Longitudinal Changes in Fasting and Glucose-Stimulated GLP-1 and GIP in Healthy Older Subjects

Hung Pham,[1] Chinmay S. Marathe,[1] Liza K. Phillips,[1,2] Laurence G. Trahair,[1] Seva Hatzinikolas,[1] Lian Huynh,[1] Tongzhi Wu,[1,2] Michael A. Nauck,[3] Christopher K. Rayner,[1,4] Michael Horowitz,[1,2] and Karen L. Jones[1,2]

[1]NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, Adelaide Medical School, The University of Adelaide, Adelaide, South Australia 5005, Australia; [2]Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, South Australia 5005, Australia; [3]Diabetes Center Bochum-Hattingen, St. Josef- Hospital, 44791 Bochum, Germany; and [4]Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, South Australia 5005, Australia

ORCiD numbers: 0000-0002-7055-3108 (H. Pham); 0000-0002-1155-5816 (K. L. Jones).

Context: It is not known whether glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) levels correlate within individuals, nor whether levels change with age. Previous studies have all been cross-sectional in design.

Objective: To evaluate longitudinal changes in fasting and glucose-stimulated incretin hormone concentrations in healthy older subjects.

Patients and Design: Forty-one healthy older subjects had measurements of plasma GLP-1 and GIP while fasting and after a 75-g oral glucose load on two occasions separated by 5.9 ± 0.1 years [mean age at the initial study: 71.2 ± 3.8 (SD) years]. Breath samples were collected to calculate the gastric 50% emptying time (T50).

Results: For GLP-1, both fasting concentrations ($P < 0.001$) and area under the curve 0 to 120 minutes ($P = 0.001$) were decreased at followup. Fasting GIP was also lower ($P = 0.03$) at follow up, but there was no change in the area under the curve 0 to 120 minutes ($P = 0.26$). The gastric emptying T50 was slower at followup ($P = 0.008$). Neither the change in T50 nor the body mass index at the initial study was a determinant of the change in incretin responses. Between the two study days, fasting GIP ($r = 0.72$, $P < 0.001$) correlated well, but not fasting GLP-1 ($r = 0.23$, $P = 0.18$). However, both glucose-stimulated GLP-1 ($r = 0.50$, $P = 0.002$) and GIP ($r = 0.60$, $P < 0.001$) showed correlations between the initial and follow-up studies.

Conclusions: Fasting GIP and glucose-stimulated GLP-1 and GIP concentrations correlate within individuals over a follow-up period of ~5.9 years. Aging is associated with reductions in fasting GLP-1 and GIP, and glucose-stimulated GLP-1, which may predispose to the development of glucose intolerance and type 2 diabetes. (***J Clin Endocrinol Metab*** **104: 6201–6206, 2019)**

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), released from the small intestine in response to nutrient exposure (GLP-1 from L cells located predominantly in the distal small intestine and GIP from K cells located more proximally) are important regulators of postprandial glycemia through glucose-dependent insulinotropic effects (1). They account for the so-called

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 4 June 2019. Accepted 2 August 2019.
First Published Online 8 August 2019

Abbreviations: AUC, area under the curve; BMI, body mass index; CV, coefficient of variation; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; T50, gastric 50% emptying time; ΔGE, change in gastric emptying.

"incretin effect," which is the markedly increased insulin response when glucose is delivered enterally when compared with an isoglycemic IV infusion (2, 3). There is a substantial interindividual variation in GLP-1 and GIP responses to nutrients (4), which is probably not systematically affected by the presence of type 2 diabetes (4). GLP-1 affects glucose homeostasis through a number of actions in addition to stimulation of insulin secretion, including suppression of glucagon, slowing of gastric emptying, and enhanced satiety to reduce food intake (5, 6). In contrast, GIP may stimulate glucagon, particularly in response to hypoglycemia (7), but has little, or no, effect on gastric emptying or appetite (7, 8), but may affect fat metabolism (6, 9). In type 2 diabetes, the incretin effect is diminished, at least in part, as a result of a marked attenuation of the insulinotropic effect of GIP (10). This observation has stimulated the development of GLP-1-based therapy (*i.e.,* GLP-1 receptor agonists and dipeptidyl peptidase 4 inhibitors) for the management of type 2 diabetes (11, 12).

It is now appreciated that the rate of gastric emptying, which varies substantially between individuals (usually in the range of 1 to 4 kcal/min), is a major determinant of the glycemic response to carbohydrate-containing meals in healthy subjects (13–17) as well as individuals with type 2 diabetes (18, 19). Gastric emptying may also affect postprandial GLP-1 and GIP secretion (20). We have demonstrated that the rate of intraduodenal glucose delivery has differential effects on GLP-1 and GIP in health (21–23) and type 2 diabetes (22, 24). Specifically, a rate of >2 kcal/min is required to stimulate GLP-1, whereas the stimulation of GIP is approximately linear, in the range of 1 to 4 kcal/min (20, 21, 23). Using the specific GLP-1 antagonist, exendin 9-39, we have also shown that endogenous GLP-1 slows gastric emptying (25).

Although gastric emptying is known to slow modestly with healthy aging (26, 27), it is not known whether baseline and/or nutrient-stimulated GLP-1 or GIP levels are subject to intraindividual variation or whether they are affected by aging. Specifically, previous studies have all been cross-sectional in design, with the inherent limitations in this approach. We have now re-evaluated a cohort of healthy older subjects after an interval of ~5.9 years and determined changes in fasting and glucose-stimulated plasma GLP-1 and GIP concentrations and their relationships with gastric emptying.

Materials and methods

Subjects

Eighty-seven healthy older individuals who took part in a study, performed between July 2010 and July 2012 evaluating the effect of gastric emptying on the glycemic and incretin hormone responses to a 75-g oral glucose load (13), were invited by mail to participate in the present follow-up study. Of the original cohort, none had died, 41 agreed to participate, 11 had medical conditions that precluded their involvement, 13 refused to participate, 21 did not respond to the letter, and, in 1 case, the invitation letter was returned and the individual considered to be lost to follow-up. Demographic information and medical history were updated. Individuals were excluded if they had a history of gastrointestinal disease or surgery, substantial respiratory or cardiac disease, alcohol intake >20 g/day, or were taking medication known to affect gastric emptying.

The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written informed consent. All experiments were carried out in accordance with the Declaration of Helsinki.

Protocol

The protocol was identical to that used in the initial study (13). Individuals attended the Royal Adelaide Hospital, at ~08.30 h after an overnight fast (14 hours for solids; 12 hours for liquids) (28). They were seated in an armchair, and a cannula was inserted into an antecubital vein for blood sampling. After a rest period of 15 to 30 minutes, each participant consumed a drink containing 75 g glucose and 150 mg ^{13}C-acetate (Cambridge Isotope Laboratories, Tewksbury, MA), made up to 300 mL with water, within 2 minutes. Time zero (t = 0) was defined as the time of completion of the drink.

Venous blood samples (~15 mL) were obtained immediately before the commencement of the drink (t = −3 minutes) and at t = 30, 60, 90, and 120 minutes. The IV cannula was then removed, and the subject offered a light lunch before leaving the laboratory.

Measurements

Blood glucose concentrations

Fasting and 2-hour blood glucose concentrations were determined using a portable glucometer (Medisense Companion 2 Meter, Medisense Inc., Waltham, MA) (29, 30). Subjects with fasting blood glucose ≥7.0 mmol/L and/or 2-hour blood glucose ≥11.1 mmol/L were classified, according to World Health Organization criteria, as having diabetes (31).

Plasma GLP-1 and GIP

Total GLP-1 was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA). The minimum detectable limit was 3 pmol/L, intra- and interassay coefficients of variation (CVs) were 8.0% and 10.0%, respectively (21). Plasma GIP was measured by radioimmunoassay. The minimum detectable limit was 2 pmol/L, interassay CV was 9.4%, and intra-assay CV was 4.4% (32). The assays used for the initial and follow-up measurements were identical, although they were not performed at the same time.

Gastric emptying

Exhaled breath samples were collected before ingestion of the drink (t = −3 minutes), every 5 minutes for the first hour (commencing at t = 5 minutes) and then every 15 minutes for the second hour, for assessment of gastric emptying. The $^{13}CO_2$ concentration in the breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific,

Crewe, UK), and the gastric 50% emptying time was calculated, using the formula described by Ghoos *et al.* (33).

Statistical analysis

Plasma GLP-1 and GIP were analyzed and presented as absolute values and changes from baseline. One-way repeated measures ANOVA was used to evaluate the effects of time (t = 0 to 120 minutes) on the change from baseline values for plasma GLP-1 and GIP at each visit.

The peak and time to peak for GLP-1 and GIP were calculated, as were areas under the curve (AUCs) from 0 to 120 minutes, the latter using the trapezoidal rule. Differences between the initial study and follow up were assessed using Student paired *t* tests.

The changes in AUCs for plasma GLP-1, plasma GIP, and gastric emptying (ΔGE) between the initial and follow-up studies were calculated. Linear regression analysis was performed to evaluate correlations for GLP-1 and GIP concentrations between the two study days. Multiple linear regression was performed to gain insights into the potential effects of ΔGE, GLP-1, GIP responses at the initial study, and body mass index (BMI) at the initial study on the changes in GLP-1 or GIP. All analyses were performed using SPSS version 24 (SPSS, Chicago, IL). A P value < 0.05 was considered significant in all analyses. Data are presented as mean values ± SEM, unless stated otherwise.

Results

Of the 41 older individuals (20 female and 21 male) who agreed to return, the mean age at the initial study was 71.2 ± 3.8 (SD) years and BMI 25.8 ± 2.7 (SD) kg/m^2. At follow-up (mean interval of 5.9 ± 0.1 years), age was 77.1 ± 3.8 (SD) years and BMI 26.5 ± 3.1 (SD) kg/m^2 (P = 0.3). There were no demographic differences at baseline between those who participated in the study and those who did not. The studies were generally well tolerated and there were no adverse events. Five subjects were shown to have diabetes and were excluded from analyses. In another six subjects, a nonlinear regression model could not be fitted to the measured $^{13}CO_2$ concentrations at the initial and/or the follow-up study. Paired plasma GLP-1 and GIP data were available in 36 subjects, whereas gastric emptying data were available in 30 subjects.

Plasma GLP-1

There was a reduction in fasting plasma GLP-1 (21.0 ± 1.0 vs 15.3 ± 0.7 pmol/L, $P < 0.001$) at follow-up compared with the initial study. Between t = 0 and 120 minutes, there was a rise ($P < 0.001$ for both) in plasma GLP-1 on both study days. The AUC for plasma GLP-1 (P = 0.001) (Fig. 1(a)) and the maximum rise in plasma GLP-1 (41.3 ± 2.6 vs 35.4 ± 2.2 pmol/L, P = 0.03) were less, and the time to peak was longer (33.4 ± 2.0 vs 47.5 ± 3.6 minutes, P = 0.001) at followup. Changes in GLP-1 were not influenced by sex (data not shown).

Plasma GIP

There was a reduction in fasting plasma GIP (19.8 ± 1.2 vs 17.1 ± 0.9 pmol/L, P = 0.03) at followup compared with the initial study. Between t = 0 and 120 minutes, there was a rise ($P < 0.001$ for both) in plasma GIP on both study days. There was no difference (P = 0.26) in the AUC for plasma GIP between the initial and follow-up studies (Fig. 1(b)), or in the maximum rise in plasma GIP (56.1 ± 2.8 vs 54.2 ± 2.4 pmol/L, P = 0.43). However, the time to peak was shorter (98.3 ± 3.7 vs 76.7 ± 5.9 minutes, P = 0.001) at follow-up. Changes in GIP were not influenced by sex (data not shown).

Gastric emptying

Gastric 50% emptying time was slower at follow-up than at the initial study (136.5 ± 4.9 vs 164.7 ± 10.6 minutes, P = 0.008).

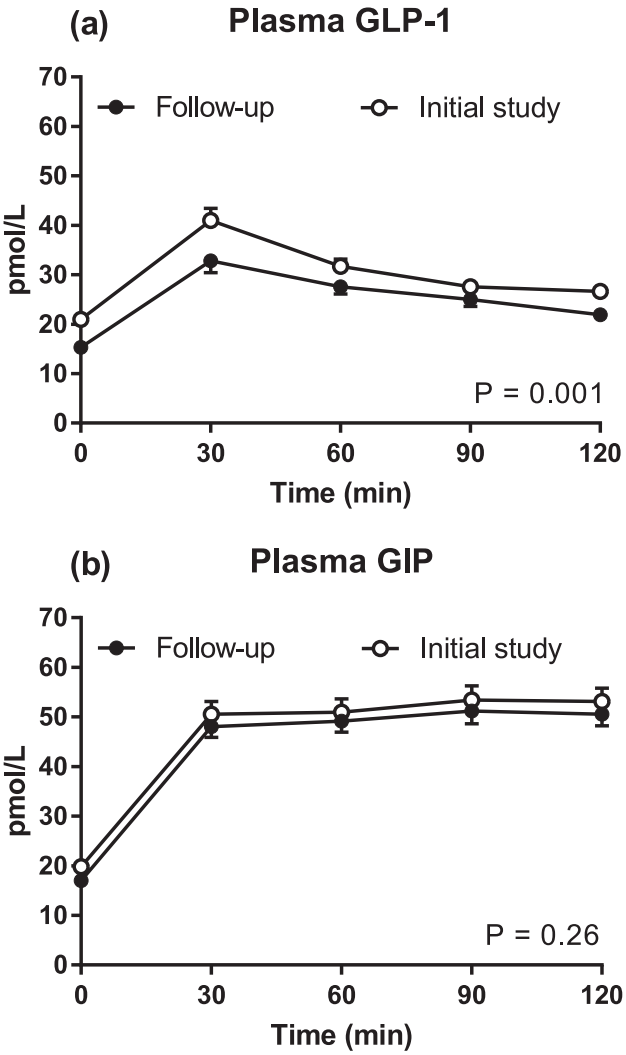

Figure 1. Plasma (a) GLP-1 and (b) GIP concentrations before and after 75 g glucose at the initial and follow-up studies in healthy older subjects (n = 36). Data are mean values ± SEM.

Relationships for GLP-1, GIP, and gastric emptying between the two study days

Fasting GLP-1 concentrations at the initial and follow-up studies were not related ($r = 0.23$, $P = 0.18$; n = 36). In contrast, there was a correlation in fasting GIP concentrations between the initial and follow-up days ($r = 0.72$, $P < 0.001$; n = 36). There were significant relationships between AUC 0 to 120 minutes for plasma GLP-1 ($r = 0.50$, $P = 0.002$; n = 36) [Fig. 2(a)], plasma GIP ($r = 0.60$, $P < 0.001$; n = 36) [Fig. 2(b)], and gastric emptying ($r = 0.38$, $P = 0.04$; n = 30) at the initial and follow-up studies.

Predictors of changes in glucose-stimulated plasma GLP-1 and GIP

Multiple linear regression analysis was performed to investigate potential predictors of the changes in AUCs for plasma GLP-1 in the subjects with complete data (n = 30). Variables in the model included the ΔGE between the two study days, GLP-1 AUC at the initial study, and BMI at the initial study. GLP-1 AUC at the initial study [$\beta = -0.47 \pm 0.17$ (SE), $P = 0\ 0.01$], but not ΔGE [$\beta = 1.5 \pm 3.4$ (SE), $P = 0.96$] or BMI at the initial study [$\beta = 35.5 \pm 62.0$ (SE), $P = 0.57$], was a significant predictor. The overall model fit was $R^2 = 0.26$, $P = 0.046$.

Similarly, multiple linear regression analysis was also performed to investigate potential predictors of the Δ.GIP-1 AUC in the subjects with complete data (n = 30). Variables in the model included the ΔGE between the two study days, GIP.AUC at the initial study, and BMI at the initial study. GIP.AUC at the initial study [$\beta = -0.48 \pm 1.4$ (SE), $P = 0.002$], but not ΔGE [$\beta = 0.38 \pm 4.7$ (SE), $P = 0.94$] or BMI at the initial study [$\beta = 37.8 \pm 93.2$ (SE), $P = 0.69$], was a significant predictor. The overall model fit was $R^2 = 0.33$, $P = 0.02$.

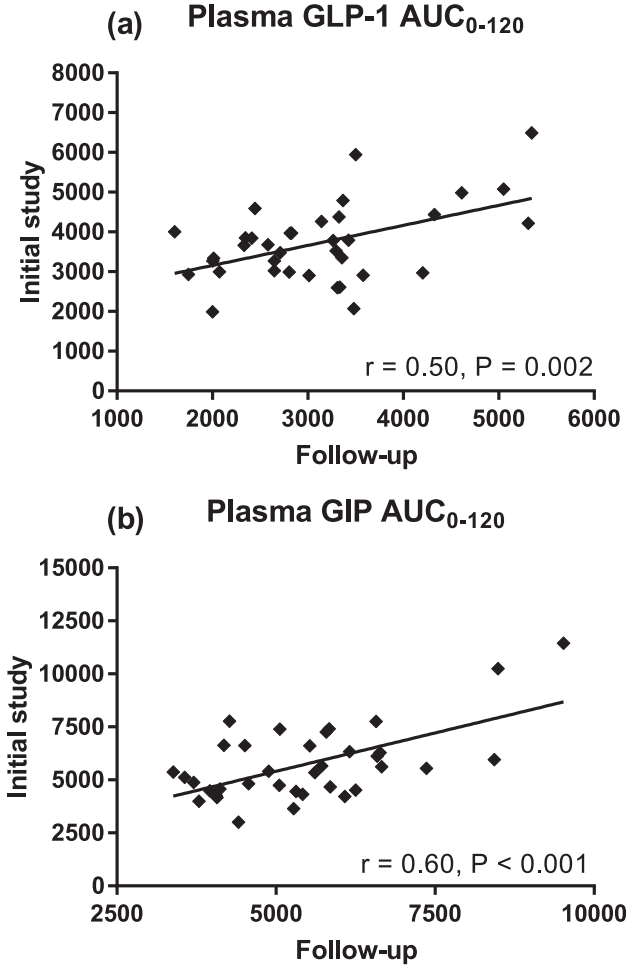

Figure 2. Relationship between AUC 0 to 120 min for plasma (a) GLP-1 and (b) GIP at the initial study and follow-up (n = 36).

Discussion

This study represents a longitudinal evaluation of fasting and oral glucose-stimulated incretin hormone responses in healthy older people. Previous studies have all been cross-sectional. Observations are that fasting GIP and GLP-1, and glucose-stimulated GLP-1, decreased significantly over a mean period of 5.9 years and there was a reasonable correlation between GLP-1 and GIP responses at baseline and at follow-up. Neither the slight slowing of gastric emptying nor BMI at the initial study predicted the changes in GLP-1 or GIP.

Existing information relating to the effects of aging on incretin hormones in healthy people and patients with type 2 diabetes is inconsistent. Some cross-sectional studies have reported that fasting and/or "postprandial" plasma GIP (34–36) and GLP-1 (36–38) concentrations do not differ between healthy young and older healthy subjects. However, in other studies, postprandial GIP (39, 40) and GLP-1 (36, 39, 41) were reported to be slightly greater in healthy older subjects. In type 2 diabetes, postprandial GIP has been reported to be greater (34, 42) or comparable (35), whereas GLP-1 has been reported to be less in older subjects with type 2 diabetes (35). The fundamental limitation of these cross-sectional studies is the inability to account for intra-individual changes over time.

Our study indicates that aging is associated with modest reductions in fasting GLP-1 and GIP, and oral glucose-stimulated GLP-1, but not GIP. The pathophysiology underlying the changes in fasting and postprandial incretin hormones is uncertain. Our focus was the incretin secretory responses and we, accordingly, measured plasma concentrations of total, rather than intact, GLP-1 and GIP which include both intact hormones and inactive metabolites (2).

The relevance of the observed changes is also uncertain. However, Færch *et al.* have suggested that a

reduction in the GLP-1 response to oral glucose could predispose to the development of type 2 diabetes (43). This is supported by recent longitudinal observations in 121 subjects (nondiabetic lean and obese adult men and women) in the Hoorn Meal Study in which a reduced GLP-1 response in an oral glucose tolerance test was associated with a greater increase in fasting glucose 7 years later (44), although an oral glucose tolerance test was not performed at follow-up, and gastric emptying was not studied. Nevertheless, our data add to the concern that a reduction in fasting and glucose-stimulated GLP-1 may predispose to impairment in glucose tolerance and type 2 diabetes.

We observed strong correlations in fasting GIP, and glucose-stimulated GLP-1 and GIP concentrations, between the initial and follow-up studies. The absence of a relationship with fasting GLP-1 may well reflect a type 2 error. Accordingly, despite the substantial interindividual variation in fasting and postprandial GLP-1 and GIP within the same individual, there is a reproducible pattern. The determinants of this phenomenon remain to be characterized. The observed slowing of gastric emptying, which may be a determinant of, as well as determined by, plasma GLP-1 concentrations, did not appear to be relevant.

Limitations of our study should be appreciated. The size of the cohort was relatively small and just less than 50% of those studied originally participated in the follow-up study with the inherent potential for selection bias. In addition, the five subjects with type 2 diabetes were excluded. Gastric emptying data of another six subjects were removed from the analyses because of technical issues, which may have reduced the predictive power of our multiple linear regression models. Furthermore, gastric emptying was assessed by the indirect breath test technique. This method has been shown to correlate closely with scintigraphy, the "gold standard" technique (45, 46), although the measurements should be regarded as notional, rather than precise (18). We used the same assays under the same conditions to measure GLP-1 and GIP at the initial study and follow-up, but it should be appreciated that inevitably the batch numbers differed given the timing of assays.

In conclusion, our study demonstrates that in healthy older people fasting GLP-1 and GIP, and glucose-stimulated GLP-1 decrease over a period of 5.9 years, and that longitudinal intraindividual fasting GIP and glucose-stimulated GLP-1 and GIP concentrations correlate. The reduction in incretin hormone responses with aging may potentially predispose to the development of glucose intolerance and type 2 diabetes.

Acknowledgments

The authors thank Kylie Lange (Centre of Research Excellence in Translating Nutritional Science to Good Health, University of Adelaide) for her assistance with the statistical analyses.

Financial Support: This work was supported by a grant from the Royal Adelaide Hospital (to K.L.J.).

Correspondence and Reprint Requests: Karen L. Jones, PhD, NHRMC Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Level 5 Adelaide Health and Medical Sciences Building, Corner of North Terrace and George Street, Adelaide, South Australia 5005, Australia. E-mail address: karen.jones@adelaide.edu.au.

Disclosure Summary: The authors have nothing to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References and Notes

1. Chang J, Rayner CK, Jones KL, Horowitz M. Diabetic gastroparesis and its impact on glycemia. *Endocrinol Metab Clin North Am*. 2010;**39**(4):745–762.
2. Nauck MA, Meier JJ. Incretin hormones: their role in health and disease. *Diabetes Obes Metab*. 2018;**20**(Suppl 1):5–21.
3. Elrick H, Stimmler L, Hlad CJ, Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. *J Clin Endocrinol Metab*. 1964;**24**(10):1076–1082.
4. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia*. 2011;**54**(1):10–18.
5. Seufert J. Incretins and their endocrine and metabolic functions. *Endocr Dev*. 2017;**32**:38–48.
6. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology*. 2007;**132**(6):2131–2157.
7. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. *J Diabetes Investig*. 2010;**1**(1-2):8–23.
8. Edholm T, Degerblad M, Gryback P, Hilsted L, Holst JJ, Jacobsson H, Efendic S, Schmidt PT, Hellstrom PM. Differential incretin effects of GIP and GLP-1 on gastric emptying, appetite, and insulin-glucose homeostasis. *Neurogastroenterol Motil*. 2010;**22**(11):1191–1200.
9. McIntosh CH, Widenmaier S, Kim SJ. Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide; GIP). *Vitam Horm*. 2009;**80**:409–471.
10. Knop FK, Vilsbøll T, Højberg PV, Larsen S, Madsbad S, Vølund A, Holst JJ, Krarup T. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? *Diabetes*. 2007;**56**(8):1951–1959.
11. Dungan K, DeSantis A. Glucagon-like peptide-1 receptor agonists for the treatment of type 2 diabetes mellitus. Available at https://www.uptodate.com/contents/glucagon-like-peptide-1-receptor-agonists-for-the-treatment-of-type-2-diabetes-mellitus. Accessed 12 August 2019.
12. Eckerle Mize DL, Salehi M. The place of GLP-1-based therapy in diabetes management: differences between DPP-4 inhibitors and GLP-1 receptor agonists. *Curr Diab Rep*. 2013;**13**(3):307–318.
13. Trahair LG, Horowitz M, Marathe CS, Lange K, Standfield S, Rayner CK, Jones KL. Impact of gastric emptying to the glycemic and insulinemic responses to a 75-g oral glucose load in older

subjects with normal and impaired glucose tolerance. *Physiol Rep*. 2014;**2**(11):e12204.
14. Marathe CS, Horowitz M, Trahair LG, Wishart JM, Bound M, Lange K, Rayner CK, Jones KL. Relationships of early and late glycemic responses with gastric emptying during an oral glucose tolerance test. *J Clin Endocrinol Metab*. 2015;**100**(9):3565–3571.
15. Horowitz M, Edelbroek MA, Wishart JM, Straathof JW. Relationship between oral glucose tolerance and gastric emptying in normal healthy subjects. *Diabetologia*. 1993;**36**(9):857–862.
16. O'Donovan D, Hausken T, Lei Y, Russo A, Keogh J, Horowitz M, Jones KL. Effect of aging on transpyloric flow, gastric emptying, and intragastric distribution in healthy humans--impact on glycemia. *Dig Dis Sci*. 2005;**50**(4):671–676.
17. Liljeberg H, Björck I. Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. *Eur J Clin Nutr*. 1998;**52**(5):368–371.
18. Jones KL, Tonkin A, Horowitz M, Wishart JM, Carney BI, Guha S, Green L. Rate of gastric emptying is a determinant of postprandial hypotension in non-insulin-dependent diabetes mellitus. *Clin Sci (Lond)*. 1998;**94**(1):65–70.
19. Stevens JE, Gilja OH, Gentilcore D, Hausken T, Horowitz M, Jones KL. Measurement of gastric emptying of a high-nutrient liquid by 3D ultrasonography in diabetic gastroparesis. *Neurogastroenterol Motil*. 2011;**23**(3):220–225.
20. Marathe CS, Rayner CK, Jones KL, Horowitz M. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. *Diabetes Care*. 2013;**36**(5):1396–1405.
21. Trahair LG, Horowitz M, Rayner CK, Gentilcore D, Lange K, Wishart JM, Jones KL. Comparative effects of variations in duodenal glucose load on glycemic, insulinemic, and incretin responses in healthy young and older subjects. *J Clin Endocrinol Metab*. 2012;**97**(3):844–851.
22. Marathe CS, Rayner CK, Bound M, Checklin H, Standfield S, Wishart J, Lange K, Jones KL, Horowitz M. Small intestinal glucose exposure determines the magnitude of the incretin effect in health and type 2 diabetes. *Diabetes*. 2014;**63**(8):2668–2675.
23. Pilichiewicz AN, Chaikomin R, Brennan IM, Wishart JM, Rayner CK, Jones KL, Smout AJ, Horowitz M, Feinle-Bisset C. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. *Am J Physiol Endocrinol Metab*. 2007;**293**(3):E743–E753.
24. Wu T, Zhang X, Trahair LG, Bound MJ, Little TJ, Deacon CF, Horowitz M, Jones KL, Rayner CK. Small intestinal glucose delivery affects the lowering of blood glucose by acute vildagliptin in type 2 diabetes. *J Clin Endocrinol Metab*. 2016;**101**(12):4769–4778.
25. Deane AM, Nguyen NQ, Stevens JE, Fraser RJL, Holloway RH, Besanko LK, Burgstad C, Jones KL, Chapman MJ, Rayner CK, Horowitz M. Endogenous glucagon-like peptide-1 slows gastric emptying in healthy subjects, attenuating postprandial glycemia. *J Clin Endocrinol Metab*. 2010;**95**(1):215–221.
26. Soenen S, Rayner CK, Horowitz M, Jones KL. Gastric emptying in the elderly. *Clin Geriatr Med*. 2015;**31**(3):339–353.
27. Pham H, Phillips L, Trahair L, Hatzinikolas S, Horowitz M, Jones KL. Longitudinal changes in the blood pressure responses to, and gastric emptying of, an oral glucose load in healthy older subjects [published online ahead of print 23 January 2019]. *J Gerontol A Biol Sci Med Sci*. doi: 10.1093/gerona/glz014.
28. Gentilcore D, Doran S, Meyer JH, Horowitz M, Jones KL. Effects of intraduodenal glucose concentration on blood pressure and heart rate in healthy older subjects. *Dig Dis Sci*. 2006;**51**(4):652–656.
29. Chan JCN, Wong RYM, Cheung C-K, Lam P, Chow C-C, Yeung VTF, Kan ECY, Loo K-M, Mong MYL, Cockram CS. Accuracy, precision and user-acceptability of self blood glucose monitoring machines. *Diabetes Res Clin Pract*. 1997;**36**(2):91–104.
30. Weitgasser R, Straberger A, Schnöll F, Sailer S. Clinical evaluation of the blood glucose monitors Accutrend, Companion 2, Glucometer 3 and One Touch II in comparison with the glucose oxidase reference method [in German]. *Wien Klin Wochenschr*. 1994;**106**(23):738–741.
31. Alberti KGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;**15**(7):539–553.
32. Wishart J, Morris HA, Horowitz M. Radioimmunoassay of gastric inhibitory polypeptide in plasma. *Clin Chem*. 1992;**38**(10):2156–2157.
33. Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology*. 1993;**104**(6):1640–1647.
34. Elahi D, Andersen DK, Muller DC, Tobin JD, Brown JC, Andres R. The enteric enhancement of glucose-stimulated insulin release. The role of GIP in aging, obesity, and non-insulin-dependent diabetes mellitus. *Diabetes*. 1984;**33**(10):950–957.
35. Geloneze B, de Oliveira MS, Vasques AC, Novaes FS, Pareja JC, Tambascia MA. Impaired incretin secretion and pancreatic dysfunction with older age and diabetes. *Metabolism*. 2014;**63**(7):922–929.
36. Korosi J, McIntosh CH, Pederson RA, Demuth H-U, Habener JF, Gingerich R, Egan JM, Elahi D, Meneilly GS. Effect of aging and diabetes on the enteroinsular axis. *J Gerontol A Biol Sci Med Sci*. 2001;**56**(9):M575–M579.
37. MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JBMJ, Morley JE, Horowitz M, Chapman IM. Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. *Am J Clin Nutr*. 1999;**69**(5):999–1006.
38. Sturm K, MacIntosh CG, Parker BA, Wishart J, Horowitz M, Chapman IM. Appetite, food intake, and plasma concentrations of cholecystokinin, ghrelin, and other gastrointestinal hormones in undernourished older women and well-nourished young and older women. *J Clin Endocrinol Metab*. 2003;**88**(8):3747–3755.
39. Ranganath L, Sedgwick I, Morgan L, Wright J, Marks V. The ageing entero-insular axis. *Diabetologia*. 1998;**41**(11):1309–1313.
40. Giezenaar C, Hutchison AT, Luscombe-Marsh ND, Chapman I, Horowitz M, Soenen S. Effect of age on blood glucose and plasma insulin, glucagon, ghrelin, CCK, GIP, and GLP-1 responses to whey protein ingestion. *Nutrients*. 2017;**10**(1):E2.
41. de Jesús Garduno-Garcia J, Gastaldelli A, DeFronzo RA, Lertwattanarak R, Holst JJ, Musi N. Older subjects with β-cell dysfunction have an accentuated incretin release. *J Clin Endocrinol Metab*. 2018;**103**(7):2613–2619.
42. Meneilly GS, Demuth HU, McIntosh CH, Pederson RA. Effect of ageing and diabetes on glucose-dependent insulinotropic polypeptide and dipeptidyl peptidase IV responses to oral glucose. *Diabet Med*. 2000;**17**(5):346–350.
43. Færch K, Torekov SS, Vistisen D, Johansen NB, Witte DR, Jonsson A, Pedersen O, Hansen T, Lauritzen T, Sandbæk A, Holst JJ, Jørgensen ME. GLP-1 response to oral glucose is reduced in prediabetes, screen-detected type 2 diabetes, and obesity and influenced by sex: the ADDITION-PRO study. *Diabetes*. 2015;**64**(7):2513–2525.
44. Koopman ADM, Rutters F, Rauh SP, Nijpels G, Holst JJ, Beulens JW, Alssema M, Dekker JM. Incretin responses to oral glucose and mixed meal tests and changes in fasting glucose levels during 7 years of follow-up: The Hoorn Meal Study. *PLoS One*. 2018;**13**(1):e0191114.
45. Zahn A, Langhans CD, Hoffner S, Haberkorn U, Rating D, Haass M, Enck P, Stremmel W, Rühl A. Measurement of gastric emptying by 13C-octanoic acid breath test versus scintigraphy in diabetics. *Z Gastroenterol*. 2003;**41**(5):383–390.
46. Szarka LA, Camilleri M, Vella A, Burton D, Baxter K, Simonson J, Zinsmeister AR. A stable isotope breath test with a standard meal for abnormal gastric emptying of solids in the clinic and in research. *Clin Gastroenterol Hepatol*. 2008;**6**(6):635–643.