic obese women. *International Journal of Obesity and Related Metabolic Disorders*, **22**, 494–499.

23 Camastra, S., Bonora, E., Del Prato, S., *et al.* (1999) Effect of obesity and insulin resistance on resting and glucose-induced thermogenesis in man. EGIR (European Group for the Study of Insulin Resistance). *International Journal of Obesity and Related Metabolic Disorders*, **23**, 1307–1313.

24 Burt, M.G., Gibney, J. & Ho, K.K. (2007) Protein metabolism in glucocorticoid excess: study in Cushing's syndrome and the effect of treatment. *American Journal of Physiology. Endocrinology and Metabolism*, **292**, E1426–E1432.

25 Petersons, C.J., Mangelsdorf, B.L., Jenkins, A.B., *et al.* (2013) Effects of low-dose prednisolone on hepatic and peripheral insulin sensitivity, insulin secretion, and abdominal adiposity in patients with inflammatory rheumatologic disease. *Diabetes Care*, **36**, 2822–2829.

26 Karpe, F., Dickmann, J.R. & Frayn, K.N. (2011) Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes*, **60**, 2441–2449.

27 Leal-Cerro, A., Jimenez, L.M., Astorga, R., *et al.* (1997) Acute pharmacological reduction of plasma free fatty acids enhances the growth hormone (GH)-releasing hormone-mediated GH secretion in patients with Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism*, **82**, 3165–3168.

28 Gathercole, L.L., Morgan, S.A., Bujalska, I.J., *et al.* (2011) Regulation of lipogenesis by glucocorticoids and insulin in human adipose tissue. *PLoS ONE*, **6**, e26223.

29 Gathercole, L.L., Bujalska, I.J., Stewart, P.M., *et al.* (2007) Glucocorticoid modulation of insulin signaling in human subcutaneous adipose tissue. *Journal of Clinical Endocrinology and Metabolism*, **92**, 4332–4339.

30 Hazlehurst, J.M., Gathercole, L.L., Nasiri, M., *et al.* (2013) Glucocorticoids fail to cause insulin resistance in human subcutaneous adipose tissue in vivo. *Journal of Clinical Endocrinology and Metabolism*, **98**, 1631–1640.

31 Guillaume-Gentil, C., Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1993) Involvement of non-esterified fatty acid oxidation in glucocorticoid-induced peripheral insulin resistance in vivo in rats. *Diabetologia*, **36**, 899–906.

32 Belfort, R., Mandarino, L., Kashyap, S., *et al.* (2005) Dose-response effect of elevated plasma free fatty acid on insulin signaling. *Diabetes*, **54**, 1640–1648.

33 Miedema, M.D., Maziarz, M., Biggs, M.L., *et al.* (2014) Plasma-free fatty acids, fatty acid-binding protein 4, and mortality in older adults (from the Cardiovascular Health Study). *American Journal of Cardiology*, **114**, 843–848.

34 Santomauro, A.T., Boden, G., Silva, M.E., *et al.* (1999) Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes*, **48**, 1836–1841.

35 van Raalte, D.H., van Genugten, R.E., Linssen, M.M., *et al.* (2011) Glucagon-like peptide-1 receptor agonist treatment prevents glucocorticoid-induced glucose intolerance and islet-cell dysfunction in humans. *Diabetes Care*, **34**, 412–417.