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Low dose prednisolone and insulin sensitivity differentially affect arterial stiffness and endothelial function: An open interventional and cross-sectional study

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ABSTRACT

Background and aims: Glucocorticoids could impair vascular function directly, or indirectly by reducing insulin sensitivity. The aim of this study was to determine the direct and indirect effects of acute and chronic low dose prednisolone on arterial stiffness and endothelial function.
Methods: Twelve subjects with inflammatory arthritis, who had not taken oral glucocorticoids for ≥6 months, and 12 subjects with inflammatory arthritis, taking chronic (>6 months) low dose (6.3 ± 2.2 mg/day) prednisolone, were studied. Patients not on glucocorticoids underwent measurement of arterial stiffness (pulse wave velocity (PWV)) and endothelial function (reactive hyperaemia index (RHI)) before and after 7–10 days of prednisolone (6 mg/day), to assess the acute effects of prednisolone. Baseline data from patients not on glucocorticoids were compared with patients on long-term prednisolone to assess the chronic effects of prednisolone. Hepatic insulin sensitivity was estimated from percentage suppression of endogenous glucose production and peripheral insulin sensitivity as glucose infusion rate (M/I) during a hyperinsulinaemic-euglycaemic clamp.
Results: There were no significant changes in PWV with acute (9.2 ± 0.8 *vs.* 8.9 ± 0.8 m/sec, $p = 0.33$) or chronic (8.9 ± 0.8 *vs.* 9.0 ± 0.7 m/sec, $p = 0.69$) prednisolone. In multiple regression analysis, PWV was negatively associated with M/I during hyperinsulinemic-euglycemic clamp ($p = 0.02$), but not with suppression of endogenous glucose production ($p = 0.15$) or glucocorticoid use ($p = 0.70$). Chronic (2.4 ± 0.2 *vs.* 1.9 ± 0.1, $p = 0.02$), but not acute (1.8 ± 0.2 *vs.* 1.9 ± 0.1, $p = 0.24$), prednisolone resulted in a higher RHI.
Conclusions: Arterial stiffness is not affected by low dose prednisolone *per se*, but is negatively associated with peripheral insulin sensitivity. Patients with rheumatoid arthritis taking long-term prednisolone had better endothelial function.

1. Introduction

Inflammatory rheumatologic disease is associated with increased cardiovascular events and mortality [1,2]. The cause of increased cardiovascular risk is not fully defined, and is likely to be multifactorial. Active inflammation *per se* is an important contributor to cardiovascular risk as it is associated with increased arterial stiffness and endothelial dysfunction [3,4]. Another potential contributor is glucocorticoid therapy, which is commonly prescribed to patients with inflammatory rheumatologic disease.

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Although higher doses can be required to treat an acute disease flare, during long-term therapy most patients are prescribed daily prednisolone doses below 10 mg [5]. These glucocorticoid doses have been associated with increased cardiovascular risk in some, but not all, epidemiologic studies [6–8].

Identifying mechanisms by which glucocorticoids alter cardiovascular risk will aid interpretation of epidemiologic studies. Increased arterial stiffness is one mechanism that could underlie an association between low dose prednisolone and cardiovascular risk. Arterial stiffness is increased in patients with atherosclerosis, but is also tonically regulated by endothelial cell nitric oxide production and the autonomic nervous system [9,10].

As vascular endothelial and smooth muscle cells contain glucocorticoid receptors [11], glucocorticoids could increase arterial stiffness directly. Alternatively, glucocorticoids could increase arterial stiffness indirectly by reducing insulin sensitivity. Acutely, reduced insulin sensitivity will attenuate signaling through the phosphatidyl ionositol-3 kinase pathway and nitric oxide-mediated vasodilatation [12]. Chronically, enhanced stimulation of the mitogen activated protein kinase pathway by hyperinsulinemia in insulin resistance can cause vascular smooth muscle proliferation and atherosclerosis [12]. Thereby, acute and chronic changes in insulin sensitivity alter vascular function by different mechanisms.

Low dose glucocorticoids reduce insulin sensitivity in patients with inflammatory rheumatologic disease [13]. However, studies investigating the effects of glucocorticoids on fasting arterial stiffness [14–16] and endothelial function [14,17–20] have produced conflicting results. Furthermore, we recently reported that low dose prednisolone reduced postprandial augmentation index, a measure of arterial stiffness, and attenuated postprandial endothelial dysfunction [21]. These beneficial changes in vascular function occurred despite a reduction in insulin sensitivity [21]. As such, the relative effects of low dose prednisolone *per se versus* changes in insulin sensitivity on vascular function require clarification.

In our previous study we employed surrogate measures to quantify insulin sensitivity and arterial stiffness [21]. Here, we report a cohort of patients with inflammatory rheumatologic disease in whom we quantified hepatic and peripheral insulin sensitivity using stable isotopes and hyperinsulinaemic-euglycaemic clamp and pulse wave velocity (PWV), the gold standard measurement of arterial stiffness [22]. We first assessed the acute effect of low dose prednisolone on arterial stiffness and endothelial function. We then investigated the relative contributions of chronic low dose prednisolone, hepatic and peripheral insulin sensitivity to arterial stiffness and endothelial function.

2. Patients and methods

The study conforms to the guidelines of the 1975 Declaration of Helsinki. It was approved by the Southern Adelaide Clinical Human Research Ethics Committee, Flinders Medical Centre, and all subjects provided written informed consent. The effects of prednisolone on insulin sensitivity in the majority of this cohort has been reported [13]. This analysis reports the relationship between insulin sensitivity and vascular function.

2.1. Subjects

Twenty-four consecutive consenting subjects with inflammatory arthritis were recruited from Repatriation General Hospital Rheumatology outpatient clinic between July 2010 and October 2011. Twelve subjects had taken a stable continuous oral prednisolone dose of 4–10 mg/day for >6 months as part of usual clinical care (GC users). The other 12 subjects had not taken oral prednisolone for at least 6 months (non-GC users). The two groups were matched for sex and age. Subjects were excluded from the study if they had clinically active synovitis, diabetes mellitus, hepatic disease, renal disease or congestive cardiac failure, or were on other medications known to affect carbohydrate metabolism [13]. Undiagnosed diabetes mellitus was excluded at a screening visit with an oral glucose tolerance test.

Hepatic insulin sensitivity could not be calculated in one GC user because of a technical problem with the basal 6,6-$^2H_2$ glucose infusion and one subject in this group did not undergo assessment of endothelial function because of severe Raynaud's disease. Twelve non-GC users underwent baseline cardiovascular assessment. For one subject, PWV and reactive hyperaemia index (RHI) could not be recorded because of technical difficulties and painful lipo-oedema respectively. Eleven non-GC users underwent repeat cardiovascular assessment after prednisolone.

2.2. Study design

To determine the acute effects of low-dose prednisolone administration, the non-GC users were studied before and after a 7–10 day course of oral prednisolone 6 mg daily. This dose of prednisolone was chosen to closely approximate the mean prednisolone dose in GC users, allowing comparison of the acute and chronic effects of prednisolone. To determine the chronic effects of low-dose prednisolone, baseline data from the non-GC users were compared with baseline data from GC users.

2.3. Study protocol

Insulin sensitivity and vascular function were assessed using a two-day protocol. At each visit, subjects attended the Endocrine Research Unit, Repatriation General Hospital at 0800 h after an overnight fast. On Day 1, subjects underwent assessment of basal endogenous glucose production, followed by a two-step hyperinsulinaemic-euglycaemic clamp study. On Day 2, subjects underwent assessment of vascular function and body composition.

2.3.1. Assessment of insulin sensitivity

Assessment of insulin sensitivity has previously been described [13]. In brief, intravenous cannulae were inserted into the antecubital fossa of one arm for administration of infusions and distally into the contralateral arm for blood sampling. After baseline blood samples were collected, subjects were administered a primed (5 mg/kg), continuous (3 mg/kg/hr) infusion of 6,6-$^2H_2$ glucose (Cambridge Isotopes Laboratories, Massachusetts, USA) for 120 min to estimate basal endogenous glucose production. Following this, a two-step hyperinsulinaemic-euglycaemic clamp study was performed. In the first step, human neutral insulin (Actrapid™, Novo Nordisk Pharmaceuticals Pty Ltd, New South Wales, Australia) was infused for 120 min at 15 mU/m^2/min. The basal 6,6-$^2H_2$ glucose infusion was continued at 50% of the initial rate (1.5 mg/kg/hr) during this step. In the second step, the basal 6,6-$^2H_2$ glucose infusion was ceased and subjects were administered a primed (320 mU/m^2/min for 2 min followed by 160 mU/m^2/min for 2 min) human neutral insulin infusion at 80 mU/m^2/min for 120 min. During the low- 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-$^2H_2$ glucose to maintain a target glucose concentration of 5 mmol/L.

Endogenous glucose production was calculated as previously described [13]. The percentage of endogenous glucose production suppression during the low-dose clamp study was considered a marker of hepatic insulin sensitivity. The mean glucose infusion rates during steady state (M) corrected for fat free mass and serum

insulin concentration (I) during the high-dose clamp study were used as a measure of peripheral tissue insulin sensitivity (M/I).

2.3.2. Assessment of cardiovascular function

Assessment of cardiovascular function was performed fasting in a standardized order. First carotid-femoral PWV was measured by one operator (CJP) by combining applanation tonometry with a SphygmoCor device (AtCor Medical, New South Wales, Australia) and high-fidelity micromanometer (SPC-301, Millar Instruments, TX, USA) with simultaneous electrocardiogram recording. Reported results are the average of 6 consecutive recordings. The average day-to-day intraclass correlation for PWV for this operator is 0.95.

Next a Taskforce Haemodynamic Monitor 3040i (CNSystems, Graz, Austria) measured heart rate and beat-by-beat arterial pressure simultaneously, which was combined with continuous electrocardiogram recording to calculate baroreceptor sensitivity by the sequence method. After subjects had rested supine for 10 min, baroreceptor sensitivity was assessed over a 20 min period.

Finally, RHI was measured by peripheral arterial tonometry using an Endo-PAT 2000 device (Itamar Medical, Caesarea, Israel). After a baseline pulse amplitude measurement was obtained, local ischaemia was induced in one arm by inflating a blood pressure cuff to supra-systolic pressures for 5 min. The period between 90 and 150 s after deflation was used for automated calculation of the RHI [23]. The average day-to-day coefficient of variation for RHI at our institution is 11.4% [24].

2.3.3. Other measurements

Fat free mass (FFM) was measured by dual energy X-ray absorptiometry on a GE Medical Systems Lunar Prodigy (GE Healthcare General Electric Company). C-reactive protein (CRP) was measured using a Tinaquant immunoturbidimetric assay (Roche Diagnostics GMBH, Mannheim, Germany) on a Roche Modular Analyser (Hitachi High-Technologies Corporation, Tokyo, Japan) with a CV <4%.

2.4. Statistical analysis

Normally distributed subject characteristic data are presented as mean ± standard deviation and other data as mean ± standard error of mean. If the distribution was not normal, data are presented as median (interquartile range). The changes in variables in non-GC users after 7–10 day prednisolone administration were assessed using the Wilcoxon Signed Rank test. Hereafter in the manuscript, these changes are reported as the acute effects of prednisolone. Difference in variables between GC users and non-GC users were assessed using Fisher's exact test for categorical variables and Mann-Whitney *U* tests for continuous variables. These differences are reported in the manuscript as the chronic effects of prednisolone. A multiple regression analysis was undertaken to determine whether chronic glucocorticoid therapy or insulin sensitivity were independently associated with PWV and RHI. The primary endpoints were the change or difference in PWV with acute and chronic prednisolone respectively. In the acute study, a sample size of 12 subjects has >80% power to detect a 1.25 m/s change in PWV. In the chronic study, a sample size of 12 subjects per group has >80% power to detect a 1.75 m/s change in PWV. These calculations assumed a standard deviation of 1.5 m/s, derived from a previous study [24].

Statistical analysis was performed using IBM SPSS version 20 for Windows (IBM, New York, USA). A p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Subject characteristics

GC users were taking a mean prednisolone dose of 6.3 ± 2.2 mg/day for 81 ± 62 months. CRP was not significantly different in GC users and non-GC users, with the median CRP in both groups within the normal reference range of 0–8 mg/L at our laboratory. There were no significant differences in age, sex, BMI, waist circumference, underlying disease, disease modifying anti-rheumatic drug use, treatment for hypertension or dyslipidemia or smoking between the two groups (Table 1). No subject in either group had a history of cardiovascular disease.

3.2. Acute effects of prednisolone

There was a reduction in percentage suppression of endogenous glucose production during the low dose clamp study (79 ± 2 *vs.* 67 ± 5%, $p = 0.04$) and M/I during the high dose clamp (0.052 ± 0.006 *vs.* 0.046 ± 0.006 mg/kg FFM/min/mU/L, $p = 0.03$) after acute prednisolone. There were no significant changes in pulse rate, systolic or diastolic blood pressure, baroreflex sensitivity or PWV after acute prednisolone (Table 2). There was no significant change in RHI after acute prednisolone ($p = 0.24$, Fig. 1A).

3.3. Chronic effects of prednisolone

GC users had a lower percentage suppression of endogenous glucose production during the low dose clamp study than non-GC users (66 ± 4 *vs.* 79 ± 2%, $p = 0.02$). There was no significant difference in M/I during the high dose clamp between GC users and non-GC users (0.045 ± 0.006 *vs.* 0.053 ± 0.006 mg/kg FFM/min/mU/L, $p = 0.38$). There were no significant differences in pulse rate, systolic or diastolic blood pressure, baroreflex sensitivity or PWV between GC users and non-GC users, although the difference in systolic blood pressure almost reached statistical significance (Table 3). GC users had a higher RHI than non-GC users ($p = 0.02$, Fig. 1B).

In a multiple regression analysis, PWV was independently and negatively associated with M/I during the high dose clamp, but not with percentage suppression of endogenous glucose production during the low dose clamp or GC use (Table 4). In contrast, RHI was

Table 1
Subject characteristics of patients with inflammatory arthritis who were not taking prednisolone (Non-GC Users) and who had been taking prednisolone for at least 6 months (GC Users).

	Non-GC[a] Users	GC[a] Users	*p* value
Number (n)	12	12	
Age (years)	59 ± 10	61 ± 8	0.57
Female (n (%))	7 (58)	6 (50)	0.68
BMI[b] (kg/m^2)	27.8 ± 6.2	27.4 ± 3.3	0.84
Waist circumference (cm)	94 ± 17	95 ± 11	0.86
RA[c]/SNA[d]/SLE[e]	6/6/0	8/3/1	0.32
DMARDs[f], n (%)	9 (75)	9 (75)	1.00
C-reactive protein, mg/L	3.3 (1.2, 8.4)	4.2 (1.8, 14.8)	0.44
Treated for hypertension, n (%)	3 (25)	2 (17)	0.62
Treated for dyslipidaemia, n (%)	1 (8)	1 (8)	1.00
Current smoker, n (%)	2 (17)	1 (8)	1.00

Data are presented as mean ± standard deviation or median (interquartile range).
[a] Glucocorticoid.
[b] Body mass index.
[c] Rheumatoid arthritis.
[d] Seronegative arthritis.
[e] Systemic *lupus erythematosus* with joint involvement.
[f] Disease modifying anti-rheumatic drugs.

Table 2
Measures of cardiovascular function in subjects with inflammatory arthritis before and after oral administration of prednisolone 6 mg per day for 7–10 days.

	Before prednisolone	After prednisolone	*p* value
Pulse (beats/min)	63 ± 2	63 ± 1	0.93
Systolic BP[a] (mmHg)	123 ± 4	122 ± 6	0.53
Diastolic BP[a] (mmHg)	80 ± 3	79 ± 3	0.29
Baroreflex sensitivity (ms/mmHg)	12.7 (9.3–14.8)	12.6 (8.4–14.5)	0.79
Pulse wave velocity (m/sec)	9.2 ± 0.8	8.9 ± 0.8	0.33

Values represent mean ± standard error or median (interquartile range).
[a] Blood pressure.

Table 3
Measures of cardiovascular function in subjects with inflammatory arthritis who have not taken prednisolone for at least 6 months (Non-GC users) and subjects who have taken low dose prednisolone for at least 6 months (GC users).

	Non-GC users	GC users	*p* value
Pulse (beats/min)	64 ± 2	67 ± 3	0.18
Systolic BP[a] (mmHg)	121 ± 4	130 ± 4	0.06
Diastolic BP[a] (mmHg)	80 ± 2	85 ± 4	0.30
Baroreflex sensitivity (ms/mmHg)	12.7 (9.3–14.8)	8.9 (7.0–12.3)	0.15
Pulse wave velocity (m/sec)	9.0 ± 0.7	8.9 ± 0.8	0.69

Values represent mean ± standard error or median (interquartile range).
[a] Blood pressure.

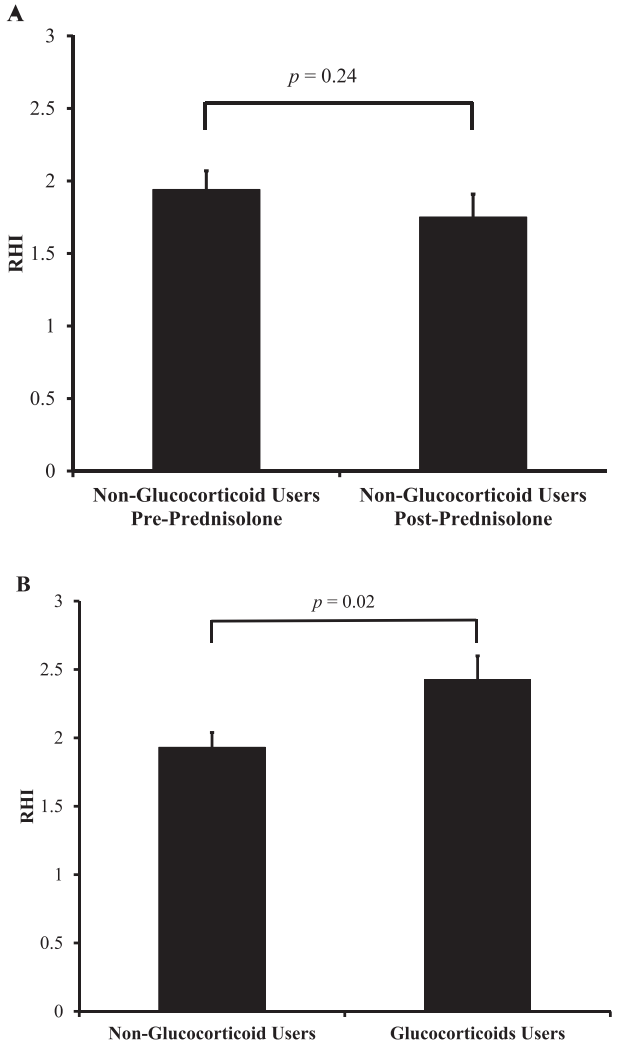

Fig. 1. Effect of acute and chronic low dose prednisolone on endothelial function. (A) Reactive hyperaemia index (RHI) in subjects who were not taking prednisolone (non-glucocorticoid users) before and after administration of low-dose prednisolone for 7–10 days. (B) RHI in non-glucocorticoid users and subjects on long-term prednisolone (glucocorticoid users). Data are mean ± standard error mean.

independently associated with GC use, but not significantly with percentage suppression of endogenous glucose production during the low dose clamp or M/I during the high dose clamp.

4. Discussion

This study sought to clarify the effects of glucocorticoids *per se* *versus* glucocorticoid-induced insulin resistance on vascular function. Low-dose prednisolone did not directly affect PWV. However, PWV was negatively associated with peripheral insulin sensitivity in a multiple regression analysis. In contrast, RHI was higher in patients on chronic low-dose prednisolone and was independently associated with glucocorticoid therapy, but not with insulin sensitivity. These findings suggest that short-term low dose prednisolone administration does not perturb vascular function, but that during long-term prednisolone therapy there is a complex interplay between the direct effects of glucocorticoids and changes in insulin sensitivity that modify different components of vascular function.

In this study there was no significant change in PWV with acute or chronic prednisolone. PWV is considered the gold-standard measure of arterial stiffness, with an increase in PWV of 1 m/sec associated with a 15% increase in relative risk of cardiovascular mortality [25]. Few studies have characterized the effects of glucocorticoids on PWV. Long-term prednisolone treatment of rheumatoid arthritis [15] and short-term increase of glucocorticoid dose in hypopituitary patients [14] did not significantly alter PWV. In contrast, administration of prednisolone to patients with an acute flare of polymyalgia rheumatica reduced PWV, with the reduction correlating with the improvement in CRP [16]. This is consistent with reports that more active inflammatory disease is associated with increased arterial stiffness [3] and suggests that glucocorticoids reduce arterial stiffness in patients with an active inflammatory process, but that in other settings they do not have an effect.

In a multiple regression analysis PWV was independently and negatively associated with peripheral insulin sensitivity, but not with hepatic insulin sensitivity or glucocorticoid use. Peripheral insulin sensitivity and uptake of glucose into skeletal muscle is the major determinant of postprandial glucose concentration. This is relevant as increased cardiovascular disease [26] and arterial stiffness [27] are more strongly associated with post glucose-load, than fasting, glucose concentration. Moreover, low dose prednisolone reduces peripheral insulin sensitivity and predominantly increases post glucose-load glucose concentration [13,28].

There have been contrasting reports of the acute effects of glucocorticoids on endothelial function. Short-term glucocorticoid excess reduced endothelial function in hypopituitary patients [14] and patients with IgA nephropathy [17], but not in healthy young adults [18]. This study suggests changes in endothelial function may become apparent during chronic glucocorticoid use, which has rarely been assessed. There was no change in flow-mediated dilatation in patients with rheumatoid arthritis treated with prednisolone for 2–5 years [19]. However, glucocorticoid therapy resulted in a sustained improvement in flow-mediated dilatation in patients with giant cell arteritis [20] and the postprandial reduction in RHI was attenuated in patients on long-term prednisolone [21]. This suggests that the acute and chronic effects of glucocorticoids on endothelial function differ. As RHI was not associated with hepatic or peripheral insulin sensitivity, we hypothesize that long-term

Table 4
Multiple regression analysis of the relationships between low dose prednisolone therapy, hepatic insulin sensitivity and peripheral insulin sensitivity with pulse wave analysis and reactive hyperemia index.

	Pulse wave velocity		Reactive hyperemia index	
	β coefficient	*p* value	β coefficient	*p* value
Glucocorticoid use	+0.10	0.70	**+0.73**	**0.003**
EGP[a] suppression low dose clamp	+0.38	0.15	+0.38	0.11
M/I[b] high dose clamp	**−0.56**	**0.02**	+0.27	0.19

[a] Endogenous glucose production.
[b] Mean glucose infusion rates during steady state (M) corrected for fat free mass and serum insulin concentration (I).

glucocorticoid therapy might act directly on endothelial cells to enhance endothelial function in patients with inflammatory rheumatologic disease.

The potential clinical implications of our findings are that therapeutic agents that reduce prednisolone-induced peripheral insulin resistance might reduce arterial stiffness, but are unlikely to affect endothelial function. Consistent with this, in a cohort of patients with rheumatoid arthritis of whom approximately 50% were prescribed glucocorticoids, pioglitazone reduced PWV, but not RHI [29]. It would be of interest to dissect whether the effect of pioglitazone was affected by prednisolone therapy or baseline insulin sensitivity. However, as glucocorticoids and thiazolidinediones both increase weight and fracture risk [30,31], the risk-benefit of pioglitazone needs to be carefully considered in prednisolone-treated patients.

There was no significant change in baroreflex sensitivity with acute or chronic prednisolone. Previous studies also reported that an increase in glucocorticoid dose did not alter baroreflex sensitivity in hypopituitary patients [14] and that low dose prednisolone did not affect heart rate variability in healthy young men [32]. However, assessment of baroreflex sensitivity by the sequence method is predominantly a marker of parasympathetic nervous system activity [33], while glucocorticoids main action is to reduce sympathetic nervous system activity [21,34].

The strengths of this study include that the subject groups comprised typical patients to whom glucocorticoids are prescribed, that the groups were well-matched for variables that affect cardiovascular risk and that by simultaneously assessing insulin sensitivity and cardiovascular risk we were able to assess direct and indirect effects of glucocorticoids on vascular function. The major limitation of the study is the small sample size, which is a particular consideration when interpreting the results of multiple regression analyses. Moreover, there was no control group in the study assessing the acute effects of prednisolone. However, the demands placed on these older subjects by the study protocol were considerable and it would be difficult to recruit a substantially larger cohort. Another limitation is that cumulative glucocorticoid exposure and duration of inflammatory disease were unable to be accurately calculated, as many subjects had been treated at more than one location and complete medical records were unavailable. 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, including use of disease modifying anti-rheumatic drugs. Finally, the results may not be applicable to patients taking higher glucocorticoid doses.

In summary, chronic, but not acute, low dose prednisolone was associated with better endothelial function. As such, endothelial dysfunction is unlikely to be a mechanism that increases cardiovascular risk in patients requiring long-term glucocorticoid therapy. In contrast, arterial stiffness was not affected by low dose prednisolone *per se*, but was negatively associated with peripheral insulin sensitivity. Future studies should explore whether, in those patients who develop glucocorticoid-induced insulin resistance, arterial stiffness and consequentially cardiovascular events are reduced by therapeutic agents such as pioglitazone that improve peripheral insulin sensitivity.

Clinical trials

Australian New Zealand Clinical Trials Registry (ANZCTR): ACTRN12610000409077.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

CJP, JRG, CHT and MGB conceived and designed the study; CJP, BLM, AP, MDS, JRG and MGB contributed to data collection; CJP and MGB analyzed the data and wrote the initial draft of the manuscript; all authors have contributed to the final version of the manuscript, with MGB taking final responsibility for the content.

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