Direct effect of incretin hormones on glucose and glycerol metabolism and hemodynamics

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Karstoft K, Mortensen SP, Knudsen SH, Solomon TP. Direct effect of incretin hormones on glucose and glycerol metabolism and hemodynamics. Am J Physiol Endocrinol Metab 308: E426–E433, 2015. First published January 6, 2015; doi:10.1152/ajpendo.00520.2014.—The objective of this study was to assess the insulin-independent effects of incretin hormones on glucose and glycerol metabolism and hemodynamics under euglycemic and hyperglycemic conditions. Young, healthy men (n = 10) underwent three trials in a randomized, controlled, crossover study. Each trial consisted of a two-stage (euglycemia and hyperglycemia) pancreatic clamp (using somatostatin to prevent endogenous insulin secretion). Glucose and lipid metabolism was measured via infusion of stable glucose and glycerol isotopic tracers. Hemodynamic variables (femoral, brachial, and common carotid artery blood flow and flow-mediated dilatation of the brachial artery) were also measured. The three trials differed as follows: 1) saline [control (CON)], 2) glucagon-like peptide (GLP-1, 0.5 pmol·kg⁻¹·min⁻¹), and 3) glucose-dependent insulinotropic polypeptide (GIP, 1.5 pmol·kg⁻¹·min⁻¹). No between-trial differences in glucose infusion rates (GIR) or glucose or glycerol kinetics were seen during euglycemia, whereas hyperglycemia resulted in increased GIR and glucose rate of disappearance during GLP-1 compared with CON and GIP (P < 0.01 for all). However, when normalized to insulin levels, no differences between trials were seen for GIR or glucose rate of disappearance. Besides a higher femoral blood flow during hyperglycemia with GIP (vs. CON and GLP-1, P < 0.001), no between-trial differences were seen for the hemodynamic variables. In conclusion, GLP-1 and GIP have no direct effect on whole body glucose metabolism or hemodynamics during euglycemia. On the contrary, during hyperglycemia, GIP increases femoral artery blood flow with no effect on glucose metabolism, whereas GLP-1 increases glucose disposal, potentially due to increased insulin levels.

pancreatic clamp; glucose disposal; glucose-dependent insulinotropic polypeptide; glucagon-like peptide-1; hyperglycemia; glucose effectiveness; glucose kinetics; somatostatin; hemodynamics

IT IS WELL ESTABLISHED that the incretin hormones, glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP), stimulate insulin secretion from pancreatic beta cells (18). It is also well known that the incretin effect on insulin secretion is glucose-dependent, such that both GLP-1 and GIP more greatly augment insulin secretion when plasma glucose is in the hyperglycemic range (19, 38, 39). Moreover, GLP-1 and GIP receptors are expressed extrapancreatically. For example, GLP-1 receptors have been identified by us and others in rat/human skeletal muscle, in muscle cell lines and primary human muscle cells (10, 16, 40), in human adipose tissue (23), and in human hepatocytes (17), although this has recently been questioned (30). GIP receptors have been identified in the endothelium (41) and in adipose tissue (32). Thus extrapancreatic effects of incretin hormones are reasonable to consider.

Cell studies in rats indicate that GLP-1 may upregulate glucose metabolism via a pathway similar to insulin in muscle and liver (1–3), a finding confirmed in human muscle cell cultures by us (16) and others (15, 22). GLP-1 infusion has also been shown to suppress endogenous glucose production (6, 29, 33) and to alter lipolysis (8), but the latter was only investigated in the presence of elevations in insulin, a powerful lipolytic inhibitor. On the other hand, GIP infusion has been shown to increase lipid metabolism and adipose blood flow in humans (4, 5). In these studies, the effects of GIP per se remain unknown, because GIP-induced insulin secretion was not controlled.

The in vivo extrapancreatic effects of both GLP-1 and GIP on glucose and lipid metabolism and hemodynamics in humans are not well understood. Moreover, since the insulinotropic effect of incretin hormones is glucose-dependent, it is prudent to also evaluate the glucose dependency of the extrapancreatic effects of GLP-1 and GIP. Previous studies of the extrapancreatic in vivo effect of incretins (6, 29, 34) have not been done under hyperglycemic pancreatic clamp conditions. Interestingly, our previous work indicated that the effects of GLP-1-stimulated glucose uptake in human primary muscle cells were blunted under hyperglycemic conditions (16).

In view of the above-mentioned findings from previous studies, we hypothesized that GLP-1 and GIP per se regulate glucose metabolism and lipolysis and hemodynamics, respectively. Therefore, the aim of this study was to assess the insulin-independent effect of incretin hormones on glucose and glycerol metabolism and hemodynamics during a pancreatic clamp under euglycemic and hyperglycemic conditions.

MATERIALS AND METHODS

Subjects. Normal-weight [18–25 kg/m² body mass index (BMI)], young (18–30 yr old), male subjects with no history of diabetes underwent medical screening, which included a physical examination, blood chemistry analysis, physical activity questionnaire (31), oral glucose tolerance test (OGTT), and dual-energy X-ray absorptiometry (Lunar Prodigy Advance, GE Healthcare, Madison, WI) scan. Exclusion criteria included compromised glucose tolerance (fasting glucose >5.6 mmol/l, 2-h OGTT glucose >7.8 mmol/l, HbA1c >5.7%) and evidence of liver, renal, or cardiopulmonary diseases. Written informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Capital
Trials. On separate days, 1–2 wk apart, subjects underwent three trials in a randomized order. Trials consisted of a baseline tracer load period followed by a two-stage (euglycemic and hyperglycemic) pancreatic clamp and were identical except for the following infusions: 1) saline [control (CON)], 2) GLP-1 (0.5 pmol·kg⁻¹·min⁻¹), and 3) GIP (1.5 pmol·kg⁻¹·min⁻¹). Subjects were instructed to avoid vigorous physical activity and alcohol for 48 h prior to the trials. Diet records were taken for 24 h prior to the first trial, and subjects were instructed to ingest the same foods prior to each trial.

Pancreatic clamp procedure. On the trial day, subjects came to the laboratory in the morning after an overnight (≥8-h) fast and were placed in a supine position for the entire trial. An antecubital venous catheter was placed for infusions, and a dorsal venous catheter was placed in the contralateral hand for blood sampling. To arterialize blood, the hand was kept warm using a heating blanket (~60°C). At ~120 min, fasting blood samples were collected, and primed, continuous infusions of [6,6-²H₂]glucose (Cambridge Isotopes, Boston, MA; 20 μmol/kg multiplied by fasting glucose divided by 5 mmol/l prime, followed by 0.15 μmol/kg⁻¹·min⁻¹) and [1,1,2,3,3-²H₂]glycerol (Cambridge Isotopes, Boston, MA; 1.5 μmol/kg prime, followed by 0.1 μmol·kg⁻¹·min⁻¹) were initiated. At 0 min, the pancreatic clamp began (Fig. 1): a mixture of somatostatin (100 ng·kg⁻¹·min⁻¹; octreotide, Hospira, Lake Forest, IL), insulin (0.15 μU·kg⁻¹·min⁻¹; Actrapid, Novo Nordisk, Bagsvaerd, Denmark), glucagon (0.5 ng·kg⁻¹·min⁻¹; GlucaGen, Novo Nordisk), and growth hormone (3 ng·kg⁻¹·min⁻¹; Humatrope, Eli Lilly, Indianapolis, IN) dissolved in 50 ml of isotonic saline spiked with 2 ml of 5% human albumin (CSL Behring, King of Prussia, PA) was infused. The pancreatic clamp continued from 0 to 180 min. Blood glucose levels were clamped at subjects’ baseline glucose from 0 to 90 min (euglycemia) by a variable-rate glucose infusion. At 90 min, a 15-min square-wave glucose infusion to increase baseline glucose concentra-

Isotopic Metabolic Tracers

- [6,6-²H₂]glucose
- [1,1,2,3,3-²H₂]glycerol

Pancreatic Clamp

- Somatostatin (Sandostatin, Novartis; 100 ng/kg/min)
- Insulin (Actrapid, Novo Nordisk; 0.15 μU/kg/min)
- Glucagon (Glucagon, Novo Nordisk; 0.5 ng·kg⁻¹·min⁻¹)
- Growth Hormone (Humatrope, Eli Lilly; 3 ng/kg/min)

Glucose

- Glucose (local [×10⁻⁵ M])
- Glucose (± 4 mM glucose base)

Trial A: Saline

Trial B: GLP-1 (0.5 pmol/kg/min)

Trial C: GIP (1.5 pmol/kg/min)

From 0 to 180 min, blood glucose was measured on a bedside platform (ABL 700, Radiometer, Herlev, Denmark) every 5 min, and glucose infusion rates (GIR) were adjusted (except during the 15-min square-wave glucose infusion at 90 min) using a computerized algorithm based on the original description by DeFronzo et al. (9). GIR, plasma glucose coefficient of variation, and plasma glucose error from the goal are reported as means of the final 30 min of each clamp stage.

Blood sampling. Blood samples for insulin and C-peptide measurements (collected in serum tubes) and for plasma glucose and glycerol tracer enrichment analyses (collected in NaF tubes) were obtained at baseline and every 10 min during the last 30 min of each clamp stage. Blood samples for measurement of plasma glucagon, GIP, and total GLP-1 [IGLP-1; collected in EDTA tubes containing apropin (500 kU/ml; Trasylol, Bayer HealthCare, Berlin, Germany) and dipeptidyl peptidase-4 inhibitor (0.01 mmol/l diprotin A; Sigma, Copenhagen, Denmark)] were obtained at the end of each clamp stage. Blood samples for plasma separation were placed on ice, while samples for serum separation were kept at room temperature, for 30 min before centrifugation (2,000 g, 15 min, 4°C). Plasma/serum samples were stored at ~80°C until analysis.

Blood chemistry analyses. Plasma [6,6-²H₂]glucose and [1,1,2,3,3-²H₂]glycerol tracer enrichments were measured simultaneously by a liquid chromatography-mass spectrometry hexabenzoyl derivative method (25). Total rates of glucose and glycerol appearance (Ra) and disappearance (Rd) were determined during the last 30 min of each clamp stage using a single-pool model (28). Endogenous rate of glucose production (EGP) was calculated as glucose Rd minus the sum of GIR and the [6,6-²H₂]glucose tracer infusion rate. Insulin and C-peptide were measured by an electrochemiluminescence immunoassay (E-modular, Roche, Basel, Switzerland). Glucagon was measured by RIA (Millipore, Darmstadt, Germany). GIP was measured by ELISA (Millipore), and IGLP-1 was measured by an electrochemiluminescence immunoassay (Meso Scale Discovery, Rockville, MD).

Arterial blood flow and flow-mediated dilation. Arterial (femoral, brachial, and carotid) blood flow and flow-mediated dilation (FMD) were measured during the baseline (~30 min), euglycemic (60 min), and hyperglycemic (150 min) stages of the pancreatic clamps using an ultrasound machine equipped with a linear probe operating at an imaging frequency of 7–9 MHz and a Doppler frequency of 4.2–5.0 MHz (Logic E9, GE Healthcare, Little Chalfont, UK). Femoral artery blood flow was measured distal to the inguinal ligament but 1–2 cm above the bifurcation. Brachial artery blood flow was measured 1–1.5 cm proximal to the bifurcation in the blood sampling arm, and common carotid artery blood flow was measured 1–2 cm below the carotid bifurcation. All recordings were obtained at the lowest possible insonation angle and always below 60°. The sample volume was maximized according to the width of the vessel and kept clear of the vessel walls. A lowvelocity (<1.8 m/s) filter rejected noises caused by turbulence at the vascular wall. Doppler traces and B-mode images were recorded continuously, and Doppler traces were averaged over eight heart cycles. Arterial diameter was calculated as one-third of the diameter during the systole and two-thirds of the diameter during the diastole. FMD was assessed in the brachial artery in the blood sampling arm with the pneumatic cuff placed proximal to the measuring site (upper arm). Baseline images were recorded for 30 s before the cuff was rapidly inflated to 250 mmHg for 3 min. Recording resumed 30 s before cuff deflation and continued for 3 min postdeflation. FMD is presented as the relative (%) rise from the preceding baseline diameter. Blood pressure (manual auscultation of 1st and 4th Korotkoff’s sound in the antecubital fossa following release of prox-
imal cuff inflation) and heart rate (manual counting) were measured immediately after each blood flow and FMD measurement.

Statistics. Baseline variables were analyzed by one-way repeated-measures ANOVA. Variables of interest were analyzed by two-way (clamp stage × trial) repeated-measures ANOVA. Multiple comparisons examined differences between clamp stages within each trial, as well as differences between trials within each clamp stage. Bonferroni-corrected post hoc tests were used to determine specific differences between means. Values are means ± SE. Statistical significance was accepted when \( P < 0.05 \). Analyses were performed using Prism version 6 (GraphPad, San Diego, CA).

RESULTS

Subjects. Ten subjects (22 ± 1 yr old, 70.0 ± 2.1 kg body wt, 21.2 ± 0.5 kg/m² BMI) with normal glucose tolerance (fasting glucose = 5.2 ± 0.4 mmol/l, fasting insulin = 52.0 ± 6.2 pmol/l, 2-h OGTT glucose = 5.6 ± 0.3 mmol/l, Hb A1c = 34.2 ± 0.7 mmol/mol) participated in the study. All subjects completed all trials. Diet records showed no between-trial differences in total energy intake or macronutrient composition (\( P > 0.05 \) for all comparisons; data not shown). Body weight was also not different between trial days (\( P > 0.05 \) for all comparisons; data not shown).

Pancreatic clamp characteristics. No baseline differences were seen for plasma glucose, serum insulin or C-peptide, or plasma glucagon, GLP-1, GIP, or glycerol levels between any of the trials (Table 1). Plasma glucose levels were successfully clamped at the desired levels during euglycemic and hyperglycemic clamp stages, with low percent coefficient of variation and percent error from the goal values in all trials (Table 1). Plasma glucose was not different between trials, but a main effect of clamp stage was noted across all trials (\( P < 0.0001 \); Fig. 2A). During euglycemia, the GIR required to clamp plasma glucose was not different between trials. GIR was increased during hyperglycemia in all trials (Fig. 2B; \( P < 0.0001 \)) and more greatly increased in the GLP-1 trial than GIP and CON trials (\( P < 0.01 \) and \( P < 0.001 \); Fig. 2B). Plasma insulin levels were not different between trials during the euglycemic stage; during the hyperglycemic stage, they were higher in the GLP-1 and GIP trials (both \( P < 0.0001 \) vs. CON; Fig. 2C). Moreover, in the GLP-1 and GIP trials, insulin levels were higher during hyperglycemia than euglycemia (\( P < 0.0001 \) for both; Fig. 2C). Plasma C-peptide levels were suppressed during euglycemia compared with baseline in all trials (\( P < 0.0001 \); Fig. 2D). During hyperglycemia, plasma C-peptide was higher during the GLP-1 and GIP trials (\( P < 0.0001 \) vs. CON; Fig. 2D), and, in all trials, C-peptide levels were higher during hyperglycemia than euglycemia (\( P < 0.0001 \); Fig. 2D). Plasma glucagon concentrations showed a main effect of time (\( P < 0.0001 \); Table 1) but were not different between trials, nor were they different between euglycemic and hyperglycemic stages (\( P > 0.05 \); Table 1). Plasma tGLP-1 and GIP levels showed a main effect of clamp stage and trial and a clamp stage × trial interaction (\( P < 0.0001 \) for all, except \( P < 0.05 \) for tGLP-1 time effect), and, as expected, tGLP-1 and GIP levels were higher during euglycemia and hyperglycemia than CON in the GLP-1 and GIP trials, respectively. Plasma glycerol was not different between trials, but a main effect of clamp stage was noted across all trials (\( P < 0.0001 \); Table 1).

Glucose kinetics. As shown in Fig. 3, glucose total \( R_T \) and \( R_d \) showed main effects of clamp stage (\( P < 0.0001 \)), trial (\( P < 0.01 \)), and clamp stage × trial interaction (\( P < 0.01 \)), while EGP showed a main effect of clamp stage (\( P < 0.01 \)). Glucose total \( R_T \) and \( R_d \) were greater during the hyperglycemic than euglycemic clamp stage and at baseline (\( P < 0.0001 \); Fig. 3, A and B). Furthermore, during hyperglycemia, total \( R_T \) and \( R_d \) were greater in the GLP-1 trial (\( P < 0.01 \) vs. CON and vs. GIP; Fig. 3, A and B). When glucose total \( R_T \) and \( R_d \) were normalized to present insulin and glucose levels, no between-trial differences were found during any clamp stage, and no differences between the euglycemic and hyperglycemic clamp stages were found for any trial. After a two-way ANOVA, no be-

### Table 1. Pancreatic clamp variables

<table>
<thead>
<tr>
<th>Trials</th>
<th>CON</th>
<th>GLP-1</th>
<th>GIP</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Euglycemia</td>
<td>Hyperglycemia</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.2 ± 0.1</td>
<td>5.2 ± 0.2</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>Glucose CV, %</td>
<td>0.9 ± 0.3</td>
<td>0.3 ± 0.9</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>Error from clamp goal, %</td>
<td>–0.9 ± 0.3</td>
<td>–1.0 ± 0.6</td>
<td>–5.4 ± 2.1</td>
</tr>
<tr>
<td>GIR, pmol·kg⁻¹·min⁻¹</td>
<td>4.9 ± 1.4</td>
<td>23.1 ± 3.4</td>
<td>7.2 ± 1.6</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>53 ± 7</td>
<td>77 ± 6</td>
<td>95 ± 6</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>54.9 ± 42</td>
<td>128 ± 11</td>
<td>136 ± 37</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>68 ± 7</td>
<td>49 ± 16</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>tGLP-1, ng/ml</td>
<td>1.3 ± 0.3</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>GIP, pg/ml</td>
<td>52 ± 7</td>
<td>14 ± 2</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Glyceral, pmol/l</td>
<td>64 ± 9</td>
<td>24 ± 2</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>120 ± 3</td>
<td>116 ± 3</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>77 ± 3</td>
<td>75 ± 3</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91 ± 3</td>
<td>88 ± 3</td>
<td>91 ± 3</td>
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</table>

Values are means ± SE. Young, healthy men underwent 3 trials in randomized order: 1) saline infusion (CON), 2) glucagon-like peptide (GLP-1) infusion (0.5 pmol·kg⁻¹·min⁻¹), and 3) glucose-dependent insulinotropic polypeptide (GIP) infusion (1.5 pmol·kg⁻¹·min⁻¹). After a 2-h basal period, a 2-stage 3-h pancreatic clamp consisting of 1.5 h of euglycemia followed by 1.5 h of hyperglycemia (5.4 mmol/l above individual basal glucose) was initiated. Plasma glucose was clamped at the required level via a variable glucose infusion rate (GIR). Data shown for plasma glucose, GIR, insulin, C-peptide, and glycerol represent means of the last 30 min of each clamp stage. Data shown for glucagon, total GLP-1 (tGLP-1), and GIP represent the final time point in each stage. CV, coefficient of variation; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
between-trial effects on EGP were found, despite a greater nonsignificant suppression of EGP during hyperglycemia in the GLP-1 trial (Fig. 3C).

Glycerol kinetics. Glycerol total Ra and Rd showed main effects of clamp stage \( (P < 0.0001; \text{Fig. 3D}) \), decreasing from baseline during euglycemia and hyperglycemia, but no between-trial differences were found.

Hemodynamic variables. No baseline differences were found for blood pressure, heart rate, femoral/brachial/common carotid artery blood flow, or FMD between any of the trials (Table 1). Systolic blood pressure showed a main effect of time \( (P < 0.05; \text{Table 1}) \), but no differences in blood pressure (systolic, diastolic, and mean arterial pressure) were seen between trials or clamp stages. Heart rate demonstrated a main effect of time \( (P < 0.01; \text{Table 1}) \) and, despite showing a tendency for elevation in the GIP trial during euglycemia, no main effect of trial or interaction. FMD was found to have a main effect of time \( (P < 0.05; \text{Fig. 4A}) \) but no differences between trials. Femoral artery blood flow showed main effects of time and trial with a significant interaction (all \( P < 0.01; \text{Fig. 4B} \)), showing that femoral artery blood flow in the GIP trial was greater during hyperglycemia than during other trials \( (P < 0.001) \) and at baseline \( (P < 0.0001) \) and during euglycemic \( (P < 0.001) \) clamp stages (Fig. 4B). However, neither common carotid (Fig. 4C) nor brachial (Fig. 4D) artery blood flow demonstrated significant effects of time or trial.

**DISCUSSION**

This study demonstrates that, under pancreatic clamp conditions at normal plasma glucose levels, neither GLP-1 nor GIP appears to affect glucose infusion rates or glucose kinetics. Conversely, under hyperglycemic conditions, physiological increases in GLP-1 augment plasma glucose disappearance and seem to suppress endogenous glucose production, effects that were not induced by physiological increases in GIP, although insulin levels were comparable between these trials. On the contrary, glycerol Ra (a marker of whole body lipolysis) and Rd were not affected by GLP-1 or GIP under euglycemic and hyperglycemic conditions. Also, under hyperglycemic pancreatic clamp conditions, physiological increases in GIP facilitated an increase in femoral artery blood flow independent of changes in heart rate. We believe that inclusion of the hyperglycemic clamp stage in our study is interesting and important, since hyperglycemic clamp results were different from euglycemic clamp results and were not previously been reported in similar studies (6, 29, 34).

Previous in vitro work showing that GLP-1 increases glucose disposal during euglycemic conditions (1–3, 15, 16, 22) appears not to be confirmed in human in vivo studies, as shown here and elsewhere (26, 37). On the contrary, GLP-1 has been reported to suppress EGP under euglycemic conditions (6, 29), which we did not find. While one of these studies was per-
formed in rodents (6), Prigeon et al. (29) used a study design much like ours to study humans. However, the study of Prigeon et al. was a within-day comparison before and during GLP-1 infusion, which reduced variance by eliminating day-to-day variance. Also, our clamp stages were 90 min long with continuous GLP-1 infusion, whereas Prigeon et al. used only a 60-min GLP-1 infusion and found suppression of EGP by GLP-1 only during the first 30 min. Consequently, it is unclear whether the EGP-suppressing effect of GLP-1 under euglycemic conditions is only temporary (29). Conversely, Seghieri et al. (33), who infused GLP-1 in physiological concentrations (0.4 pmol·kg\(^{-1}\)·min\(^{-1}\)), found a sustained reduction of EGP during hyperglycemia, which we also found (although our results were statistically insignificant). Thus, sustained activation of the GLP-1 receptors in the liver (17) may be responsible for reducing glucose output in a glucose-dependent manner independent of insulin and glucagon (33). However, in the study of Seghieri et al., hyperglycemia was reached via higher glucagon and lower insulin concentrations than were used in our study. This resulted in greater EGP in the study of Seghieri et al. than in our study, thereby allowing for greater impact of GLP-1 on EGP. Moreover, the insignificant reduction of EGP during hyperglycemia in the GLP-1 trial in our study may be related to a twofold increase in GLP-1 levels compared with a threefold increase reported by Seghieri et al. The mechanisms by which GLP-1 may decrease EGP have recently been challenged, since the presence of GLP-1 receptors on hepatocytes has been questioned (30). However, GLP-1 may affect EGP indirectly via neural mechanisms (27). Finally, under normal physiological circumstances, the most important mechanism by which the incretins affect EGP is via alterations in insulin and glucagon concentrations (20).

The finding that GLP-1 may directly increase glucose disappearance during hyperglycemic pancreatic clamp conditions is interesting, because it potentially provides additional clinical relevance to the use of GLP-1 analogs in the treatment of hyperglycemic patients. While the effects of GLP-1 during hyperglycemic pancreatic clamp conditions, consequently, are intriguing, the mechanism explaining these effects requires further examination, particularly in view of our findings that the insulin secretory responses (which are reflected by plasma C-peptide levels) during hyperglycemia were not totally suppressed. This precludes us from definitively stating that GLP-1 and GIP have direct extrapancreatic effects during hyperglycemia. Nevertheless, the endogenous insulin secretion and plasma insulin levels were not significantly different between GLP-1 and GIP trials during hyperglycemia. Thus the larger GIR and glucose \(R_d\) and apparently lower EGP during the GLP-1 than the GIP trial seem to be mediated independent of insulin.

Dimitriadis et al. (11) also showed effects of hyperglycemia on somatostatin-induced suppression of pancreatic endocrine secretion, and Toft-Nielsen et al. (36) documented a small rise in plasma insulin following administration of an intravenous 25-g glucose bolus during infusion of 50 pmol·kg\(^{-1}\)·h\(^{-1}\) GLP-1 and 500 (but not 1,000) \(\mu\)g/h somatostatin. The failure of somatostatin to suppress endogenous insulin secretion during hyperglycemic conditions is possibly due to persistent insulin biosynthesis that would occur as a result of acute elevations in blood glucose (12, 21); however, the mechanism is not understood. Future studies should try to overcome this problem, e.g., by investigating the direct effects of the incretin hormones under hyperglycemic conditions in subjects with minimal endogenous insulin secretion capacity, such as in patients with type 1 diabetes.

Neither GLP-1 nor GIP affected glycerol metabolism. Whereas this lack of in vivo effect on lipolysis has previously been shown for GLP-1 (33), GIP has previously been shown to increase fatty acid reesterification in subcutaneous abdominal adipose tissue (4) and to stimulate lipolysis in perfused rat adipose tissue (4) and to stimulate lipolysis in perfused rat.

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**Fig. 3.** Effects of GLP-1 and GIP on glucose and glycerol kinetics. In a randomized, crossover design, subjects underwent three trials: saline infusion (CON), 0.5 pmol·kg\(^{-1}\)·min\(^{-1}\) GLP-1 infusion, and 1.5 pmol·kg\(^{-1}\)·min\(^{-1}\) GIP infusion. After a 2-h basal period, a 3-h pancreatic clamp consisting of 1.5 h of euglycemia followed by 1.5 h of hyperglycemia (5.4 mmol/l above individual basal glucose) was conducted. Plasma glucose and glycerol kinetics were determined via infusions of \([6,6-\text{H}_2]\)glucose and \([1,1,2,3,3-\text{H}_2]\)glycerol. Rates of total plasma glucose appearance (Ra; \(A\)) and glucose disappearance (Rd; \(B\)), endogenous glucose production (EGP; \(C\)), total glycerol appearance (\(D\)), and glycerol disappearance (\(E\)) were determined. Values are means ± SE. Statistically significant post hoc comparisons are as follows: ***P < 0.0001, hyperglycemia vs. euglycemia and hyperglycemia vs. basal within all trials; ##P < 0.01, GLP-1 vs. CON and GLP-1 vs. GIP in hyperglycemic clamp stage.
adipocytes (14). However, this difference is likely explained by the fact that these prior studies show local effects on specific adipose depots, whereas we have studied whole body metabolism.

Another intriguing finding of our study is that GIP increased femoral artery blood flow during hyperglycemic pancreatic clamp conditions. While this may be a secondary consequence of insulin elevation, this was also present during GLP-1 infusion, where no increment in blood flow was seen. Since no changes in carotid and brachial arm flow were seen within or between any of the trials, it is unclear whether the increased femoral artery blood flow during hyperglycemia in the GIP trial is a result of a statistical type 1 error. Still, if the finding is accepted as true, GIP may directly increase femoral artery blood flow during hyperglycemia. Accordingly, Asmar et al. (4) found that 1.5 pmol·kg⁻¹·min⁻¹ GIP infusion during hyperinsulinemic hyperglycemic conditions increased adipose tissue blood flow, as measured by ¹³³Xe washout. In their study, while glucose levels were a little lower (7 mM) than those in the present study, the insulin levels were identical (150 pM) to those found during our hyperglycemic pancreatic clamp. While endothelial cells express GIP receptors (41), neither the underlying mechanism nor the clinical relevance of these findings is known. Interestingly, GLP-1 has been shown to increase forearm blood flow as measured by venous occlusion plethysmography (7) and both brachial artery diameter and blood flow (35), as well as microvascular perfusion measured by ultrasound Doppler (34), during euglycemic conditions. In our study, however, we found no effects of GLP-1 on hemodynamic variables. Differences in administration of GLP-1 between our study (systemic infusions, 0.5 pmol·kg⁻¹·min⁻¹) and other studies [direct infusion into the femoral artery at 1.2 pmol·kg⁻¹·min⁻¹ (7) and 1 pmol·kg⁻¹·min⁻¹ (34)], GLP-1 infusion rate differences [1.2 pmol·kg⁻¹·min⁻¹ (35)], and the younger age and lower BMI of our subjects than other cohorts (7) are likely to explain these different findings.

Because somatostatin-induced suppression of insulin secretion was impaired under hyperglycemic conditions in the GLP-1 and GIP trials, a limitation of our study is that accurate conclusions regarding the insulin-independent effect of incretins during hyperglycemia cannot be made. Nevertheless, this effect is interesting and occurred despite the fact that somatostatin was infused at the highest permissible dose in humans (24). Thus the insulinotropic effect of the incretins during hyperglycemia is very strong in young, healthy men. Of further interest is the finding that suppression of glucagon secretion was normal under hyperglycemic conditions and not different between the trials, suggesting that only pancreatic beta cells, and not alpha cells, were affected by this phenomenon. Despite this limitation, we saw differential effects between GLP-1 and GIP on glucose Rd and EGP. The strengths of our study are the randomized crossover design using complex metabolic methods, the add-on of a hyperglycemic clamp stage, and the successful clamping of euglycemic and hyperglycemic glucose levels during the somatostatin-induced pancreatic clamps.
In summary, under euglycemic pancreatic clamp conditions, incretin hormones have no effect on whole body glucose metabolism or lipolysis. Under hyperglycemic pancreatic clamp conditions, GLP-1 has no effect on glycerol metabolism but increases glucose disposal and suppresses EGP, while GIP increases femoral artery blood flow but has no effect on glucose or glycerol metabolism. The failure of somatostatin to prevent endogenous insulin secretion during hyperglycemic conditions in the presence of GLP-1 and GIP may explain the effect of these incretin hormones during hyperglycemia; however, this phenomenon warrants further investigation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K., S.P.M., and T.P.S. developed the concept and designed the research; K.K., S.P.M., S.H.K., and T.P.S. performed the experiments; K.K., S.P.M., and T.P.S. analyzed the data; K.K., S.P.M., S.H.K., and T.P.S. interpreted the results of the experiments; K.K. and T.P.S. prepared the figures; K.K. and T.P.S. wrote the paper; K.K., S.P.M., S.H.K., and T.P.S. edited and revised the manuscript; K.K., S.P.M., S.H.K., and T.P.S. approved the final version of the manuscript.

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