Supplementary Table 1. Baseline characteristics of CAMERA participants

Characteristic	Metformin (n=86)	Placebo (n=87)
Males	70 (81%)	63 (72%)
Age (years)	63 (8)	64 (8)
Weight (kg)	87.9 (14.1)	86.8 (15.0)
BMI (kg/m^2)	30.2 (4.0)	30.5 (4.4)
Fasting plasma glucose (mmol/L)	5.4 (0.6)	5.3 (0.5)
HOMA2-IR*†	1.36 (1.23-1.51)	1.40 (1.25-1.56)
HbA1c (mmol/mol)	38.7 (3.6)	38.2 (3.3)
HbA1c (%)	5.6 (0.3)	5.6 (0.3)
Fasting leptin (ng/mL)*	16.1 (13.6-19.1)	18.7 (15.8-22.1)
Fasting total GLP-1 (pg/mL)*	11.6 (10.1-13.3)	12.4 (10.9-14.2)

Data presented as mean (SD) or n (%) except where indicated (*Geometric mean and 95%CI)
† calculated using the HOMA2-IR calculator (https://www.dtu.ox.ac.uk/homacalculator/)

Supplementary Table 2. Review of previous studies investigating the effect of metformin on circulating GLP-1

REFERENCE	Study summary	N, duration	Methods	Metformin effect on GLP-1
Kappe et al. (2014)	To determine if high fat diet (HFD) and metformin lowers number of entero-endocrine L cells and/or GLP-1 plasma levels	-, -	C57/B16 mice received control/HFD for 12 weeks and oral metformin/ saline for last 14 days. ELISA used to measure GLP-1 before and after metformin. Immunohistochemistry used to quantify GLP-1 positive cells in intestinal cells.	- ↓ GLP positive cells in HFD mice - improved incretin response - intestinal expression of GLP-1R mRNA upregulation - improved metformin response in mice on HFD
Wu et al. (2014)	Study of Caucasian T2DM men treated with placebo or metformin to investigate effects on DPP-4 and total intact GLP-1	N=12, 2 X 7 days	Crossover study with Intra-duodenal glucose infusion on day 5 and 8 then C-terminally directed assay and sandwich ELISA	- ↓ plasma fasting DPP-4 activity - ↑ plasma intact GLP-1 - no significant difference in total GLP-1
Solis-Herrera et al. (2013)	Study assessing glucose lowering mechanisms of sitagliptin and/or metformin in patients with T2DM	N=16, 4 X 6 weeks	Cross-over study with meal tolerance testing, radioimmunoassay, glucose oxidase method and ELISA	- ↑ GLP-1 secretion and β cell function in metformin and sitagliptin combined (2 to 3 fold ↑ in basal plasma GLP-1 concentration - no significant ↑ with metformin alone
Vardarli et al. (2013)	Effect of metformin, sitagliptin or both on GLP-1 responses of overweight/obese patients with T2DM	N=20, 4 X 6 days	Cross-over study with oral glucose challenge on day 5 and IV glucose infusion on day 6 then sandwich ELISA or C-terminally detected assay	- metformin ↑ fasting total GLP-1 by ↑ insulin secretory responses. -Whereas, DPP-4 inhibitor ↑ plasma intact GLP-1 and ↓ total GLP-1
Kappe et al. (2012)	Effect of metformin on regulation of GLP-1 secreting cells	-, -	Used murine GLUTag cell line, DNA-fragment assay, ELISA, RT-PCR etc.	- Regulates GLP-1 receptor expression in pancreas - Protects GLP-1 cells against lipoapoptosis - ↑ secretion of pre-proglucagon
Thondam et al. (2012)	Effect of 3 months of metformin monotherapy on GLP-1, ghrelin and DPP-4 in obese T2DM patients	N=8, 3 months	Prospective, observational study using ELISA	-↑ postprandial active GLP-1 levels - after 3 months, mean fasting GLP-1 didn't significantly change
Mulherin et al. (2011)	Assess direct effects of metformin on GLP-1 secretion from intestinal L cells and assess indirect actions that increase plasma GLP-1.	-, -	In vivo and in vitro studies using murine human NCI-H716 and rat FRIC cells	- ↑ GLP-1 in vivo only (M3 muscarinic dependent effects) - activity of DPP-4 not affected - ↑ total GLP-1 over 24 hours
Cuthbertson et al. (2011)	Acute effect of metformin and GLP-1 alone or in combination on DPP-4 activity in overweight/obese patients with T2DM	N=10, 1 day	Overnight fast then blood tested for DPP-4 activity, insulin, GLP-1, glucose and C-peptide concentrations using biochemical assays	- DPP-4 only inhibited by 7% - insulin sensitizing effects important in glucose lowering by GLP-1

Maida et al. (2011)	To assess if metformin exerts glucoregulatory actions via modulation of the incretin axis using knock-out GLP-1R versus obese hyperglycaemic wild-type mice with/without exendin	-, -	Assessed incretin receptor expression, glucose tolerance, gastric emptying and food intake	- ↑ GLP-1 levels and improved glucose tolerance in knock-out mice. - ↑ GLP-1R in INS-1β CELLS via PPAR-α dependent and AMPK independent pathways
Migoya et al. (2010)	Effect of metformin in healthy men and women	N=16, 4 X 2 days	Four period (two day) cross-over study including placebo and metformin interventions; Day 2 active and total GLP-1 and GIP and glucose plasma concentrations measured pre-meal and post-meal	- ↑ postprandial total GLP-1 concentrations in plasma - ↑ postprandial active GLP-1 concentrations - no effect on total or active GIP concentrations - no effect on postprandial DPP-4 activity
Svendsen et al. (2009)	Effect of metformin in women with polycystic ovarian syndrome	N=40 (22 completed treatment), 8 months	Uncontrolled interventional study with 180min oral glucose loading tests and comparison to baseline	- ↑ area under GLP-1 curve
Green et al. (2006)	Effect of metformin on DPP-4 activity in normal and obese diabetic mice	-, -	Radioimmunoassay using blood samples taken 30 minutes post-intraperitoneal injection of glucose and GLP-1 or GLP-1+ metformin	- in vivo metformin ↓ DPP-4 activity in ob/ob mice with improved glucose lowering and insulin release from GLP-1 - ↑ circulating GLP-1 (7-36) amide levels
Mannucci et al. (2004)	Effect of metformin on 22 obese T2DM versus 12 placebo controls	N=34, 4 weeks	GLP-1 measured before and after 100g glucose load after 4 weeks of 850mg metformin	- Single dose didn't modify GLP-1 - fasting GLP-1 ↑ after 4 weeks of metformin
Hinke et al. (2002)	Investigates whether metformin acts as a DPP-4 inhibitor to increase GLP-1 in obese non-diabetic patients	-, -	In vitro analysis of 20% human serum, porcine kidney and recombinant human DPP-4 using mass spectrometry and surface plasmon resonance	- Metformin does not act directly on DPP-4 - Instead, it may ↑ GLP-1 and glucagon secretion from pancreatic α cells and intestinal L cells
Mannucci et al. (2001)	Effect of metformin versus placebo on GLP-1 and leptin in obese non-diabetic men before and after 14 days of treatment	N=10, 14 days	GLP-1 measured using ELISA in fasting state and after oral glycaemic load during euglycaemic hyperinsulinaemic clamp	- significant GLP-1 ↑ at 30 and 60 minutes after oral glucose load -no GLP-1 variation in controls

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