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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