The use of metformin to reduce metabolic complications and inflammation in patients on systemic glucocorticoid therapy: a randomised, double-blind, placebo-controlled, phase 2 clinical trial

Short title: Metformin to reduce metabolic complications of systemic glucocorticoids: RCT

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ABSTRACT

Background: There is an urgent need to limit the metabolic side-effects of glucocorticoid overexposure as these can lead to Cushing's syndrome, associated with high morbidity. We have explored the potential for metformin to ameliorate such effects whilst sparing the anti-inflammatory benefits of glucocorticoids.

Methods: In this double-blind, phase 2 proof-of-concept trial, 53 patients without known diabetes established on mid-to-high doses of glucocorticoids, administered as treatment for a chronic inflammatory disease, were randomised to receive 2550mg/day metformin (n=26) or an identical placebo (n=27) for 12 weeks. The primary endpoint was the change in visceral to truncal subcutaneous fat ratio assessed by computed tomography; secondary endpoints involved metabolic, bone, cardiovascular and inflammatory parameters.

Findings: Nineteen patients on metformin and 21 on placebo completed the study. The groups received equivalent cumulative dose of glucocorticoids (1860mg (IQR 1060 to 2810) vs. 1770mg (IQR 1020-2356) prednisolone equivalent; p=0.76). There was no change in the visceral-to-subcutaneous fat ratio (0.11 (95%CI -0.02 to 0.24); p=0.09) between the treatment groups but metformin-treated patients lost truncal subcutaneous fat (-3835mm^2 (95%CI -6781 to -888); p=0.01) compared to placebo. Improvements in markers of carbohydrate, lipid, liver and bone metabolism were observed on metformin. Additionally, metformin-treated patients had improved fibrinolysis, carotid intima-media thickness, inflammatory parameters and clinical markers of disease activity. The frequency of pneumonia (1 vs. 7 events; p=0.01), overall rate of moderate-to-severe infections (2 vs. 11; p=0.001), and all-cause hospital admissions due to adverse events (1 vs. 9; p=0.001) were lower in the metformin group compared with placebo. Metformin-treated patients experienced more diarrhoea initially.

Interpretation: Metformin administration improved the metabolic profile of glucocorticoid-treated patients with inflammatory disease, favourably modifying cardiovascular risk surrogates, reducing inflammation and hospitalisation.

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Research in context

Evidence before this study

Glucocorticoid excess, due to endogenous causes or to glucocorticoid treatment, can lead to Cushing's syndrome, a phenotype associated with an increased morbidity and mortality. The higher morbidity risk lasts even after removal of glucocorticoid excess.

Our team have previously shown that several metabolic changes associated with glucocorticoid overexposure correspond to metabolic steps regulated by 5'AMP-activated-protein-kinase (AMPK). AMPK is one of the mediators of metformin's action: metformin was able to reverse the glucocorticoid effect on AMPK *in vitro* and to prevent glycaemic deterioration in non-diabetic patients when initiated simultaneously with glucocorticoid treatment.

Metformin treatment was shown to be associated with metabolic benefits on weight, blood pressure, lipids, coagulation, inflammation, and endothelium in some, but not all, human studies. We wondered whether metformin administration for patients with glucocorticoid treatment might result in beneficial metabolic effects without adversely affecting their anti-inflammatory actions.

Added value of this study

This randomised, double-blind, placebo-controlled, phase 2 clinical trial evaluated patients established on glucocorticoid treatment, without diabetes, for the potential beneficial effects of metformin to diminish multisystem adverse effects of glucocorticoid treatment. Our data do not just show improvement in several surrogates of metabolic and cardiovascular risks for patients receiving metformin, but the intervention also appeared to reduce inflammation and number of infections and hospitalisation of this vulnerable patient cohort. The results are clinically relevant, and could be applied efficiently and economically to a large number of patients worldwide.

Implications of all the available evidence

Although glucocorticoids are the most common cause of medication-induced hyperglycaemia, and rising glycaemia has been reported as the number one concern when prescribing glucocorticoid treatment, there is no international consensus on the screening and management of glucocorticoid-induced hyperglycaemia. Based on experimental data, metformin's mode of action may interact with the glucocorticoid pathway. We propose that metformin, irrespective of the diabetic status, could be a good agent to attenuate adverse effects of glucocorticoid treatment, reducing inflammation and cost, and improving patient outcomes. In view of limitations of lifestyle intervention for patients in need of glucocorticoid therapy, based on this study we recommend patients be considered for concomitant metformin treatment early and that larger studies are initiated to evaluate hard cardiovascular endpoints.

INTRODUCTION

Glucocorticoid treatment is prescribed long-term in up to 3% of the adult population[1,2]. Chronic exposure to glucocorticoid excess can lead to distinctive truncal obesity, hypertension, hyperglycaemia, dyslipidaemia, hypercoagulability, fatty liver, osteoporosis, increased infections and other complications[3]. The developing phenotype, known as iatrogenic Cushing's syndrome, is associated with increased morbidity and mortality, especially from cardiovascular causes and infections[3,4] and the raised morbidity persists even after abrogation of glucocorticoid excess[5,6]. Separating the desired anti-inflammatory effects of glucocorticoids from their unwanted metabolic action has so far proved difficult.

In patients with Cushing's syndrome, the activity of one of the enzymatic mediators of metformin's action, 5'AMP-activated-protein-kinase (AMPK)[7], was decreased in visceral adipose tissue[8] while metformin reversed the glucocorticoid effect on AMPK in adipose and hypothalamic tissues *in vitro*[9], and improved glucose levels in patients who were starting glucocorticoid treatment[10]. Metformin was also associated with a favourable immune response in several animal models of autoimmune diseases[7]. We hypothesised that metformin might alleviate a plethora of metabolic features of glucocorticoid overexposure without adversely affecting their anti-inflammatory benefits. Therefore, we designed a randomised, double-blind, placebo-controlled, phase 2 proof-of-concept trial of the addition of metformin therapy in patients with chronic inflammatory diseases already established on long-term glucocorticoid treatment. We wished to evaluate the potential of metformin to reverse multisystem glucocorticoid metabolic side-effects. There is clearly an urgent need for such a resolution but no current solution. We aimed to improve the metabolic profile associated with glucocorticoid treatment and demonstrate that metabolically vulnerable patients with substantial cumulative glucocorticoid exposure significantly benefited from the administration of metformin.

METHODS

Participants

Eligible patients in this randomised, double-blind, placebo-controlled, phase 2 proof-of-concept trial were adults (≥18 and ≤75 years) with an inflammatory disease with ongoing ≥20mg/day prednisolone treatment for ≥4 weeks (or its cumulative dose-equivalent), remaining on ≥10mg/day prednisolone-equivalent for ≥12 subsequent weeks. Cumulative prednisolone equivalent was calculated using recognized conversion tables and both oral and parenteral glucocorticoids were taken into account. Minimum duration of continuous glucocorticoid exposure prior enrolment was 4 weeks, minimum cumulative dose: 560mg prednisolone equivalent. Key exclusion criteria were known pre-existing diabetes mellitus, prior therapy with metformin over the previous 6 months, ALT and/or AST ≥2.5×upper limit of normal, or serum creatinine ≥135μmol/L (males) and ≥110μmol/L (females) (further details: Supplementary Methods).

Study design, randomisation and masking

After assessing 849 patients attending respiratory and rheumatology outpatient clinics regarding the presence of current glucocorticoid treatment and other study-entry criteria, 53 patients were randomised to receive metformin (n=26 subjects) or placebo (n=27) for 12 weeks (Figure 1). Participants were allocated to metformin or placebo treatments (in a 1:1 ratio) according to a pre-specified, computer-generated randomisation table, using blocks of four, stratified according to age (≤45 years or >45) and BMI (≤27kg/m^2 or >27). Sequentially numbered containers with metformin or placebo tablets of identical appearance, size, weight, and taste were issued to the eligible patients directly by the Pharmacy. Participants, investigators and treating physicians were blinded to the treatment allocation.

Identical metformin (850mg; *Merck Serono*) or placebo tablets (850mg; *Delpharm Laboratories*) were administered in escalating doses to reduce gastrointestinal adverse events, aiming for 3×850mg/day: 850mg/day for the first 5 days, 2×850mg/day for the next 5 days and full-dose subsequently. Study drug was administered for 12 weeks. The glucocorticoids were titrated by the treating specialists, independently of the study, based on patients' clinical needs, with glucocorticoids dose reduced as soon as clinical condition would allow. Four

patients withdrew due to gastrointestinal intolerance of the minimum acceptable dose (2×850mg) in the metformin group. Two patients withdrew from the placebo group due to the development of overt diabetes mellitus, and one for the glucocorticoid dose falling outside of inclusion criteria. Three patients in each group discontinued due to inability to adhere to the appointment schedule. This left 19 subjects on metformin and 21 on placebo for analysis (Figure 1).

The study was approved by an independent Ethics Committee (South East Research Ethics Committee, REC09/H1102/82) and conducted according to the Declaration of Helsinki. All patients gave written informed consent.

Assessments

The primary outcome was the between-group change in the visceral-to-subcutaneous fat area ratio over 12 weeks, assessed by computed tomography (CT)[11], as this parameter, a recognised surrogate for metabolic risk[12], was found to be markedly raised in patients with Cushing's syndrome compared to matched subjects with simple obesity[11], with the cortisol burden corresponding to a reduced AMPK activity in visceral fat[8] being reversed by metformin *in vitro*[9]. Secondary outcomes involved changes in the following parameters: anthropometric measurements, insulin resistance and β-cell function by the homeostasis model assessment (HOMA2)[13], fasting and post 75g sucrose challenge fibroblast growth factor 21 (FGF21), glucose, and tumour necrosis factor-alpha (TNFα), lipid profile, high sensitivity C-reactive protein (hsCRP), fibrin clot properties, β-C-terminal telopeptide (βCTX), procollagen type 1 N-terminal propeptide (P1NP), and osteocalcin concentrations, bone density by dual-energy X-ray absorptiometry (DEXA), and carotid intima-media thickness (IMT) by Doppler ultrasound. Appetite was examined using visual analogue scales (VAS) and the 'Three factor eating questionnaire'[14]. Physical activity was assessed via VAS and the National Audit of Cardiac Rehabilitation Minimum Dataset Short Physical Activity Questionnaire[15]. Symptom severity/intrinsic disease activity were evaluated by relevant VAS, and safety and clinical impact by types and the occurrence of adverse events.

Imaging and carbohydrate challenge were performed at 0 and 12 weeks. Physical examination, VAS and fasting blood sampling were conducted at 4-week intervals. Details of the imaging and assay protocols are described in the Supplementary Methods.

Statistical analysis

Sample size calculation for the primary outcome was based on differences between patients with endogenous Cushing's syndrome and matched subjects with simple obesity[11]. Considering that the difference in visceral-to-subcutaneous adipose tissue area ratio was 0.845±0.525 (n=24) for patients with Cushing's syndrome and 0.28±0.17 (n=10) for obese controls, this results in a population mean difference of 0.57 with a standard deviation using pooled estimate of variance of 0.45. 90% power at 5% significance, the estimated sample sizes were 15 experimental subjects and 15 controls. Allowing for the short duration of treatment and heterogeneity of the study population with inflammation on exogenous glucocorticoids, the target was 20 patients completing the study in each arm. Our analysis followed the modified intention-to-treat principle in which individuals completing the study were analysed in the groups to which they were randomised (Supplementary Material). For continuous data, normality was assessed by the Shapiro-Wilk normality test and comparison of variances by the F-test; two-sample t-test for equal variances or Mann-Whitney tests were used for between-group analysis; paired t-test or Wilcoxon signed-rank test were conducted for within-group comparisons. Parametric data are reported as a mean ± standard deviation, non-parametric as a median and interquartile range (IQR). Group analysis was performed by repeated-measures two-way ANOVA. Multivariate regression model was used for adjustment for baseline variables as required. Mixed-effect regression was done for assessment of covariants at different time-points. Categorical data were examined by chi-square or Fisher's exact tests in cases of few events. Pre-determined relevant correlations were computed by Pearson or Spearman rank-tests (correlation strength was defined by r-value: 0.3 to 0.69 (moderate), ≥0.7 (strong)). Given small group sizes, analysis reflects all available data without adjustment for missing data and the results were not adjusted for multiple testing. A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

Patients in the two treatment groups, although heterogeneous, were well-balanced in terms of age, sex, BMI and underlying diagnosis both at randomisation (Table 1) and from those completing the study (Table 2, Table S1&S3-12). There were no statistically significant differences in glucocorticoid exposure prior and during the study between the treatment groups (Table 1, Table S1). For patients completing the study (Table S1), the duration of continuous glucocorticoid treatment at recruitment was 36 (IQR 10-156) vs. 36 (IQR 7.5-126) months (metformin vs. placebo; p=0.67) and the cumulative exposure over the study period was 1860mg prednisolone-equivalent (IQR 1060-2810) vs. 1770mg (IQR 1020-2356) (metformin vs. placebo; p=0.76), having received continuous glucocorticoid treatment.

There was no between-group difference for participants' baseline physical activity (Table 1, Table S1), which was very low. Although all participants received the same advice on benefits of healthy eating and physical activity at the start, only the placebo-treated patients reported a mild increase in physical activity over the course of the study (Figure S1). Metformin and placebo treated patients did not match at randomisation and throughout the study for one aspect of feeding behaviour, the cognitive restraint of eating (Table 1, Table S6), where placebo-treated patients scored significantly higher (suggestive they could more easily follow dietary restrictions). We have included these lifestyle factors, which in theory could favour the placebo-treated arm, in an adjusted model for our primary outcome. The treatment adherence was similar between patients taking metformin and the placebo, 88±3% vs. 88±2%, metformin vs. placebo, p=0.85.

Main findings

Effect of metformin on body composition

The primary outcome, visceral-to-subcutaneous ratio, did not change in metformin-treated patients (Table 2). The truncal subcutaneous fat area decreased in metformin-treated patients relative to placebo (Figure 2a, Table 2) over 12 weeks, but not visceral adiposity (+2501±4891mm^2 vs. +443±6810, metformin vs. placebo, p=0.28). Adjustment for baseline variables (difference in self-reported exercise, attitude to diet and cumulative glucocorticoid exposure, ethnicity, BMI and FGF21) in a multivariate regression model did not alter the above conclusions (Table S2).

Changes in weight and waist circumference did not reach statistical significance (Table 2, Table S3). On physical examination, two (10%) metformin-treated vs. 11 (52%) placebo-treated patients were noted to have more pronounced characteristic facial adiposity associated with Cushing's syndrome, a so-called 'moon face'. (p=0.007).

Effect of metformin on carbohydrate and lipid metabolism and the liver function

Glucose and HbA1c decreased in metformin-treated subjects (Figure 2b, Table 2), reducing the number of patients with dysglycaemia (defined as fasting glucose ≥6.1mmol/L or any 2h post-challenge glucose ≥7.8[16]). At baseline, 4 patients in each group had dysglycaemia, whilst at the end of the study none of the metformin-treated and 7 (33%) of the placebo-treated patients were dysglycaemic (p=0.009). Metformin prevented worsening of insulin resistance (HOMA2IR) (Figure 2c, Table 2) and improved β-cell function, estimated as the HOMA2%B/HOMA2IR disposition index (Figure 2d, Table 2), accounting for a compensatory increase in insulin secretion. The lipid profile (Figure 2e, Table 2, Table S4) and liver function tests (Figure 2f, Table S5) improved in metformin-treated subjects. Changes in AST/ALT (r=-0.47, p=0.02) and GGT (r=0.55, p=0.008) correlated with insulin resistance. Considering metabolic syndrome risk factors, the worse were the initial parameters, the greater were the improvements in waist circumference (r=-0.57, p=0.01), HOMA2IR (r=-0.54, p=0.02), glucose (r=-0.62, p=0.005), triglycerides (r=-0.63, p=0.004), LDL (r=-0.46, p=0.046) and HDL (r=-0.49, p=0.001) with metformin over the study period. Metformin had no effect on blood pressure.

Effect of metformin on appetite

Hunger (Figure 2g, Table S6), sugar craving (Figure 2h) and disinhibition of eating (p=0.02) (Table S6) were reduced in metformin-treated subjects. Glucocorticoid treatment doses correlated positively to hunger (r=0.33, p=0.04) and sugar craving (r=0.40, p=0.02). The hunger scores, sugar craving and disinhibition correlated positively to fat body composition changes and insulin resistance (Supplementary Material).

Effect of metformin on FGF21 and adiponectin

Fasting FGF21 and adiponectin concentrations increased over 12 weeks in metformin-treated patients (Table 2). We identified an increase in FGF21 in response to sucrose challenge (76.2pg/mL (IQR 33.3-168.0) to 154.8 (IQR 50.8-472.6), baseline to peak, n=50, p=0.003), but there was no between-group post-challenge difference (Table 2). FGF21 and adiponectin changes correlated significantly with nearly all favourable study outcomes (Supplementary Material).

Effect of metformin on fibrinolysis and intima-media thickness

Fibrin clot lysis time (Figure 3a, Table 2), an assay measuring fibrinolytic potential, was reduced in metformin-treated subjects while there was no significant effect on maximum absorbance, a parameter assessing clot density and fibre thickness (Table S7). Progression of IMT was attenuated in metformin-treated subjects (Table 2). The higher the entry IMT, the greater was any IMT reduction on metformin (r=-0.46, p=0.048).

Effect of metformin on bone turnover and bone mass

The bone resorption marker βCTX decreased in metformin-treated patients (Figure 3e-f). P1NP, representing bone formation, and osteocalcin, reflecting both formation and resorption, did not change significantly (Table S8-S12). The treatment groups were matched for bisphosphonate treatment and vitamin D concentrations (Table S12). Bone mineral density (BMD) at the hip (Figure 3d, Table 2&S8-S9) increased in metformin-treated patients. This increase persisted after adjustment for changes in hip circumference and weight in a multivariate regression model (p=0.02). Three (19%) metformin-treated subjects reduced their hip BMD compared to 14 (67%) patients on placebo (p=0.007). No changes were identified at the spine (Table S8-S9).

Effect of metformin on inflammation and clinical safety outcomes

hsCRP decreased in metformin-treated subjects compared to placebo (Figure 3b, Table 2). Total white cell-count and neutrophil count reduced from baseline within the metformin group but the between-group difference did not reach statistical significance (Table 2). Carbohydrate-challenged TNFα levels increased significantly compared to pre-treatment in the placebo cohort (p=0.04), but not in the metformin group (Figure S2). In a mixed effect regression model incorporating glucocorticoid dose change during the study, white cell-count decreased with decreasing glucocorticoid dose by 0.07×10^9/L/mg prednisolone-equivalent decrease (p<0.001), with no statistically significant difference between metformin and placebo. Neutrophils decreased with decreasing glucocorticoid dose by 0.06×10^9/L/mg prednisolone-equivalent decrease (p=0.003) with no statistically significant difference between metformin and placebo (difference 0.49; p=0.60). hsCRP did not change with decreasing glucocorticoid dose (0.00006 increase per unit decrease in glucocorticoid dose; p=0.69) and there was a statistically significant difference between metformin and placebo (difference -0.1; p=0.03).

Metformin-treated patients with respiratory conditions reported reduced dyspnoea (Figure 3c). Patients with rheumatic diseases taking metformin improved their global disease activity score (Table 2). There were more episodes of pneumonia, more severe infections and more respiratory and overall serious adverse events in the placebo arm (Table 3). Diarrhoea occurred more frequently in metformin-treated subjects (Table 3).

DISCUSSION

In this study, metformin administration showed superiority to placebo in improving the metabolic profile and clinical outcomes in patients without known diabetes established on systemic glucocorticoid treatment for a chronic inflammatory disease.

While no change was observed for the visceral-to-subcutaneous fat ratio (primary outcome) or the visceral fat mass during the 3-months study period, a modest reduction in truncal subcutaneous fat was seen in the metformin-treated patients. Metformin also appeared to prevent progression of facial adiposity. Truncal subcutaneous fat is known to expand in hypercortisolaemia[17], alongside visceral fat[11,17], and higher truncal subcutaneous fat contributes to the adverse cardiometabolic risk profile associated with central adiposity[18]. The visceral to subcutaneous ratio has been associated with cardiometabolic risk above and beyond body mass index and visceral adiposity in the Framingham Heart Study[12]. Metformin has been shown to qualitatively improve visceral fat irrespective of fat mass[19] and to reduce abdominal subcutaneous fat in overweight patients with type 2 diabetes (T2DM)[20], a cohort with the best cardiovascular outcome in the United Kingdom Prospective Diabetes Study[7,21]. Whilst glucocorticoid effects on adipose functionality are insufficiently known, our data suggest a multisystem metabolic advantage with glucocorticoid-metformin co-administration. The AMPK-pathway may represent one of the mediators of glucocorticoid-metformin interaction[8,9]. Metformin may interact with glucocorticoid metabolism via 11β-hydroxysteroid dehydrogenase-1[22], although this may not play a role in patients with supraphysiological doses of synthetic glucocorticoid treatment.

Insulin resistance, β-cell function and glucose levels were improved in metformin-treated subjects compared to placebo which is clinically relevant, given the cohort's propensity to diabetes. These findings together with improved liver function are in keeping with the reduced conversion from pre-diabetes to T2DM and improved liver function in metformin-treated subjects in the Diabetes Prevention Program[23,24]. Glucocorticoids increase glucose levels both at non-diabetic as well as diabetic glucose ranges[25]. Metformin's ability to antagonise this glucose rise is important, as cardiovascular events increase linearly with rising glucose even in the non-diabetic

glucose range[26]. Moreover, increasing glucose often associates with poor outcomes[16] and metformin has been shown to improve clinical outcomes where glucose parameters decreased in non-diabetic patients[7,21].

Glucocorticoid therapy, which can paradoxically induce a pro-inflammatory phenotype overlapping with the metabolic syndrome, is associated with increased cardiovascular morbidity and mortality[5,6]. The more florid the initial metabolic disturbances in our patients, the greater the detected benefits of metformin. Our finding of 19% lower LDL in statin-naïve metformin-treated patients, in line with reports from pre-diabetic cohorts[7], may represent a considerable clinical benefit. Every 1% reduction in LDL by statins was associated with up to 2.5% lower cardiovascular events in inflammatory disease where glucocorticoid-related lipid side-effects can be confounded by their anti-inflammatory action[27].

Appetite stimulation by glucocorticoids represents a major drive for obesity[28]. Here we show that metformin, a drug with anorectic effects[7], was associated with suppressed appetite and sugar craving, promoting abdominal fat loss, even when co-administered with glucocorticoids. Supporting this, glucocorticoids increased appetite in rodents, recruiting AMPK, an effect mitigated by metformin *in vitro*[9].

Fructose overconsumption, associated with adverse metabolic effects and altered glucocorticoid signalling, could contribute to a Cushingoid phenotype[29]. FGF21, a biomarker of fructose metabolism and crucial in cardiometabolic protection, robustly and rapidly increases in response to fructose ingestion differentially from glucose[29]. Here we identified a rise in FGF21 after a sucrose challenge in all groups. Metformin prevented a drop in fasting FGF21 and increased adiponectin, with the changes correlating favourably with nearly all study outcomes. We hypothesise that metformin may reduce glucocorticoid-related metabolic dysfunction and systemic inflammation by enhancing the FGF21-adiponectin axis.

Both poorly-treated inflammatory disease and glucocorticoid overexposure represent prothrombotic phenotypes, associated with impaired fibrinolysis and an accelerated atherosclerosis[6,30]. Prolonged fibrin clot lysis time is an independent predictor of cardiovascular mortality[31]; here we demonstrate enhanced fibrinolysis in metformin-treated patients. Metformin is believed to improve the hypofibrinolytic environment in T2DM, with this effect

proposed to contribute substantially to the reduced cardiovascular risk attributed to metformin in the United Kingdom Prospective Diabetes Study[32]. Our data mark potentially a major advantage for metformin in reducing thrombosis risk in glucocorticoid-treated patients.

IMT is a widely accepted surrogate marker for a generalised clinical/subclinical atherosclerosis, correlating to the risk of development of atherosclerotic plaques and coronary events in the majority of trials[33]. Metformin treatment was associated with a reduced progression of IMT in this heterogeneous cohort, similar to the benefits identified in subjects with the metabolic syndrome of T1DM & T2DM[7,33,34] unlike in non-diabetic patients established on statins for coronary disease where metformin did not reduce LDL[35]. A longer treatment study is warranted to probe into the mechanistic pathways that are responsible for the protective effects of metformin in patients on glucocorticoids.

There was a reduction in inflammation (hsCRP) and improvement in patient-oriented clinical markers of disease activity in metformin-treated patients who also appeared to have a better infection profile. This is clinically relevant, given the participants' severe immunosuppression. There were fewer episodes of pneumonia, milder infective events, no severe asthma exacerbation and less overall serious adverse events in metformin-treated patients. Although warranting an exploration in a larger study, these results point to up to 30% absolute risk reduction of moderate-to-severe infections or all-cause hospital admissions, which corresponds to treating 3 patients with metformin to prevent one case. The beneficial effect of lower glucose levels on immunomodulation is likely. Nonetheless, there is growing evidence that metformin has discrete anti-inflammatory properties beyond this[36]. In experimental models, metformin prevented lung infections independently of glycaemia and reduced the inflammation of chronic asthma, uveitis and rheumatoid arthritis[7,37,38]. Metformin appeared to be superior to other glucose-lowering agents at reducing infections in T2DM[38,39] and compared to placebo in PCOS[40], independently of glycaemia. Moreover, others linked a greater burden of adverse events with a higher disease activity and reduced remissions[41]. As an acute treatment for chronic obstructive pulmonary disease exacerbations, metformin did not benefit non-diabetic individuals in a very short trial[37], while in the chronic setting prescribing metformin in T2DM was associated with lower hospitalisation in patients with asthma or

chronic obstructive pulmonary disease[37]. Glucocorticoid effects on the immune system are complex, and metformin's interactions would deserve further exploration with immune profiling in a dedicated study[42].

Glucocorticoids are renowned for adverse bone effects[6]. In our metformin-treated patients there was a modest reduction in the bone resorption marker βCTX, with the overall bone turnover consistent with a bone mass increase, and a subtle bone mass increase was identified at the hip. This effect appeared to persist irrespective of the body composition assessed, vitamin D levels or bisphosphonate treatment, warranting further studies with greater patient numbers and using other assessment modalities to characterise the effect of confounding factors in more detail, and to explore the clinical significance of these signal findings. Metformin prevented glucocorticoid-induced bone loss in the femur of an animal model, differentially to the mode of action of alendronate[43]. Advantageous effects of metformin on fracture risk or bone mass were noted in subjects with T2DM[44] and in PCOS[40], possibly by modulating metabolic-immune interplay[7].

Glucocorticoid-related metabolic dysfunction offsets the drug's profound anti-inflammatory benefits, contributing to the lasting increased morbidity associated with inflammatory diseases[5,6]. Metformin is effective in treating the endothelial dysfunction and the metabolic syndrome of PCOS[7,45] and preventing glycaemic deterioration when initiated simulataneously with glucocorticoids in non-diabetic individuals[10]. Here we show wider metabolic and clinical benefits in subjects already established on glucocorticoid treatment. While metformin has shown favourable effects in different insulin resistant pathological states, no other studies have been conducted to explore the role of this agent on vascular, thrombotic, inflammatory and bone markers following glucocorticoid treatment. The morbidity of the underlying disease and the glucocorticoid-driven increased appetite often limit intervention by lifestyle change. We suggest that metformin used in glucocorticoid-treated patients without diabetes may have the potential for improving treatment-related complications and cardiovascular prognosis.

Many patients on glucocorticoid tretament develop diabetes. Our recent study, for example, showed worsening metabolic parameters (basal and 2h AUC glucose and cholesterol) in glucocortioid-treated patients (previously not on glucocorticoids) on placebo compared to metformin treatment[10]. Our current study population on chronic

glucococortcoid treatment, who did not have overt diabetes at study entry might, therefore, be somewhat less predisposed to diabetes. We did not expect in our placebo group that metabolic parameters would worsen during the 12-week study period, especially in face of reducing glucocorticoid doses over the 12-weeks (Table 1).

Indeed, only a few parameters showed modest worsening in the placebo group (Table 1 and Supplementary Material). However, our data showed significant improvement in various parameters in the metformin-treated group. These results raise the intriguing possibility to treat chronic inflammatory disease patients with metformin even without concomitant glucocorticoid medication.

Strength and weaknesses

The strength of our study is its randomised, double-blind design in largely well-balanced treatment cohorts. Selected patients typify subjects with chronic active inflammation at a high risk for metabolic complications of iatrogenic Cushing's syndrome not offered metformin in real-life practice. Our results are clinically pertinent and the cohort heterogeneity adds to the data generalizability. Metformin is available worldwide and could improve metabolic status of large number of patients; according to a recent study 3% of the overall general population, with even higher percentages in the older population (7% of 60-79 and 10% of over 80 years old subjects)[2].

Our study has several limitations, the main ones being the small sample size, heterogeneity of patients and the relatively short treatment duration. The initial sample size calculation was derived from records on patients with endogenous Cushing's syndrome[11], although data in patients with rheumatoid arthritis and other conditions supported our calculations[46-48]. While various biases may affect parameters at different time points impacting on the final conclusions, the direction of changes favouring metformin in the assessments appears consistent. Given the small sample size, we report unadjusted findings; selection bias due our drop-out patients cannot be fully excluded, although the remaining groups remained well-balanced and the drop-out rate was similar in both groups. The cohort size, heterogeneity and treatment duration limit the scope for exploring disease-specific and long-term clinical outcomes of interest. Given the sample size and relatively large number of secondary

outcomes, cautious interpretation is warranted; nevertheless, our data are concordant and contributory to the growing evidence of metformin's benefits involving robust numbers of patients, especially those at diabetes risk. A larger study may help to both identify an optimal cohort of patients who would benefit most from this intervention and explore the subsequent clinical benefits suggested by this trial.

In summary: We have carried out a randomised, double-blind, placebo-controlled, phase 2 proof-of-concept trial exploring the potential of metformin to improve the metabolic profile of established glucocorticoid treatment in patients without known diabetes burdened with chronic active inflammation. We have shown that metformin-treated patients showed a significant improvement in several prognostic parameters and clinical outcomes. Compared to placebo, metformin-treated participants had reduced appetite, truncal and facial subcutaneous fat and insulin resistance, improved β-cell function, glycaemia, lipid profile, liver function, fibrinolysis, subclinical atherosclerosis, bone metabolism, infection risk, inflammation, disease activity and symptom severity, as well as fewer hospital admissions. These results may indicate the possibilty of a major improvement in patient care and justify further research into the concomitant use of metformin in patients on glucocorticoid treatment.

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Registration: ClinicalTrials.gov number NCT01319994

Data sharing

Study protocol and individual participant data that underlie the results reported in this article will be available after de-identification (text, tables, figures, and appendices) 9-36 months after publication to researchers who have signed data access agreement and provide a methodologically sound proposal for individual participant data meta-analysis. Proposals should be directed to the corresponding author.

Table 1: Baseline characteristics

Reported as a mean ± standard deviation or as median (IQR: interquartile range) or as number (% out of randomized patients)

	Metformin	Placebo	P-value
Allocated number of patients	26	27	0.99
Age	47±15	45 ±15	0.63
Sex – female (%)	14 (54%)	15 (56%)	0.99
Ethnicity	Mixed	Mixed	0.31
- White	12 (46%)	17 (63%)	
- South Asian	6 (23%)	5 (18%)	
- Black/African American	5 (19%)	5 (18%)	
- Other (oriental; mixed White/South Asian)	3 (12%)	0	
BMI (kg/m^2)	27.3 (IQR 23.3 to 36.1)	28.5 (IQR 24.7 to 35.8)	0.64
Current smoker	6 (23%)	2 (7%)	0.14
Smoking pack history	0 (IQR 0 to 6.3)	0 (IQR 0 to 5.0)	0.94
Main indication for GC treatment	Mixed	Mixed	0.21
- Asthma	7 (27%)	9 (33%)	
- Vasculitis (GCA/PNA/Wegener's, non-specified)	7 (27%)	3 (11%)	
- Sarcoidosis	5 (19%)	1 (4%)	
- SLE	4 (15%)	5 (19%)	
- RA	1(4%)	2 (7%)	
- Interstitial lung disease (other than connective tissue disease-related)	1 (4%)	2 (7%)	
- Other (mixed connective tissue disease; isolated uveitis/scleritis/retinitis)	1 (4%)	1 (4%)	
- Myositis (other than SLE-related)	0	4 (15%)	
Duration of continuous systemic glucocorticoid treatment by the study entry (months)	24 (IQR 6 to 129)	36 (IQR 5 to 120)	0.98
Estimated cumulative dose of glucocorticoids by the study entry (equivalent mg of prednisolone)	16 643 (IQR 2643 to 103275)	20 860 (IQR 8360 to 64400)	0.92
Cumulative dose of glucocorticoid last 3 months prior to the study entry (equivalent mg prednisolone)	1845 (IQR 1273 to 2700)	2010 (IQR 1295 to 2930)	0.45
Cumulative dose of glucocorticoids during the study (equivalent mg of prednisolone)	1782 ± 1092	1718 ± 805	0.82
Prednisolone equivalent dose (mg) at the initial study visit	23 (IQR 12 to 40)	25 (IQR 12 to 40)	0.83
Prednisolone-equivalent dose (mg) at 4 weeks	20 (IQR 10 to 28)	20 (IQR 10 to 29)	0.98
Prednisolone-equivalent dose (mg) at 8 weeks	20 (IQR 10 to 30)	18 (10 to 22)	0.53
Prednisolone-equivalent dose (mg) at 12 weeks	13 (IQR 10-25)	15 (IQR 10-25)	0.62
Waist circumference (cm)	96.9 ± 17.3	100.2 ± 19.4	0.52
Hip circumference (cm)	106.5 ± 16.1	108.9 ± 14.2	0.48
Systolic BP (mm Hg)	121 ± 16	129 ± 20	0.14

	Metformin	Placebo	P-value
Diastolic BP (mm Hg)	79 ± 12	83 ± 12	0.26
Visceral abdominal fat (mm2)	22646 (IQR 16378 to 30746)	25984 (IQR 17211 to 35355)	0.36
Subcutaneous abdominal fat (mm2)	48715 (IQR 36614 to 65124)	48485 (IQR 33278 to 62939)	0.98
Visceral-to-subcutaneous fat ratio	0.44 (IQR 0.33 to 0.67)	0.58 (IQR 0.34 to 0.84)	0.49
Fasting glucose (mmol/L)	4.7 (IQR 4.5 to 5.7)	4.9 (IQR 4.5 to 5.8)	0.38
*AUC glucose (mmol/L*min) post 75g sucrose challenge*	826 (IQR 643 to 1054)	806 (IQR 717 to 1179)	0.87
HbA1c (mmol/mol)	40 ± 6	39 ± 7	0.69
HOMA2IR	4.7 (IQR 2.2 to 5.5)	4.1 (IQR 2.8 to 5.4)	0.97
HOMA2%B	282.8 ± 132.9	254.9 ± 124.6	0.43
HOMA2%B/HOMA2IR	68.3 ± 21.0	63.8 ± 24.8	0.48
Total cholesterol (mmol/L)	5.6 (IQR 4.7 to 6.4)	5.4 (IQR 4.7 to 6.2)	0.66
LDL (mmol/L)	2.8 (IQR 2.3 to 3.4)	2.9 (IQR 2.5 to 3.4)	0.71
HDL (mmol/L)	1.86 ± 0.52	1.81 ± 0.46	0.67
Triglycerides (mmol/L)	1.5 (IQR 1.0 to 2.3)	1.3 (IQR 1.1 to 1.8)	0.29
ALT(U/L)	17 (IQR 14 to 25)	19 (IQR 14 to 26)	0.53
ALP(U/L)	62 ±15	59 ±19	0.55
FGF21 (pg/mL)	56.5 (IQR 27.3 to 156.3)	96.0 (IQR 41.5 to 256.8)	0.25
Adiponectin (ng/mL)	22.0 (IQR 11.6 to 44.9)	21.8 (IQR 8.6 to 41.5)	0.60
hsCRP (mg/L)	0.033 (IQR 0.009 to 0.049)	0.025 (IQR 0.010 to 0.050)	0.96
Haemoglobin	13.2 ± 0.3	13.1 ± 0.3	0.89
White cell count	10.2 ± 0.7	8.7 ± 0.8	0.18
Neutrophils	7.4 (IQR 5.2 to 8.7)	5.8 (IQR 3.5 to 8.5)	0.15
Lymphocytes	1.7 (IQR 1.2 to 2.8)	1.3 (IQR 0.8 to 2)	0.08
Monocytes	0.6 ± 0.1	0.6 ± 0.1	0.91
Eosinophils	0.1 (IQR 0.1 to 0.2)	0.1 (IQR 0 to 0.2)	0.23
Basophils	0 (IQR 0 to 0)	0 (IQR 0 to 0.03)	0.40
Platelets	254 (IQR 203 to 327)	233 (IQR 185 to 280)	0.46
Intima-media thickness (mm)	0.50 (IQR 0.46 to 0.55)	0.51 (IQR 0.46 to 0.56)	0.77
Lysis time (s)	354 (IQR 276 to 426)	366 (IQR 297 to 444)	0.53
Lysis area (AU)	92.0 (IQR 60.7 to 126.7)	120.7 (IQR 72.9 to 218.5)	0.15
Total hip BMD (g/cm2)	0.965 ± 0.145	0.988 ± 0.195	0.63
Spine BMD (g/cm2)	0.981 ± 0.150	0.997 ± 0.168	0.73
Vitamin D (nmol/L)	45.5.0 (IQR 29.0 to 58.3)	32.0 (IQR 25.0 to 71.5)	0.62
βCTX (µg/L)	0.21 (IQR 0.17 to 0.33)	0.17 (IQR 0.08 to 0.31)	0.35
P1NP (µg/L)	22.3 (IQR 13.9 to 34.7)	20.5 (IQR 12.9 to 39.1)	0.79
Osteocalcin (µg/L)	9.3 (IQR 6.7 to 16.3)	9.6 (IQR 5.2 to 14.6)	0.64

	Metformin	Placebo	P-value
Physical activity on the day of assessment (virtual scale 0-10. 10 is the highest)	2 (IQR 1 to 3.3)	2 (IQR 1 to 3)	0.20
Reported frequency of at least 15min. of strenuous physical activity in 7 days (National Audit of Cardiac Rehabilitation (NACR) Minimum Dataset Short Physical Activity questionnaire)	0 (IQR 0 to 0)	0 (IQR 0 to 0)	0.28
Reported frequency of at least 15min of moderate physical activity in 7days (NACR questionnaire)	0 (IQR 0 to 0.5)	0 (IQR 0 to 2)	0.84
Reported frequency of at least 15min of minimal effort physical activity in 7 days (NACR questionnaire)	3.5 (IQR 0 to 7)	3 (IQR 2 to 7)	0.95
Cognitive restraint of eating ^	6 ± 4	9 ± 4	0.007
Disinhibition of eating ^	5 (IQR 3 to 10)	5 (IQR 2 to 8)	0.77
*Hunger^*	4 (IQR 2 to 9)	4. (IQR 2 to 8)	0.99
Medications			
Prednisolone	25 (96%)	27 (100%)	0.49
Methylprednisolone	0	1 (4%)	0.99
Beclomethasone	1 (4%)	1 (4%)	0.99
Fluticasone	4 (15%)	5 (19%)	0.99
Dexamethasone	2 (8%)	0	0.22
Clobetasone	1 (4%)	0	0.99
Methotrexate	2 (8%)	3 (11%)	0.99
Hydroxychloroquine	2 (8%)	4 (15%)	0.67
Azathioprine	1 (4%)	1 (4%)	0.99
Mycophenolate	1 (4%)	4 (15%)	0.35
Sulphasalazine	1 (4%)	0	0.49
Ustekinumab	0	1 (4%)	0.99
Tocilizumab	0	1 (4%)	0.99
Omaluzimab	1 (4%)	1 (4%)	0.99
Cyclophosphamide	0	2 (7%)	0.49
Ciclosporin	1 (4%)	1 (4%)	0.99
Bronchodilators	11 (42%)	11 (41%)	0.99
Bisphosphonates	12 (46%)	9 (33%)	0.41
HRT/OCP	3 (12%)	0	0.11
TB treatment	2 (8%)	1 (4%)	0.61
Warfarin/LMWH	1 (4%)	2 (7%)	0.99
Aspirin/clopidogrel	3 (12%)	5 (19%)	0.70
Statins	4 (15%)	1 (4%)	0.19
Antihypertensives	12 (46%)	11 (41%)	0.79
Beta-blockers	3 (12%)	1 (4%)	0.61
ACE-inhibitors/Sartans	9 (35%)	8 (30%)	0.77

MF, metformin; PL, placebo; BMI, body mass index; GC, glucocorticoid(s); GCA, giant cell arteritis; PNA, polyarteritis nodosa; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; ALP, alkaline phosphatase; FGF21, fibroblast growth factor 21; ßCTX, ß-C-terminal telopeptide; P1NP, procollagen type 1 N-terminal propeptide; HRT, hormone replacement therapy; OCT, oral contraceptive therapy; LMWH, low molecular weight heparin; BMD, bone mineral density; AU, arbitrary units; ^Components of the Three factor eating questionnaire

Table 2. Outcome data

Expressed as mean ± standard deviation or median and interquartile range (IQR)

	Metformin (MF)	Placebo (PL)	Difference between groups (P-value)
Visceral-to-subcutaneous fat area (ratio)			
Baseline (MF n=19; PL n=21)	0.45 (IQR 0.32 to 0.77)	0.60 (IQR 0.36 to 0.89)	0.34
At 12 weeks	0.56 (IQR 0.33 to 0.79)	0.59 (IQR 0.37 to 0.81)	0.92
Change over 12 weeks	0.08 ± 0.19	-0.03 ± 0.22	0.09
Difference within groups (P-value)	0.11	0.57	
Truncal subcutaneous fat area (mm^2)			
Baseline (MF n=19; PL n=21)	48640 (IQR 33786 to 73959)	41753 (IQR 32760 to 58873)	0.70
At 12 weeks	42643 (IQR 30519 to 72160)	45214 (IQR 34394 to 57371)	0.61
Change over 12 weeks	-2035 ± 4283	1799 ± 4859	0.01
Difference within groups (P-value)	0.053	0.12	
Weight (kg)			
Baseline (MF n=19, PL n=21)	75(IQR 59.4 to 99)	79 (IQR 68 to 97)	0.56
At 12 weeks	71 (IQR 58 to 103)	80 (IQR 69 to 99)	0.42
Change over 12 weeks	-1.1±0.8	0.5±0.5	0.09
Difference within groups (p-value)	0.11	0.47	
Fasting glucose (mmol/L)			
Baseline (MF n=19; PL n=21)	5.0 (IQR 4.5 to 5.9)	4.9 (IQR 4.5 to 5.4)	0.82
At 12 weeks	4.3 (IQR 4.1 to 4.6)	5.0 (IQR 4.4 to 5.6)	0.03
Change over 12 weeks	-0.5 (IQR -0.8 to -0.3)	0 (IQR -0.4 to 0.3)	0.005
Difference within groups (P-value)	0.001	0.89	
AUC glucose (mmol/L*min) post 75g sucrose challenge			
Baseline (MF n=19; PL n=20)	837 (IQR 636 to 1140)	806 (IQR 712 to 1077)	0.80
At 12 weeks	711 (IQR 552 to 816)	821 (IQR 749 to 1097)	0.01
Change over 12 weeks	-121.5 (IQR -306 to -24)	30.8 (IQR -60.4 to 108.4)	0.002

Difference within groups (P-value)	0.003	0.23	
HOMA2IR (Mass Units)			
Baseline (MF n=19; PL n=21)	4.63 (IQR 2.09 to 4.88)	3.82 (IQR 2.48 to 4.99)	0.95
At 12 weeks	4.53 ± 2.1	6.20 ± 2.6	0.03
Change over 12 weeks	0.22 ± 3.3	2.35 ± 3.2	0.04
Difference within groups (P-value)	0.61	0.003	
Disposition index (HOMA2%B/HOMA2IR)			
Baseline (MF n=19; PL n=21)	66.5 ± 21.3	69.5 ± 23.2	0.68
At 12 weeks	83.7 ± 21.9	63.2 ± 25.5	0.01
Change over 12 weeks	17.1 ± 22.6	-6.3 ± 20.6	0.001
Difference within groups (P-value)	0.004	0.18	
LDL cholesterol (mmol/L)			
Baseline (MF n=19, PL n=20)	2.6 (IQR 2.3 to 3.3)	2.9 (IQR 2.6 to 3.3)	0.33
At 12 weeks	2.3 (IQR 1.9 to 2.7)	3.0 (IQR 2.4 to 3.8)	0.01
Change over 12 weeks	-0.4 (IQR -0.9 to 0.3)	0.2 (IQR -0.4 to 0.5)	0.08
Difference within groups (P-value)	0.04	0.68	
Cholesterol:HDL ratio (Atherogenic index) change over 12 weeks (MF n=19; PL n=21)	-0.26 ± 0.85	0.27 ± 0.71	0.04
hsCRP (mg/L)			
Baseline (MF n=19, PL n=21)	0.031 ± 0.025	0.033 ± 0.024	0.86
At 12 weeks	0.016 (IQR 0.006 to 0.029)	0.036 (IQR 0.021 to 0.050)	0.02
Change over 12 weeks	-0.010 ± 0.025	0.003 ± 0.026	0.09
Difference within groups (P-value)	0.08	0.5731	
% change over 12 weeks	-40.9 (IQR -82.9 to 45.4)	8.5 (IQR -30.3 to 128.6)	0.09
AUC of 4 visits over 12 weeks	0.08±0.01	0.12±0.02	0.02
White cell-count (10^9/L)			
Baseline (MF n=19 , PL n=19)	9.9 ± 0.9	8.9 ± 0.9	0.40

At 12 weeks	8.4 (IQR 6.6 to 10.3)	7.4 (IQR 5.9 to 10.9)	0.95
Change over 12 weeks	-1.0 (IQR -2.4 to -0.1)	-0.6 (IQR -2.0 to 0.8)	0.25
Difference within groups (P-value)	0.01	0.15	
Neutrophils			
Baseline (MF n=16; PL=17)	7.4 (IQR 4.5 to 9.6)	5.8 (IQR 3.8 to 8.1)	0.33
At 12 weeks	4.6 (IQR 4 to 6.4)	4.2 (IQR 3.4 to 8.3)	0.69
Change over 12 weeks	-2.1 ± 0.6	-0.7 ± 0.5	0.08
Difference within groups (P-value)	0.003	0.43	
Intima-media thickness (IMT) (mm)			
Baseline (MF n=19; PL n=20)	0.53 (IQR 0.48 to 0.55)	0.51 (IQR 0.48 to 0.56)	0.75
At 12 weeks	0.53 (IQR 0.48 to 57)	0.54 (IQR 0.50 to 0.60)	0.67
Change over 12 weeks	-0.02 ± 0.08	0.02 ± 0.05	0.049
Difference within groups (P-value)	0.62	0.04	
Lysis time (sec)			
Baseline (MF n=13; PL n=16)	342.0 (IQR 267.0 to 393.0)	357.0 (IQR 285.0 to 400.5)	0.43
At 12 weeks	300 (IQR 234 to 324)	360 (IQR 313 to 632)	0.001
Change over 12 weeks	-48.0 (IQR -111.0 to 3.0)	33.0 (IQR -13.5 to 192.0)	0.004
Difference within groups (P-value)	0.03	0.06	
Bone mineral density (BMD) – Total hip (g/cm2)			
Baseline (MF n=16; PL n=21)	1.002 ± 0.150	0.977 ± 0.181	0.66
At 12 weeks	1.021 ± 0.148	0.974 ± 0.179	0.40
Change over 12 weeks	0.014 (IQR 0.002 to 0.027)	-0.008 (IQR -0.015 to 0.010)	0.005
Difference within groups (P-value)	0.02	0.44	
FGF21 (pg/mL)			
Baseline fasting MF n=19; PL n=19	53.1 (IQR 29.9 to 154.6)	118.9 (IQR 68.8 to 268.6)	0.06
At 12 weeks	77.0 (IQR 40.0 to 208.0)	34.1 (IQR 18.3 to 147.0)	0.05

Change over 12 weeks	7.6 (IQR -30.5 to 169.0)	-78.5 (IQR -234.4 to -1.13)	0.03
Difference within groups (P-value)	0.43	0.03	
%change over 12 weeks	5.0 (IQR -23.6 to 252.1)	-66.4(IQR -87.3 to -5.0)	0.01
Maximum post-challenge increment from fasting (change over 12 weeks)(MF n=18; PL n=18)	167.2 (IQR -124.4 to 483.7)	109.2 (IQR -291 to 1067)	0.89
Adiponectin (ng/mL)			
Baseline (MF n=19, PL n= 21)	15.0 (IQR 9.1 to 29.7)	24.1 (IQR 12.8 to 49.5)	0.18
At 12 weeks	44.9 (IQR 22.7 to 78.1)	15.9 (IQR 7.3 to 39.7)	0.02
Change over 12 weeks	27.6 ± 36.0	-5.5 ± 32.2	0.004
Difference within groups (P-value)	0.004	0.14	
Global disease activity VAS (mm) - Rheumatology cohort (pre-specified)			
Baseline (MF n=11; PL n=11)	48 (IQR 28 to 74)	68 (IQR 53 to 80)	0.34
At 12 weeks	32 ± 27	57 ± 23	0.03
Change over 12 weeks	-17 ± 22	-4 ± 32	0.27
Difference within groups (P-value)	0.02	0.69	

Table 3. Safety evaluation

	Metformin	Placebo	P-value
Adverse events (AE) – numbers of episodes reported			
Nausea	19	12	0.13
Vomiting	8	4	0.22
Diarrhoea[^]	**18**	**8**	**0.01**
Flatulence	4	2	0.57
Abdominal discomfort	14	12	0.78
Constipation	1	3	0.49
Indigestion	4	3	0.99
Exacerbation of asthma	8	7	0.99
Pneumonia[+]	**1**	**7**	**0.01**
Exacerbation of bronchiectasis	1	0	0.99
Upper airway viral illness	6	3	0.35
Gastrointestinal viral illness	2	0	0.33
Candidosis	0	2	0.33
Herpes zoster	0	1	0.99
Ear infection	0	1	0.99
Diverticulitis	0	1	0.99
Dental issue (root canal)	1	0	0.99
Ulcer	2	1	0.99
Dysglycaemia/osmotic symptoms[♦]	5	11	0.08
Ischemic heart disease/Atrial fibrillation/atypical chest pain[#]	2	2	0.99
B12 deficiency	0	2	0.33
Other (affecting <4% patients: fall, car incident, incidental imaging findings, dog bite, schiatica, headache, hematoma etc.)	14	12	0.78
Serious adverse events (SAE)* – numbers of episodes reported			
Exacerbation of asthma	0	3	0.10
Pneumonia	0	1	0.99
Diverticulitis	0	1	0.99
Ischemic heart disease	1	0	0.99
Atypical chest pain	0	1	0.99
Severe Raynaud's	0	1	0.99
Severe osmotic symptoms	0	2	0.33
All SAEs	***1***	***9***	***0.001***

AE and SAE were defined and reported as per Good Clinical Practice: Here SAE equated to inpatient hospitalizations (≥24h stay). The degree of severity was assessed separately to the definition of SAE. The study drugs were not deemed causative of the SAE. The reporting period ended 30 days post cessation of treatment.

[^]10(38%) metformin-treated patients compared to 2 (7%) on placebo reported diarrhoea during the first 4 weeks after treatment initiation (p=0.009) with a median symptom resolution of 3 days (IQR 2 to 28) in the metformin cohort and no between-group difference in diarrhoea reports during subsequent follow-ups.

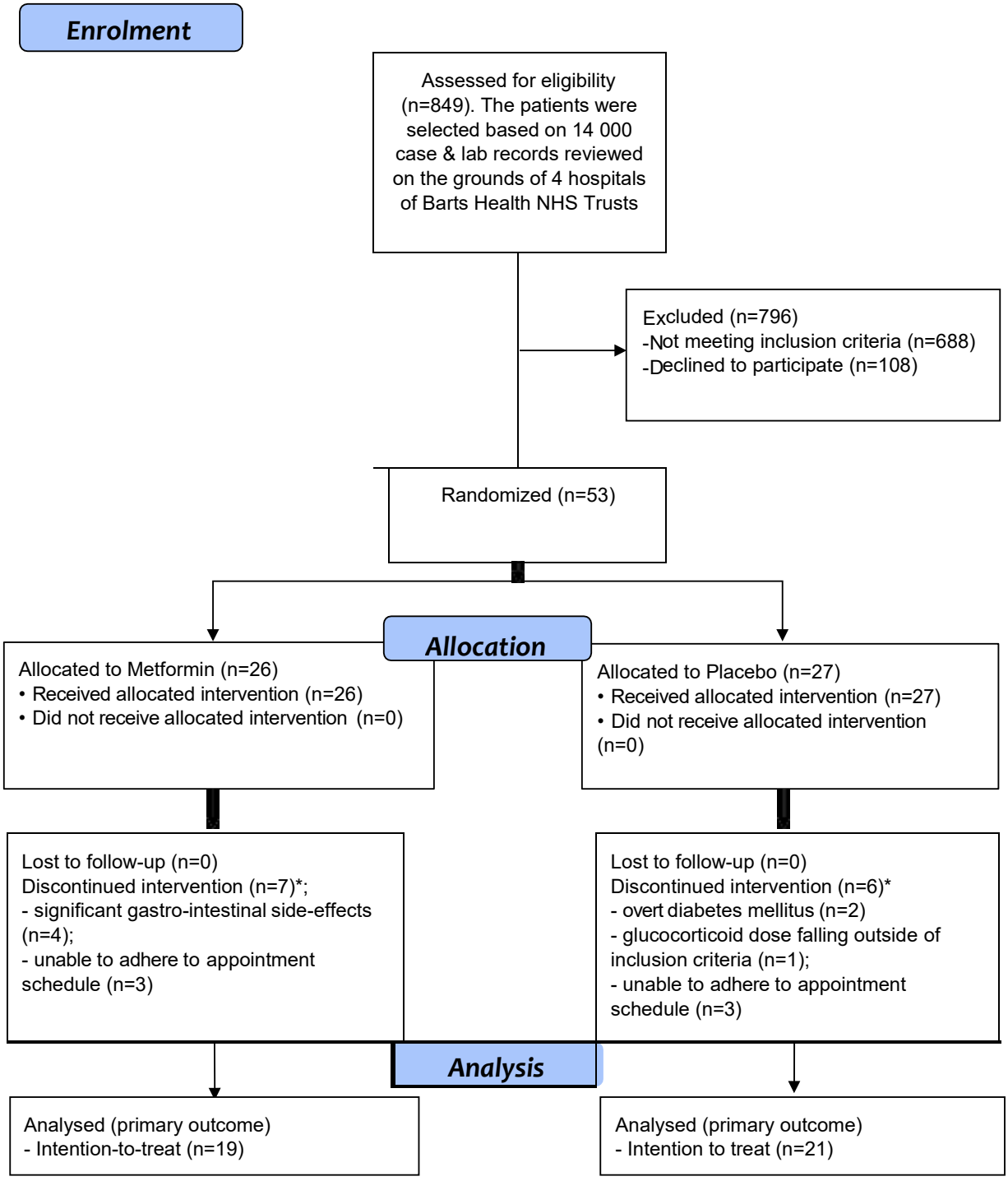
[+]The total number of infective episodes was not significantly different between the treatment groups (15 on metformin vs. 21 on placebo, p=0.24), but their severity differed. There were 2 moderate or severe infective episodes on metformin compared to 11 on placebo (p=0.001); the incidence of mild

infective episodes was similar (13 on metformin compared to 10 on placebo, p=0.56). This suggested 33% (95%CI 12 to 54) absolute risk reduction of moderate and severe infective events on metformin (p=0.009) where the number needed to treat was 3 (95%CI 2 to 8).

♦During hospital encounters and admissions, glycaemia was monitored and managed as per routine clinical practice, independently of the study. Apart from the two subjects developing severe osmotic symptoms, withdrawn from the study, no other patient was deemed to require a glucose-lowering pharmacological intervention.

#Ischemic heart disease diagnosed incidentally in an elderly heavy smoker (>40 pack year smoking history) treated with a coronary stenting off the investigational medicinal product.

*There were less serious adverse events affecting the respiratory system in the metformin arm (p=0.03). The number of episodes of asthma exacerbations was similar; out of these, the proportional rate of severe episodes seemed higher on placebo (p=0.08). Overall, there was a 30% (95%CI 10 to 49) absolute risk reduction of serious adverse events on metformin (p=0.01) which suggests about 3 (95% CI 2 to10) patients needing treatment to prevent 1 hospital admission.



*Drop-out rates were not different in the metformin and placebo groups.

Figure 1. Trial flow diagram

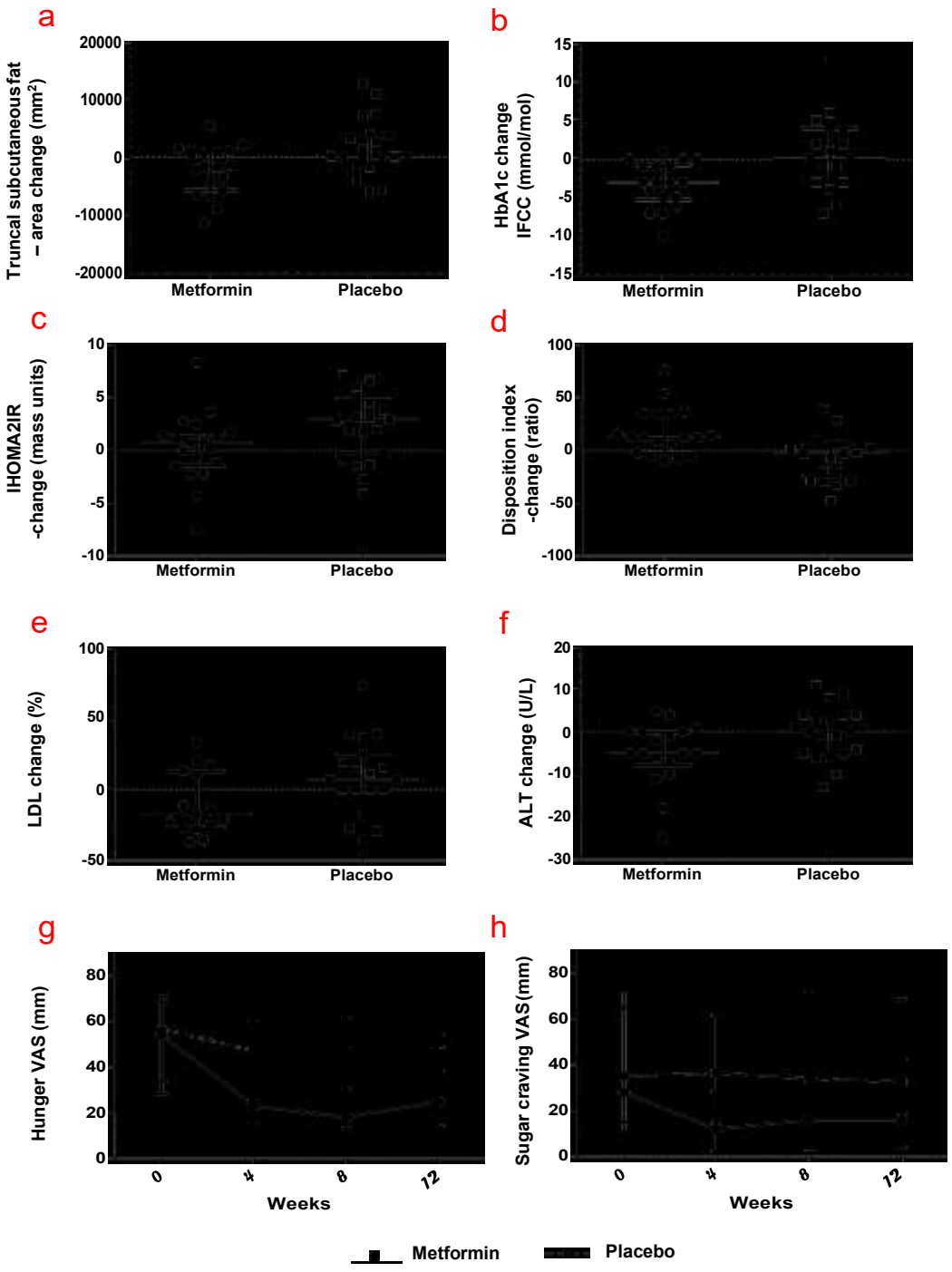


Figure 2. Metformin effects on appetite, pancreas, fat and liver

Figure 2. legend: Changes over 12 weeks between metformin- and placebo-treated groups in a) truncal subcutaneous fat area (p=0.01), b) HbA1c (p=0.007), c) insulin resistance (p=0.04), d) HOMA2%B/HOMA2IR disposition index (p=0.001), e) LDL in statin-naïve patients (p=0.03), f) ALT (p=0.03), g) hunger (p=0.04) and h) sugar craving (p=0.01) by 100mm

visual analogue scales (VAS). Data are presented as median and interquartile range; data points (a-f) represent individual patients. P-values were assessed by T-test/Mann-Whitney (a-f), repeated-measures two-way ANOVA (g, h).

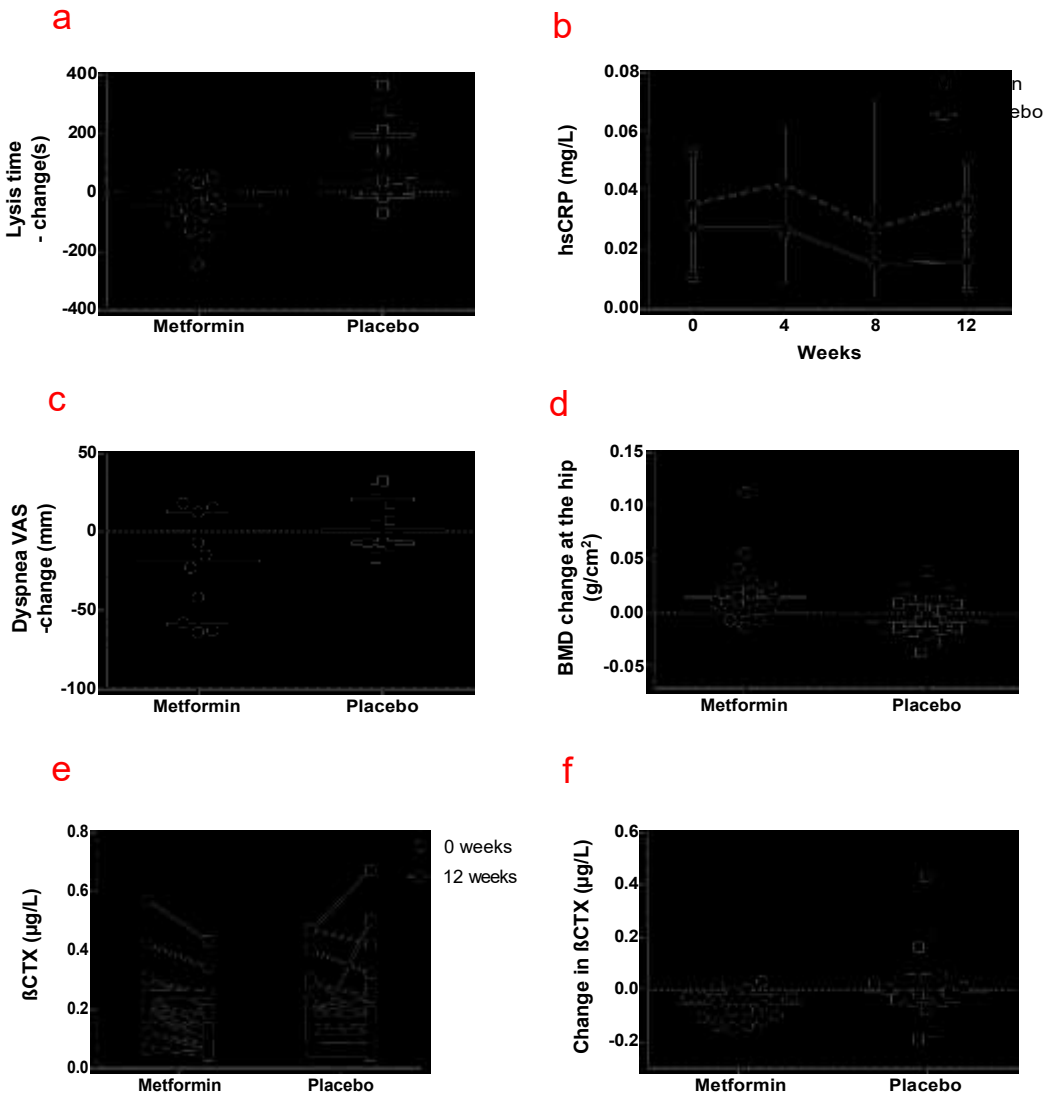


Figure 3. Metformin effects on fibrinolysis, inflammation and bone metabolism

Figure 3 legend: Changes over 12 weeks between treatment groups in a) lysis time (p=0.004), b) hsCRP (p=0.04), c) dyspnoea 100mm VAS in patients with diseases affecting the respiratory system (p=0.03), d) BMD at the hip (p=0.005), e) ßCTX showing individual patient data: changes within-metformin group (p=0.001); changes within-placebo group (p=0.82), f) βCTX: between-group difference (p=0.01). Medians with interquartile error bars are presented (a-d, f); data points (a, c, d, e, f) represent individual patients. P-values were assessed by T-test/Mann-Whitney (a, c-f) and repeated-measures two-way ANOVA (b).
hsCRP, high sensitivity C-reactive protein; VAS, visual analogue scale; βCTX, β-C-terminal telopeptide; BMD, bone mineral density

Supporting information

Supplementary Material text, tables and figures

CONSORT checklist

Study protocol

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