Supplementary Material

For Pernicova et al. *'Metformin to reduce metabolic complications and inflammation in patients on systemic glucocorticoid therapy: a randomised, double-blind, placebo-controlled, phase 2 clinical trial'*

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Supplementary Methods

Inclusion criteria

- patients suffering from an inflammatory disease and treated with prednisolone at a dose more than or equal to 20mg/d (or equivalent) for at least 4 weeks or its cumulative dose equivalent.**
- minimal duration of prospective therapy 12weeks
- predicted prospective treatment: prednisolone more than or equal to 10mg/d (or equivalent glucocorticoid)
- ambulatory patients
- patients more than or equal to 18 years old and less than or equal to 75 at the start of the trial
- ability to understand verbal and written instructions and informed consent

Exclusion criteria

- prior therapy with metformin during the last 6 months
- pre-existing diabetes*
- pregnancy
- breastfeeding
- liver impairment: ALT and/or AST more than or equal to 2.5×upper limits of normal
- renal impairment: serum creatinine levels more than or equal to 135µmol/L in males and more than or equal to 110µmol/L in females
- clinically relevant current malignancy
- patients unable to give written informed consent or patients not understanding English

*Patients with known pre-existing diabetes (WHO criteria) or with osmotic symptoms were excluded from participation in the study. Patients were re-assessed at each visit and, following independent consultation, newly diagnosed cases where medical treatment was offered, were withdrawn from the study. This strategy enabled our cohort to reflect real-life population not routinely offered metformin. All patients received verbal and written dietary and lifestyle advice.

** Cumulative prednisolone-equivalent was calculated based on oral and parenteral glucocorticoids[1].

Most patients not meeting eligibility would either not be on glucocorticoid treatment or received lower doses of glucocorticoids or were prescribed intermittent glucocorticoid treatment or developed diabetes mellitus. Most patients declining to participate felt excessively burdened by their current hospital appointments related to their disease severity.

The recruitment and follow-up were conducted at Barts Health NHS Trust, London, U.K. from 2012 to 2014. There were no major changes to the study protocol after recruitment initiation.

Patients were consulted during the planning of the study and they were updated on the recruitment progress and eventually the results.

Blood testing

Patients were asked to fast for 10-12 hours prior to blood testing. An oral sucrose tolerance test (75g sucrose, Silver Spoon UK) with blood sampling every 30 min for 2 h was conducted at baseline and after 12 weeks of treatment. Routine blood tests were analysed at the Royal London Hospital, London, UK, CPA accredited laboratory; C-terminal telopeptide (CTX), procollagen type 1 N-terminal propeptide (P1NP) and osteocalcin were analysed at the Norfolk and Norwich University Hospitals NHS Foundation Trust as previously published[2]. Commercial assays used for a batch analysis:

Assay	Inter assay CV	Intra assay CV	Company
Insulin	<6.0%	<2.6%	IBL International GmbH, Hamburg, Germany
FGF21	3.3%	2%	BioVendor, Brno, Czech Republic,
Adiponectin	<7.4%	<8.4%	EMD Millipore, Billerica, Minneapolis, USA
hsCRP	12.7%	5.8%	Invitron Ltd, UK,
TNF-alpha	5.3%	8.7%	R&D systems, Abingdon, UK

CV, coefficient of variation

Fibrin clot assessment

Fibrin clot properties and resistance to fibrinolysis were assessed using turbidimetric analysis described elsewhere[3,4]. The following parameters were recorded in turbidimetric analyses: lysis time, calculated as the time from full clot formation to 50% lysis, which indicates fibrinolytic potential and is associated with adverse vascular outcome[5,6], and maximum absorbance, which assesses fibrin fibre thickness and clot density and is also related to atherothrombotic risk[4]. Haemolysed specimens and samples from patients on warfarin or heparin were excluded from the analysis.

Computed tomography (CT)

CT was used to assess the proportion of visceral to subcutaneous fat ratio, building up on previous work in glucocorticoid excess[7]. Non-contrast CT (1 × 10mm slice), going through the body of L4, was performed at Siemens Definition Flash (Siemens Healthcare, Germany) and Siemens Somaton Sensation (Siemens Healthcare, Germany) scanners. The images were re-checked to confirm the entire skin surface was included in the scan area. The protocol for reporting followed previously published work from our institution[7,8]. The total fat area and visceral fat area were delineated by manual tracing as a contour of each region, using GE software[7]. Scans were reported by a consultant radiologist. A subset of measurements was repeated by the same observer. The coefficient of variability, calculated as (standard deviation/mean) × 100, was 0.3% for the total fat area, 1.3% for the visceral fat area, and 0.6% for the subcutaneous fat area measurements.

Ultrasound (US) carotid Doppler

US carotid imaging of the common carotid artery intima-media thickness (IMT) was conducted at baseline and after 12 weeks of treatment by three trained and blinded clinical vascular scientists using Philips iU22 (USA). With the subject's head turned to an angle of 45%, the carotid scan was carried out with 7-4 MHz transducer using Carotid preset. B mode longitudinal images were taken covering the distal 3 cm of the common carotid artery and the proximal 1 cm of the internal carotid artery over

at least 3 cardiac cycles during diastole. IMT measurements were performed on the stored images using the Philips QLAB IMT package as automated measurements; where there was a suspicion of an error, a vascular scientist could reposition the calliper for it to attempt another automated measurement. Measurements from the lateral projection of the right & left side (i.e. up to 6 measurements per patient's visit depending on the image quality) were reported as the mean IMT[9]. The inter-operator variability (CV%) for IMT measurements was 3.36%, the average intra-operator CV% was 2.96%.

Bone densitometry

Dual-energy X-ray absorptiometry (DEXA) scanning was performed of the lumbar spine and right hip by a single operator. The examination was done on a single Hologic Discovery A scanner (system number 80933). Daily assessments of stability were conducted using an anthropomorphic spine phantom, each showing stable long-term performance (CV<0.5%); the in vivo precisions of the lumbar spine and of the hip were 0.69% and 0.84% respectively. Prior to unblinding, the images were re-reviewed by two observers. Images with confounders (e.g. inadequate positioning of the patient under the scanner, severe scoliosis and extreme obesity) were excluded from the relevant analysis.

Physical examination

Physical examination was conducted by a single blinded investigator, experienced clinical endocrinologist, and any changes in the features of the Cushing's phenotype were noted every 4 weeks.

Statistical analysis

Statistical analysis followed the study protocol with addition of standard exploratory analysis as clinically relevant. We have reported all available study outcomes, described in our study protocol, where there was a statistically significant between-group difference. Other prespecified secondary outcomes were liver fat content, left ventricular mass index, skinfold measurement, lipoprotein-A, homocysteine, glucagon, testosterone, dehydroepiandrosterone sulphate, androstenedione, oestrogen,

SHBG, LH/FSH, thyroid function tests, cardiac dimensions, diastolic function, Hospital Anxiety and Depression score, quality of life with Cushing-QoL questionnaire, polymorphisms in genes involved in metformin cellular uptake and excretion; these will be reported in a future publication. Given the small sample size, we have chosen to do a modified intention-to-treat analysis rather than per-protocol which would inflict more loss in statistical power. However, we have computed per-protocol analysis for the primary outcome and the CT parameters. The conclusions are unaltered.

Sample size calculation is included in the man text. Similar number of patients were assessed in metformin treatment studies in other metabolic disorders[10,11].

GraphPad-Prism5 (GraphPad Software, La Jolla, California) and Stata13 (StataCorp, College Station, Texas) were used for data analysis and figures. Statistical analysis was performed by I Pernicova and J Bestwick.

Supplementary Results and Tables

Presented as mean ± standard deviation or median and interquartile range (IQR) or as number (% out of randomized patients).

Baseline characteristics for patients completing the study

(Other parameters are included within the specific sections of the main manuscript (e.g. Table 2 or Supplementary-Material)

Table S1 Baseline characteristics for patients completing the study

	Metformin	**Placebo**	**P-value**
Allocated number of patients	19	21	
Age	49±15	47±15	0.70
Sex – female (%)	10 (52%)	10 (48%)	0.99
Ethnicity	Mixed	Mixed	0.32
- White	9 (47%)	14 (67%)	
- South Asian	3 (16%)	4 (19%)	
- Black/African American	4 (21%)	3 (14%)	
- Other (oriental; mixed white/Asian)	3 (16%)	0	
BMI (kg/m^2)	27.1 (IQR 22.1 to 36.2)	26.6(IQR 24 to 31.9)	0.93
Current smoker	3 (16%)	2 (10%)	0.65
Smoking pack history	0 (IQR 0 to 5)	0 (IQR 0 to 5)	0.91
Main indication for GC treatment	Mixed	Mixed	0.31
- Asthma	5	6	

	Metformin	Placebo	P-value
- Vasculitis (GCA/PNA/Wegener's, non-specified)	6	3	
- Sarcoidosis	4	1	
- SLE	3	4	
- RA	1	2	
- Interstitial lung disease (other than connective tissue disease-related)	0	2	
- Other (scleritis)	0	1	
- Myositis (other than SLE-related)	0	2	
Duration of continuous systemic glucocorticoid treatment by the study entry (months)	36 (IQR 10 to 156)	36 (IQR 7.5 to 126)	0.67
Estimated cumulative dose of glucocorticoids by the study entry (equivalent mg of prednisolone)	55000 (IQR 9500 to 110000)	21430 (IQR 9605 to 72070)	0.69
Cumulative dose of glucocorticoid last 3 months prior to the study entry (equivalent mg prednisolone)	1800 (IQR 1300 to 2700)	2175 (IQR 1515 to 3128)	0.40
Cumulative dose of glucocorticoids during the study (equivalent mg of prednisolone)	1860 (IQR 1060 to 2810)	1770 (IQR 1020 to 2356)	0.76
Prednisolone equivalent dose (mg) at initial visit	25 (IQR 13 to 40) ; range [10 to 60mg]	25 (IQR 14 to 36); range [10-70mg]	0.90
Prednisolone equivalent dose (mg) at 4 weeks	20 (IQR 10 to 30)	20 (IQR 10 to 25)	0.64
Prednisolone equivalent dose (mg) at 8 weeks	20 (IQR 10 to 30)	18 (IQR 10 to 22)	0.80
Prednisolone equivalent dose (mg) at 12 weeks	13 (IQR 10 to 25)	15 (10 to 25)	0.62
Neutrophils	7.4 (IQR 4.5 to 9.6)	5.8 (IQR 3.8 to 8.1)	0.33
Lymphocytes	1.7 (IQR 1.3 to 2.3)	1.4 (0.8 to 2)	0.28
Monocytes	0.6±0.1	0.6±0.1	0.56
Eosinophils	0.1 (IQR 0.1 to 0.2)	0.1 (IQR 0.0 to 0.2)	0.35
Basophils	0 (IQR 0-0.1)	0 (IQR 0-0.1)	0.98
Physical activity on the day (virtual scale 0 to 10; 10 is the highest)	2 (IQR 1 to 3)	2 (IQR 1 to 3.5)	0.62
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)	0.53
Reported frequency of at least 15min of moderate physical activity in 7days (NACR questionnaire)	0 (IQR 0 to 0)	0 (IQR 0 to 2.5)	0.30
Reported frequency of at least 15min of minimal effort physical activity in 7 days (NACR questionnaire)	3 (IQR 0 to 7)	3.5 (IQR 2 to 7)	0.42
Medications			
Prednisolone	19 (100%)	21 (100%)	0.99
Methylprednisolone	0	1	0.99
Beclomethasone	1	1	0.99
Fluticasone	3	4	0.99
Dexamethasone	1	0	0.99
Clobetasone	1	0	0.99
Methotrexate	2	3	0.99
Hydroxychloroquine	1	3	0.61
Azathioprine	1	1	0.99
Mycophenolate	1	4	0.35
Sulphasalazine	1	0	0.99
Ustekinumab	0	1	0.99
Tocilizumab	0	1	0.99
Omaluzimab	0	1	0.99
Cyclophosphamide	0	1	0.99
Ciclosporin	1	1	0.99
Bisphosphonates	12	9	0.22
HRT/OCP	0	3	0.23
TB treatment	1	1	0.99
Warfarin/low molecular weight heparin	0	1	0.99

	Metformin	Placebo	P-value
Statins	3	1	0.33
Antihypertensives	10	9	0.99
Beta-blockers	2	1	0.99
ACE-inhibitors/Sartans	7	6	0.73

Physical activity during the study

The exercise activity at the study initiation and at weeks 4, 8, and 12 was assessed via a visual analogue scale and National Audit of Cardiac Rehabilitation (NACR) Minimum Dataset Short Physical Activity Questionnaire[12]. The overall physical activity was low. Patients matched in all tested parameters at baseline; however, during the study patients receiving placebo increased the frequency of moderate exercise (e.g. fast walking, mowing the lawn, tennis, easy cycling, badminton, easy swimming, ballroom dancing, fast or high step ups) relative to the patients on metformin (p=0.043), as well as they reported slightly higher physical activity on the day, prior to their appointments (visual analogue scale, p=0.003 (Figure S1). There were no statistically significant differences in the reported amount of physical activity judged as 'minimal' (e.g. easy walking, slow dancing, standing active fishing, bowling, golf, low step-ups) and 'strenuous' (e.g. running, jogging, vigorous long-distance cycling, circuit training, aerobic dance, skipping, football, squash, basketball, roller skating, vigorous swimming).

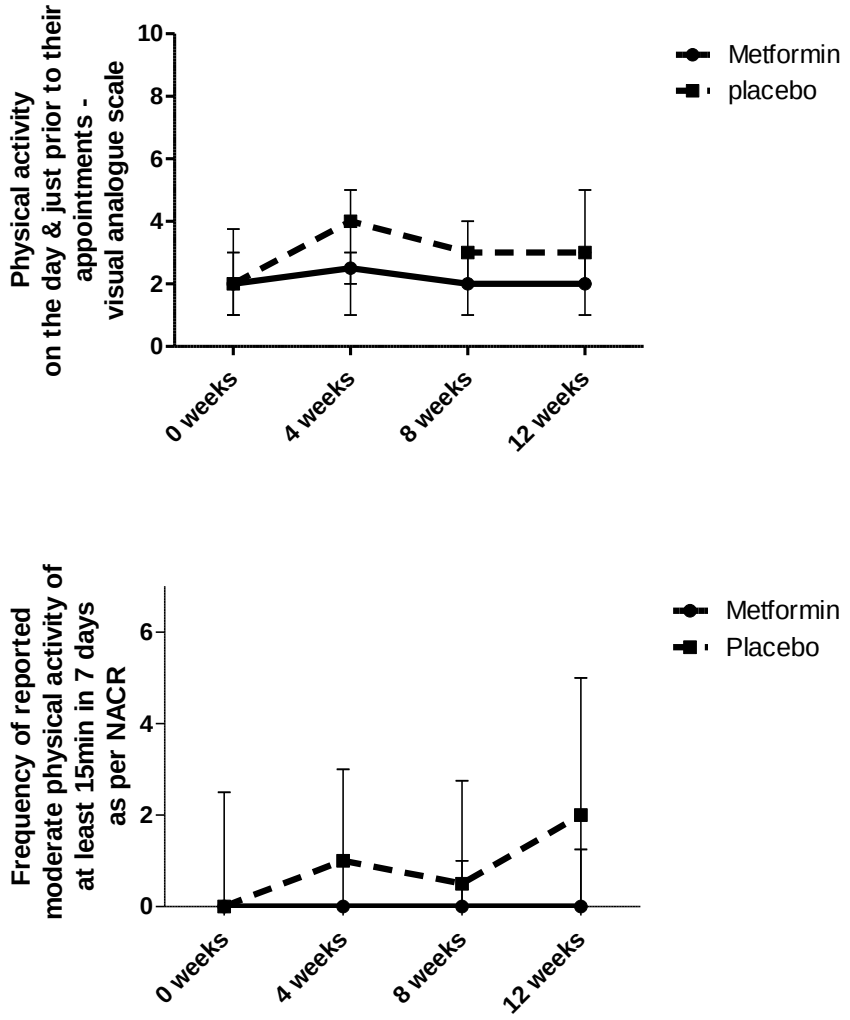

Figure S1 Higher panel: reported physical activity on the day on a 10-point virtual scale (10 represents the highest activity: difference between the treatment groups p=0.003). Lower panel: weekly frequency of reported moderate physical activity (difference between the treatment groups p=0.043); Repeated measures two way ANOVA (Median (IQR).

Effects on CT parameters of body composition

Adjustment for baseline variables deemed particularly influential or suspected for a degree of dysbalance based on baseline between-group difference (p-value <1).

Table S2 Differences in CT parameters adjusted for variables

Difference in visceral to subcutaneous fat ratio (Metformin-Placebo) unadjusted and adjusted* for baseline variables

	Visceral to subcutaneous fat ratio		Variable adjusted for	
Adjustment variable	Difference	p-value	Coefficient	p-value
-	0.11	0.09	-	-
Ethnicity (white v non-white)	0.12	0.09	-0.02	0.76
BMI	0.11	0.10	0.0026	0.56
Cumulative dose (per 1000mg)	0.12	0.07	-0.00078	0.18
Cognitive restraint of eating	0.10	0.20	-0.0028	0.75
Mean NACR during study	0.13	0.06	0.02	0.25
Mean exercise during study (scale 1 to 10)	0.11	0.11	-0.004	0.85
FGF21 (per 100 pg/mL)	0.12	0.10	-0.000017	1.00

*Adjusted using a multivariate regression model

Difference in visceral fat (Metformin-Placebo) unadjusted and adjusted* for baseline variables

	Visceral fat		Variable adjusted for	
Adjustment variable	Difference	p-value	Coefficient	p-value
-	2058	0.28	-	-
Ethnicity (white v non-white)	2075	0.29	-103	0.96
BMI	2051	0.29	69	0.59
Cumulative dose (per 1000mg)	2357	0.22	-21	0.22
Cognitive restraint of eating	1138	0.61	-207	0.43
Mean NACR during study	2011	0.31	-52	0.92
Mean exercise during study (scale 1 to 10)	1867	0.34	-432	0.52
FGF21 (per 100 pg/mL)	2143	0.28	135	0.70

*Adjusted using a multivariate regression model

Difference in subcutaneous fat (Metformin-Placebo) unadjusted and adjusted* for baseline variables

	Subcutaneous fat		Variable adjusted for	
Adjustment variable	Difference	p-value	Coefficient	p-value
-	-3835	0.01	-	-
Ethnicity (white v non-white)	-4305	0.01	2437	0.10
BMI	-3808	0.01	-57	0.57
Cumulative dose (per 1000mg)	-3918	0.01	6.3	0.63
Cognitive restraint of eating	-4726	0.01	-201	0.31
Mean NACR during study	-4360	<0.01	-684	0.08
Mean exercise during study (scale 1 to 10)	-3920	0.01	-163	0.75
FGF21 (per 100 pg/mL)	-3872	0.02	274	0.32

*Adjusted using a multivariate regression model

Effects on waist and hip circumference

Table S3. Effects on waist and hip circumference

	Metformin	Placebo	p-value
Waist (cm)			
Baseline (MF n=19, PL n=21)	91.7(IQR 82.6 to 110)	96.8 (IQR 88.9 to 106.5)	0.65
At 12 weeks	89.2(IQR 82 to 107.4)	97(IQR 89.7 to 105.6)	0.54
Change over 12 weeks	-0.9±0.4	0.3±0.4	0.07
Difference within groups (p-value)	0.06	0.95	
Hip (cm)			
Baseline (MF n=19, PL n=21)	105±3.3	106.6±2.4	0.70
At 12 weeks	103.7±3.4	107.7±2.5	0.35
Change over 12 weeks	-1(IQR -3.2 to 0.5)	0.5 (IQR -0.3 to 2)	0.02
Difference within groups (p-value)	0.05	0.04	

Effects on lipids

Table S4. Effects on lipids

	Metformin	Placebo	p-value
Total cholesterol (mmol/L)			
Baseline (MF n=19, PL n=21)	5.4 (IQR 4.7 to 6.4)	5.7 (IQR 4.7 to 6.2)	0.56
At 12 weeks	4.8 (IQR 4.0 to 5.3)	5.5 (IQR 4.8 to 6.6)	0.06
Change over 12 weeks	-0.45 ± 0.77	-0.15 ± 0.97	0.29
Difference within groups (p-value)	0.02	0.97	
% change over 12 weeks	-8 (IQR -19 to 2)	4 (IQR -8 to 9)	0.08
Triglycerides (mmol/L)			
Baseline (MF n=19, PL n=21)	1.4 (IQR 1 to 2.4)	1.3 (IQR 1.1 to 1.8)	0.88
At 12 weeks	1.3 (IQR 0.9 to 1.6)	1.5 (IQR 1.1 to 1.9)	0.36
Change over 12 weeks	-0.3 ± 0.6	0 ± 0.5	0.07
Difference within groups (p-value)	0.30	0.60	
% change over 12 weeks	-17 (IQR -32 to 17)	12 (IQR -16 to 32)	0.06
HDL (mmol/L)			
Baseline (MF n=19, PL n=21)	1.86 ± 0.54	1.92 ± 0.42	0.72
At 12 weeks	1.78 ± 0.47	1.74 ± 0.40	0.75
Change over 12 weeks	-0.08 ± 0.31	-0.18 ± 0.36	0.36
Difference within groups (p-value)	0.28	0.04	
% change over 12 weeks	-2 ± 16	-8 ± 19	0.35
Change over 12 weeks (patients fulfilling the International Diabetes Federation (IDF) metabolic syndrome criteria[13]) (Post-hoc; interaction: p=0.41)	0.16 ± 0.27	-0.38 ± 0.23	0.002
Non-HDL cholesterol (mmol/L)			
Baseline (MF n=19, PL n=21)	3.22 (IQR 2.66 to 4.13)	3.64 (IQR 3.08 to 4.03)	0.79
At 12 weeks	2.69 (IQR 2.41 to 3.91)	3.65 (IQR 3.11 to 4.67)	0.03
Change over 12 weeks	-0.37 ± 0.82	0.03 ± 0.92	0.16
Difference within groups (p-value)	0.07	0.50	
% change over 12 weeks	-11.7 (IQR -25 to 9.4)	8.3 (IQR 10.6 to 18.1)	0.054
% change over 12 weeks (statin-naïve patients) (post-hoc)	-12 (IQR -25 to 9)	9 (IQR -5 to 18)	0.04

Effects on the liver function

Table S5. Liver function tests

	Metformin	Placebo	p-value
ALP (U/L) - alkaline phosphatase			
Baseline (MF n=18, PL n=21)	62 ± 15	57 ± 19	0.48
At 12 weeks	58 ± 18	59 ± 21	0.87
Change over 12 weeks	-3.9 ± 7.2	1.2 ± 8.8	0.06
Difference within groups (p-value)	0.03	0.54	
GGT (U/L) - gamma glutamyl transferase			
Baseline (MF n=14, PL n=13)	34 (IQR 19 to 50)	29 (IQR 16 to 40)	0.38
At 12 weeks	24 (IQR 15 to 33)	25 (IQR 19 to 47)	0.64
Change over 12 weeks	-7.0 (IQR -26.8 to -0.8)	0.0 (IQR -4.0 to 3.5)	0.007
Difference within groups (p-value)	0.005	0.99	
AST/ALT ratio			
Baseline (MF n=12; PL n=12)	1.1 ± 0.4	0.9 ± 0.4	0.23
At 12 weeks	1.3 ± 0.5	1.0 ± 0.4	0.09
Change over 12 weeks	0.2 ± 0.3	0.1 ± 0.3	0.31
Difference within groups (p-value)	0.02	0.27	

Effects on appetite

Table S6. The three-factor eating questionnaire

Cognitive restraint of eating (scoring[14]: higher value means greater restraint)*			
Baseline (MF n=19; PL n=21)	5 ± 4	9 ± 4	<0.001
At 12 weeks	5 ± 3	9 ± 4	<0.001
Change over 12 weeks	-0.3 ± 2.7	-0.1 ± 2.6	0.58
Difference within groups (p-value)	0.68	0.87	
Disinhibition (scoring[14]: higher value means greater disinhibition)*			
Baseline (MF n=19; PL n=21)	5 (IQR 3 to 10)	5 (IQR 2 to 8)	0.56
At 12 weeks	3 (IQR 3 to 6)	4 (IQR 2 to 8)	0.76
Change over 12 weeks	-1 (IQR -3 to 0)	-1 (IQR -2 to 1)	0.25
Difference within groups (p-value)	0.01	0.41	
Hunger (scoring[14]: higher value means greater hunger)			
Baseline (MF n=19; PL n=21)	4 (IQR 3 to 9)	4 (IQR 2 to 8)	0.99
At 12 weeks	2 (IQR 1 to 5)	5 (IQR 2 to 8)	0.17
Change over 12 weeks	-1.6 ± 2.9	0.2 ± 2.3	0.04
Difference within groups (p-value)	0.04	0.65	

*Cognitive restraint was previously described to be protective against the effects of disinhibition, a factor considered a major risk for visceral fat accumulation in the general population[15].

Correlations of hunger scores, sugar craving, disinhibition

The hunger scores correlated positively with changes in weight (r=0.44, p=0.004), waist (r=0.37, p=0.02), hip (r=0.36, p=0.02), truncal fat (r=0.41, p=0.009) and insulin resistance (r=0.31, p=0.049). Similarly, the dynamics of sugar craving associated positively with waist (r=0.45, p=0.004), truncal subcutaneous fat (r=0.36, p=0.03) and insulin resistance (r=0.38, p=0.03). Changes in disinhibition correlated with the changes in weight (r=0.38, p=0.03), truncal subcutaneous (r=0.39, p=0.01) and visceral (r=0.32, p=0.04) fat depots. Overall, hunger, sugar craving and disinhibition appeared to promote parameters related to weight gain and fat accumulation.

Effects on fibrin clot properties

Table S7. Fibrin clot properties

	Metformin	Placebo	p-value
Maximum absorbance (AU)			
Baseline (MF n=13; PL n=16)	0.12 (IQR 0.11 to 0.20)	0.18 (IQR 0.12 to 0.27)	0.10
At 12 weeks	0.15 ± 0.06	0.23 ± 0.12	0.03
Change over 12 weeks	0.00 ± 0.07	0.04 ± 0.11	0.38
Difference within groups (p-value)	0.74	0.18	

Effects on inflammation

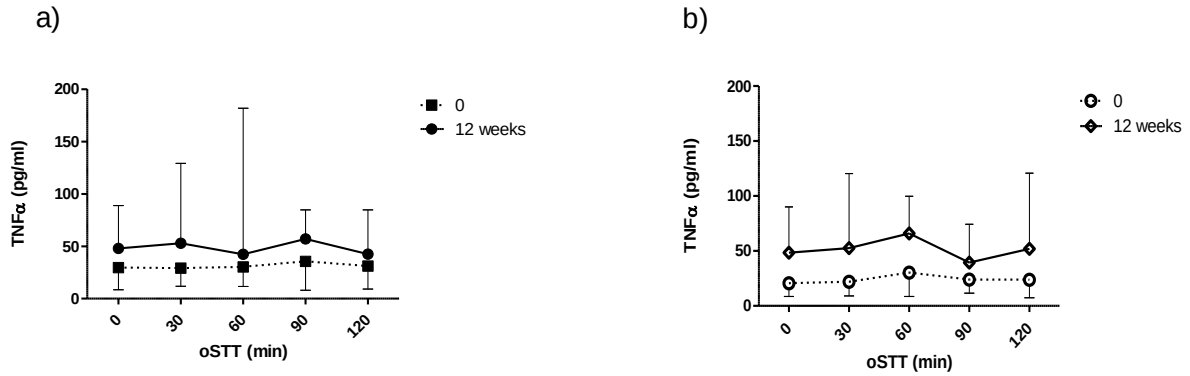

Figure S2. TNFα

TNFα levels in response to a 75g-sucrose challenge at study entry and after 12 weeks of treatment within treatment groups: a) on metformin (p=0.46); b) on placebo (p=0.04) (repeated measures two-way ANOVA; medians with interquartile ranges). Between-group differences were not statistically significant.

Effects on the bone metabolism

Table S8 Total hip: T- and Z-scores

	Metformin	Placebo	p-value
Total hip – T-score			
Baseline (MF n=16; PL n=21)	-0.1 ± 1.1	-0.2 ± 1.1	0.68
At 12 weeks	0.0 ± 1.1	-0.3 ± 1.0	0.36
Change over 12 weeks	0.1 (IQR 0 to 0.1)	-0.1 (IQR -0.1 to 0.1)	0.007
Difference within groups (p-value)	0.01	0.26	
Total hip Z-score			
Baseline (MF n=16; PL n=21)	0.0 (IQR -0.6 to 0.5)	0.0 (IQR -0.5 to 0.6)	0.85
At 12 weeks	0.4 ± 1.2	0.1± 1.0	0.45

Change over 12 weeks	0.1 (IQR 0 to 0.2)	-0.1 (IQR -0.1 to 0.1)	0.002
Difference within groups (p-value)	0.006	0.41	

Table S9 Spine BMD, T- and Z-scores

	Metformin	Placebo	p-value
Spine BMD (g/cm^2)			
Baseline (MF n=15; PL n=20)	1.002 ± 0.170	0.987 ± 0.158	0.78
At 12 weeks	1.000 ± 0.162	0.988 ± 0.158	0.85
Change over 12 weeks	-0.002 ± 0.031	0.001 ± 0.018	0.66
Difference within groups (p-value)	0.77	0.74	
Spine – T score			
Baseline	-0.8 ± 1.3	-0.9 ± 1.3	0.84
At 12 weeks	-0.8 ± 1.3	-0.9 ± 1.3	0.85
Change over 12 weeks	0.0 ± 0.3	0.0 ± 0.2	0.54
Difference within groups (p-value)	0.71	0.63	
Spine – Z score			
Baseline (MF n=15; PL n=20)	-0.2± 1.5	-0.3± 1.3	0.86
At 12 weeks	-0.3 ± 1.4	-0.3± 1.3	0.95
Change over 12 weeks	-0.1 (IQR -0.2 to 0.1)	0.0 (IQR -0.1 to 0.2)	0.26
Difference within groups (p-value)	0.15	0.95	

Table S10. Effects on bone parameters P1NP and osteocalcin

	Metformin	Placebo	p-value
P1NP (µg/L)			
Baseline (MF n=18; PL n=21)	20.6 (IQR 9.3 to 37.2)	17.0 (IQR 8.2 to 24.9)	0.38
At 12 weeks	19.7 (IQR 8.4 to 36.3)	16.5 (IQR 11.2 to 29.1)	0.69
Change over 12 weeks	-1.5 (IQR -4.0 to 2.88)	-2.5 (IQR -5.3 to 7.2)	0.34
Difference within groups (p-value)	0.47	0.92	
Osteocalcin (µg/L)			
Baseline	8.9 (IQR 6.3 to 14)	8.5 (IQR 4.7 to 12.7)	0.42
At 12 weeks	8.0 (IQR 6.7 to 15.7)	9.1 (IQR 4.9 to 14)	0.89
Change over 12 weeks	-0.4 ± 3.4	1.8 ± 5.1	0.13
Difference within groups (p-value)	0.54	0.12	

Table S11. Vitamin D concentrations and calcium and vitamin D supplementation

	Metformin	Placebo	p-value
Vitamin D levels (25-Hydroxyvitamin D3) (nmol/L)*			
Baseline (MF n=16, PL n=19)	46.0 ± 18.6	51.6 ± 27.4	0.49
At 12 weeks	40.3 ± 16.5	49.3 ± 22.1	0.19
Change over 12 weeks	-5.8 ± 11.2	-2.3 ± 19.2	0.53
Difference within groups (p-value)	0.06	0.62	
**Reflecting seasonal changes and dietary sources*			
	Metformin	**Placebo**	
Calcium and vitamin D supplements -number of patients (out of 19 on metformin and 21 on placebo completing the study)			
Calcium carbonate+colecalciferol (500mg-3000mg + 200-800 I.U.)	15	11	
Alfacalcidol 500ng	0	1	
Colecalciferol 1000-1600 I.U.	2	1	

Table S12. Bisphosphonate treatment & bone effects in patients not taking bisphosphonates

	Metformin	Placebo

Bisphosphonate treatment - number of patients (out of 19 on metformin and 21 on placebo completing the study)			
Alendronic acid 70mg once weekly	9	9	
Risedronate 35mg once weekly	3	0	
Patients not taking bisphosphonates – changes over 12 weeks (post hoc) (DEXA: MF n=7; PL n=11); (Bone markers: MF n=8; PL n=11)			
Total hip BMD change	0.008±0.024	-0.003±0.020	0.29
Total hip T-score change	0.1±0.2	-0.1±0.4	0.33
Total hip Z–score change	0.1 (IQR 0.0 to 0.4)	0.0 (IQR -0.1 to 0.1)	0.04
βCTX change (µg/L) *__within group change__ (p=0.01)	-0.07±0.06*	0.01±0.11	0.12
P1NP change (µg/L)	3.0 (IQR -9.6 to 13.6)	2.5 (IQR -2.3 to 7.3)	0.90

Fibroblast growth factor 21 (FGF21) and adiponectin

Changes in FGF21 levels correlated with changes in the following parameters, favourably modified in metformin-treated subjects: **1)** body composition (weight: r=-0.39, p=0.01; waist: r=-0.38, p=0.02; hip: r=-0.49, p=0.002; truncal subcutaneous fat: r=-0.34, p=0.04), **2)** glucose (r= -0.35, p=0.03), **3)** lipids (total cholesterol: r=-0.50, p=0.03; LDL: r=-0.43, p=0.02; triglycerides: r=-0.32 p=0.08) **4)** liver function tests (ALT: r=-0.54, p<0.001; GTT: r=-0.46, p=0.02; AST: r=-0.47, p=0.02), **5)** sugar craving (r=-0.36, p=0.02), **6)** hsCRP (r=-0.43, p=0.01), **7)** IMT (r=-0.41, p=0.01), **8)** bone resorption (ßCTX: r=-0.35, p=0.04) **9)** insulin release (HOMAB: r=0.37, p=0.02). In metformin-treated patients, FGF21 changes correlated with adiponectin changes (r=0.64, p=0.004).

Changes in adiponectin correlated with changes in the following (favourably for patients on metformin): 1) glucose (r=-0.34, p=0.03), 2) inflammation (hsCRP: r=-0.42, p=0.01; TNFα: r=-0.55, p<0.001; ESR: r=-0.34, p=0.03;), 3) lipids (triglycerides: r=-0.48, p=0.005; chol:HDL ratio: r=-0.51, p=0.003), 4) liver function tests (ALT: r=-0.39, p=0.01; GGT: r=-0.43, p=0.02; AST/ALT change: r=0.41, p=0.047), 5) fibrinolysis: lysis time (r=-0.46, p=0.01), maximum absorbance (r=-0.40, p=0.03), lysis area (r=-0.39, p=0.03) 6) intima-media thickness (r=-0.48, p=0.002).

Cumulative glucocorticoid exposure during the study correlated negatively with changes in adiponectin levels (r=-0.34, p=0.04) and fasting FGF21 after 12 weeks of placebo treatment (r=-0.48, p=0.03).

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