Title:

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

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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.

ABSTRACT

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

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

Abbreviations:

DPPIV	Dipeptidyl peptidase IV
GLP-1	Glucagon-like peptide-1
iAUC	Incremental area under the curve

INTRODUCTION

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

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

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

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

METHODS

Ethical approval

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

Participants and study design

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

Oral glucose tolerance test, blood sampling and analysis

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

3500 *g*) for 10-min (Heraeus Biofuge Primo R, Kendro Laboratory Products Plc., UK) to obtain plasma. DPP4 inhibitors were not added to the sample collection tubes since the aim of this study was to assess total GLP-1 concentrations, rather than GLP-$1_{7\text{-}36}$. Participants then rested for 60 min in a semi-supine position, before consuming 75 g glucose dissolved in 300 mL water. During the postprandial period, participants remained at rest in a semi-supine position with no further food intake. Water intake was permitted *ad libitum* on the first trial, and was replicated on the second trial. Blood samples were analysed for GLP-1 and insulin concentrations at baseline, immediately prior to the OGTT, and at 30, 60 and 120 min post-OGTT. GLP-1 and insulin concentrations were determined by ELISA (Merck Millipore, Watford, UK and Mercodia AB, Uppsala, Sweden, respectively; intra-assay CV both <7%). The antibodies in the GLP-1 assay employed are specific to both GLP-$1_{7\text{-}36}$ and GLP-$1_{9\text{-}36}$ and thus this assay captures total GLP-1 concentrations. Furthermore, the recovery is 90-110% and there is no significant cross-reactivity with GLP-2, GIP, glucagon or oxyntomodulin (Bak *et al.*, 2014). The intra-assay and inter-assay coefficients of variation are <5% and <12%, respectively.

Statistical analysis

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

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

RESULTS

Venous-arterialised differences in GLP-1 concentrations

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

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

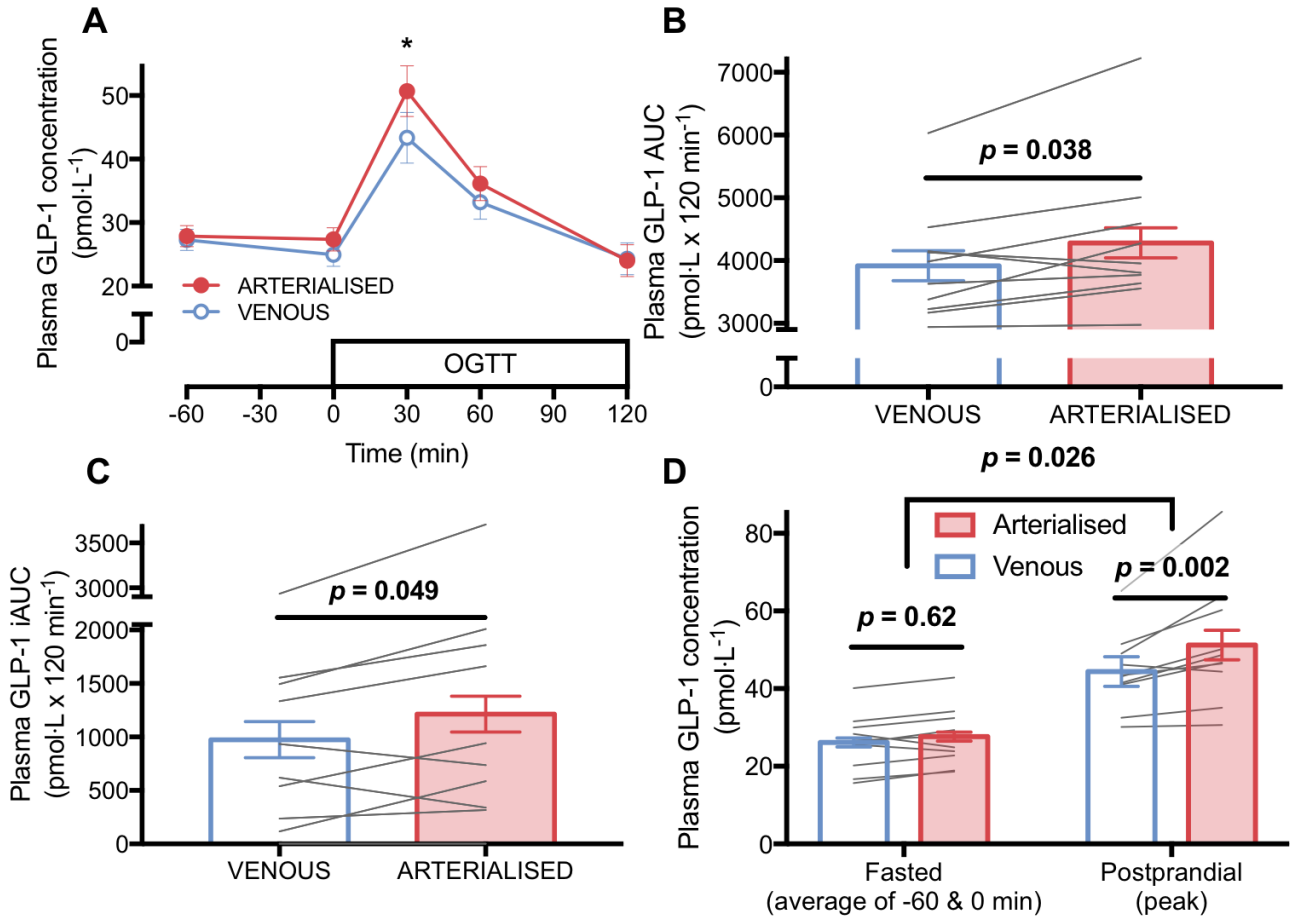

Figure 1. (A) Plasma glucagon-like peptide-1 (GLP-1) concentrations, (**B**) total area under the curve (AUC), and (**C**) incremental area under the curve (iAUC) determined in arterialised and non-arterialised venous blood before and during an oral glucose tolerance test (OGTT). (**D**) Interaction effect of blood sampling method (venous *versus* arterialised) and feeding status (fasted *versus* peak postprandial) on plasma GLP-1 concentrations during oral glucose tolerance test. Data are means ± 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).

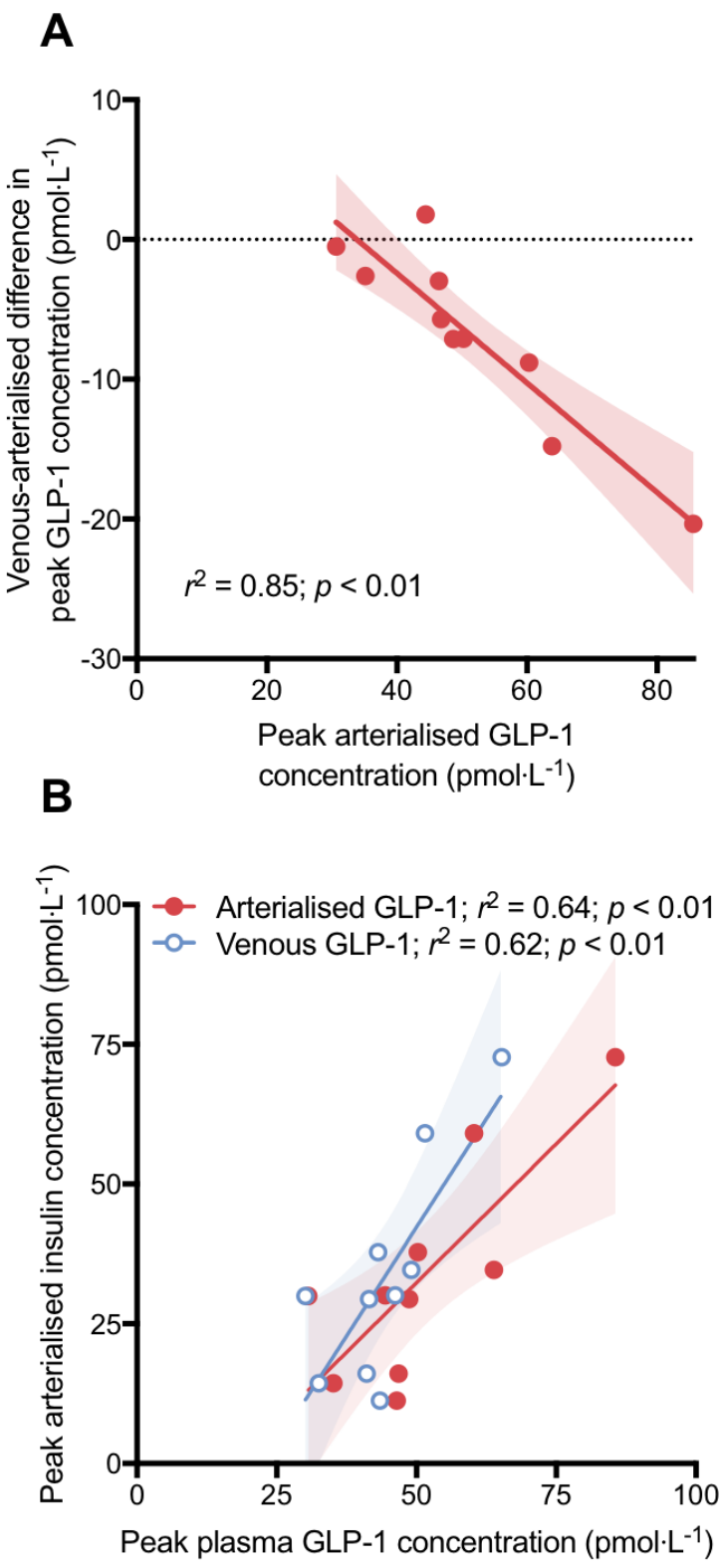

Figure 2. (**A**) Relationship between peak plasma glucagon-like peptide-1 (GLP-1) concentrations determined in arterialised blood and the venous-arterialised difference in peak plasma GLP-1 concentrations. (**B**) Relationship between plasma GLP-1 concentrations determined in arterialised or venous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

DISCUSSION

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

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

GLP-1 exists in at least two forms in humans, with $GLP\text{-}1_{7\text{-}36}$ known as the primary activator of the classical GLP-1 receptor. We chose to assess total GLP-1 rather than $GLP\text{-}1_{7\text{-}36}$ concentrations for two reasons: first, total GLP-1 concentrations are the best indication of GLP-1 secretion in humans *in vivo* (Holst,

2007); second, to rule-out changes in GLP-1 concentrations due to activity of dipeptidyl peptidase IV (DPPIV) across tissue beds. In humans, GLP-1 is almost entirely secreted as GLP-$1_{7\text{-}36}$ by the enteroendocrine cells of the intestine (Orskov *et al.*, 1994; Holst, 2007). GLP-$1_{7\text{-}36}$ is subsequently cleaved by DPPIV which acts on GLP-$1_{7\text{-}36}$ in the endothelium, liver and circulating plasma (Holst, 2007). Furthermore, DPPIV is secreted by adipose tissue (Lamers *et al.*, 2011; Sell *et al.*, 2013), therefore venous-arterialised differences in GLP-$1_{7\text{-}36}$ could merely represent DPPIV action. By detecting changes in total GLP-1 concentrations between arterialised and venous blood, we can rule out DPPIV as a mechanism for this response. Consequently, our data suggest that differences in GLP-1 concentrations between arterialised and venous blood are likely due to uptake/entrapment in peripheral tissues in the postprandial state.

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

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

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

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

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

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

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COMPETING INTERESTS

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

AUTHOR CONTRIBUTIONS

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

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