



University of Dundee

The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with and without type 2 diabetes

Preiss, David; Dawed, Adem; Welsh, Paul; Heggie, Alison; Jones, Angus G.; Dekker, Jacqueline

Published in:
Diabetes, Obesity & Metabolism

DOI:
[10.1111/dom.12826](https://doi.org/10.1111/dom.12826)

Publication date:
2017

Document Version
Peer reviewed version

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):

Preiss, D., Dawed, A., Welsh, P., Heggie, A., Jones, A. G., Dekker, J., Koivula, R., Hansen, T. H., The DIRECT consortium, Stewart, C., Holman, R. R., Franks, P. W., Walker, M., Pearson, E. R., & Sattar, N. (2017). The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with and without type 2 diabetes. *Diabetes, Obesity & Metabolism*, 19(3), 356-363. <https://doi.org/10.1111/dom.12826>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with and without type 2 diabetes

Journal:	<i>Diabetes, Obesity and Metabolism</i>
Manuscript ID	DOM-16-0604-OP.R1
Manuscript Type:	Original Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Preiss, David; University of Oxford, CTSU Dawed, Adem; University of Dundee, Medical Research Institute Welsh, Paul; University of Glasgow, BHF GCRC Heggie, Alison; Newcastle University Jones, Angus; University of Exeter Dekker, Jacqueline; VU University Medical Center, EMGO Institute for Health and Care Research; VU University Medical Center, Department of Epidemiology and Biostatistics Koivula, Robert; Lunds Universitet Hansen, Tue; Kobenhavns Universitet Stewart, Caitlin; University of Glasgow Holman, Rury R; Oxford Centre for Diabetes, Endocrinology, and Metabolism, Diabetes Trial Unit Franks, Paul; Lunds Universitet Walker, Mark; Newcastle University Pearson, Ewan; University of Dundee, Medical Research Institute Sattar, Naveed; University of Glasgow ,
Key Words:	antidiabetic drug, GLP-1, metformin

SCHOLARONE™
Manuscripts

This is the peer reviewed version of the following article: 'The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with and without type 2 diabetes', *Diabetes, Obesity & Metabolism* which has been published in final form at <http://dx.doi.org/10.1111/dom.12826>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

1
2
3 **The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with**
4 **and without type 2 diabetes**
5
6

7 *Running title:* metformin and circulating GLP-1 levels
8

9 Dr David Preiss (PhD)¹, Adem Dawed (MPH)², Dr Paul Welsh (PhD)³, Dr Alison Heggie (MRCP)⁴,
10 Dr Angus G Jones (PhD)⁵, Prof Jacqueline Dekker (PhD)⁶, Mr Robert Koivula⁷, Dr Tue H Hansen
11 (MD)⁸, The DIRECT consortium⁹, Ms Caitlin Stewart (MSc)³, Prof Rury R Holman (FRCP)¹⁰, Prof
12 Paul W Franks (PhD)⁷, Prof Mark Walker (PhD)⁴, Prof Ewan R Pearson (PhD)^{2*}, Prof Naveed Sattar
13 (PhD)^{3*}
14
15
16

17 ¹ MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit,
18 University of Oxford, Oxford, UK
19

20 ² Molecular and Clinical Medicine, University of Dundee, Dundee, UK
21

22 ³ Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK
23

24 ⁴ Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
25

26 ⁵ NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter, UK
27

28 ⁶ VU Medical Center, Dept Epidemiology and Biostatistics, Amsterdam, Netherlands.
29

30 ⁷ Department of Clinical Sciences, Lund University, Genetic and Molecular Epidemiology, Malmö, Sweden
31

32 ⁸ The Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty
33 of Health Sciences, University of Copenhagen, Denmark
34

35 ⁹ Consortium members listed in Supplementary data
36

37 ¹⁰ Diabetes Trials Unit, University of Oxford, Oxford, UK
38

39 *joint senior authors
40

41 *Correspondence to:*
42

43 David Preiss, CTSU, Richard Doll Building, Old Road Campus, University of Oxford, Oxford, OX3
44 7LF, UK
45

46 Email: david.preiss@ndph.ox.ac.uk
47

48 Phone: +44 1865 743527
49

50 OR
51

52 Ewan R Pearson, Division of Molecular and Diabetes Medicine, School of Medicine, University of
53 Dundee, DD1 9SY, UK
54

55 Email: e.z.pearson@dundee.ac.uk
56

57 Tel: +44 1382 383387
58

59 Word count: 3,491 (abstract 249) with 29 references
60

Tables: 3 (and 3 supplementary tables)

Figures: 1

Abstract

Aims: Small, short studies suggest metformin influences the glucagon-like peptide (GLP)-1 axis in individuals with and without type 2 diabetes (T2DM). In the Carotid Atherosclerosis: Metformin for insulin ResistAnce (CAMERA) trial ([NCT00723307](https://clinicaltrials.gov/ct2/show/study/NCT00723307)) we investigated whether this effect is sustained and related to changes in glycaemia or weight. In the cross-sectional DIabetes REsearCh on patient stratiFication (DIRECT) study, we investigated basal and post-meal GLP-1 levels in diabetic patients.

Materials and Methods: CAMERA was a double-blinded randomized placebo-controlled trial of metformin in 173 participants without diabetes. Using six-monthly fasted total GLP-1 levels over 18 months, we evaluated metformin's effect on total GLP-1 with repeated-measures and ANCOVA analyses. In DIRECT, we examined active and total fasting and 60-minute post-meal GLP-1 levels in 775 patients recently diagnosed with T2DM treated with metformin or diet, using Student's T-tests and linear regression.

Results: In CAMERA, metformin increased total GLP-1 at 6 (+20.7%, [95% confidence intervals 4.7-39.0%]), 12 (+26.7% [10.3-45.6%]) and 18 months (+18.7% [3.8-35.7%]), an overall increase of 23.4% (11.2-36.9%; $p < 0.0001$) versus placebo. Adjustment for changes in glycaemia and adiposity, individually or combined, did not attenuate this effect. In DIRECT, metformin was associated with higher fasting active (39.1% [21.3-56.4%]) and total GLP-1 (14.1% [1.2-25.9%]) but not post-meal incremental GLP-1. These changes were independent of potential confounders including age, sex, adiposity and HbA1c.

Conclusions: In non-diabetic individuals, metformin increases total GLP-1 in a sustained manner and independently of changes in weight or glycaemia. Metformin-treated diabetic patients also have higher fasted GLP-1 independent of weight and glycaemia.

Introduction

Metformin is recommended as first-line therapy for the majority of individuals with type 2 diabetes mellitus (T2DM)¹. This is based on evidence of cardiovascular benefit and also its capacity to maintain or reduce weight. In the United Kingdom Prospective Diabetes Study, metformin monotherapy led to a 39% reduction in the risk of myocardial infarction compared to conventional dietary therapy over 10 years, a finding not explained by the drug's effect on glycaemia². Metformin has also been shown to reduce the risk of developing T2DM. In the Diabetes Prevention Program, metformin therapy reduced new-onset T2DM by 31% and also led to 2.1kg weight loss compared to placebo over 2.8 years^{3,4}.

The glucagon-like peptide 1 (GLP-1) axis remains at the forefront of T2DM and cardiovascular research. Major outcomes trials of dipeptidyl peptidase-4 (DPP-4) inhibitors and the first completed outcome trial of a GLP-1 receptor agonist in T2DM patients indicated cardiovascular safety, though not benefit⁵⁻⁸. However, it was recently reported that the potent GLP-1 receptor agonist, liraglutide, has demonstrated cardiovascular benefit⁹. Furthermore, it has been reported that another GLP-1 receptor agonist, semaglutide, has also provided cardiovascular benefit in a major trial¹⁰. This is supported by recently published results from a Mendelian randomization study of a GLP-1 genetic variant (Ala316Thr; rs10305492) strongly associated with lower fasting glucose levels which demonstrated a lower risk of cardiovascular disease¹¹, supporting the concept that GLP-1 may indeed be protective against cardiovascular disease. In addition, GLP-1 receptor agonists can yield modest weight loss¹² and blood pressure reduction, important goals in the management of T2DM.

1
2
3 It is unclear whether some of metformin's benefits may be mediated via GLP-1. To explore
4 this, it is important to robustly establish the effect of metformin on GLP-1, and whether any
5 effect is mediated by changes in related parameters such as weight or glycaemia. Various
6 small studies of short duration have investigated the effect of metformin therapy on
7 circulating GLP-1 levels in individuals with and without T2DM¹³⁻²⁰. While results have been
8 inconsistent, some have shown increases in active GLP-1 and total GLP-1 in both the fasting
9 and post-prandial states. To date, however, no suitable studies have been conducted to
10 robustly investigate whether metformin therapy influences circulating GLP-1 levels in
11 individuals with and without T2DM, whether any observed effect is sustained in the longer
12 term (i.e. beyond a few weeks), and whether any effect is related to changes in other variables
13 which metformin is known to impact on, such as weight and glycaemia. To address these
14 questions, we performed complementary studies namely an ancillary study using data from a
15 randomized placebo-controlled repeated measures study with 18 months follow-up, the
16 Carotid Atherosclerosis: Metformin for insulin ResistAnce (CAMERA)²¹ and a cross-
17 sectional study from the Diabetes Research on Patient StraTification (DIRECT)
18 consortium²².

41 **Materials and Methods**

42
43
44
45 CAMERA was a randomized double-blinded placebo-controlled trial designed to investigate
46 the effect of metformin on surrogate markers of cardiovascular disease in patients without
47 diabetes, aged 35 to 75, with established coronary heart disease and a large waist
48 circumference (≥ 94 cm in men, ≥ 80 cm in women) ([NCT00723307](#)). This single-centre trial
49 enrolled 173 adults who were followed up for 18 months each. Patients attended the research
50 centre every 6 months in a fasted state. A detailed description of the trial and its results has
51
52
53
54
55
56
57
58
59
60

1
2
3 been published previously²¹. Participants were randomized 1:1 to 850mg metformin or
4
5 matched placebo twice daily with meals though they could reduce the dose to once daily
6
7 based on side-effects for the duration of the trial. Weight was measured in light clothing
8
9 using a bio-impedance scale. While bio-impedance body fat results were available from the
10
11 trial, we opted to measure circulating leptin levels as a better marker of body fat.
12
13

14
15 The DIRECT (DIabetes Research on Patient StraTification) study (www.direct-diabetes.org)
16
17 is part of a European Union Innovative Medicines Initiative project, with the overarching aim
18
19 to discover and validate biomarkers of rapid diabetes development, progression and drug
20
21 response²². It involves four industrial partners and 21 academic institutes within Europe. As
22
23 part of Work Package 2 that aimed to identify predictive biomarkers of glycaemic
24
25 deterioration, deep phenotyping and biochemical assays were performed in 836 people
26
27 recently diagnosed with T2D who had been on either metformin or life-style therapy alone at
28
29 baseline. The 18 months follow up data are being collected. For this study, complete cross-
30
31 sectional data were analysed from the baseline visit in 775 participants from all six clinical
32
33 centres.
34
35
36

37 38 *Sample assays*

39
40 In CAMERA, participants attended six monthly visits after overnight fasts and before taking
41
42 their morning dose of metformin. Blood samples collected during the trial were centrifuged at
43
44 4 degrees Celsius soon after sampling, separated and stored at -80°C at the Western
45
46 Infirmary's Clinical Research Facility, Glasgow, for subsequent analyses. Six monthly
47
48 fasting plasma glucose, fasting insulin and HbA1c were analysed as previously described²¹.
49
50 We calculated the Homeostasis Model Assessment for Insulin Resistance (HOMA2-IR) index
51
52 using the HOMA Calculator (v2.2.3, <https://www.dtu.ox.ac.uk/homacalculator/>). Using
53
54 available stored EDTA plasma samples, six monthly total GLP-1 levels (Meso scale
55
56
57
58
59
60

1
2
3 discovery, Maryland, USA) were measured with commercially available
4
5 electrochemiluminescence assay (Meso scale discovery, Maryland, USA). Leptin levels were
6
7 measured with a commercially available enzyme-linked immunosorbent assay (R&D systems
8
9 Oxon, UK). For total GLP-1, the mean inter-assay and intra-assay coefficients of variation
10
11 (CVs) were 2.6% and 17.3% respectively. For leptin, the mean inter-assay and intra-assay
12
13 CVs were 10.1% and 6.3%. All time points for an individual participant were run on the same
14
15 plate, blinded to treatment arm.
16
17

18
19
20 For the DIRECT study, blood samples were collected in the morning after a 10 hour
21
22 overnight fast. Metformin was stopped for the 24 hours preceding the study visit and restarted
23
24 immediately thereafter. For a Mixed Meal Test (MMT), participants drank 250mL Fortisip
25
26 liquid drink (18.4g carbohydrate/100mL) over 2-5 minutes. Blood samples were taken
27
28 immediately prior to the drink (time 0) and then every 30 minutes up to 120 minutes.
29
30 Samples for GLP-1 measurement were collected using P800 (for active GLP-1) and EDTA
31
32 tubes (for total GLP-1) (Becton Dickenson, UK) at 0 and 60 minutes. The same commercial
33
34 kits were used to measure GLP-1 levels as in CAMERA. In DIRECT, the mean intra- and
35
36 inter-assay CVs for active GLP-1 were 9% and 10%, respectively. For total GLP-1, these
37
38 CVs were 6% and 9%, respectively.
39
40
41
42
43
44

45 *Ethics and consent*

46
47 All participants provided written informed consent for participation in both studies. For the
48
49 CAMERA study, this included permission for biochemical assays that were not planned at
50
51 the time of the trial. The CAMERA trial was approved by the Medicines and Healthcare
52
53 Products Regulatory Agency and West Glasgow Research Ethics Committee. In DIRECT,
54
55
56
57
58
59
60

1
2
3 each partner clinical centre obtained approval from their respective research ethics review
4
5 boards.
6
7

8 9 *Statistics*

10
11 Normality was assessed for all variables and non-normally distributed data were transformed
12
13 using the natural log value where relevant (specifically for active GLP-1, total GLP-1, leptin
14
15 and HOMA2-IR).
16
17

18
19
20 In the CAMERA study, analyses were performed for the modified intention-to-treat
21
22 population (i.e. participants with a baseline total GLP-1 and at least one subsequent total
23
24 GLP-1 result). The effect of metformin on total GLP-1 was investigated using two different
25
26 approaches. First, repeated-measures analysis was carried out, allowing a comparison of
27
28 metformin-treated and placebo-treated participants over the entire trial (assuming a general
29
30 covariance structure). Repeated-measures analyses were only performed after demonstrating
31
32 that there was no significant treatment-by-visit interaction (i.e. that any observed effect was
33
34 stable over the trial). Secondly, analyses of covariance (ANCOVA) were carried out to
35
36 determine the effect of metformin versus placebo on total GLP-1 at 6, 12 and 18 months
37
38 respectively. Additional on-treatment analyses were performed to assess whether any change
39
40 in total GLP-1 due to metformin was related to simultaneous changes in weight, HOMA2-IR,
41
42 HbA1c, leptin or all four variables combined by adding these as cofactors.
43
44
45
46
47
48

49
50 In DIRECT, fasting active and total GLP-1, and 60-minute post-meal total GLP-1 levels were
51
52 compared between metformin and lifestyle groups using Student's T-tests. Anthropometric
53
54 measures (age, sex, waist to hip ratio [WHR], BMI), lifestyle factors (smoking and alcohol
55
56
57
58
59
60

1
2
3 use), HbA1c, fasting glucose and centre were investigated regarding any influence of
4
5 metformin on GLP-1 levels using linear regression models.
6
7

8
9
10 Due to the natural log transformation for GLP-1 measures, results are presented as the
11
12 percentage differences in geometric means of GLP-1 measures on metformin vs. placebo or
13
14 metformin vs. lifestyle to aid interpretation. The same approach was taken to present leptin
15
16 results. Statistical analyses were carried out using the statistical packages SPSS (version 22,
17
18 SPSS Inc., Chicago, Ill) and R (version 3.0.1). A two-sided p-value of 0.05 was used as the
19
20 threshold for statistical significance.
21
22

23 24 25 **Results**

26
27
28
29 Baseline characteristics for the CAMERA and DIRECT participants are summarized in
30
31 **Supplementary Table 1** and **Table 1**, respectively. It has previously been reported that
32
33 metformin led to falls in HbA1c (1.4mmol/mol), fasting insulin (21%), Homeostasis Model
34
35 Assessment of Insulin Resistance (HOMA-IR; 26%) and weight (3.2kg) compared to placebo
36
37 over 1.5 years in CAMERA.
38
39

40
41
42
43 In DIRECT there was no significant difference in age, sex, BMI, duration of diabetes or
44
45 HbA1c between the metformin and non-metformin treated groups. Metformin treated
46
47 individuals had a higher fasting glucose (<0.001) and a slightly higher WHR than those on no
48
49 treatment (p=0.045) in DIRECT.
50
51

52
53
54 *CAMERA results: metformin increases fasting total GLP-1 over 18 months*
55
56
57
58
59
60

1
2
3 The geometric mean for total GLP-1 was 11.6pg/mL in metformin recipients and 12.4pg/mL
4 in placebo recipients at baseline. Metformin therapy led to significant increases in fasting
5 total GLP-1 compared to placebo at each of the 6, 12 and 18-month study visits (see **Table 2**
6 and **Figure 1A**). The increases in total GLP-1 at these visits were 21% (p=0.010), 27%
7 (p=0.001) and 19% (p=0.012) respectively. In repeated-measures analysis, metformin
8 increased total GLP-1 by 23.4% (p<0.0001) across the entire duration of the 18 month
9 follow-up with no evidence of heterogeneity between study visits (p=0.74).
10
11
12
13
14
15
16
17
18
19

20
21 Leptin levels fell with metformin treatment in keeping with a reduction in body fat (see
22 **Table 2**). Overall, metformin therapy reduced leptin by 25% (p<0.0001) compared to placebo
23 with similar changes observed at each visit.
24
25
26
27
28
29

30 Adjustment for the observed changes in weight, HOMA2-IR, HbA1c and leptin at each visit,
31 whether individually or combined, did not attenuate metformin's effect on total GLP-1 (see
32 **Table 3**). Adjusted comparisons (for all four parameters) at 6, 12 and 18 months showed
33 increased in total GLP-1 of 32% (p=0.001), 35% (p≤0.001) and 26% (p=0.002) respectively
34 for metformin compared to placebo therapy.
35
36
37
38
39
40
41
42

43 *DIRECT results: the association of metformin with fasting and post-meal GLP-1*

44
45 The geometric mean for total fasted GLP-1 was 7.9pg/mL in metformin recipients and
46 6.9pg/mL in lifestyle-treated patients. Metformin users had higher basal fasted active GLP-1
47 (+25.5% [95%CI 17.0-35.5%], p<0.001) and fasted total GLP-1 (+14.5% [95%CI 8.4-
48 21.0%], p=0.0097) than individuals who were on lifestyle therapy (see **Table 1** and **Figure**
49 **1B**). These differences persisted after controlling for anthropometric measures (age, sex,
50 waist to hip ratio, BMI), lifestyle factors (smoking and alcohol), study centre and HbA1c for
51
52
53
54
55
56
57
58
59
60

1
2
3 both fasted active and fasted total GLP-1 (+39.1% [21.3-56.4%]; p=1.35e-05 and +14.1%
4 [1.2-25.9%] respectively; p=0.03). Replacing HbA1c with fasting glucose in these models did
5
6 not materially alter these results. There was no difference in the 60 minute total GLP-1
7
8 concentration between metformin users and non-metformin users after adjusting for these
9
10 covariates and baseline total GLP-1 (4.4% [95%CI -0.5-9.4%]; p=0.27).
11
12
13
14
15

16 **Discussion**

17
18
19
20 In these complementary studies we sought further information regarding the relationship
21
22 between metformin therapy and circulating GLP-1. We demonstrate that daily metformin
23
24 therapy for 18 months led to a 25% increase in circulating total GLP-1 levels in individuals
25
26 without diabetes but with elevated waist circumferences, and this increase was sustained
27
28 across the entire duration of the study and did not appear to be related to any changes in
29
30 glycaemia or adiposity. In recently diagnosed T2D individuals, metformin treatment was
31
32 associated with higher fasted active and fasted total, but not incremental, GLP-1 levels. In
33
34 both studies, these differences in GLP-1 levels occurred despite the previous dose of
35
36 metformin having been taken the day before each visit (>24 hours in DIRECT), suggesting
37
38 that circulating GLP-1 levels probably remain consistently elevated in patients on metformin
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

Active GLP-1 is secreted by gastro-intestinal L cells in response to the presence of nutrients in the small intestine leading to an increase in glucose-stimulated insulin secretion and suppressed glucagon secretion. GLP-1 also delays gastric emptying and promotes satiety. This bioactive form of the hormone is rapidly metabolized by the enzyme DPP-4 with the result that its half-life in the circulation is less than two minutes. Understanding the incretin

1
2
3 pathway led to the development of related medications, namely GLP-1 receptor agonists
4
5 (incretin mimetics) and DPP-4 inhibitors (incretin enhancers)²³, which are designed to
6
7 directly or indirectly increase the in vivo activity of GLP-1.
8
9

10
11 Previous small studies with various designs have produced mixed, often null, results but with
12
13 some suggesting that metformin therapy increases circulating GLP-1 levels by various
14
15 mechanisms²⁴ (see Supplementary Table 2). In a study of 10 obese participants without
16
17 diabetes and 10 controls who were given metformin 2.55g/d for two weeks, GLP-1 levels at
18
19 30 and 60 minutes after a glucose load were increased though baseline GLP-1 levels (and
20
21 leptin) were unchanged on metformin¹³. An uncontrolled study of metformin therapy (2g/d)
22
23 in 40 women with polycystic ovarian syndrome over 8 months, albeit with substantial loss to
24
25 follow up with only 22 women completing metformin therapy, produced similar findings to
26
27 our own with a 25% increase in area-under-the-curve GLP-1 levels over 180 minutes during
28
29 oral glucose loading compared to baseline¹⁴. A crossover study of 10 individuals with T2DM
30
31 given three single dose interventions on three different days a week apart (either metformin
32
33 1g plus placebo subcutaneous injection; or placebo tablet plus subcutaneous GLP-1; or
34
35 metformin 1g plus subcutaneous GLP-1)¹⁵. Glucose was infused to achieve a concentration of
36
37 approximately 15mmol/L. Analyses showed that metformin therapy inhibited DPP-4 activity
38
39 and also increased active GLP-1 levels. In a further crossover study conducted in 20
40
41 participants with T2DM who were treated for 6 days with each of four respective regimens
42
43 (placebo or metformin or sitagliptin or the combination) with washout periods in between
44
45 interventions, metformin therapy led to an increase in fasted and post-challenge total GLP-1
46
47 levels though no change in intact GLP-1 levels¹⁶. And in a crossover study of 12 participants
48
49 with T2DM treated with placebo or metformin for seven days respectively and then
50
51 investigated during intraduodenal catheter infusion of glucose, DPP-4 activity fell modestly
52
53
54
55
56
57
58
59
60

1
2
3 while intact and total GLP-1 levels rose at baseline and during the infusion after metformin¹⁷.
4
5 By contrast, a crossover study of 16 participants with T2DM treated for 4 weeks respectively
6
7 with placebo, metformin, sitagliptin and combined metformin / sitagliptin yielded no increase
8
9 in active GLP-1 on metformin¹⁸. Other studies have suggested no effect on DPP-4 activity. In
10
11 a study of eight drug-naïve participants with T2DM treated with metformin for three months,
12
13 the area-under-the-curve for active GLP-1 over 6 hours following a standard mixed meal rose
14
15 though DPP-4 activity was unchanged¹⁹. It is therefore apparent that most studies in this area
16
17 have been limited by small sample size (and therefore reduced power) and that most have
18
19 focused on the acute effect of metformin therapy as opposed to its longer term effects.
20
21
22 Animal studies have produced similarly mixed results including evidence of an acute increase
23
24 in GLP-1 with metformin treatment and of DPP-4 inhibition in some studies but not all²⁵⁻²⁷.
25
26
27 In contrast our results have examined the relationship of metformin with circulating GLP-1 in
28
29 large cohorts with and without type 2 diabetes and addressed long term effects of metformin
30
31 over 18 months in non-diabetic individuals.
32
33
34
35

36
37 In individuals without diabetes, our finding that the increase in GLP-1 was not related to the
38
39 observed 3.2kg decrease in weight or the 25% improvement in insulin sensitivity is in
40
41 keeping with a direct effect of metformin on the incretin axis. The sustained nature of the
42
43 GLP-1 increase suggests the possibility that metformin may in part provide cardio-metabolic
44
45 benefit, even in a non-diabetic population and beyond reducing the risk of developing T2DM,
46
47 by increasing exposure of treated individuals to GLP-1 in the longer term. This is supported
48
49 by findings from both recently completed outcomes trials of GLP-1 receptor agonists^{9,10} and
50
51 from a Mendelian¹¹ randomization study of a GLP-1 receptor variant associated with lower
52
53 fasting glucose which was also associated with lower risk of coronary heart disease. It also
54
55 provides further rationale to test these potential benefits of metformin in a population without
56
57
58
59
60

1
2
3 diabetes. The Glucose Lowering In Non-diabetic hyperglycaemia Trial (GLINT;
4
5 ISRCTN34875079) is studying whether metformin reduces cardiovascular risk as well as
6
7 cancer and other outcomes in non-diabetic participants.
8
9

10
11
12 Our study has numerous strengths. The CAMERA study is by far the largest and longest trial
13
14 to address the question of metformin's impact on circulating GLP-1 levels and its randomized
15
16 design minimises the possibility of unmeasured or unaccounted for confounding. Though
17
18 cross-sectional and therefore unable to directly address causality, DIRECT is the largest
19
20 study to investigate the association of metformin with GLP-1 levels in T2DM individuals,
21
22 and was able to adjust for a range of potential confounding factors. The CAMERA trial was
23
24 specifically conducted in participants without T2DM (though with elevated waist
25
26 circumferences) which enabled us to avoid the potential effects of other glucose-lowering
27
28 agents and also to provide novel data on a group at high risk of T2DM in whom metformin is
29
30 being investigated in a major trial, GLINT. Samples were available at 6 month intervals,
31
32 providing data on the sustained effect of metformin on GLP-1 levels. An important weakness
33
34 of the CAMERA trial was that we did not have access to suitably prepared samples to allow
35
36 the measurement of active GLP-1 levels and only fasted samples were available. However, in
37
38 the DIRECT study in which we had access to both active and total fasted GLP-1 levels,
39
40 metformin recipients demonstrated clearly higher levels of both, in particular active GLP-1.
41
42 Notably, however, in both studies, higher GLP-1 levels were noted despite the last metformin
43
44 dose having been taken the day before blood sampling which, in the context of the limited
45
46 bioavailability of metformin (less than 60%), suggests that some of this effect may reflect the
47
48 impact of the drug in the distal small intestine and colon as highlighted in other studies²⁸.
49
50 Consistent with this, the apparent impact of metformin in DIRECT was on fasting GLP-1
51
52 rather than meal stimulated GLP-1. In addition, the fact that some CAMERA participants
53
54
55
56
57
58
59
60

1
2
3 reduced their metformin dose and, in some cases, stopped trial medication suggests that our
4
5 results are likely to be an underestimation of the true effect of metformin on fasting total
6
7 GLP-1 in this population.
8
9

10
11 Further studies are needed to determine the longitudinal effect of metformin on GLP-1 levels
12
13 in diabetic individuals. Additional research on the mechanism by which metformin increases
14
15 GLP-1 would also be useful, including whether this effect is largely a direct of metformin on
16
17 L-cells or is mediated indirectly via metformin's many other effects on the gastro-intestinal
18
19 tract such as altering the microbiome or decreasing bile acid reabsorption²⁹.
20
21
22
23
24

25 In summary, we report evidence from two major studies demonstrating that metformin
26
27 treatment leads to a sustained and long term increase in circulating total GLP-1 levels in non-
28
29 diabetic individuals independent of changes in weight and glycaemia, while metformin
30
31 therapy is also associated with higher fasted total and active GLP-1 in diabetic patients,
32
33 independent of weight and glycaemia. These complementary findings support a potential
34
35 direct role for the incretin axis on the cardiometabolic benefits of metformin.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Acknowledgements:** DP is guarantor for CAMERA data and ERP for DIRECT data.

4
5 CAMERA was funded by the Chief Scientist Office, Scotland (CZB/4/613). We are grateful
6
7 to Sara Jane Duffus and Elaine Butler for analysing GLP-1 on the CAMERA samples. The
8
9 work leading to this publication has received support from the Innovative Medicines
10
11 Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which
12
13 are composed of financial contribution from the European Union's Seventh Framework
14
15 Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. ERP holds a
16
17 Wellcome Trust New Investigator Award (ref 102820/Z/13/Z).
18
19

20
21
22 **Contributors:** DP had the idea for and designed the analysis, analysed and interpreted data,
23
24 wrote the first draft and revised later drafts of the article. AD analysed the DIRECT data and
25
26 contributed to the manuscript. ERP, PWF, AJ and MW participated in the design and sample
27
28 collection of the DIRECT diabetes progression study, interpreted the data and revised the
29
30 manuscript. PW co-ordinated the laboratory work for GLP-1 and leptin measurements,
31
32 interpreted data and revised the article. CS analysed leptin, analysed and interpreted the data
33
34 and revised the article. RRH interpreted the data and revised the article. NS had the idea for
35
36 and designed the analysis, interpreted data, revised the article, and supervised the analysis.
37
38
39
40
41 DIRECT collaborators are listed in Supplementary Table 3.
42
43

44
45 **Conflicts of interest:** DP, PW, CS, AJ, THH, JD, and RK report no conflicts of interest.

46
47 RRH has received research support from Amylin, Bayer, Merck, and Novartis; participated in
48
49 advisory boards for Amylin, Lilly, Merck, Novartis, and Novo Nordisk; and received
50
51 compensation for lectures from Bayer, Lilly, Merck, and Merck Serono. ERP has received
52
53 lecture fees from Eli Lilly, Novo Nordisk, Astra Zeneca and Sanofi. NS has consulted for Eli
54
55 Lilly, Bristol-Myers Squibb, Merck Sharp & Dohme, AstraZeneca, Sanofi, and Boehringer
56
57
58
59
60

1
2
3 Ingelheim; and received research support from Merck. PWF has received consulting
4
5 honoraria from Sanofi Aventis and Eli Lilly Inc, and research support from Novo Nordisk.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only

REFERENCES

1. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2012; **55**(6): 1577-96.
2. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**(9131): 854-65.
3. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**(6): 393-403.
4. Diabetes Prevention Program Research G. Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. *Diabetes Care* 2012; **35**(4): 731-7.
5. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**(14): 1317-26.
6. White WB, Cannon CP, Heller SR, et al. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**(14): 1327-35.
7. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**(3): 232-42.
8. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med* 2015; **373**(23): 2247-57.
9. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; **375**(4): 311-22.
10. Marso SP, Bain SC, Consoli A, et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016. (online ahead of print)
11. Scott RA, Freitag DF, Li L, et al. A genomic approach to therapeutic target validation identifies a glucose-lowering GLP1R variant protective for coronary heart disease. *Sci Transl Med* 2016; **8**(341): 341ra76.
12. Potts JE, Gray LJ, Brady EM, Khunti K, Davies MJ, Bodicoat DH. The Effect of Glucagon-Like Peptide 1 Receptor Agonists on Weight Loss in Type 2 Diabetes: A Systematic Review and Mixed Treatment Comparison Meta-Analysis. *PLoS One* 2015; **10**(6): e0126769.

- 1
2
3 13. Mannucci E, Ognibene A, Cremasco F, et al. Effect of metformin on glucagon-like
4 peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care* 2001; **24**(3):
5 489-94.
6
- 7
8 14. Svendsen PF, Nilas L, Madsbad S, Holst JJ. Incretin hormone secretion in women
9 with polycystic ovary syndrome: roles of obesity, insulin sensitivity, and treatment with
10 metformin. *Metabolism* 2009; **58**(5): 586-93.
11
- 12
13 15. Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous
14 glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations
15 and greater glucose lowering in type 2 diabetes mellitus. *Metabolism* 2011; **60**(1): 52-6.
16
- 17
18 16. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and
19 metformin treatment on incretin hormone and insulin secretory responses to oral and
20 "isoglycemic" intravenous glucose. *Diabetes* 2014; **63**(2): 663-74.
21
- 22
23 17. Wu T, Thazhath SS, Bound MJ, Jones KL, Horowitz M, Rayner CK. Mechanism of
24 increase in plasma intact GLP-1 by metformin in type 2 diabetes: stimulation of GLP-1
25 secretion or reduction in plasma DPP-4 activity? *Diabetes Res Clin Pract* 2014; **106**(1): e3-6.
26
- 27
28 18. Solis-Herrera C, Triplitt C, Garduno-Garcia Jde J, Adams J, DeFronzo RA,
29 Cersosimo E. Mechanisms of glucose lowering of dipeptidyl peptidase-4 inhibitor sitagliptin
30 when used alone or with metformin in type 2 diabetes: a double-tracer study. *Diabetes Care*
31 2013; **36**(9): 2756-62.
32
- 33
34 19. Thondam SK, Cross A, Cuthbertson DJ, Wilding JP, Daousi C. Effects of chronic
35 treatment with metformin on dipeptidyl peptidase-4 activity, glucagon-like peptide 1 and
36 ghrelin in obese patients with Type 2 diabetes mellitus. *Diabet Med* 2012; **29**(8): e205-10.
37
- 38
39 20. Migoya EM, Bergeron R, Miller JL, et al. Dipeptidyl peptidase-4 inhibitors
40 administered in combination with metformin result in an additive increase in the plasma
41 concentration of active GLP-1. *Clin Pharmacol Ther* 2010; **88**(6): 801-8.
42
- 43
44 21. Preiss D, Lloyd SM, Ford I, et al. Metformin for non-diabetic patients with coronary
45 heart disease (the CAMERA study): a randomised controlled trial. *Lancet Diabetes*
46 *Endocrinol* 2014; **2**(2): 116-24.
47
- 48
49 22. Koivula RW, Heggie A, Barnett A, et al. Discovery of biomarkers for glycaemic
50 deterioration before and after the onset of type 2 diabetes: rationale and design of the
51 epidemiological studies within the IMI DIRECT Consortium. *Diabetologia* 2014; **57**(6):
52 1132-42.
53
- 54
55 23. Ahren B, Schmitz O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment
56 of type 2 diabetes. *Horm Metab Res* 2004; **36**(11-12): 867-76.
57
58
59
60

- 1
2
3 24. Bahne E, Hansen M, Bronden A, Sonne DP, Vilsboll T, Knop FK. Involvement of
4 glucagon-like peptide-1 in the glucose-lowering effect of metformin. *Diabetes Obes Metab*
5 2016; **18**(10): 955-61.
6
7
8 25. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor
9 axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice.
10 *Diabetologia* 2011; **54**(2): 339-49.
11
12 26. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms
13 underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell.
14 *Endocrinology* 2011; **152**(12): 4610-9.
15
16 27. Green BD, Irwin N, Duffy NA, Gault VA, O'Harte F P, Flatt PR. Inhibition of
17 dipeptidyl peptidase-IV activity by metformin enhances the antidiabetic effects of glucagon-
18 like peptide-1. *Eur J Pharmacol* 2006; **547**(1-3): 192-9.
19
20 28. Buse JB, DeFronzo RA, Rosenstock J, et al. The Primary Glucose-Lowering Effect of
21 Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic
22 and 12-Week Dose-Ranging Studies. *Diabetes Care* 2016; **39**(2): 198-205.
23
24 29. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract.
25 *Diabetologia* 2016; **59**(3): 426-35.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Characteristics of DIRECT participants

Characteristics	Metformin (n=270)	Lifestyle (n=505)	P
Males (%)	151 (55.9%)	295 (58.4%)	0.70
Age (years)*	63 (35-75)	64 (35-75)	0.064
Duration of diabetes (years)	1.18 (0.82)	1.25 (0.75)	0.10
Weight (kg)	88.9 (16.7)	89.7 (17.0)	0.53
BMI (kg/m ²)	30.1 (4.7)	30.7 (5.1)	0.11
WHR	0.97 (0.08)	0.96 (0.08)	0.045
HbA1c (%)	6.40 (0.60)	6.34 (0.59)	0.20
HbA1c (mmol/mol)	46.44 (6.57)	45.81 (6.39)	0.20
Fasting plasma glucose (mmol/L)	7.49 (1.47)	6.94 (1.37)	<0.001
Fasting active GLP-1 (pg/ml) †	0.45 (0.40-0.51)	0.36 (0.33-0.39)	<0.001
Fasting total GLP-1 (pg/ml) †	7.9 (7.2-8.5)	6.9 (6.4-7.3)	0.0097
60-min total GLP-1 (pg/ml) †	16.0 (14.9 – 17.3)	14.3 (13.3-15.3)	0.031

Data presented as mean (SD) or n (%) except where indicated (*median [range]; †geometric mean [95% CI])

Table 2: Change in GLP-1 and leptin levels on metformin vs. placebo over 18 months in CAMERA

	Visit (nr of paired samples)	Metformin vs. Placebo *	Average treatment effect (Metformin – Placebo) †		p-value for interaction across visits
			Effect (95% CI)	p-value	
GLP-1 (natural log units)	<u>6 months</u> (n=150)	0.188 (0.046, 0.329)	0.210 (0.106, 0.314)	<0.0001	0.74
	<u>12 months</u> (n=146)	0.237 (0.098, 0.376)			
	<u>18 months</u> (n=157)	0.172 (0.038, 0.305)			
GLP-1 (%) ‡	<u>6 months</u> (n=150)	20.7% (4.7, 39.0%)	23.4% (11.2, 36.9%)	<0.0001	0.74
	<u>12 months</u> (n=146)	26.7% (10.3, 45.6%)			
	<u>18 months</u> (n=157)	18.7 (3.8, 35.7%)			
Leptin (natural log units)	<u>6 months</u> (n=152)	-0.262 (-0.403, -0.120)	-0.286 (-0.419; -0.153)	<0.0001	0.80
	<u>12 months</u> (n=146)	-0.293 (-0.467, -0.118)			
	<u>18 months</u> (n=157)	-0.237 (-0.405, -0.069)			
Leptin (%)^c	<u>6 months</u> (n=152)	-23.1% (-33.2, -11.3%)	-24.9% (-34.2, -14.2%)	<0.0001	0.80
	<u>12 months</u> (n=146)	-25.4% (-37.3, -11.1%)			
	<u>18 months</u> (n=157)	-21.1% (-33.3, -6.7%)			

* ANCOVA analysis for visits at 6, 12 and 18 months respectively

† Repeated measures analysis for the overall treatment effect over 18 months

‡ Percentage difference in geometric means

Table 3. Effects of metformin on total GLP-1 without and with on-treatment adjustments for changes in key variables

Variable	Adjustment	Metformin-Placebo Mean % change (95%CI) ^a	P- value
GLP-1, 6 months	No adjustment	20.7 (4.7, 39.0)	0.010
	Weight	25.0 (7.6, 45.3)	0.004
	HOMA2-IR	24.6 (8.0, 43.7)	0.003
	HbA1c	26.1 (8.4, 46.8)	0.003
	Leptin	22.9 (6.4, 41.9)	0.005
	Combined†	32.4 (13.0, 55.1)	0.001
GLP-1, 12 months	No adjustment	26.7 (10.3, 45.6)	0.001
	Weight	35.0 (15.9, 57.3)	<0.001
	HOMA2-IR	27.2 (10.4, 46.7)	0.001
	HbA1c	28.7 (11.0, 49.2)	0.001
	Leptin	33.0 (15.7, 53.0)	<0.001
	Combined †	35.4 (15.8, 58.1)	<0.001
GLP-1, 18 months	No adjustment	18.7 (3.8, 35.7)	0.012
	Weight	23.5 (7.0, 42.5)	0.004
	HOMA2-IR	21.4 (6.2, 39.0)	0.005
	HbA1c	20.9 (5.3, 38.8)	0.007
	Leptin	20.8 (5.6, 38.2)	0.006
	Combined †	26.0 (9.1, 45.6)	0.002

The unadjusted result at each time point is provided, followed by the result adjusted for changes in weight, HOMA2-IR, HbA1c and leptin respectively; this is followed by the result adjusted for all these variables combined (indicated by †)

HOMA2-IR: Homeostasis Model Assessment of Insulin Resistance

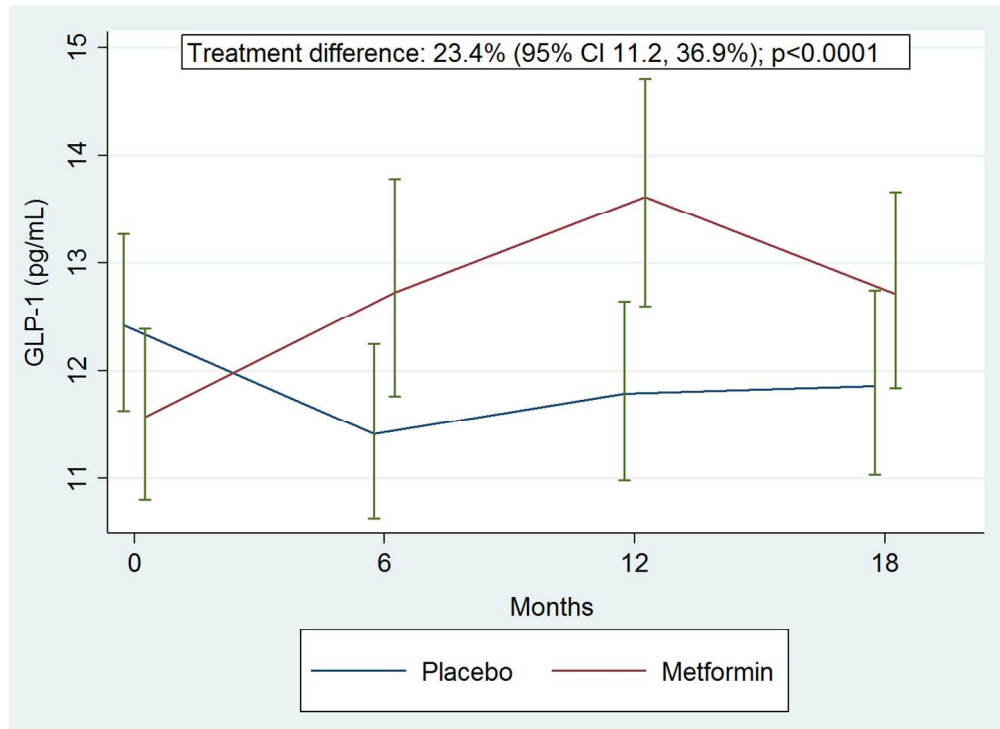
* Results displayed as percentage change in geometric mean (95% CI)

1
2
3 **Figure Legend**
4

5
6 **Figure 1.** Metformin and GLP-1 (A) total GLP-1 levels on metformin vs. placebo over 18
7
8 months in the CAMERA study (B) The association of metformin therapy vs. lifestyle
9
10 treatment with fasting active GLP-1, fasting total GLP-1 and incremental total GLP-1 in
11
12 DIRECT
13

14
15
16 *Footnote:* Data displayed as geometric mean (1SE) (A) and geometric mean (95% CI) (B)
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only



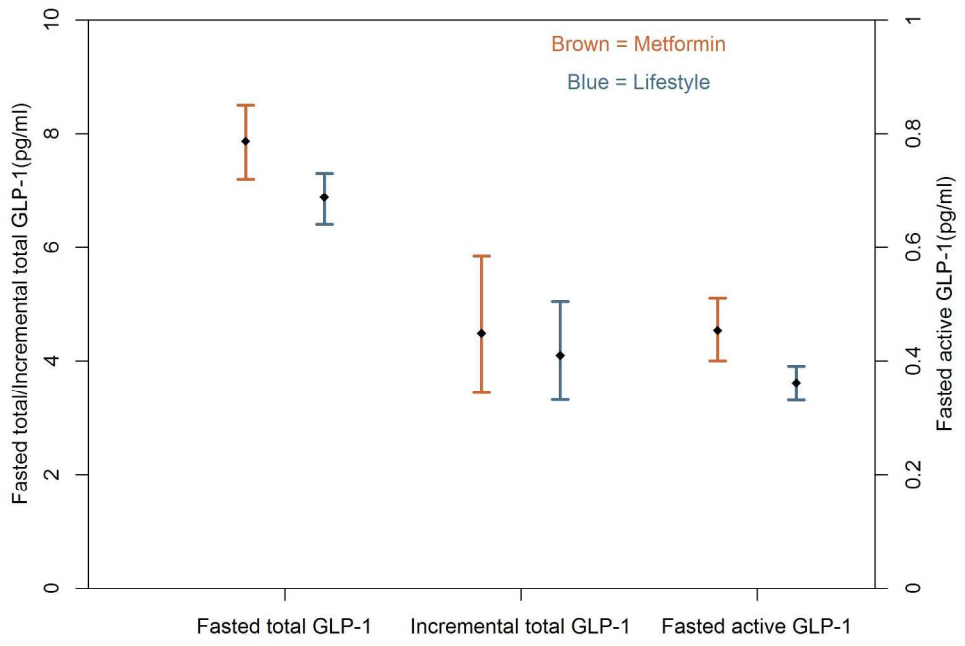
Metformin and GLP-1 (A) total GLP-1 levels on metformin vs. placebo over 18 months in the CAMERA study
(B) The association of metformin therapy vs. lifestyle treatment with fasting active GLP-1, fasting total GLP-1 and incremental total GLP-1 in DIRECT

512x372mm (72 x 72 DPI)

Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Metformin and GLP-1 (A) total GLP-1 levels on metformin vs. placebo over 18 months in the CAMERA study
(B) The association of metformin therapy vs. lifestyle treatment with fasting active GLP-1, fasting total GLP-1 and incremental total GLP-1 in DIRECT

203x139mm (300 x 300 DPI)

Only

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.	<ul style="list-style-type: none"> - ↓ 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	<ul style="list-style-type: none"> - ↓ 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - 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.	<ul style="list-style-type: none"> - Regulates GLP-1 receptor expression in pancreas - Protects GLP-1 cells against lipopoptosis - ↑ 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - ↑ 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	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - 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	<ul style="list-style-type: none"> - significant GLP-1 ↑ at 30 and 60 minutes after oral glucose load -no GLP-1 variation in controls

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

References for Supplementary Table 2

1. Kappe C, Zhang Q, Nystrom T, Sjöholm A. Effects of high-fat diet and the anti-diabetic drug metformin on circulating GLP-1 and the relative number of intestinal L-cells. *Diabetol Metab Syndr* 2014; **6**: 70.
2. Wu T, Thazhath SS, Bound MJ, Jones KL, Horowitz M, Rayner CK. Mechanism of increase in plasma intact GLP-1 by metformin in type 2 diabetes: stimulation of GLP-1 secretion or reduction in plasma DPP-4 activity? *Diabetes Res Clin Pract* 2014; **106**(1): e3-6.
3. Solis-Herrera C, Triplitt C, Garduno-Garcia Jde J, Adams J, DeFronzo RA, Cersosimo E. Mechanisms of glucose lowering of dipeptidyl peptidase-4 inhibitor sitagliptin when used alone or with metformin in type 2 diabetes: a double-tracer study. *Diabetes Care* 2013; **36**(9): 2756-62.
4. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and metformin treatment on incretin hormone and insulin secretory responses to oral and "isoglycemic" intravenous glucose. *Diabetes* 2014; **63**(2): 663-74.
5. Kappe C, Holst JJ, Zhang Q, Sjöholm A. Molecular mechanisms of lipoapoptosis and metformin protection in GLP-1 secreting cells. *Biochem Biophys Res Commun* 2012; **427**(1): 91-5.
6. Thondam SK, Cross A, Cuthbertson DJ, Wilding JP, Daousi C. Effects of chronic treatment with metformin on dipeptidyl peptidase-4 activity, glucagon-like peptide 1 and ghrelin in obese patients with Type 2 diabetes mellitus. *Diabet Med* 2012; **29**(8): e205-10.
7. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. *Endocrinology* 2011; **152**(12): 4610-9.
8. Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations and greater glucose lowering in type 2 diabetes mellitus. *Metabolism* 2011; **60**(1): 52-6.
9. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice. *Diabetologia* 2011; **54**(2): 339-49.
10. Migoya EM, Bergeron R, Miller JL, et al. Dipeptidyl peptidase-4 inhibitors administered in combination with metformin result in an additive increase in the plasma concentration of active GLP-1. *Clin Pharmacol Ther.* 2010; **88**(6): 801-8.
11. Svendsen PF, Nilas L, Madsbad S, Holst JJ. Incretin hormone secretion in women with polycystic ovary syndrome: roles of obesity, insulin sensitivity, and treatment with metformin. *Metabolism* 2009; **58**(5): 586-93.
12. Green BD, Irwin N, Duffy NA, Gault VA, O'Harte F P, Flatt PR. Inhibition of dipeptidyl peptidase-IV activity by metformin enhances the antidiabetic effects of glucagon-like peptide-1. *Eur J Pharmacol* 2006; **547**(1-3): 192-9.
13. Mannucci E, Tesi F, Bardini G, et al. Effects of metformin on glucagon-like peptide-1 levels in obese patients with and without Type 2 diabetes. *Diabetes Nutr Metab* 2004; **17**(6): 336-42.
14. Hinke SA, Kuhn-Wache K, Hoffmann T, Pederson RA, McIntosh CH, Demuth HU. Metformin effects on dipeptidylpeptidase IV degradation of glucagon-like peptide-1. *Biochem Biophys Res Commun* 2002; **291**(5): 1302-8.
15. Mannucci E, Ognibene A, Cremasco F, et al. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care* 2001; **24**(3): 489-94.

Supplementary Table 3. DIRECT collaborators

First Name	Surname	Initials	Institution Address
Christopher	Jennison	C.	Department of Mathematical Sciences, University of Bath, Bath, UK
Patrick	Baum	P.	Boehringer Ingelheim Pharma GmbH & Co. KG, Translational Medicine & Clinical Pharmacology, Birkendorferstr.65, 88397 Biberach an der Riss, Germany
Corinna	Schoelsch	C.	Boehringer Ingelheim Pharma GmbH & Co. KG, Translational Medicine & Clinical Pharmacology, Birkendorferstr.65, 88397 Biberach an der Riss, Germany
Jan	Freijer	J.I.	Boehringer Ingelheim Pharma GmbH & Co. KG, Translational Medicine & Clinical Pharmacology, Birkendorferstr.65, 88397 Biberach an der Riss, Germany
Rolf	Grempler	R.	Boehringer Ingelheim Pharma GmbH & Co. KG, Translational Medicine & Clinical Pharmacology, Birkendorferstr.65, 88397 Biberach an der Riss, Germany
Ulrike	Graefe-Mody	U.	Boehringer Ingelheim Pharma GmbH & Co. KG, Medicine Therapeutic Area Metabolism 1, Binger Strasse 173, 55216 Ingelheim am Rhein, Germany
Anita	Hennige	A.M.	Boehringer Ingelheim Pharma GmbH & Co. KG, Medicine Therapeutic Area Metabolism 1, Birkendorferstr.65, 88397 Biberach an der Riss, Germany
Christiane	Dings	C.	Clinical Pharmacy, Saarland University, Campus C2.2, 66123 Saarbrücken, Germany
Thorsten	Lehr	T.	Clinical Pharmacy, Saarland University, Campus C2.2, 66123 Saarbrücken, Germany
Nina	Scherer	N.	Clinical Pharmacy, Saarland University, Campus C2.2, 66123 Saarbrücken, Germany
Francois	Pattou	F.	Centre Hospitalier Régional Universitaire de Lille 2, Av Oscar Lambret 59037 Lille Cedex
Violeta	Raverdi	V.	Centre Hospitalier Régional Universitaire de Lille 2, Av Oscar Lambret 59037 Lille Cedex
Robert	Caiazzo	R.	Centre Hospitalier Régional Universitaire de Lille 2, Av Oscar Lambret 59037 Lille Cedex
Fanelly	Torres	F.	Centre Hospitalier Régional Universitaire de Lille 2, Av Oscar Lambret 59037 Lille Cedex
Helene	Verkindt	H.	Centre Hospitalier Régional Universitaire de Lille 2, Av Oscar Lambret 59037 Lille Cedex
Andrea	Mari	A.	Institute of Neuroscience, National Research Council, Corso Stati Uniti 4, 35127 Padova, Italy
Andrea	Tura	A.	Institute of Neuroscience, National Research Council, Corso Stati Uniti 4, 35127 Padova, Italy
Toni	Giorgino	T.	Institute of Neuroscience, National Research Council, Corso Stati Uniti 4, 35127 Padova, Italy
Loic	Yengo	L.	Centre National de la Recherche Scientifique CNRS, Délégation Nord Pas-de-Calais et Picardie du CNRS Espace Recherche et Innovation, 2, rue des Canonniers, 59046 LILLE CEDEX, France
Philippe	Froguel	P.	Centre National de la Recherche Scientifique CNRS, Délégation Nord Pas-de-Calais et Picardie du CNRS Espace Recherche et Innovation, 2, rue des Canonniers, 59046 LILLE CEDEX, France IMPERIAL COLLEGE LONDON, Department of Genomics of Common Disease, School Of Public Health, Hammersmith Hospital Campus, Imperial College Faculty of Medicine, Burlington-Danes building, Du Cane Road, London, W12 0NN , United Kingdom
Amelie	Bonneford	A.	Centre National de la Recherche Scientifique CNRS, Délégation Nord Pas-de-Calais et Picardie du CNRS Espace Recherche et Innovation, 2, rue des Canonniers, 59046 LILLE CEDEX, France
Mickael	Canouil	M.	Centre National de la Recherche Scientifique CNRS, Délégation Nord Pas-de-Calais et Picardie du CNRS Espace Recherche et Innovation, 2, rue des Canonniers, 59046 LILLE CEDEX, France
Veronique	Dhennin	V.	Centre National de la Recherche Scientifique CNRS, Délégation Nord Pas-de-Calais et Picardie du CNRS Espace Recherche et Innovation, 2, rue des Canonniers, 59046 LILLE CEDEX, France
Caroline	Brorsson	C.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Soren	Brunak	S.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Peter	Daavidsen	P.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Federico	De Masi	F.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Valborg	Gudmundsdóttir	V.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Ramneek	Gupta	R.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Helle	Krogh Pedersen	H.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Kristoffer	Rapacki	K.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Emil	Rydza	E.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Hans-Henrik	Staerfeldt	H.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Karina	Banasik	K.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark

Cecilia	Engel Thomas	C.E.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Peter	Sackett	P.W.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Piotr	Chmura	P.J.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Sanna	Herrgard	S.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Mia	Dybkjær Rønsholdt	M.	Center for Biological Sequence Analysis, Dept. of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark
Andreas	Fritsche	A.	Medizinische Universitätsklinik Tübingen, Eberhard Karls Universität Tübingen, Otfried Müller Straße 10, 72076 Tübingen, Germany
Annette	Peters	A.	Institute of Epidemiology II, Research Unit of Diabetes Epidemiology, Helmholtz Zentrum München Research Center for Environmental Health, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany German Center for Diabetes Research (DZD), Neuherberg, Germany; Helmholtz Zentrum München, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany
Barbara	Thorand	B.	Institute of Epidemiology II, Research Unit of Diabetes Epidemiology, Helmholtz Zentrum München Research Center for Environmental Health, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany German Center for Diabetes Research (DZD), Neuherberg, Germany; Helmholtz Zentrum München, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany
Jurek	Adamski	J.	Institute of Experimental Genetics, Genome Analysis Center, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany German Center for Diabetes Research (DZD), 85764 München-Neuherberg, Germany
Harald	Grallert	H.	Institute of Epidemiology II, Research Unit of Molecular Epidemiology, Helmholtz Zentrum München Research Center for Environmental Health, Neuherberg, Germany German Center for Diabetes Research (DZD), Neuherberg, Germany; Clinical Cooperation Group Type 2 Diabetes, Helmholtz Zentrum München, Neuherberg, Germany; Clinical Cooperation Group Nutrigenomics and Type 2 Diabetes, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany Technische Universität München, 85350 Freising-Weihenstephan, Germany
Mark	Haid	M.	Institute of Experimental Genetics, Genome Analysis Center, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany; German Center for Diabetes Research (DZD), 85764 München-Neuherberg, Germany
Tonia	Herasgala	T.	Institute of Epidemiology II, Research Unit of Diabetes Epidemiology, Helmholtz Zentrum München Research Center for Environmental Health, Ingolstaedter Landstr. 1, 85764 Neuherberg, Germany;
Jorge	Ferrer	J.	Institut d'Investigacions Biomediques August Pi i Sunye, Centre Esther Koplowitz, c/Rosselló 153, Barcelona 08036, Spain
			Imperial Centre for Translational and Experimental Medicine, Du Cane Road, London W12 0NN, United Kingdom
Elin	Birgersson	E.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Mun-gwan	Hong	M.G.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Peter	Nilsson	P.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Sanna	Byström	S.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Jochen	Schwenk	J.M.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Mathias	Uhlen	M.	Affinity Proteomics, Science for Life Laboratory, School of Biotechnology, KTH - Royal Institute of Technology, Box 1031, SE-171 21 Solna, Sweden
Melissa	Thomas	M.K.	Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, 46285, USA
Han	Wu	H.	Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, 46285, USA
Leen	't Hart	L.M.	Leiden University Medical Center, Dept. of Molecular Cell Biology, Albinusdreef 2, 2333ZA Leiden, The Netherlands Leiden University Medical Center, Dept. of Molecular Epidemiology, Albinusdreef 2, 2333ZA Leiden, The Netherlands VU University Medical Center, Dept. of Epidemiology & Biostatistics, De Boelelaan 1089a, 1081 HV Amsterdam, The Netherlands
Nienke	van Leeuwen	N.	Leiden University Medical Center, Dept. of Molecular Cell biology, Albinusdreef 2, 2333ZA Leiden, The Netherlands
Karla	Allebrandt	K.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Francesca	Frau	F.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Johann	Gassenhuber	J.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Mathias	Gebauer	M.	DSAR, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Bernd	Jablonka	B.	Strategy & Innovation, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Katharina	Michalik	K.	DSAR, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Petra	Musholt	P.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Elizabeth	Ramos Lopez	E.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Hartmut	Ruetten	H.	Diabetes Division, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main

Joachim	Tillner	J.	Clinical Operations, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany, 65926 Frankfurt am Main
Tania	Baltauss	T.	Translational & Clinical Research, Metabolism Innovation Pole- Institut de Recherches Internationales Servier- 92284 Suresnes Cedex- FRANCE
Oana	Bernard Poenaru	O.	Translational & Clinical Research, Metabolism Innovation Pole- Institut de Recherches Internationales Servier- 92284 Suresnes Cedex- FRANCE
Nathalie	de Preville	N.	Translational & Clinical Research, Metabolism Innovation Pole- Institut de Recherches Internationales Servier- 92284 Suresnes Cedex- FRANCE
Marianne	Rodriquez	M.	Biotech&Biomarkers Research Department-Institut de Recherches Internationales Servier, 78290 Croissy sur Seine - FRANCE
Manimozhiyan	Arumugam	M.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Kristine	Allin	K.H.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Line	Engelbrechtsen	L.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Neils	Grarup	N.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Torben	Hansen	T.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Tue	Hansen	T.H.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Anna	Jonsson	A.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Nikolaj	Krarup	N.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Oluf	Pedersen	O.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Mathilde	Svendstrup	M.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Trine	Nielsen	T.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Henrik	Vestergaard	H.	The Novo Nordisk Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark, DK-2100
Markku	Laakso	M.	Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, 70210 Kuopio, Finland
Johanna	Kuusisto	J.	Institute of Clinical Medicine, Internal Medicine, University of Eastern Finland, 70210 Kuopio, Finland
Paul	Franks	P.W.	Genetic and Molecular Epidemiology, Department of Clinical Science, Lund University, Skåne University Hospital Malmö, CRC, 91-10, 205 02, Malmö, Sweden
Leif	Groop	L.	Department of Clinical Sciences, Diabetes & Endocrinology Unit, Lund University, Skåne University Hospital Malmö, CRC, 91-12, 205 02, Malmö, Sweden
Robert	Koivula	R.W.	Genetic and Molecular Epidemiology, Department of Clinical Science, Lund University, Skåne University Hospital Malmö, CRC, 91-10, 205 02, Malmö, Sweden
Azra	Kurbasic	A.	Genetic and Molecular Epidemiology, Department of Clinical Science, Lund University, Skåne University Hospital Malmö, CRC, 91-10, 205 02, Malmö, Sweden
Martin	Ridderstrale	M.	Department of Clinical Sciences, Diabetes & Endocrinology Unit, Lund University, Skåne University Hospital Malmö, CRC, 91-12, 205 02, Malmö, Sweden
Naeimeh	Atabaki Pasdar	N.	Genetic and Molecular Epidemiology, Department of Clinical Science, Lund University, Skåne University Hospital Malmö, CRC, 91-10, 205 02, Malmö, Sweden
Harshal	Deshmukh	H.A.	Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, UK
Alison	Heggie	A.J.	Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, UK
Dianne	Wake	D.	Diabetes Research Network, Royal Victoria Infirmary, Newcastle upon Tyne, UK
Mark	Walker	M.	Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, UK
Andrew	Hattersley	A.T.	NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter EX25DW
Anita	Hill	A.V.	NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter EX25DW
Angus	Jones	A.G.	NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter EX25DW
Timothy	McDonald	T.J.	Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Barrck Rd, Wonford, Exeter, EX2 5DW, UK
Mandy	Perry	M.H.	Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Barrck Rd, Wonford, Exeter, EX2 5DW, UK

1				
2				
3				
4				
5	Adrian	Cudmore	A.P.	Blood Sciences, Royal Devon and Exeter NHS Foundation Trust, Barrack Rd, Wonford, Exeter, EX2 5DW, UK
6	Andrew	Brown	A.A.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
7	Olivier	Delaneau	O.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
8	Emmanouil	Dermitzakis	E.T.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
9	Cedric	Howald	C.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
10	Halit	Ongen	H.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
11	Ana	Viñuela	A.	Department of Genetic Medicine and Development, University of Geneva Medical School, 1211 Geneva
12	Louise	Cabrelli	L.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
13	Helen	Colhoun	H.M.	formerly Dundee, recently moved to University of Edinburgh
14	Adem	Dawed	A.Y.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
15	Louise	Donnelly	L.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
16	Ian	Forge	I.M.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
17	Mike	Loneragan	M.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
18	Ewan	Pearson	E.R.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
19	Colin	Palmer	C.N.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
20	Kaixen	Zhou	K.	Molecular and Clinical Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland, United Kingdom, DD1 9SY
21	Moustafa	Abdalla	M.	Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, OX3 7BN
22	Juan	Fernandez	J.	Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, OX3 7BN
23	Stephen	Gough	S.	Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, UK, OX3 7LJ
24	Chris	Groves	C.	Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, UK, OX3 7LJ
25	Jane	Kaye	J.	Nuffield Department of Population Health, Centre for Health, Law and Emerging Technologies (HeLEX), University of Oxford, Oxford, United Kingdom, OX2 7DD
26	Anubha	Mahajan	A.	Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK, OX3 7BN
27	Mark	McCarthy	M.I.	Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, UK, OX3 7LJ
28	Harriet	Teare	H.	Nuffield Department of Population Health, Centre for Health, Law and Emerging Technologies (HeLEX), University of Oxford, Oxford, United Kingdom, OX2 7DD
29	Martijn	Vandebunt	M.	Oxford Centre for Diabetes Endocrinology and Metabolism, University of Oxford, Oxford, UK, OX3 7LJ
30	Victoria	Coathup	V.	Nuffield Department of Population Health, Centre for Health, Law and Emerging Technologies (HeLEX), University of Oxford, Oxford, United Kingdom, OX2 7DD
31	Reinhard	Holl	R.W.	University of Ulm, Institute for Epidemiology and medical Biometry, ZIBMT, Albert-Einstein-Allee 41, D-89081 Ulm, Germany
32	Julia	Stingl	J.C.	Federal Institute for Drugs and Medical Devices, Research group Pharmacogenomics, Kurt-Georg-Kiesinger-Allee 3, 53175 Bonn, Germany
33	Jacqueline	Dekker	J.M.	Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands
34	Anitra	Koopman	A.D.	Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands
35	Femke	Rutters	F.	Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands
36	Simone	Rauh	S.	Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands
37	Joline	Beulens	J.W.	Department of Epidemiology and Biostatistics, VUMC, de Boelelaan 1089a, 1081 HV, Amsterdam, the Netherlands
38	Jimmy	Bell	J.D.	Research Centre for Optimal Health, Department of Life Sciences, University of Westminster, London UK
39	Louise	Thomas	E.L.	Research Centre for Optimal Health, Department of Life Sciences, University of Westminster, London UK
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

The sustained influence of metformin therapy on circulating GLP-1 levels in individuals with and without type 2 diabetes

Running title: metformin and circulating GLP-1 levels

Dr David Preiss (PhD)¹, Adem Dawed (MPH)², Dr Paul Welsh (PhD)³, Dr Alison Heggie (MRCP)⁴, Dr Angus G Jones (PhD)⁵, Prof Jacqueline Dekker (PhD)⁶, Mr Robert Koivula⁷, Dr Tue H Hansen (MD)⁸, The DIRECT consortium⁹, Ms Caitlin Stewart (MSc)³, Prof Rury R Holman (FRCP)¹⁰, Prof Paul W Franks (PhD)⁷, Prof Mark Walker (PhD)⁴, Prof Ewan R Pearson (PhD)^{2*}, Prof Naveed Sattar (PhD)^{3*}

¹ MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, University of Oxford, Oxford, UK

² Molecular and Clinical Medicine, University of Dundee, Dundee, UK

³ Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK

⁴ Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK

⁵ NIHR Exeter Clinical Research Facility, University of Exeter Medical School, Exeter, UK

⁶ VU Medical Center, Dept Epidemiology and Biostatistics, Amsterdam, Netherlands.

⁷ Department of Clinical Sciences, Lund University, Genetic and Molecular Epidemiology, Malmö, Sweden

⁸ The Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health Sciences, University of Copenhagen, Denmark

⁹ Consortium members listed in Supplementary data

¹⁰ Diabetes Trials Unit, University of Oxford, Oxford, UK

*joint senior authors

Correspondence to:

David Preiss, CTSU, Richard Doll Building, Old Road Campus, University of Oxford, Oxford, OX3 7LF, UK

Email: david.preiss@ndph.ox.ac.uk

Phone: +44 1865 743527

OR

Ewan R Pearson, Division of Molecular and Diabetes Medicine, School of Medicine, University of Dundee, DD1 9SY, UK

Email: e.z.pearson@dundee.ac.uk

Tel: +44 1382 383387

Word count: 3,491 (abstract 249) with 29 references

Tables: 3 (and 3 supplementary tables)

Figures: 1

Abstract

Aims: Small, short studies suggest metformin influences the glucagon-like peptide (GLP)-1 axis in individuals with and without type 2 diabetes (T2DM). **In the Carotid Atherosclerosis: Metformin for insulin ResistAnce (CAMERA) trial ([NCT00723307](#)) we investigated whether this effect is sustained and related to changes in glycaemia or weight. In the cross-sectional DIabetes REsearCh on patient stratiFication (DIRECT) study, we investigated basal and post-meal GLP-1 levels in diabetic patients.**

Materials and Methods: CAMERA was a double-blinded randomized placebo-controlled trial of metformin in 173 participants without diabetes. Using six-monthly fasted total GLP-1 levels over 18 months, we evaluated metformin's effect on total GLP-1 with repeated-measures and ANCOVA analyses. In DIRECT, we examined active and total fasting and 60-minute post-meal GLP-1 levels in 775 patients recently diagnosed with T2DM treated with metformin or diet, using Student's T-tests and linear regression.

Results: In CAMERA, metformin increased total GLP-1 at 6 (+20.7%, [95% confidence intervals 4.7-39.0%]), 12 (+26.7% [10.3-45.6%]) and 18 months (+18.7% [3.8-35.7%]), an overall increase of 23.4% (11.2-36.9%; $p < 0.0001$) versus placebo. Adjustment for changes in glycaemia and adiposity, individually or combined, did not attenuate this effect. In DIRECT, metformin was associated with higher fasting active (39.1% [21.3-56.4%]) and total GLP-1 (14.1% [1.2-25.9%]) but not post-meal incremental GLP-1. These changes were independent of potential confounders including age, sex, adiposity and HbA1c.

Conclusions: **In non-diabetic individuals, metformin increases total GLP-1 in a sustained manner and independently of changes in weight or glycaemia. Metformin-treated diabetic patients also have higher fasted GLP-1 independent of weight and glycaemia. These complementary studies provide the strongest evidence for a sustained effect of metformin on fasting GLP-1 levels.**

Introduction

Metformin is recommended as first-line therapy for the majority of individuals with type 2 diabetes mellitus (T2DM)¹. This is based on evidence of cardiovascular benefit and also its capacity to maintain or reduce weight. In the United Kingdom Prospective Diabetes Study, metformin monotherapy led to a 39% reduction in the risk of myocardial infarction compared to conventional dietary therapy over 10 years, a finding not explained by the drug's effect on glycaemia². Metformin has also been shown to reduce the risk of developing T2DM. In the Diabetes Prevention Program, metformin therapy reduced new-onset T2DM by 31% and also led to 2.1kg weight loss compared to placebo over 2.8 years^{3,4}.

The glucagon-like peptide 1 (GLP-1) axis remains at the forefront of T2DM and cardiovascular research. Major outcomes trials of dipeptidyl peptidase-4 (DPP-4) inhibitors and the first completed outcome trial of a GLP-1 receptor agonist in T2DM patients indicated cardiovascular safety, though not benefit⁵⁻⁸. However, it was recently reported that the potent GLP-1 receptor agonist, liraglutide, has demonstrated cardiovascular benefit⁹. Furthermore, it has been reported that another GLP-1 receptor agonist, semaglutide, has also provided cardiovascular benefit in a major trial¹⁰. ~~Publications from these studies are awaited.~~ This is supported by recently published results from a Mendelian randomization study of a GLP-1 genetic variant (Ala316Thr; rs10305492) strongly associated with lower fasting glucose levels which demonstrated a lower risk of cardiovascular disease¹¹, supporting the concept that GLP-1 may indeed be protective against cardiovascular disease. In addition, GLP-1 receptor agonists can yield modest weight loss¹² and blood pressure reduction, important goals in the management of T2DM.

1
2
3 It is unclear whether some of metformin's benefits may be mediated via GLP-1. To explore
4 this, it is important to robustly establish the effect of metformin on GLP-1, and whether any
5 effect is mediated by changes in related parameters such as weight or glycaemia. Various
6 small studies of short duration have investigated the effect of metformin therapy on
7 circulating GLP-1 levels in individuals with and without T2DM¹³⁻²⁰. While results have been
8 inconsistent, some have shown increases in active GLP-1 and total GLP-1 in both the fasting
9 and post-prandial states. To date, however, no suitable studies have been conducted to
10 robustly investigate whether metformin therapy influences circulating GLP-1 levels in
11 individuals with and without T2DM, whether any observed effect is sustained in the longer
12 term (i.e. beyond a few weeks), and whether any effect is related to changes in other variables
13 which metformin is known to impact on, such as weight and glycaemia. To address these
14 questions, we performed **complementary studies namely** an ancillary study using data from a
15 randomized placebo-controlled repeated measures study with 18 months follow-up, the
16 Carotid Atherosclerosis: Metformin for insulin ResistAnce (CAMERA)²¹ and a cross-
17 sectional study from the Diabetes Research on Patient StraTification (DIRECT)
18 consortium²².

41 **Materials and Methods**

42
43
44
45
46 CAMERA was a randomized double-blinded placebo-controlled trial designed to investigate
47 the effect of metformin on surrogate markers of cardiovascular disease in patients without
48 diabetes, aged 35 to 75, with established coronary heart disease and a large waist
49 circumference (≥ 94 cm in men, ≥ 80 cm in women) ([NCT00723307](#)). This single-centre trial
50 enrolled 173 adults who were followed up for 18 months each. Patients attended the research
51 centre every 6 months in a fasted state. A detailed description of the trial and its results has
52
53
54
55
56
57
58
59
60

1
2
3 been published previously²¹. Participants were randomized 1:1 to 850mg metformin or
4
5 matched placebo twice daily with meals though they could reduce the dose to once daily
6
7 based on side-effects for the duration of the trial. Weight was measured in light clothing
8
9 using a bio-impedance scale. While bio-impedance body fat results were available from the
10
11 trial, we opted to measure circulating leptin levels as a better marker of body fat.
12
13

14
15 The DIRECT (DIabetes Research on Patient StraTification) study (www.direct-diabetes.org)
16
17 is part of a European Union Innovative Medicines Initiative project, with the overarching aim
18
19 to discover and validate biomarkers of rapid diabetes development, progression and drug
20
21 response²². It involves four industrial partners and 21 academic institutes within Europe. As
22
23 part of Work Package 2 that aimed to identify predictive biomarkers of glycaemic
24
25 deterioration, deep phenotyping and biochemical assays were performed in 836 people
26
27 recently diagnosed with T2D who had been on either metformin or life-style therapy alone at
28
29 baseline. The 18 months follow up data are being collected. For this study, complete cross-
30
31 sectional data were analysed from the baseline visit in 775 participants from all six clinical
32
33 centres.
34
35
36

37 38 *Sample assays*

39
40 In CAMERA, participants attended six monthly visits after overnight fasts and before taking
41
42 their morning dose of metformin. Blood samples collected during the trial were centrifuged at
43
44 4 degrees Celsius soon after sampling, separated and stored at -80°C at the Western
45
46 Infirmary's Clinical Research Facility, Glasgow, for subsequent analyses. Six monthly
47
48 fasting plasma glucose, fasting insulin and HbA1c were analysed as previously described²¹.
49
50 We calculated the Homeostasis Model Assessment for Insulin Resistance (HOMA2-IR) index
51
52 using the HOMA Calculator (v2.2.3, <https://www.dtu.ox.ac.uk/homacalculator/>). Using
53
54 available stored EDTA plasma samples, six monthly total GLP-1 levels (Meso scale
55
56
57
58
59
60

1
2
3 discovery, Maryland, USA) were measured with commercially available
4
5 electrochemiluminescence assay (Meso scale discovery, Maryland, USA). Leptin levels were
6
7 measured with a commercially available enzyme-linked immunosorbent assay (R&D systems
8
9 Oxon, UK). For total GLP-1, the mean inter-assay and intra-assay coefficients of variation
10
11 (CVs) were 2.6% and 17.3% respectively. For leptin, the mean inter-assay and intra-assay
12
13 CVs were 10.1% and 6.3%. All time points for an individual participant were run on the same
14
15 plate, blinded to treatment arm.
16
17
18
19

20
21 For the DIRECT study, blood samples were collected in the morning after a 10 hour
22
23 overnight fast. Metformin was stopped for the 24 hours preceding the study visit and restarted
24
25 immediately thereafter. For a Mixed Meal Test (MMT), participants drank 250mL Fortisip
26
27 liquid drink (18.4g carbohydrate/100mL) over 2-5 minutes. Blood samples were taken
28
29 immediately prior to the drink (time 0) and then every 30 minutes up to 120 minutes.
30
31 Samples for GLP-1 measurement were collected using P800 (for active GLP-1) and EDTA
32
33 tubes (for total GLP-1) (Becton Dickenson, UK) at 0 and 60 minutes. The same commercial
34
35 kits were used to measure GLP-1 levels as in CAMERA. In DIRECT, the mean intra- and
36
37 inter-assay CVs for active GLP-1 were 9% and 10%, respectively. For total GLP-1, these
38
39 CVs were 6% and 9%, respectively.
40
41
42
43
44

45 *Ethics and consent*

46
47 All participants provided written informed consent for participation in both studies. For the
48
49 CAMERA study, this included permission for biochemical assays that were not planned at
50
51 the time of the trial. The CAMERA trial was approved by the Medicines and Healthcare
52
53 Products Regulatory Agency and West Glasgow Research Ethics Committee. In DIRECT,
54
55
56
57
58
59
60

1
2
3 each partner clinical centre obtained approval from their respective research ethics review
4
5 boards.
6
7

8 9 *Statistics*

10
11 Normality was assessed for all variables and non-normally distributed data were transformed
12
13 using the natural log value where relevant (specifically for active GLP-1, total GLP-1, leptin
14
15 and HOMA2-IR).
16
17

18
19
20 In the CAMERA study, analyses were performed for the modified intention-to-treat
21
22 population (i.e. participants with a baseline total GLP-1 and at least one subsequent total
23
24 GLP-1 result). The effect of metformin on total GLP-1 was investigated using two different
25
26 approaches. First, repeated-measures analysis was carried out, allowing a comparison of
27
28 metformin-treated and placebo-treated participants over the entire trial (assuming a general
29
30 covariance structure). Repeated-measures analyses were only performed after demonstrating
31
32 that there was no significant treatment-by-visit interaction (i.e. that any observed effect was
33
34 stable over the trial). Secondly, analyses of covariance (ANCOVA) were carried out to
35
36 determine the effect of metformin versus placebo on total GLP-1 at 6, 12 and 18 months
37
38 respectively. Additional on-treatment analyses were performed to assess whether any change
39
40 in total GLP-1 due to metformin was related to simultaneous changes in weight, HOMA2-IR,
41
42 HbA1c, leptin or all four variables combined by adding these as cofactors.
43
44
45
46
47

48
49 In DIRECT, fasting active and total GLP-1, and 60-minute post-meal total GLP-1 levels were
50
51 compared between metformin and lifestyle groups using Student's T-tests. Anthropometric
52
53 measures (age, sex, waist to hip ratio [WHR], BMI), lifestyle factors (smoking and alcohol
54
55
56
57
58
59
60

1
2
3 use), HbA1c, fasting glucose and centre were investigated regarding any influence of
4
5 metformin on GLP-1 levels using linear regression models.
6
7

8
9
10 Due to the natural log transformation for GLP-1 measures, results are presented as the
11
12 percentage differences in geometric means of GLP-1 measures on metformin vs. placebo or
13
14 metformin vs. lifestyle to aid interpretation. The same approach was taken to present leptin
15
16 results. Statistical analyses were carried out using the statistical packages SPSS (version 22,
17
18 SPSS Inc., Chicago, Ill) and R (version 3.0.1). A two-sided p-value of 0.05 was used as the
19
20 threshold for statistical significance.
21
22

23 24 25 **Results**

26
27
28
29 Baseline characteristics for the CAMERA and DIRECT participants are summarized in
30
31 **Supplementary Table 1** and **Table 1**, respectively. It has previously been reported that
32
33 metformin led to falls in HbA1c (1.4mmol/mol), fasting insulin (21%), Homeostasis Model
34
35 Assessment of Insulin Resistance (HOMA-IR; 26%) and weight (3.2kg) compared to placebo
36
37 over 1.5 years in CAMERA.
38
39

40
41
42
43 **In DIRECT** there was no significant difference in age, sex, BMI, duration of diabetes or
44
45 HbA1c between the metformin and non-metformin treated groups in ~~DIRECT~~. Metformin
46
47 treated individuals had a higher fasting glucose (<0.001) and a slightly higher WHR than
48
49 those on no treatment (p=0.045) in DIRECT.
50
51

52
53
54 *CAMERA results: metformin increases fasting total GLP-1 over 18 months*
55
56
57
58
59
60

1
2
3 The geometric mean for total GLP-1 was 11.6pg/mL in metformin recipients and 12.4pg/mL
4 in placebo recipients at baseline. Metformin therapy led to significant increases in fasting
5 total GLP-1 compared to placebo at each of the 6, 12 and 18-month study visits (see **Table 2**
6 and **Figure 1A**). The increases in total GLP-1 at these visits were 21% (p=0.010), 27%
7 (p=0.001) and 19% (p=0.012) respectively. In repeated-measures analysis, metformin
8 increased total GLP-1 by 23.4% (p<0.0001) across the entire duration of the 18 month
9 follow-up with no evidence of heterogeneity between study visits (p=0.74).
10
11
12
13
14
15
16
17
18
19

20
21 Leptin levels fell with metformin treatment in keeping with a reduction in body fat (see
22 **Table 2**). Overall, metformin therapy reduced leptin by 25% (p<0.0001) compared to placebo
23 with similar changes observed at each visit.
24
25
26
27
28
29

30 Adjustment for the observed changes in weight, HOMA2-IR, HbA1c and leptin at each visit,
31 whether individually or combined, did not attenuate metformin's effect on total GLP-1 (see
32 **Table 3**). Adjusted comparisons (for all four parameters) at 6, 12 and 18 months showed
33 increased in total GLP-1 of 32% (p=0.001), 35% (p≤0.001) and 26% (p=0.002) respectively
34 for metformin compared to placebo therapy.
35
36
37
38
39
40
41
42

43 *DIRECT results: the association of metformin with fasting and post-meal GLP-1*

44
45 The geometric mean for total fasted GLP-1 was 7.9pg/mL in metformin recipients and
46 6.9pg/mL in lifestyle-treated patients. Metformin users had higher basal fasted active GLP-1
47 (+25.5% [95%CI 17.0-35.5%], p<0.001) and fasted total GLP-1 (+14.5% [95%CI 8.4-
48 21.0%], p=0.0097) than individuals who were on lifestyle therapy (see **Table 1** and **Figure**
49 **1B**). These differences persisted after controlling for anthropometric measures (age, sex,
50 waist to hip ratio, BMI), lifestyle factors (smoking and alcohol), study centre and HbA1c for
51
52
53
54
55
56
57
58
59
60

1
2
3 both fasted active and fasted total GLP-1 (+39.1% [21.3-56.4%]; p=1.35e-05 and +14.1%
4 [1.2-25.9%] respectively; p=0.03). Replacing HbA1c with fasting glucose in these models did
5
6
7 not materially alter these results. There was no difference in the 60 minute total GLP-1
8
9
10 concentration between metformin users and non-metformin users after adjusting for these
11
12 covariates and baseline total GLP-1 (4.4% [95%CI -0.5-9.4%]; p=0.27).
13

14 15 16 **Discussion**

17
18
19
20
21 In these complementary studies we sought further information regarding the relationship
22
23 between metformin therapy and circulating GLP-1. We demonstrate that daily metformin
24
25 therapy for 18 months led to a 25% increase in circulating total GLP-1 levels in individuals
26
27 without diabetes but with elevated waist circumferences, and this increase was sustained
28
29 across the entire duration of the study and did not appear to be related to any changes in
30
31 glycaemia or adiposity. In recently diagnosed T2D individuals, metformin treatment was
32
33 associated with higher fasted active and fasted total, but not incremental, GLP-1 levels. In
34
35 both studies, these differences in GLP-1 levels occurred despite the previous dose of
36
37 metformin having been taken the day before each visit (>24 hours in DIRECT) in both
38
39 studies, suggesting that circulating GLP-1 levels probably remain consistently elevated in
40
41 patients established on metformin therapy.
42
43
44
45
46

47 Active GLP-1 is secreted by gastro-intestinal L cells in response to the presence of nutrients
48
49 in the small intestine leading to an increase in glucose-stimulated insulin secretion and
50
51 suppressed glucagon secretion. GLP-1 also delays gastric emptying and promotes satiety.
52
53 This bioactive form of the hormone is rapidly metabolized by the enzyme DPP-4 with the
54
55 result that its half-life in the circulation is less than two minutes. Understanding the incretin
56
57
58
59
60

1
2
3 pathway led to the development of related medications, namely GLP-1 receptor agonists
4
5 (incretin mimetics) and DPP-4 inhibitors (incretin enhancers)²³, which are designed to
6
7 directly or indirectly increase the in vivo activity of GLP-1.
8
9

10
11 Previous small studies with various designs have produced mixed, often null, results but with
12
13 some suggesting that metformin therapy increases circulating GLP-1 levels by various
14
15 mechanisms²⁴ (see Supplementary Table 2). In a study of 10 obese participants without
16
17 diabetes and 10 controls who were given metformin 2.55g/d for two weeks, GLP-1 levels at
18
19 30 and 60 minutes after a glucose load were increased though baseline GLP-1 levels (and
20
21 leptin) were unchanged on metformin¹³. An uncontrolled study of metformin therapy (2g/d)
22
23 in 40 women with polycystic ovarian syndrome over 8 months, albeit with substantial loss to
24
25 follow up with only 22 women completing metformin therapy, produced similar findings to
26
27 our own with a 25% increase in area-under-the-curve GLP-1 levels over 180 minutes during
28
29 oral glucose loading compared to baseline¹⁴. A crossover study of 10 individuals with T2DM
30
31 given three single dose interventions on three different days a week apart (either metformin
32
33 1g plus placebo subcutaneous injection; or placebo tablet plus subcutaneous GLP-1; or
34
35 metformin 1g plus subcutaneous GLP-1)¹⁵. Glucose was infused to achieve a concentration of
36
37 approximately 15mmol/L. Analyses showed that metformin therapy inhibited DPP-4 activity
38
39 and also increased active GLP-1 levels. In a further crossover study conducted in 20
40
41 participants with T2DM who were treated for 6 days with each of four respective regimens
42
43 (placebo or metformin or sitagliptin or the combination) with washout periods in between
44
45 interventions, metformin therapy led to an increase in fasted and post-challenge total GLP-1
46
47 levels though no change in intact GLP-1 levels¹⁶. And in a crossover study of 12 participants
48
49 with T2DM treated with placebo or metformin for seven days respectively and then
50
51 investigated during intraduodenal catheter infusion of glucose, DPP-4 activity fell modestly
52
53
54
55
56
57
58
59
60

1
2 while intact and total GLP-1 levels rose at baseline and during the infusion after metformin¹⁷.
3
4 By contrast, a crossover study of 16 participants with T2DM treated for 4 weeks respectively
5
6 with placebo, metformin, sitagliptin and combined metformin / sitagliptin yielded no increase
7
8 in active GLP-1 on metformin¹⁸. Other studies have suggested no effect on DPP-4 activity. In
9
10 a study of eight drug-naïve participants with T2DM treated with metformin for three months,
11
12 the area-under-the-curve for active GLP-1 over 6 hours following a standard mixed meal rose
13
14 though DPP-4 activity was unchanged¹⁹. It is therefore apparent that most studies in this area
15
16 have been limited by small sample size (and therefore reduced power) and that most have
17
18 focused on the acute effect of metformin therapy as opposed to its longer term effects.
19
20

21
22 Animal studies have produced similarly mixed results including evidence of an acute increase
23
24 in GLP-1 with metformin treatment and of DPP-4 inhibition in some studies but not all²⁵⁻²⁷.
25
26

27 In contrast our results have examined the relationship of metformin with circulating GLP-1 in
28
29 large cohorts with and without type 2 diabetes and addressed long term effects of metformin
30
31 over 18 months in non-diabetic individuals.
32
33

34
35 In individuals without diabetes, our finding that the increase in GLP-1 was not related to the
36
37 observed 3.2kg decrease in weight or the 25% improvement in insulin sensitivity is in
38
39 keeping with a direct effect of metformin on the incretin axis. The sustained nature of the
40
41 GLP-1 increase suggests the possibility that metformin may in part provide cardio-metabolic
42
43 benefit, even in a non-diabetic population and beyond reducing the risk of developing T2DM,
44
45 by increasing exposure of treated individuals to GLP-1 in the longer term. This is supported
46
47 by findings from both recently completed outcomes trials of GLP-1 receptor agonists^{9,10} and
48
49 from a Mendelian¹¹ randomization study of a GLP-1 receptor variant associated with lower
50
51 fasting glucose which was also associated with lower risk of coronary heart disease. It also
52
53 provides further rationale to test these potential benefits of metformin in a population without
54
55
56
57
58
59
60

1
2
3 diabetes. The Glucose Lowering In Non-diabetic hyperglycaemia Trial (GLINT;
4
5 ISRCTN34875079) is studying whether metformin reduces cardiovascular risk as well as
6
7 cancer and other outcomes in **non-diabetic participants** ~~with HbA1c 5.5–6.5%.~~
8
9

10
11 Our study has numerous strengths. The CAMERA study is by far the largest and longest trial
12
13 to address the question of metformin's impact on circulating GLP-1 levels and its randomized
14
15 design minimises the possibility of unmeasured or unaccounted for confounding. Though
16
17 cross-sectional **and therefore unable to directly address causality**, DIRECT is the largest
18
19 study to investigate the association of metformin with GLP-1 levels in T2DM individuals,
20
21 and was able to adjust for a range of potential confounding factors. The CAMERA trial was
22
23 specifically conducted in participants without T2DM (though with elevated waist
24
25 circumferences) which enabled us to avoid the potential effects of other glucose-lowering
26
27 agents and also to provide novel data on a group at high risk of T2DM in whom metformin is
28
29 being investigated in a major trial, GLINT. Samples were available at 6 month intervals,
30
31 providing data on the sustained effect of metformin on GLP-1 levels. An important weakness
32
33 of the CAMERA trial was that we did not have access to suitably prepared samples to allow
34
35 the measurement of active GLP-1 levels and only fasted samples were available. However, in
36
37 the DIRECT study in which we had access to both active and total fasted GLP-1 levels,
38
39 metformin recipients demonstrated clearly higher levels of both, in particular active GLP-1.
40
41 Notably, however, in both studies, **higher GLP-1 levels were noted** despite the last metformin
42
43 dose having been taken the day before blood sampling which, in the context of the limited
44
45 bioavailability of metformin (less than 60%), suggests that some of this effect may reflect the
46
47 impact of the drug in the distal small intestine and colon as highlighted in other studies²⁸.
48
49 Consistent with this, the apparent impact of metformin in DIRECT was on fasting GLP-1
50
51 rather than meal stimulated GLP-1. In addition, the fact that some CAMERA participants
52
53
54
55
56
57
58
59
60

1
2
3 reduced their metformin dose and, in some cases, stopped trial medication suggests that our
4
5 results are likely to be an underestimation of the true effect of metformin on fasting total
6
7 GLP-1 in this population.
8
9

10
11 Further studies are needed to determine the longitudinal effect of metformin on GLP-1 levels
12
13 in diabetic individuals. Additional research on the mechanism by which metformin increases
14
15 GLP-1 would also be useful, including whether this effect is largely a direct of metformin on
16
17 L-cells or is mediated indirectly via metformin's many other effects on the gastro-intestinal
18
19 tract such as altering the microbiome or decreasing bile acid reabsorption²⁹.
20
21
22
23

24
25 In summary, we report the most robust evidence from two major studies demonstrating that
26
27 metformin treatment leads to a sustained and long term increase in circulating total GLP-1
28
29 levels in non-diabetic individuals independent of changes in weight and glycaemia, while
30
31 metformin therapy is also associated with higher fasted total and active GLP-1 in diabetic
32
33 patients, independent of weight and glycaemia. These complementary findings support a
34
35 potential direct role for the incretin axis on the cardiometabolic cardiovascular and glycaemic
36
37 benefits of metformin.
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Acknowledgements:** DP is guarantor for CAMERA data and ERP for DIRECT data.
4

5 CAMERA was funded by the Chief Scientist Office, Scotland (CZB/4/613). We are grateful
6
7 to Sara Jane Duffus and Elaine Butler for analysing GLP-1 on the CAMERA samples. The
8
9 work leading to this publication has received support from the Innovative Medicines
10
11 Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which
12
13 are composed of financial contribution from the European Union's Seventh Framework
14
15 Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. ERP holds a
16
17 Wellcome Trust New Investigator Award (ref 102820/Z/13/Z).
18
19

20
21
22 **Contributors:** DP had the idea for and designed the analysis, analysed and interpreted data,
23
24 wrote the first draft and revised later drafts of the article. AD analysed the DIRECT data and
25
26 contributed to the manuscript. ERP, PWF, AJ and MW participated in the design and sample
27
28 collection of the DIRECT diabetes progression study, interpreted the data and revised the
29
30 manuscript. PW co-ordinated the laboratory work for GLP-1 and leptin measurements,
31
32 interpreted data and revised the article. CS analysed leptin, analysed and interpreted the data
33
34 and revised the article. RRH interpreted the data and revised the article. NS had the idea for
35
36 and designed the analysis, interpreted data, revised the article, and supervised the analysis.
37
38
39 DIRECT collaborators are listed in Supplementary Table 3.
40
41
42
43
44

45 **Conflicts of interest:** DP, PW, CS, AJ, THH, JD, and RK report no conflicts of interest.
46

47 RRH has received research support from Amylin, Bayer, Merck, and Novartis; participated in
48
49 advisory boards for Amylin, Lilly, Merck, Novartis, and Novo Nordisk; and received
50
51 compensation for lectures from Bayer, Lilly, Merck, and Merck Serono. ERP has received
52
53 lecture fees from Eli Lilly, Novo Nordisk, Astra Zeneca and Sanofi. NS has consulted for Eli
54
55 Lilly, Bristol-Myers Squibb, Merck Sharp & Dohme, AstraZeneca, Sanofi, and Boehringer
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Ingelheim; and received research support from Merck. PWF has received consulting honoraria from Sanofi Aventis and Eli Lilly Inc, and research support from Novo Nordisk.

For Review Only

REFERENCES

1. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2012; **55**(6): 1577-96.
2. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**(9131): 854-65.
3. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**(6): 393-403.
4. Diabetes Prevention Program Research G. Long-term safety, tolerability, and weight loss associated with metformin in the Diabetes Prevention Program Outcomes Study. *Diabetes Care* 2012; **35**(4): 731-7.
5. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**(14): 1317-26.
6. White WB, Cannon CP, Heller SR, et al. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**(14): 1327-35.
7. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**(3): 232-42.
8. Pfeffer MA, Claggett B, Diaz R, et al. Lixisenatide in Patients with Type 2 Diabetes and Acute Coronary Syndrome. *N Engl J Med* 2015; **373**(23): 2247-57.
9. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2016; **375**(4): 311-22.
10. Marso SP, Bain SC, Consoli A, et al. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 2016. (online ahead of print)
11. Scott RA, Freitag DF, Li L, et al. A genomic approach to therapeutic target validation identifies a glucose-lowering GLP1R variant protective for coronary heart disease. *Sci Transl Med* 2016; **8**(341): 341ra76.
12. Potts JE, Gray LJ, Brady EM, Khunti K, Davies MJ, Bodicoat DH. The Effect of Glucagon-Like Peptide 1 Receptor Agonists on Weight Loss in Type 2 Diabetes: A Systematic Review and Mixed Treatment Comparison Meta-Analysis. *PLoS One* 2015; **10**(6): e0126769.

- 1
2
3 13. Mannucci E, Ognibene A, Cremasco F, et al. Effect of metformin on glucagon-like
4 peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care* 2001; **24**(3):
5 489-94.
6
- 7
8 14. Svendsen PF, Nilas L, Madsbad S, Holst JJ. Incretin hormone secretion in women
9 with polycystic ovary syndrome: roles of obesity, insulin sensitivity, and treatment with
10 metformin. *Metabolism* 2009; **58**(5): 586-93.
11
- 12
13 15. Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Addition of metformin to exogenous
14 glucagon-like peptide-1 results in increased serum glucagon-like peptide-1 concentrations
15 and greater glucose lowering in type 2 diabetes mellitus. *Metabolism* 2011; **60**(1): 52-6.
16
- 17
18 16. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA. Effects of sitagliptin and
19 metformin treatment on incretin hormone and insulin secretory responses to oral and
20 "isoglycemic" intravenous glucose. *Diabetes* 2014; **63**(2): 663-74.
21
- 22
23 17. Wu T, Thazhath SS, Bound MJ, Jones KL, Horowitz M, Rayner CK. Mechanism of
24 increase in plasma intact GLP-1 by metformin in type 2 diabetes: stimulation of GLP-1
25 secretion or reduction in plasma DPP-4 activity? *Diabetes Res Clin Pract* 2014; **106**(1): e3-6.
26
- 27
28 18. Solis-Herrera C, Triplitt C, Garduno-Garcia Jde J, Adams J, DeFronzo RA,
29 Cersosimo E. Mechanisms of glucose lowering of dipeptidyl peptidase-4 inhibitor sitagliptin
30 when used alone or with metformin in type 2 diabetes: a double-tracer study. *Diabetes Care*
31 2013; **36**(9): 2756-62.
32
- 33
34 19. Thondam SK, Cross A, Cuthbertson DJ, Wilding JP, Daousi C. Effects of chronic
35 treatment with metformin on dipeptidyl peptidase-4 activity, glucagon-like peptide 1 and
36 ghrelin in obese patients with Type 2 diabetes mellitus. *Diabet Med* 2012; **29**(8): e205-10.
37
- 38
39 20. Migoya EM, Bergeron R, Miller JL, et al. Dipeptidyl peptidase-4 inhibitors
40 administered in combination with metformin result in an additive increase in the plasma
41 concentration of active GLP-1. *Clin Pharmacol Ther* 2010; **88**(6): 801-8.
42
- 43
44 21. Preiss D, Lloyd SM, Ford I, et al. Metformin for non-diabetic patients with coronary
45 heart disease (the CAMERA study): a randomised controlled trial. *Lancet Diabetes*
46 *Endocrinol* 2014; **2**(2): 116-24.
47
- 48
49 22. Koivula RW, Heggie A, Barnett A, et al. Discovery of biomarkers for glycaemic
50 deterioration before and after the onset of type 2 diabetes: rationale and design of the
51 epidemiological studies within the IMI DIRECT Consortium. *Diabetologia* 2014; **57**(6):
52 1132-42.
53
- 54
55 23. Ahren B, Schmitz O. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment
56 of type 2 diabetes. *Horm Metab Res* 2004; **36**(11-12): 867-76.
57
58
59
60

- 1
2
3 24. Bahne E, Hansen M, Bronden A, Sonne DP, Vilsboll T, Knop FK. Involvement of
4 glucagon-like peptide-1 in the glucose-lowering effect of metformin. *Diabetes Obes Metab*
5 2016; **18**(10): 955-61.
6
7
8 25. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor
9 axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice.
10 *Diabetologia* 2011; **54**(2): 339-49.
11
12 26. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms
13 underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell.
14 *Endocrinology* 2011; **152**(12): 4610-9.
15
16 27. Green BD, Irwin N, Duffy NA, Gault VA, O'Harte F P, Flatt PR. Inhibition of
17 dipeptidyl peptidase-IV activity by metformin enhances the antidiabetic effects of glucagon-
18 like peptide-1. *Eur J Pharmacol* 2006; **547**(1-3): 192-9.
19
20 28. Buse JB, DeFronzo RA, Rosenstock J, et al. The Primary Glucose-Lowering Effect of
21 Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic
22 and 12-Week Dose-Ranging Studies. *Diabetes Care* 2016; **39**(2): 198-205.
23
24 29. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract.
25 *Diabetologia* 2016; **59**(3): 426-35.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Characteristics of DIRECT participants

Characteristics	Metformin (n=270)	Lifestyle (n=505)	P
Males (%)	151 (55.9%)	295 (58.4%)	0.70
Age (years)*	63 (35-75)	64 (35-75)	0.064
Duration of diabetes (years)	1.18 (0.82)	1.25 (0.75)	0.10
Weight (kg)	88.9 (16.7)	89.7 (17.0)	0.53
BMI (kg/m ²)	30.1 (4.7)	30.7 (5.1)	0.11
WHR	0.97 (0.08)	0.96 (0.08)	0.045
HbA1c (%)	6.40 (0.60)	6.34 (0.59)	0.20
HbA1c (mmol/mol)	46.44 (6.57)	45.81 (6.39)	0.20
Fasting plasma glucose (mmol/L)	7.49 (1.47)	6.94 (1.37)	<0.001
Fasting active GLP-1 (pg/ml) †	0.45 (0.40-0.51)	0.36 (0.33-0.39)	<0.001
Fasting total GLP-1 (pg/ml) †	7.9 (7.2-8.5)	6.9 (6.4-7.3)	0.0097
60-min total GLP-1 (pg/ml) †	16.0 (14.9 – 17.3)	14.3 (13.3-15.3)	0.031

Data presented as mean (SD) or n (%) except where indicated (*median [range]; †geometric mean [95% CI])

Table 2: Change in GLP-1 and leptin levels on metformin vs. placebo over 18 months in CAMERA

	Visit (nr of paired samples)	Metformin vs. Placebo *	Average treatment effect (Metformin – Placebo) †		p-value for interaction across visits
			Effect (95% CI)	p-value	
GLP-1 (natural log units)	<u>6 months</u> (n=150)	0.188 (0.046, 0.329)	0.210 (0.106, 0.314)	<0.0001	0.74
	<u>12 months</u> (n=146)	0.237 (0.098, 0.376)			
	<u>18 months</u> (n=157)	0.172 (0.038, 0.305)			
GLP-1 (%) ‡	<u>6 months</u> (n=150)	20.7% (4.7, 39.0%)	23.4% (11.2, 36.9%)	<0.0001	0.74
	<u>12 months</u> (n=146)	26.7% (10.3, 45.6%)			
	<u>18 months</u> (n=157)	18.7 (3.8, 35.7%)			
Leptin (natural log units)	<u>6 months</u> (n=152)	-0.262 (-0.403, -0.120)	-0.286 (-0.419; -0.153)	<0.0001	0.80
	<u>12 months</u> (n=146)	-0.293 (-0.467, -0.118)			
	<u>18 months</u> (n=157)	-0.237 (-0.405, -0.069)			
Leptin (%) ^c	<u>6 months</u> (n=152)	-23.1% (-33.2, -11.3%)	-24.9% (-34.2, -14.2%)	<0.0001	0.80
	<u>12 months</u> (n=146)	-25.4% (-37.3, -11.1%)			
	<u>18 months</u> (n=157)	-21.1% (-33.3, -6.7%)			

* ANCOVA analysis for visits at 6, 12 and 18 months respectively

† Repeated measures analysis for the overall treatment effect over 18 months

‡ Percentage difference in geometric means

Table 3. Effects of metformin on total GLP-1 without and with on-treatment adjustments for changes in **key variables**

Variable	Adjustment	Metformin-Placebo Mean % change (95%CI) ^a	P- value
GLP-1, 6 months	No adjustment	20.7 (4.7, 39.0)	0.010
	Weight	25.0 (7.6, 45.3)	0.004
	HOMA2-IR	24.6 (8.0, 43.7)	0.003
	HbA1c	26.1 (8.4, 46.8)	0.003
	Leptin	22.9 (6.4, 41.9)	0.005
	Combined†	32.4 (13.0, 55.1)	0.001
GLP-1, 12 months	No adjustment	26.7 (10.3, 45.6)	0.001
	Weight	35.0 (15.9, 57.3)	<0.001
	HOMA2-IR	27.2 (10.4, 46.7)	0.001
	HbA1c	28.7 (11.0, 49.2)	0.001
	Leptin	33.0 (15.7, 53.0)	<0.001
	Combined †	35.4 (15.8, 58.1)	<0.001
GLP-1, 18 months	No adjustment	18.7 (3.8, 35.7)	0.012
	Weight	23.5 (7.0, 42.5)	0.004
	HOMA2-IR	21.4 (6.2, 39.0)	0.005
	HbA1c	20.9 (5.3, 38.8)	0.007
	Leptin	20.8 (5.6, 38.2)	0.006
	Combined †	26.0 (9.1, 45.6)	0.002

The unadjusted result at each time point is provided, followed by the result adjusted for changes in weight, HOMA2-IR, HbA1c and leptin respectively; this is followed by the result adjusted for all these variables combined (indicated by †)

HOMA2-IR: Homeostasis Model Assessment of Insulin Resistance

* Results displayed as percentage change in geometric mean (95% CI)

1
2
3 **Figure Legend**
4

5
6 **Figure 1.** Metformin and GLP-1 (A) total GLP-1 levels on metformin vs. placebo over 18
7
8 months in the CAMERA study (B) The association of metformin therapy vs. lifestyle
9
10 treatment with fasting active GLP-1, fasting total GLP-1 and incremental total GLP-1 in
11
12 DIRECT
13

14
15
16 *Footnote:* Data displayed as geometric mean (1SE) (A) and geometric mean (95% CI) (B)
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Review Only