

Citation for published version:

Chen, Y-C, Edinburgh, R, Hengist, A, Smith, H, Walhin, J-P, Betts, J, Thompson, D & Gonzalez, J 2018, 'Venous blood provides lower glucagon-like peptide-1 concentrations than arterialised blood in the postprandial but not the fasted state: Consequences of sampling methods', *Experimental Physiology*, vol. 103, no. 9, pp. 1200-1205. <https://doi.org/10.1113/EP087118>

DOI:

[10.1113/EP087118](https://doi.org/10.1113/EP087118)

Publication date:

2018

Document Version

Peer reviewed version

[Link to publication](#)

This is the peer reviewed version of the following article: Chen, Y. , Edinburgh, R. M., Hengist, A. , Smith, H. A., Walhin, J. , Betts, J. A., Thompson, D. and Gonzalez, J. T. (2018), Venous blood provides lower GLP1 concentrations than arterialised blood in the postprandial, but not fasted state: Consequences of sampling methods. *Exp Physiol.*, which has been published in final form at <https://doi.org/10.1113/EP087118>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

University of Bath

General rights

Copyright and moral rights for the publications made accessible in the public 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.

Title:

Venous blood provides lower GLP-1 concentrations than arterialised blood in the postprandial, but not fasted state: consequences of sampling methods.

Authors:

Yung-Chih Chen, Robert M. Edinburgh, Aaron Hengist, Harry A. Smith, Jean-Philippe Walhin, James A. Betts, Dylan Thompson, Javier T. Gonzalez

Author Affiliations:

Department for Health, University of Bath, Bath, United Kingdom.

Corresponding Author:

Javier T. Gonzalez, Department for Health, University of Bath, BA2 7AY, United Kingdom. Tel: 0(+44) 1225 38 5518; E-mail: J.T.Gonzalez@bath.ac.uk

Running Title:

Blood sampling methods and GLP-1

NEW FINDINGS

What is the central question of this study?

Glucagon-like peptide-1 (GLP-1) is an important obesity/diabetes target, with effects dependent on circulating GLP-1 concentrations. Peripheral tissues extract GLP-1, therefore sampling venous versus arterialised blood may provide different GLP-1 concentrations. This study examined whether arterialisation alters GLP-1 concentrations during fasting and feeding.

What is the main finding and its importance?

This study demonstrates that venous blood provides lower postprandial, but not fasting, GLP-1 concentrations versus arterialised blood. Therefore, when accurate assessment of postprandial peripheral availability of GLP-1 is required, blood sampling methods should be carefully considered, clearly reported, and arterialisation is recommended.

1 **ABSTRACT**

2 Glucagon-like peptide-1 (GLP-1) displays concentration-dependent effects on
3 metabolism, appetite and angiogenesis, so accurate determination of circulating
4 GLP-1 concentrations is important. This study compared GLP-1 concentrations in
5 venous *versus* arterialised blood under both fasted and fed conditions. Venous and
6 arterialised blood samples were simultaneously drawn from ten, young, healthy men
7 before, and 30, 60 and 120 min after, ingestion of 75 g glucose. Plasma GLP-1
8 concentrations increased in response to glucose ingestion (time effect: $p < 0.01$) and
9 to a lesser extent in venous *versus* arterialised plasma (time x arterialisation
10 interaction: $p < 0.01$). Accordingly, the plasma incremental area under the curve was
11 lower in venous *versus* arterialised plasma (974 ± 88 *versus* 1214 ± 115 pmol·L x
12 120 min^{-1} , respectively, $p = 0.049$). In the postprandial state, there was a positive
13 relationship between arterialised GLP-1 concentrations and the venous-arterialised
14 difference in GLP-1 concentrations ($r^2 = 0.51$; $p < 0.01$). Both arterialised and venous
15 peak GLP-1 concentrations showed positive relationships with peak arterialised
16 insulin concentrations (both $r^2 > 0.6$, $p < 0.01$). Venous sampling results in lower
17 concentrations of GLP-1 in the postprandial, but not the fasted state compared to
18 arterialised blood. This absolute difference is biologically meaningful and is
19 magnified when GLP-1 availability is high. Therefore, sampling from arterialised
20 blood may provide a better chance of detecting small differences in postprandial
21 GLP-1 availability with interventions and if absolute GLP-1 concentrations are of
22 interest, the blood sampling method should be carefully considered and clearly
23 reported.

24

25 **Keywords:** glucagon-like peptide-1; incretins; oral glucose tolerance test;
26 metabolism; insulin

27

28 **Abbreviations:**

29 DPPIV Dipeptidyl peptidase IV

30 GLP-1 Glucagon-like peptide-1

31 iAUC Incremental area under the curve

32

33

34 **INTRODUCTION**

35

36 Postprandial glucagon-like peptide-1 (GLP-1) concentrations appear to
37 mediate many of the beneficial changes in metabolism and feeding behaviour
38 following bariatric surgery (Drucker, 2018). Consequently, GLP-1 is a major target in
39 diabetes research (Drucker, 2018) and various nutritional, pharmacological and
40 surgical methods are explored with the intention to elevate circulating GLP-1
41 availability and/or action (Edwards *et al.*, 2001; Gonzalez *et al.*, 2013; Nauck *et al.*,
42 2017; Jirapinyo *et al.*, 2018). Whilst much of the evidence supporting mechanistic
43 roles of GLP-1 involve exogenous agonists or DPP-IV inhibitors, there is also
44 evidence that endogenous GLP-1 regulates insulin secretion and appetite via studies
45 using GLP-1 receptor antagonists or physiological infusions (Aulinger *et al.*, 2014;
46 Tan *et al.*, 2017). Given that effects of GLP-1 are concentration-dependent (Nauck *et al.*,
47 1993; Verdich *et al.*, 2001; Aronis *et al.*, 2013; Gonzalez & Stevenson, 2014;
48 Gonzalez *et al.*, 2015), accurate determination of peripheral exposure to GLP-1 is
49 especially important.

50 In the fasted state, arterio-venous differences in GLP-1 concentrations across
51 the leg are have been reported during intravenous GLP-1 infusion (Asmar *et al.*,
52 2017). Venous sampling, therefore, may not accurately determine tissue exposure to
53 GLP-1. Since GLP-1 is the major incretin hormone (Holst, 2007; Drucker, 2018),
54 these arterio-venous differences could alter inferences regarding the relationship
55 between postprandial GLP-1 and insulinaemia. Moreover, if differences between
56 venous and arterialised blood are not consistent, then comparisons between studies
57 using different blood sampling methods would be problematic. Indeed, blood
58 sampling methods for GLP-1 measurement commonly vary between studies, which
59 may sample from capillaries, arterial, arterialised or venous blood (Gonzalez &

60 Stevenson, 2014; Green *et al.*, 2014; Gonzalez *et al.*, 2015; Asmar *et al.*, 2017),
61 albeit arterial blood is much less frequently sampled due being more technically
62 challenging. However, to date, no study has ever examined whether postprandial
63 GLP-1 concentrations differ in venous compared to arterialised blood, and thus
64 whether the method of blood sampling has implications for determining the
65 relationship between GLP-1 and insulinemia (i.e. the incretin effect).

66 The primary aim of the present study was to assess whether venous blood
67 exhibits concentrations of GLP-1 that are representative of arterialised blood, with a
68 secondary aim to consider whether any apparent discrepancy affects the expected
69 relationship between postprandial GLP-1 and insulinaemia. We hypothesised that
70 postprandial GLP-1 concentrations would be lower in venous compared to
71 arterialised blood and that this would alter the relationship between GLP-1 and
72 insulinaemia.

73

74

75 **METHODS**

76 **Ethical approval**

77 The data presented in this study include plasma samples collected as part of
78 a wider study (registered on clinicaltrials.gov as NCT02852044) but all data reported
79 here are novel and have not been previously published, other than the participant
80 characteristics and mean insulin concentrations (Edinburgh *et al.*, 2017). The study
81 protocols adhered to the second Declaration of Helsinki and were approved by the
82 University of Bath Research Ethics Approval Committee for Health (ref: EP 15/16
83 44). All participants provided informed written consent prior to participating.

84

85 **Participants and study design**

86 Ten young, healthy men (mean \pm SD; age: 23 ± 3 years; body mass index: $23.3 \pm$
87 $1.8 \text{ kg}\cdot\text{m}^2$) undertook a 75-g oral glucose tolerance test (OGTT) after an hour of rest
88 with blood sampled from each arm, comprising one arterialised and one non-
89 arterialised vein, simultaneously (Edinburgh *et al.*, 2017). Inclusion criteria included:
90 aged 18 to 49 years, healthy, males. Exclusion criteria included: diagnosis of any
91 bleeding disorder or taking medication which impacts blood coagulation, diagnosis of
92 metabolic disease, or any other contraindications to the procedures in the present
93 study.

94

95 **Oral glucose tolerance test, blood sampling and analysis**

96 After a 10-h overnight fast and 24 h of dietary and physical activity control,
97 participants arrived in the laboratory at $08:00 \pm 01:00$ h. Height was measured to the
98 nearest 0.1 cm with a stadiometer (Seca Ltd, Birmingham, UK), with participants
99 barefoot in the Frankfurt plane. Body mass was measured in light clothing, to the
100 nearest 0.1 kg using electronic weighing scales (BC543 Monitor, Tanita, Tokyo,
101 Japan). Participants placed their dominant hand into a heated-air box with the
102 temperature set at 55°C (Mass Spectrometry Facility; The University of Vermont &
103 the University of Vermont Medical Center). Following a 20-min acclimation period, an
104 intravenous catheter was inserted – retrograde – into a dorsal hand vein of the
105 heated hand for sampling of arterialised blood. A second catheter was inserted –
106 antegrade – into a vein in the antecubital fossa of the contralateral arm for sampling
107 of venous blood. A baseline blood sample was drawn simultaneously from both
108 arterialised and non-arterialised veins by two members of the research team and
109 dispensed into EDTA-coated tubes (BD, Oxford, UK) before centrifugation 4°C and

110 3500 g) for 10-min (Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., UK)
111 to obtain plasma. DPP4 inhibitors were not added to the sample collection tubes
112 since the aim of this study was to assess total GLP-1 concentrations, rather than
113 GLP-1₇₋₃₆. Participants then rested for 60 min in a semi-supine position, before
114 consuming 75 g glucose dissolved in 300 mL water. During the postprandial period,
115 participants remained at rest in a semi-supine position with no further food intake.
116 Water intake was permitted *ad libitum* on the first trial, and was replicated on the
117 second trial. Blood samples were analysed for GLP-1 and insulin concentrations at
118 baseline, immediately prior to the OGTT, and at 30, 60 and 120 min post-OGTT.
119 GLP-1 and insulin concentrations were determined by ELISA (Merck Millipore,
120 Watford, UK and Mercodia AB, Uppsala, Sweden, respectively; intra-assay CV both
121 <7%). The antibodies in the GLP-1 assay employed are specific to both GLP-1₇₋₃₆
122 and GLP-1₉₋₃₆ and thus this assay captures total GLP-1 concentrations. Furthermore,
123 the recovery is 90-110% and there is no significant cross-reactivity with GLP-2, GIP,
124 glucagon or oxyntomodulin (Bak *et al.*, 2014). The intra-assay and inter-assay
125 coefficients of variation are <5% and <12%, respectively.

126

127 **Statistical analysis**

128 Statistical analyses were performed in GraphPad Prism (Version 7, GraphPad
129 Software, San Diego, USA) and SPSS (Version 24, IBM SPSS Statistics, Armonk,
130 NY, USA). Data are presented as means \pm SD in text, whereas error bars on figures
131 are 95% confidence intervals normalized to remove between subject variance for
132 within-subject designs (Loftus & Masson, 1994). The area under the curve (AUC)
133 and incremental area under the curve (iAUC) were calculated using the trapezoidal
134 rule. The Matsuda insulin sensitivity index (ISI_{Matsuda}) was calculated as previously

135 described (Matsuda & DeFronzo, 1999; Edinburgh *et al.*, 2017). Prior to statistical
136 analyses, residuals of comparisons were checked for normality using the Shapiro-
137 Wilk test in addition to visual inspection of histograms. There were no clear
138 indications of non-normal distribution and therefore parametric statistics were
139 employed for all variables. Plasma GLP-1 concentrations were assessed by a two-
140 way (time x arterialisation) repeated measures ANOVA. Where an interaction effect
141 was present, post-hoc comparisons were adjusted for multiple comparisons using
142 the Bonferroni correction. Fasting values represent the average of time points -60
143 min and 0 min. Pearson correlations were employed to determine the relationships
144 between venous-arterialised differences and the absolute GLP-1 concentration, and
145 between peak GLP-1 concentrations (from each vein) and peak insulinaemia.
146 Differences in the AUC and iAUC between venous versus arterialised blood
147 samples, were assessed by paired *t*-tests. A *p* value of ≤ 0.05 was considered
148 statistically significant.

149

150

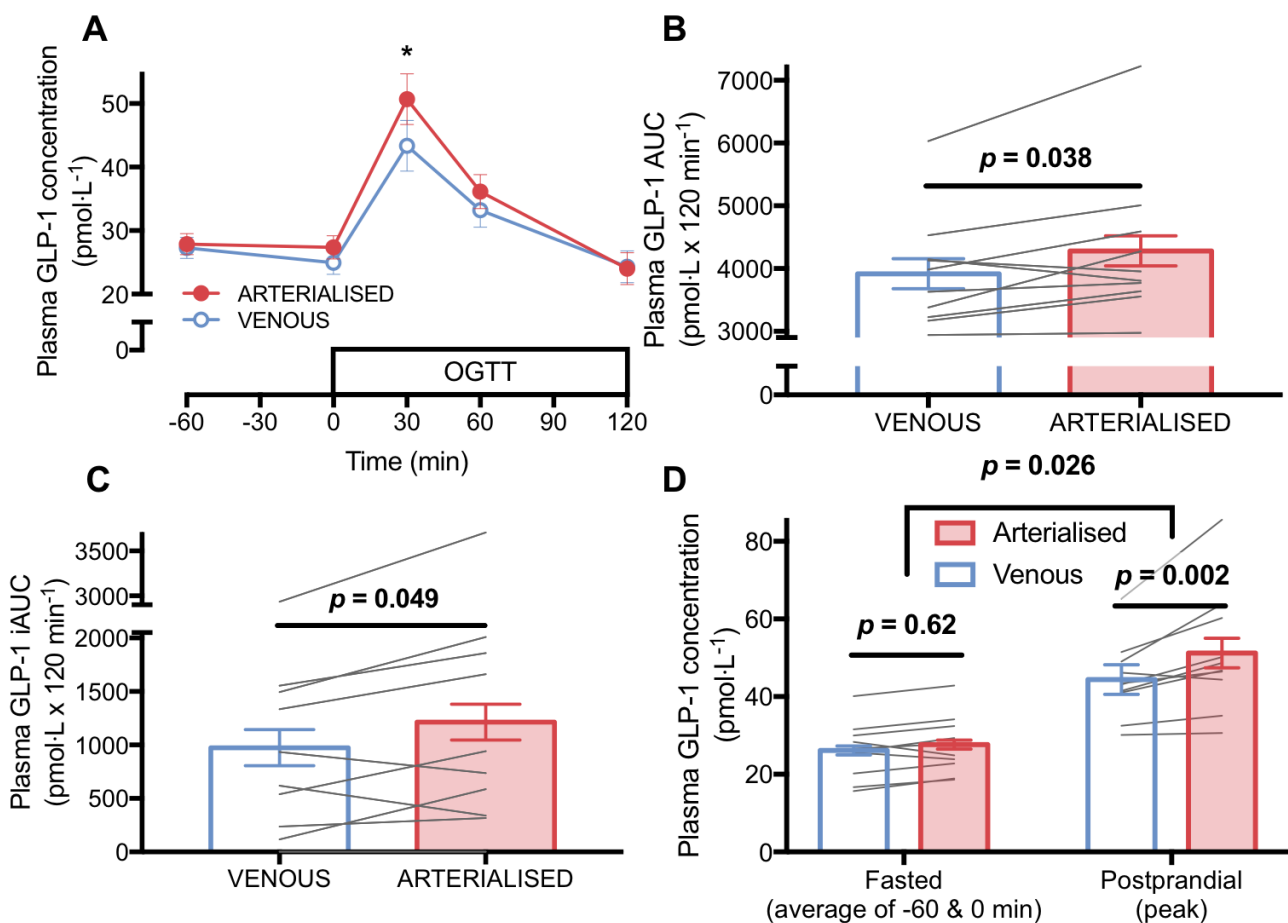
151 **RESULTS**

152 **Venous-arterialised differences in GLP-1 concentrations**

153 After glucose ingestion, plasma GLP-1 concentrations increased (time effect: $p <$
154 0.01), and to a lesser extent in venous *versus* arterialised plasma (time x
155 arterialisation interaction: $p < 0.01$; **Figure 1A**), which was most clear at peak GLP-1
156 concentrations. This resulted in a plasma AUC and iAUC that were both lower in
157 venous *versus* arterialised plasma (**Figures 1B** and **1C**, respectively).

158

159 An interaction effect of blood sampling method (venous *versus* arterialised) and
 160 feeding status (fasted *vs* peak postprandial) on plasma GLP-1 concentrations was
 161 detected ($p = 0.026$), whereby plasma GLP-1 concentrations did not differ between
 162 venous and arterialised blood samples in the fasted state (average of -60 and 0 min;
 163 mean difference: $-1.52 \text{ pmol}\cdot\text{L}^{-1}$; 95%CI: $-5.30 \text{ to } 2.27 \text{ pmol}\cdot\text{L}^{-1}$; **Figure 1D**).
 164 However, in the postprandial state, peak plasma GLP-1 concentrations were lower in



178 **Figure 1.** (A) Plasma glucagon-like peptide-1 (GLP-1) concentrations, (B) total area
 179 under the curve (AUC), and (C) incremental area under the curve (iAUC) determined in
 180 arterialised and non-arterialised venous blood before and during an oral glucose
 181 tolerance test (OGTT). (D) Interaction effect of blood sampling method (venous *versus*
 182 arterialised) and feeding status (fasted *versus* peak postprandial) on plasma GLP-1
 183 concentrations during oral glucose tolerance test. Data are means \pm n95%CI; $n = 10$.

184

185

186 Relationships between variables

187 Peak arterialised GLP-1 concentrations demonstrated a strong negative correlation
188 with the venous arterialised difference in peak GLP-1 concentrations ($r^2 = 0.85$; $p <$
189 0.001 ; **Figure 2A**). However, the relationship between ISI_{Matsuda} and the venous-
190 arterialised difference in GLP-1 AUC was weak ($r^2 = 0.03$; $p = 0.62$).

191

192 Postprandial peak GLP-1 concentrations determined in arterialised plasma displayed
193 a strong relationship with postprandial peak arterialised insulin concentrations
194 (**Figure 2B**; $r^2 = 0.64$, $p < 0.01$). Similarly, postprandial peak GLP-1 concentrations
195 determined in venous plasma also displayed a strong relationship with postprandial
196 peak arterialised insulin concentrations (**Figure 2B**; $r^2 = 0.62$, $p < 0.01$).
197 Furthermore, there was no evidence that the relationships between postprandial
198 GLP-1 and insulinaemia differed depending on the blood sampling method when
199 GLP-1 and insulin concentrations were expressed as the AUC (venous: $r^2 = 0.13$, p
200 $= 0.31$; arterialised: $r^2 = 0.12$, $p = 0.32$) or the iAUC (venous: $r^2 = 0.16$, $p = 0.26$;
201 arterialised: $r^2 = 0.18$, $p = 0.22$).

202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223

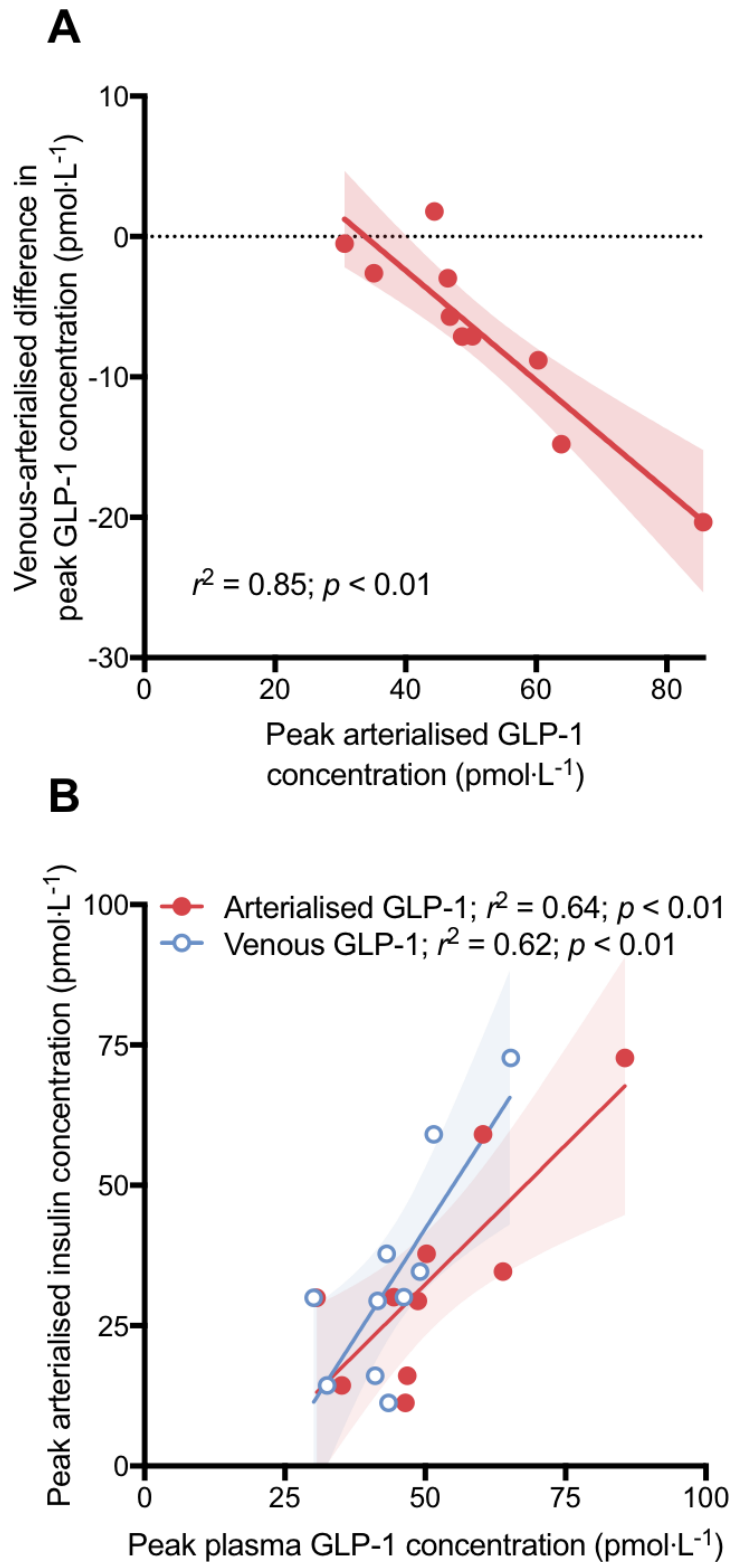


Figure 2. (A) Relationship between peak plasma glucagon-like peptide-1 (GLP-1) concentrations determined in arterialised blood and the venous-arterialised difference in peak plasma GLP-1 concentrations. (B) Relationship between plasma GLP-1 concentrations determined in arterialised or venous blood and peak arterialised insulin concentrations during an oral glucose tolerance test (OGTT). Data are individual data points and shaded areas are means \pm 95%CI; $n = 10$.

224 **DISCUSSION**

225 The present study provides the first evidence that venous plasma provides
226 lower postprandial, but not fasting, GLP-1 concentrations compared to arterialised
227 plasma, with the absolute magnitude of these differences amplified at higher GLP-1
228 concentrations. Therefore, future studies should carefully consider the sample
229 method for determination of GLP-1 availability. The difference in GLP-1
230 concentrations between arterialised and non-arterialised blood samples does not,
231 however, appear to have implications for determining the relationship between GLP-
232 1 availability and postprandial insulinaemia.

233 Determination of the circulating concentrations of GLP-1 is essential for
234 appropriate inferences regarding peripheral actions of GLP-1. GLP-1 displays
235 concentration-dependent effects on satiety (Verdich *et al.*, 2001), glucose-induced
236 insulin secretion (Nauck *et al.*, 1993) and angiogenesis (Aronis *et al.*, 2013). The
237 difference in measured GLP-1 concentrations between venous and arterialised
238 plasma in the present data ranged from -20 to 7 pmol·L⁻¹. These differences are
239 biologically meaningful, as they are greater than the biological and technical variation
240 (~5.3 and ~3.8 pmol·L⁻¹, respectively; *unpublished observations*), and equate to
241 more than 10 units on a typical visual analogue scale for hunger, satiety or appetite
242 (Verdich *et al.*, 2001). Therefore, interpretations drawn from studies reporting effects
243 on GLP-1 availability should be made with careful consideration of the blood
244 sampling method in mind.

245 GLP-1 exists in at least two forms in humans, with GLP-1₇₋₃₆ known as the
246 primary activator of the classical GLP-1 receptor. We chose to assess total GLP-1
247 rather than GLP-1₇₋₃₆ concentrations for two reasons: first, total GLP-1
248 concentrations are the best indication of GLP-1 secretion in humans *in vivo* (Holst,

249 2007); second, to rule-out changes in GLP-1 concentrations due to activity of
250 dipeptidyl peptidase IV (DPPIV) across tissue beds. In humans, GLP-1 is almost
251 entirely secreted as GLP-1₇₋₃₆ by the enteroendocrine cells of the intestine (Orskov
252 *et al.*, 1994; Holst, 2007). GLP-1₇₋₃₆ is subsequently cleaved by DPPIV which acts on
253 GLP-1₇₋₃₆ in the endothelium, liver and circulating plasma (Holst, 2007).
254 Furthermore, DPPIV is secreted by adipose tissue (Lamers *et al.*, 2011; Sell *et al.*,
255 2013), therefore venous-arterialised differences in GLP-1₇₋₃₆ could merely represent
256 DPPIV action. By detecting changes in total GLP-1 concentrations between
257 arterialised and venous blood, we can rule out DPPIV as a mechanism for this
258 response. Consequently, our data suggest that differences in GLP-1 concentrations
259 between arterialised and venous blood are likely due to uptake/entrapment in
260 peripheral tissues in the postprandial state.

261 Peripheral tissue extraction of GLP-1 in the postprandial state could be
262 increased due to either a direct increase in cellular uptake of GLP-1 and/or increased
263 tissue perfusion. The insulin response to feeding stimulates microvascular
264 recruitment and increases tissue perfusion (Vincent *et al.*, 2002). This may explain
265 why we observed a greater venous-arterialised difference in GLP-1 in the
266 postprandial state. We also found a positive correlation between the venous-
267 arterialised difference in GLP-1 and GLP-1 availability, but did not observe any
268 meaningful relationship between an index of insulin sensitivity and the venous-
269 arterialised difference in GLP-1. Therefore, peripheral GLP-1 extraction could be due
270 to increased tissue perfusion and/or increased GLP-1 availability, but at present,
271 seems not to be related to insulin sensitivity.

272 One of the primary actions of GLP-1 is to potentiate glucose-induced insulin
273 secretion (Holst, 2007; Drucker, 2018), i.e., the incretin effect. In order to assess

274 whether arterialisation of the veins has implications for inferences of the incretin
275 effect, we assessed the relationship between peak arterialised insulin concentrations
276 and GLP-1 concentrations determined in arterialised or venous plasma. Both
277 arterialised and venous peak GLP-1 concentrations strongly and positively correlated
278 with peak arterialised insulin concentrations (both displaying an $r^2 > 0.6$). This
279 suggests that both GLP-1 and insulin are removed from arterial blood at a similar
280 rate, and thus, arterialisation does not appear to influence the interpretation of the
281 incretin effect. Whether this relationship holds under differing populations, remains to
282 be seen.

283 The present study was limited by the small sample size (which can
284 overestimate effect sizes) and the relatively homogenous cohort of males. Therefore,
285 further work in larger cohorts with females, and individuals differing in weight status
286 will be useful to make these data more generalisable. There is little physiological
287 rationale to expect that other human cohorts would differ in the direction of venous-
288 arterialised difference observed in the present study, however the magnitude of this
289 venous arterialised difference could be altered in other cohorts.

290 To conclude, venous blood provides lower GLP-1 concentrations than
291 arterialised blood in the postprandial, but not the fasted state. This difference is
292 biologically meaningful and is magnified under conditions when GLP-1 availability is
293 elevated. Therefore, comparison of findings from studies using venous and
294 arterialised blood samples should be performed with care, and sampling from
295 arterialised blood may provide a better chance of detecting small differences in
296 postprandial GLP-1 availability with interventions. However, the relationship between
297 GLP-1 and insulinaemia does not appear to depend on whether blood samples are
298 arterialised. Thus, if absolute GLP-1 concentrations are of interest, it should be

299 carefully considered and reported whether samples are drawn from an artery,
300 arterialised-vein or vein.

301

302 **ACKNOWLEDGEMENTS**

303 The authors thank the volunteers for their time and effort in participating in this study,
304 and also Iain Templeman, Oliver J. Perkin, and Harriet A. Carroll for assistance with
305 blood sampling during some trials.

306

307 **FUNDING**

308 This work was funded by the University of Bath and R.M.E. receives a studentship
309 that is funded in part by a The Rank Prize Funds award to J.T.G.

310

311 **COMPETING INTERESTS**

312 The authors declare there are no competing interests associated with this
313 manuscript.

314

315 **AUTHOR CONTRIBUTIONS**

316 R.M.E., J.T.G., J.A.B., D.T. and Y-C.C. conceived and designed the study. R.M.E.,
317 H.A.S., A.H., Y-C.C. and J-P.W. performed the data collection and analysis. Y-C.C.
318 and J.T.G. interpreted the data. All authors contributed to drafting the article or
319 revising the article critically for intellectual content. All authors also approved the final
320 version of the manuscript to be published.

321

322 **REFERENCES**

323

324 Aronis KN, Chamberland JP & Mantzoros CS. (2013). GLP-1 promotes angiogenesis
325 in human endothelial cells in a dose-dependent manner, through the Akt, Src
326 and PKC pathways. *Metabolism* **62**, 1279-1286.

327
328 Asmar A, Asmar M, Simonsen L, Madsbad S, Holst JJ, Hartmann B, Sorensen CM &
329 Bulow J. (2017). Glucagon-like peptide-1 elicits vasodilation in adipose tissue
330 and skeletal muscle in healthy men. *Physiological Reports* **5**.
331
332 Aulinger BA, Bedorf A, Kutscherauer G, de Heer J, Hols JJ, Göke B & Schirra J.
333 (2014). Defining the role of GLP-1 in the enteroinsular axis in type 2 diabetes
334 using DPP-4 inhibition and GLP-1 receptor blockade. *Diabetes* **63**, 1079-
335 1092.
336
337 Bak MJ, Wewer Albrechtsen NJ, Pedersen J, Knop FK, Visbøll T, Jørgensen NB,
338 Hartmann B, Deacon CF, Dragsted LO & Holst JJ. (2014). Specificity and
339 sensitivity of commercially available assays for glucagon-like peptide-1 (GLP-
340 1) implications for GLP-1 measurements in clinical studies. *Diabetes, Obesity*
341 *and Metabolism* **16**, 1155-1164.
342
343 Drucker DJ. (2018). Mechanisms of action and therapeutic application of glucagon-
344 like peptide-1. *Cell Metabolism* **27**, 740-756.
345
346 Edinburgh RM, Hengist A, Smith HA, Betts JA, Thompson D, Walhin JP & Gonzalez
347 JT. (2017). Prior exercise alters the difference between arterialised and
348 venous glycaemia: implications for blood sampling procedures. *British Journal*
349 *of Nutrition* **117**, 1414-1421.
350
351 Edwards CM, Stanley SA, Davis R, Brynes AE, Frost GS, Seal LJ, Ghatei MA &
352 Bloom SR. (2001). Exendin-4 reduces fasting and postprandial glucose and
353 decreases energy intake in healthy volunteers. *American Journal of*
354 *Physiology - Endocrinology and Metabolism* **281**, E155-161.
355
356 Gonzalez JT, Green BP, Brown MA, Rumbold PL, Turner LA & Stevenson EJ.
357 (2015). Calcium ingestion suppresses appetite and produces acute
358 overcompensation of energy intake independent of protein in healthy adults.
359 *Journal of Nutrition* **145**, 476-482.
360
361 Gonzalez JT & Stevenson EJ. (2014). Calcium co-ingestion augments postprandial
362 glucose-dependent insulinotropic peptide1-42, glucagon-like peptide-1 and
363 insulin concentrations in humans. *European Journal of Nutrition* **53**, 375-385.
364
365 Gonzalez JT, Veasey RC, Rumbold PL & Stevenson EJ. (2013). Breakfast and
366 exercise contingently affect postprandial metabolism and energy balance in
367 physically active males. *British Journal of Nutrition* **110**, 721-732.
368
369 Green BP, Gonzalez JT, Thomas K, Stevenson EJ & Rumbold PL. (2014).
370 Agreement between finger-tip capillary and antecubital-venous appetite-
371 related peptides. *Endocrine Connections* **3**, 233-242.
372
373 Holst JJ. (2007). The physiology of glucagon-like peptide 1. *Physiological Reviews*
374 **87**, 1409-1439.
375

376 Jirapinyo P, Jin DX, Qazi T, Mishra N & Thompson CC. (2018). A Meta-Analysis of
377 GLP-1 After Roux-En-Y Gastric Bypass: Impact of Surgical Technique and
378 Measurement Strategy. *Obesity Surgery* **28**, 615-626.
379

380 Lamers D, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, Eckardt K,
381 Kaufman JM, Ryden M, Muller S, Hanisch FG, Ruige J, Arner P, Sell H &
382 Eckel J. (2011). Dipeptidyl peptidase 4 is a novel adipokine potentially linking
383 obesity to the metabolic syndrome. *Diabetes* **60**, 1917-1925.
384

385 Loftus GR & Masson ME. (1994). Using confidence intervals in within-subject
386 designs. *Psychonomic Bulletin & Review* **1**, 476-490.
387

388 Matsuda M & DeFronzo RA. (1999). Insulin sensitivity indices obtained from oral
389 glucose tolerance testing: comparison with the euglycemic insulin clamp.
390 *Diabetes Care* **22**, 1462-1470.
391

392 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R & Creutzfeldt W. (1993).
393 Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of
394 synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes
395 mellitus. *Journal of Clinical Investigation* **91**, 301-307.
396

397 Nauck MA, Meier JJ, Cavender MA, Abd El Aziz M & Drucker DJ. (2017).
398 Cardiovascular Actions and Clinical Outcomes With Glucagon-Like Peptide-1
399 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors. *Circulation* **136**, 849-
400 870.
401

402 Orskov C, Rabenhøj L, Wettergren A, Kofod H & Holst JJ. (1994). Tissue and
403 plasma concentrations of amidated and glycine-extended glucagon-like
404 peptide I in humans. *Diabetes* **43**, 535-539.
405

406 Sell H, Bluher M, Kloting N, Schlich R, Willems M, Ruppe F, Knoefel WT, Dietrich A,
407 Fielding BA, Arner P, Frayn KN & Eckel J. (2013). Adipose dipeptidyl
408 peptidase-4 and obesity: correlation with insulin resistance and depot-specific
409 release from adipose tissue in vivo and in vitro. *Diabetes Care* **36**, 4083-4090.
410

411 Tan T, Behary P, Tharakan G, Minnion J, Al-Najim W, Albrechtsen NJW, Holst JJ &
412 Bloom SR. (2017). The effect of a subcutaneous infusion of GLP-1, OXM and
413 PYY on energy intake and expenditure in obese volunteers. *Journal of Clinical
414 Endocrinology and Metabolism* **102**, 2364-2372.
415

416 Verdich C, Flint A, Gutzwiller JP, Naslund E, Beglinger C, Hellstrom PM, Long SJ,
417 Morgan LM, Holst JJ & Astrup A. (2001). A meta-analysis of the effect of
418 glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans.
419 *Journal of Clinical Endocrinology and Metabolism* **86**, 4382-4389.
420

421 Vincent MA, Dawson D, Clark AD, Lindner JR, Rattigan S, Clark MG & Barrett EJ.
422 (2002). Skeletal muscle microvascular recruitment by physiological
423 hyperinsulinemia precedes increases in total blood flow. *Diabetes* **51**, 42-48.
424
425