GIP and GLP-1 Potentiate Sulfonylurea-Induced Insulin Secretion in Hepatocyte Nuclear Factor 1α Mutation Carriers

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Sulfonylureas (SUs) provide an efficacious first-line treatment in patients with hepatocyte nuclear factor 1α (HNF1A) diabetes, but SUs have limitations due to risk of hypoglycemia. Treatment based on the incretin hormones glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) is characterized by their glucose-dependent insulinotropic actions without risk of hypoglycemia. The effect of SUs together with GIP or GLP-1, respectively, on insulin and glucagon secretion in patients with HNF1A diabetes is currently unknown. To investigate this, 10 *HNF1A* mutation carriers and 10 control subjects without diabetes were recruited for a double-blinded, placebo-controlled, crossover study including 6 experimental days in a randomized order involving 2-h euglycemic-hyperglycemic clamps with coadministration of: *1*) SU (glimepiride 1 mg) or placebo, combined with *2*) infusions of GIP (1.5 pmol/kg/min), GLP-1 (0.5 pmol/kg/min), or saline (NaCl). In *HNF1A* mutation carriers, we observed: *1*) hypoinsulinemia, *2*) insulinotropic effects of both GIP and GLP-1, *3*) additive to supra-additive effects on insulin secretion when combining SU+GIP and SU+GLP-1, respectively, and *4*) increased fasting and arginine-induced glucagon levels compared with control subjects without diabetes. Our study suggests that a combination of SU and incretin-based treatment may be efficacious in patients with HNF1A diabetes via potentiation of glucose-stimulated insulin secretion.

Hepatocyte nuclear factor 1α (HNF1A) diabetes is a monogenic subtype of diabetes, also known as maturity-onset diabetes of the young (MODY) type 3 (MODY3 or HNF1A-MODY). *HNF1A* mutation carriers are characterized by an impaired insulin response to a glucose stimulus (1). A mutation in the transcription factor HNF1A causes impaired insulin secretion due to decreased expression of proteins involved in insulin gene transcription, glucose uptake (GLUT2), and metabolism (glycolysis and citric acid cycle) in β-cells (2). The disrupted glucose uptake and metabolism result in reduced intracellular levels of ATP, which under normal circumstances plays a vital role in glucose-stimulated insulin secretion. ATP binds to and closes K_{ATP} channels, which in turn causes membrane depolarization, initiating a cascade of events that results in secretion of insulin (2,3). Sulfonylureas (SUs) stimulate insulin secretion by enhancing ATP-independent closure of the K_{ATP} channel (4,5) and thus bypassing the low level of ATP in the pancreatic β-cells. In mechanistic and clinical studies, *HNF1A* mutation carriers have been demonstrated to be highly sensitive to SUs due to robust increments in insulin secretion (4,5). Clinically, this translates into a potent glucose-lowering effect when using SUs, which is why they are recommended as first-line treatment of HNF1A-diabetes (6,7). The main limitation of SU treatment in patients with HNF1A diabetes is that treatment intensification with additional glucose-lowering drugs is often needed in the long run to provide glycemic control (8).

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Additional limitations are problems with recurrent hypoglycemia with SUs in some patients (9,10) and that SUs may also induce body weight gain, as observed in patients with type 2 diabetes (11).

Cross-sectional studies indicate that patients with HNF1A-diabetes suffer from both microvascular and macrovascular complications to the same extent as patients with type 1 and type 2 diabetes (12,13). Thus, investigating add-on treatment to SUs is important to prevent diabetic complications. We have previously shown that *HNF1A* mutation carriers have impaired insulinotropic effects of the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) compared with control subjects without diabetes (14) but, in contrast, that treatment with pharmacological doses of a GLP-1 receptor agonist in patients with HNF1A diabetes has glucose-lowering properties almost similar to SUs, with an 18-fold lower risk of hypoglycemia (9).

In the current study, we hypothesized that administration of a single dose of an SU (glimepiride) and exogenous infusions of GLP-1 and GIP, respectively, have additive effects on insulin secretion in *HNF1A* mutation carriers and control subjects without diabetes. The infusion rates of GLP-1 and GIP in this study were chosen to imitate postprandial plasma levels of endogenous GLP-1 and GIP during treatment with dipeptidyl peptidase 4 inhibitor (DPP-4i).

RESEARCH DESIGN AND METHODS

Ethical Approval

This study was approved by the Scientific Ethical Committee of the Capital Region (protocol number H-16038140) and the Data Protection Agency (HGH-2017–050, I-Suite 05657) and registered at ClinicalTrials.gov (NCT03081676). The study was conducted in accordance with the Declaration of Helsinki, and all participants gave oral and written consent before inclusion.

Participants

Ten carriers of mutations in *HNF1A* (7 treated with glucose-lowering therapies) and 10 control subjects without diabetes were individually matched 1:1 according to age, sex, and BMI (Table 1). *HNF1A* mutations had previously been established with heterozygous loss-of-function mutations verified by Sanger sequencing (Table 2). Mutations were considered pathogenic if they met at least one of the following criteria: *1*) previously published reports on disease-causing effect of the specific mutation, *2*) the presence

Table 1—Participant characteristics

	HNF1A mutation carriers	Control subjects	Difference (*P* value)
Sex (male/female)	4/6	4/6	
Age (years)	35.3 (8.1)	33.9 (8.0)	
Weight (kg)	67.4 (7.0)	67.9 (14.3)	
Height (m)	1.74 (0.1)	1.74 (0.1)	
BMI (kg/m^2)	22.4 (1.5)	22.2 (2.4)	
Waist circumference (cm)	76.3 (7.3)	76.2 (7.2)	0.9757
Hip circumference (cm)	93.5 (4.6)	90.7 (8.0)	0.3495
Waist-to-hip ratio	0.82 (0.08)	0.85 (0.08)	0.4684
HbA_{1c} (%)	6.0 (0.6)	5.1 (0.3)	<0.0001
HbA_{1c} (mmol/mol)	42.3 (6.1)	31.9 (2.9)	<0.0001
Fasting plasma glucose (mmol/L)	6.6 (2.1)	5.1 (0.4)	0.0113
HOMA-IR	1.4 (0.6)	1.4 (0.6)	0.7972
Patients with diabetes (%)*	80 (n = 8)		
Diabetes duration (years)†	18 (6)		
Complications			
Retinopathy (%)	40 (n = 4)		
Neuropathy (%)	10 (n = 1)		
Treatment			
Diet (%)	30 (n = 3)		
Glimepiride (%)	30 (n = 3)		
Glimepiride + linagliptin (%)	10 (n = 1)		
Glimepiride + liraglutide (%)	10 (n = 1)		
Linagliptin (%)	10 (n = 1)		
Repaglinide + metformin (%)	10 (n = 1)		

Data are mean (SD) unless otherwise indicated. Participants were matched according to sex, age, and BMI. Participants met fasting (10 h) without morning medication on screening day. Diabetes is defined as HbA_{1c} ≥48 mmol/mol (n = 7) or prior gestational diabetes mellitus (n = 1) diagnosed with an oral glucose tolerance test. HOMA-IR, HOMA of insulin resistance. *Two *HNF1A* mutation carriers had never presented an HbA_{1c} ≥48 mmol/mol. †*HNF1A* mutation carriers treated with glucose-lowering agents only.

Table 2—Overview of *HNF1A* mutations, treatment, and diabetes status

Subject	Mutation chromosome 12	Treatment	Diabetes status
HNF1A-1	Cys241Gly	Glimepiride	Diabetes
HNF1A-2	IVSnt-2 a->g	Diet	No diabetes
HNF1A-3	c.1623+1 g->t	Glimepiride + linagliptin	Diabetes
HNF1A-4	Pro291fsinsC	Glimepiride + liraglutide	Diabetes
HNF1A-5	Ala559fsinsA	Linagliptin	Diabetes
HNF1A-6	Deletion (exon2–10)	Glimepiride	Diabetes
HNF1A-7	Pro291fsinsC	Repaglinide + metformin	Diabetes
HNF1A-8	Pro291fsinsC	Glimepiride	Diabetes
HNF1A-9	Pro379fsdelCT	Diet	No diabetes
HNF1A-10	Glu234Term	Diet	Diabetes*

"Diabetes" is defined as one measurement of HbA_{1c} ≥48 mmol/mol at any time. *Patient diagnosed with gestational diabetes mellitus during two pregnancies verified with oral glucose tolerance tests.

of a truncating mutation, and/or *3*) cosegregation of the mutation with diabetes within the family. Participants attended a screening visit after an overnight fast (10 h). Medical history, anthropometric data, and blood samples were obtained. *HNF1A* mutation carriers were recruited either from the diabetic outpatient clinic at Steno Diabetes Center Copenhagen, Gentofte Hospital, University of Copenhagen, or via letter to an *HNF1A* mutation registry located at the University of Copenhagen. Inclusion criteria for *HNF1A* mutation carriers were: *1*) pathogenic *HNF1A* mutation verified by genetic testing, *2*) treated with diet, SU monotherapy, or noninsulin treatments with or without SU, *3*) aged ≥18 years, and *4*) informed consent. Inclusion criteria for control subjects without diabetes were: *1*) fasting plasma glucose ≤6 mmol/L, *2*) glycated hemoglobin A_{1c} (HbA_{1c}) ≤6.1% (43 mmol/mol), *3*) no family history of type 1 or type 2 diabetes, *4*) aged ≥18 years, and *5*) informed consent. Exclusion criteria in both groups were pregnancy, breastfeeding, and abnormal blood or urine biochemistry (hemoglobin, liver enzymes [alanine and aspartate aminotransferases], plasma creatinine, and urine albumin-to-creatinine ratio). Apart from the antidiabetic drugs (Table 2), none of the participants were treated with drugs suspected to influence the plasma/serum levels of glucose, insulin, C-peptide, glucagon, or incretin hormones.

Peptides

Synthetic GIP and GLP-1 (Bachem, Bubendorf, Switzerland) were subjected to sterile filtration and microbiological testing and dispensed into vials by the Capital Region Pharmacy (Herlev, Denmark). The peptides were dissolved in sterilized water containing 0.5% human albumin (Statens Serum Institut, Copenhagen, Denmark). All infusions (GIP, GLP-1, and NaCl) had an identical transparent appearance.

Tablets

Tablets with 1 mg glimepiride (1 mg Amaryl) (Sanofi Denmark A/S, Copenhagen, Denmark) and placebo had identical appearance and were provided by the hospital pharmacy of the Capital Region (Herlev, Denmark). Both glimepiride and placebo were gelatin capsules containing trace amounts of lactose monohydrate, potato starch, talc, and magnesium stearate.

Study Design

This study was a double-blinded, crossover study with 6 experimental days (separated by a minimum of 4 days) performed in randomized order over a period of at least 3 months. Employees who were not otherwise involved in the study prepared all interventions to ensure blinding of both investigators and participants. Antidiabetic treatments were discontinued prior to each experimental day (repaglinide 24 h, glimepiride 72 h, and metformin/linagliptin/liraglutide 14 days before). After an experimental day, patients recommenced their antidiabetic treatments only if the time interval before the next experimental day was greater than the treatment-specific washout period. Participants were instructed to continue their usual diet (with at least 250 g carbohydrates the day prior to an experimental day) and avoid strenuous exercise and alcohol consumption 24 h before experimental days. After an overnight fast (10 h), the participants rested in a recumbent position, and a cannula was inserted in a cubital vein of each arm, one for infusions and one for collection of arterialized blood samples. Arterialized venous blood was obtained by a modified heated-hand technique by wrapping the forearm and hand with a heating pad (50°C) throughout the experiment (15). A tablet of 1 mg glimepiride or placebo was administered 90 min before the clamp procedures. The two-step glucose clamp consisted of: step 1 at time 0–60 min with a glucose level targeted at the fasting plasma glucose (determined as mean plasma glucose measured at time −105, −100, and −90 min) and step 2 at time 60–125 min at 1.5 × fasting plasma glucose (mimicking postprandial plasma glucose levels). From time 0 to 125 min, GIP (1.5 pmol/kg/min), GLP-1 (1.5 pmol/kg/min), or saline was infused. Infusion of glucose (200 mg/mL) was given from time 0 to 125 min at a rate adjusted according to bedside measurements of plasma glucose,

performed every 5th minute. A bolus of 20% glucose was given at time 60 min for 30 s to increase plasma glucose levels to a target of 1.5 × fasting plasma glucose. At the end of the clamp (time 120 min), a bolus of 5 g L-arginine (given as 10% arginine HCl) was infused for 30 s, and from time 120 to 125 min, the rate of the glucose infusion was not changed.

Data Collection

Plasma glucose was measured at time −115, −100, and −90 min and every 5th minute from time 0 to 120. For bedside measurement of plasma glucose, blood was collected into fluoride tubes and centrifuged immediately for 30 s at room temperature and 7,500*g*. For the analysis of plasma glucagon, GIP, and GLP-1, blood was collected in chilled tubes (on ice) containing EDTA and a specific DPP-4i (valine pyrrolidine, 0.01 mmol/L) (a gift from Novo Nordisk, Måløv, Denmark). For analyses of serum insulin and C-peptide, blood was sampled in plain tubes for coagulation (20 min at room temperature). EDTA tubes and plain tubes were centrifuged for 15 min at 2,900*g* and 4°C. Plasma samples for glucagon, GIP, and GLP-1 were stored at −20°C and serum samples for insulin and C-peptide at −80°C until analysis.

Laboratory Methods

Plasma glucose was measured bedside by the glucose oxidase method (Model 2900 Series Biochemistry Analyzers; YSI Incorporated, Yellow Springs, OH). Serum insulin and C-peptide concentrations were measured with a two-sided electrochemiluminescence immunoassay (Siemens Healthcare, Ballerup, Denmark). Plasma concentrations of total GIP (16), total GLP-1 (17), and glucagon (18) were measured by radioimmunoassays as described previously. For the GIP, GLP-1, and glucagon assays, plasma samples (EDTA) were extracted with ethanol (70% v/v) to eliminate unspecific interference.

Statistical Analyses and Calculations

All results in the text and figures are presented as mean ± SEM unless stated otherwise. Differences resulting in P values of <0.05 were considered significant. Area under the curve (AUC) and baseline-subtracted AUC (bsAUC) values were calculated using the trapezoidal rule. Time −105, −100, and −90 min were defined as baseline values for calculations of $bsAUC_{0–60\ min}$, $bsAUC_{60–120\ min}$, and $bsAUC_{0–120\ min}$. Primary end points were differences between interventions in $bsAUC_{0–60\ min}$, $bsAUC_{60–120\ min}$, and $bsAUC_{0–120\ min}$ for C-peptide. For calculation of incremental $AUC_{120–125\ min}$ ($iAUC_{120–125\ min}$), values were subtracted from the value at time 120 min. Insulin secretion rate (ISR) was calculated based on C-peptide elimination rates and deconvolution as previously described (19,20). To check whether the targeted plasma glucose levels were obtained, the $AUC_{0–120\ min}$ for the plasma glucose/fasting plasma glucose ratio was calculated (optimal value: 150 mmol/L/mmol/L/min). Statistical analyses were carried out within each group using linear mixed models with an unrestricted covariance structure and the Kenward-Roger approximation of the df using the algorithm of $y = SU \times infusion \times SU^{*}infusion$, in which y is the variant of interest, subject ID is random effect, infusion (GIP, GLP-1, or NaCl) and SU (SU or placebo) are fixed effects, and SU*infusion to test for interaction. To test for differences between groups, we added "group" to the algorithm ($y = SU \times infusion \times group \times SU^{*}infusion$) and tested the significance level of group. When calculating the total amount of glucose given, we adjusted for fasting plasma glucose levels for *HNF1A* mutation carriers. To guard against false positives, all comparisons including primary end points were adjusted for multiple testing using the Tukey multiple-comparison test. Extreme outliers were identified according to Tukey fences (21), and extreme outliers are presented explicitly in the RESULTS. All analyses were performed in SAS Studio 9.4M5 (SAS Institute, Cary, NC) and graphical presentations in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

Data and Resource Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

RESULTS

Participant Characteristics

HNF1A mutation carriers and control subjects without diabetes were well matched according to sex, age, and BMI, and the two groups had similar HOMA of insulin resistance but differed, as expected, on fasting plasma glucose and HbA_{1c} (Table 1). One study day in a control subject without diabetes (SU+GLP-1) and one study day in an *HNF1A* mutation carrier (SU+placebo) were excluded from the analysis because the randomization sequence had not been followed, resulting in erroneousness infusions (human error). Regarding glucagon, one participant without diabetes qualified as an extreme outlier and was excluded from glucagon data analyses. This participant had extraordinarily high fasting glucagon concentrations (mean 54 pmol/L, range 30–101 pmol/L) compared with other control subjects without diabetes (mean 9.4 pmol/L) but was kept in other analyses because glucagon concentrations were suppressed and insulin/C-peptide response was comparable to other control subjects without diabetes.

Plasma Levels of GIP and GLP-1

Mean baseline concentrations of GIP (*HNF1A* mutation carriers, 18 ± 3 pmol/L, and control subjects without diabetes, 18 ± 1 pmol/L; $P = 0.9167$) and GLP-1 (*HNF1A* mutation carriers, 9 ± 1 pmol/L, and control subjects without diabetes, 11 ± 1 pmol/L; $P = 0.2821$) did not differ between groups (Fig. 1*A–D*). GIP peak concentrations were similar on study days with GIP infusion in *HNF1A* mutation carriers (131 ± 7 pmol/L [placebo+GIP] and 123 ± 10 pmol/L [SU+GIP]; $P = 0.2821$) and in the group

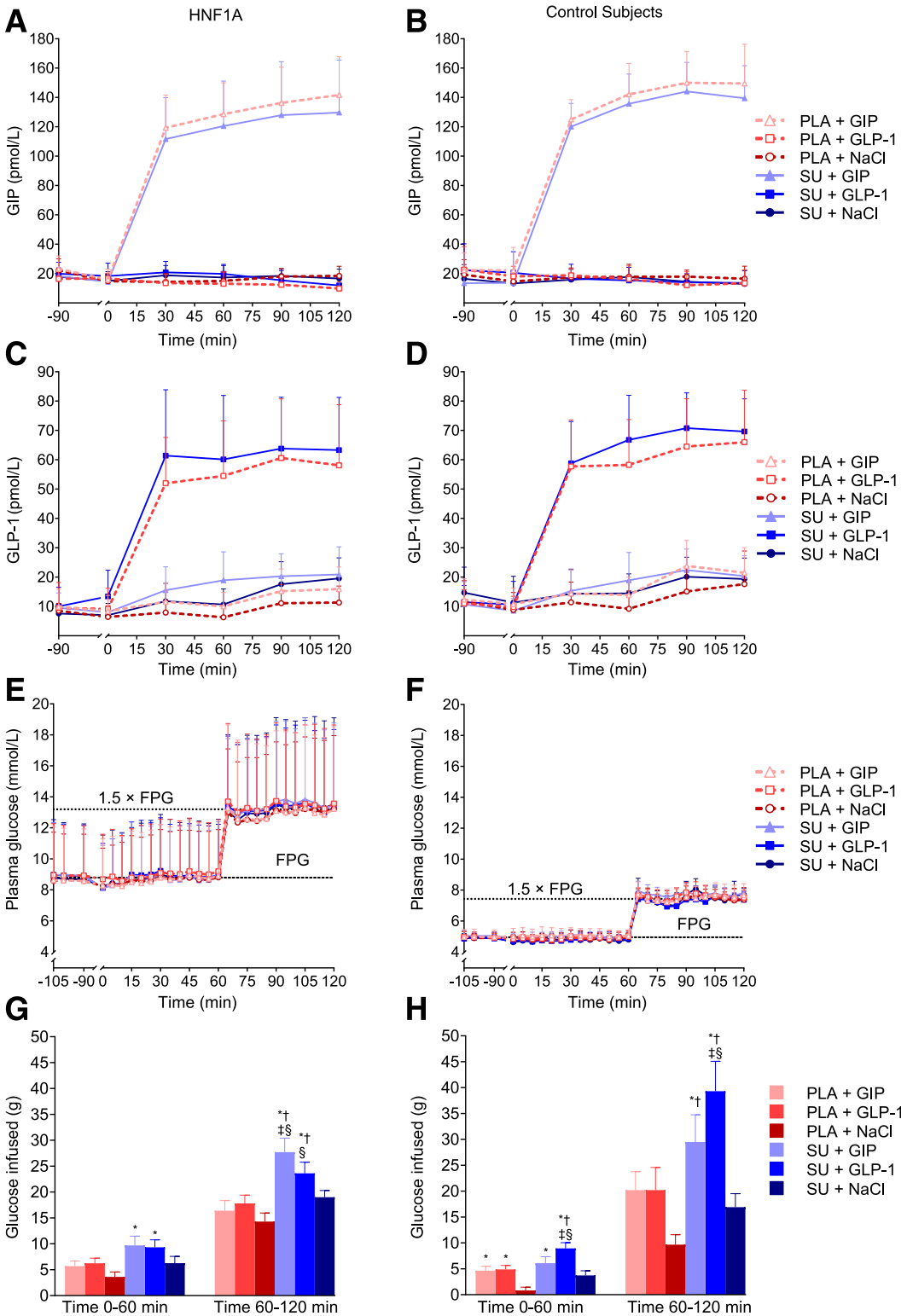

Figure 1—GIP, GLP-1, plasma glucose, and glucose infused. Plasma GIP (*A* and *B*), GLP-1 (*C* and *D*), and glucose (*E* and *F*) vs. time during the 2-h two-step glucose clamp in 10 *HNF1A* mutation carriers and 10 control subjects without diabetes. Accumulated glucose infused at time 60 min and 120 min (*G* and *H*) (subtracted bolus given at time 60 min) in *HNF1A* mutation carriers (*G*) and control subjects without diabetes (*H*). Data are presented as mean ± SD (*A*–*F*) and mean ± SEM (*G* and *H*). Continuous infusions of saline (NaCl), GIP, or GLP-1 were started at time 0 min preceded by either single-dose SU 1 mg glimepiride or placebo (PLA) at time −90 min. Symbols show significant differences ($P < 0.05$) between interventions: *significantly greater than PLA+NaCl; †significantly greater than SU+NaCl; ‡significantly greater than PLA+GIP; §significantly greater than PLA+GLP-1. FPG, fasting plasma glucose.

of control subjects without diabetes (124 ± 6 pmol/L [placebo+GIP] and 117 ± 6 pmol/L [SU+GIP]; $P = 0.4438$). Likewise, GLP-1 peak concentrations were similar on days with GLP-1 infusion in *HNF1A* mutation carriers (56 ± 6 pmol/L [placebo+GLP-1] and 62 ± 6 pmol/L [SU+GLP-1]; $P = 0.2620$) and in control subjects without diabetes (60 ± 5 pmol/L [placebo+GLP-1] and 66 ± 3 pmol/L [SU+GLP-1]; $P = 0.5830$).

Plasma Glucose and Glucose Infused

Fasting plasma glucose was 3.7 ± 1.1 mmol/L higher in *HNF1A* mutation carriers compared with control subjects without diabetes ($P = 0.0041$), with no difference in fasting plasma glucose between experimental days within each group (Table 3). The targeted glucose concentrations (expressed as the $AUC_{0–120\ min}$ for plasma glucose/fasting plasma glucose) during the glucose clamp procedure were achieved without differences between groups ($P = 0.6135$) or between study days within the two groups (Fig. 1*E* and *F* and Table 3). The amount of glucose (grams) needed to maintain the plasma glucose concentrations during the experimental days was greatest with the combination of SU+GIP and SU+GLP-1, respectively, in both *HNF1A* mutation carriers and control subjects without diabetes (Fig. 1*G* and *H* and Table 3).

C-Peptide, Insulin, and ISR

Mean fasting C-peptide concentrations in *HNF1A* mutation carriers were significantly lower than in control subjects without diabetes (308 ± 16.8 vs. 387 ± 31.7 pmol/L; $P = 0.0442$), even though their plasma glucose was higher (Table 3). When looking across all indices of insulin secretion (C-peptide, insulin, ISR, C-peptide/glucose, insulin/glucose, and ISR/glucose), the overall trends were the same (Figs. 2 and 3, Table 3, and Supplementary Table 1); below, detailed results for C-peptide are presented. In *HNF1A* mutation carriers, combinations of SU+GIP and SU+GLP-1, respectively, were significantly more insulinotropic (based on C-peptide $bsAUC_{0–60\ min}$, $bsAUC_{60–120\ min}$, and $bsAUC_{0–120\ min}$) compared with administration of placebo+GIP, placebo+GLP-1, placebo+NaCl, and SU+NaCl (Table 3). In *HNF1A* mutation carriers, both placebo+GIP and placebo+GLP-1, respectively, compared with placebo+NaCl resulted in significantly greater C-peptide $bsAUC_{0–120\ min}$ values. Other analyses (insulin, insulin/glucose, ISR, and ISR/glucose) demonstrated an insignificant insulinotropic trend. SU+NaCl was not significantly more insulinotropic compared with placebo+NaCl (in all insulin secretion parameters). In control subjects without diabetes, SU+GLP-1 was more insulinotropic (C-peptide $bsAUC_{0–120\ min}$) compared with all other interventions, while SU+GIP was the second most insulinotropic intervention (Table 3). In control subjects without diabetes, placebo+GLP-1 and placebo+GIP alone were more insulinotropic (C-peptide $bsAUC_{0–120\ min}$) compared with placebo+NaCl, while SU+NaCl was not significantly different from placebo+NaCl.

Supra-additive Effect of Combining SU With an Incretin Hormone

We observed a significant interaction between SU (SU or placebo) and infusions (GIP, GLP-1, or NaCl) for C-peptide ($bsAUC_{60–120\ min}$ and $bsAUC_{0–120\ min}$) in both *HNF1A* mutation carriers ($P = 0.0190$ and $P = 0.0294$, respectively) and control subjects without diabetes ($P = 0.0097$ and $P = 0.0078$, respectively), which is indicative of a supra-additive effect of combining SU and GIP and/or GLP-1, respectively (Table 3). When looking at $bsAUC_{0–60\ min}$ for C-peptide, no interaction was observed in *HNF1A* mutation carriers ($P = 0.1617$), while an interaction was observed in control subjects without diabetes ($P = 0.0233$). Regarding C-peptide/glucose, an interaction was present across all time periods ($bsAUC_{0–60\ min}$, $bsAUC_{60–120\ min}$, and $bsAUC_{0–120\ min}$) in both groups (Table 3). The magnitude of the interaction for C-peptide $bsAUC_{0–120\ min}$ and C-peptide/glucose is depicted in Fig. 4. In *HNF1A* mutation carriers, the supra-additive effect on C-peptide was rather small (~5–10%); however, it was substantially higher when adjusted for glucose concentrations and C-peptide/glucose (~25–45%).

Arginine-Induced C-Peptide Secretion

The arginine-induced maximal secretion test (Table 4 and insets in Fig. 2*A* and *B*) displayed a significantly attenuated C-peptide response in *HNF1A* mutation carriers compared with control subjects without diabetes. In *HNF1A* mutation carriers, the greatest peak and $AUC_{120–125\ min}$ for C-peptide were observed after administration of arginine on experimental days with SU+GIP and SU+GLP-1, while in control subjects without diabetes, SU+GLP-1 was the most potent stimuli. The concentration of C-peptide at the time of the arginine administration was the most important determinant of the C-peptide response given that the difference of the $iAUC_{120–125\ min}$ (subtracted the C-peptide level at time 120 min) is small in both groups across all study days.

Glucagon

Fasting glucagon concentrations were higher in *HNF1A* mutation carriers compared with control subjects without diabetes (11.8 ± 0.5 vs. 9.5 ± 0.8 pmol/L; $P = 0.0163$) (Table 3 and Fig. 3*E* and *F*). Glucagon concentrations decreased from baseline (time −100 min and −90 min) to time 0 min regardless of SU or placebo administration, and the difference between groups was abolished at time 0 min (*HNF1A* mutation carriers, 8.9 ± 0.9 pmol/L, vs. control subjects without diabetes, 7.4 ± 1.0 pmol/L; $P = 0.2777$). The glucagon concentrations decreased with increasing glucose concentrations in both *HNF1A* mutation carriers and control subjects without diabetes. There were no significant differences in $bsAUC_{0–120\ min}$ for glucagon between interventions in any of the groups. We observed an insignificant trend toward a greater decrease of glucagon concentrations on days with placebo+GLP-1 and SU+GLP-1, while the smallest decrements in glucagon levels were

Table 3—Glucose, C-peptide, C-peptide/glucose, and glucagon

Intervention	PLA+NaCl	PLA+GIP	PLA+GLP-1	SU+NaCl	SU+GIP	SU+GLP-1	Interaction *P* value
Plasma glucose							
HNF1A mutation carriers							
Baseline (mmol/L)	8.6 ± 1.1	8.9 ± 1.0	8.6 ± 1.0	8.9 ± 1.0	8.9 ± 1.1	8.7 ± 1.1	
$AUC_{0–120}$ min (mol/L × min)	1.28 ± 0.15	1.33 ± 0.15	1.28 ± 0.15	1.34 ± 0.16	1.31 ± 0.16	1.28 ± 0.16	
PG/FPG $AUC_{0–120}$ (min^{-1})	150 ± 1	149 ± 1	149 ± 1	149 ± 1	147 ± 1	147 ± 1	
Control subjects without diabetes							
Baseline (mmol/L)	4.8 ±0.1	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	
$AUC_{0–120\ min}$ (mol/L × min)	0.72 ± 0.02	0.75 ± 0.02	0.73 ± 0.02	0.75 ± 0.02	0.73 ± 0.01	0.74 ± 0.01	
PG/FPG $AUC_{0–120\ min}$ (min)	149 ± 1	149 ± 1	149 ± 1	150 ± 1	148 ± 1	148 ± 1	
Glucose infused							
HNF1A mutation carriers							
Time 0–60 min (g)	3.6 ± 1.0	5.7 ± 1.4	6.2 ± 1.0	6.5 ± 1.3	9.7 ± 1.8*	9.3 ± 1.5*	0.6745
Time 60–120 min (g)	14 ± 1.0	16 ± 2.0	18 ± 1.6	19 ± 1.3	28 ± 2.7*†‡§	24 ± 2.2*‡§	0.0144
Time 0–120 min (g)	18 ± 1.7	22 ± 3.2	24 ± 2.3	26 ± 2.1	37 ± 4.2*†‡§	33 ± 3.3*‡§	0.0472
Control subjects without diabetes							
Time 0–60 min (g)	0.8 ± 0.6	4.6 ± 0.9*	4.9 ± 0.8*	3.7 ± 0.9	6.1 ± 1.3*	9.3 ± 1.2*†‡§‖	0.1237
Time 60–120 min (g)	9.7 ± 1.9	20 ± 3.6	20 ± 4.4	17 ± 2.6	30 ± 5.3*†	39 ± 5.8*†‡§	0.0180
Time 0–120 min (g)	11 ± 2.3	25 ± 4.3	25 ± 5.0	21 ± 3.2	36 ± 6.3*†	48 ± 6.7*†‡§	0.0205
C-peptide							
HNF1A mutation carriers							
Baseline (pmol/L)	302 ± 14	299 ± 21	314 ± 23	299 ± 24	314 ± 18	310 ± 19	
$bsAUC_{0–60\ min}$ (nmol/L × min)	0.7 ± 0.9	7.5 ± 1.7	5.8 ± 1.4	8.0 ± 2.4	19 ± 3.8*†‡§	18 ± 3.4*†‡§	0.1617
$bsAUC_{60–120\ min}$ (nmol/L × min)	9.6 ± 1.6	20 ± 3.3	26 ± 3.1*	21 ± 4.3	45 ± 6.5*†‡	46 ± 5.6*†‡§	0.0190
$bsAUC_{0–120\ min}$ (nmol/L × min)	10 ± 2.1	28 ± 4.7*	32 ± 4.0*	29 ± 6.5	64 ± 10*†‡§	63 ± 8.6*†‡§	0.0294
Control subjects without diabetes							
Baseline (pmol/L)	358 ± 42	415 ± 35	370 ± 27	394 ± 50	372 ± 34	426 ± 39	
$bsAUC_{0–60\ min}$ (nmol/L × min)	−2.7 ± 2.4	5.5 ± 2.2	5.1 ± 2.4	3.9 ± 1.8	12 ± 2.9*	24 ± 3.9*†‡§‖	0.0233
$bsAUC_{60–120\ min}$ (nmol/L × min)	17 ± 5.8	48 ± 6.7	60 ± 11*	40 ± 7.7	80 ± 14*†	131 ± 17*†‡§‖	0.0097
$bsAUC_{0–120\ min}$ (nmol/L × min)	14 ± 7.8	53 ± 8.2	65 ± 13	43 ± 8.9	93 ± 16*†	156 ± 20*†‡§‖	0.0078
C-peptide/glucose							
HNF1A mutation carriers							
Baseline (pmol/L)	41 ± 6.4	40 ± 7.3	42 ± 6.3	36 ± 5.1	39 ± 6.1	42 ± 6.5	
$bsAUC_{0–60\ min}$ (nmol/L × min)	−0.0 ± 0.12	0.8 ± 0.2*	0.7 ± 0.2*	0.9 ± 0.3	2.5 ± 0.4*†‡§	2.2 ± 0.4*†‡§	0.0398
$bsAUC_{60–120\ min}$ (nmol/L × min)	0.1 ± 0.1	1.0 ± 0.3	1.6 ± 0.3*	1.0 ± 0.3	3.1 ± 0.5*†‡	3.3 ± 0.5*†‡§	0.0042
$bsAUC_{0–120\ min}$ (nmol/L × min)	0.1 ± 0.2	1.8 ± 0.4*	2.3 ± 0.5*	2.0 ± 0.5	5.6 ± 0.9*†‡§	5.5 ± 0.8*†‡§	0.0077
Control subjects without diabetes							
Baseline (pmol/L)	75 ± 9.2	83 ± 6.8	75 ± 5.9	78 ± 8.5	75 ± 7.3	75 ± 7.0	
$bsAUC_{0–60\ min}$ (nmol/L × min)	−0.7 ± 0.52	1.2 ± 0.5	1.1 ± 0.5	0.8 ± 0.4	2.6 ± 0.6*	5.0 ± 0.9*†‡§‖	0.0260
$bsAUC_{60–120\ min}$ (nmol/L × min)	0.8 ± 0.9	4.7 ± 0.9	6.6 ± 1.6	3.5 ± 1.0	9.3 ± 1.9*†	16 ± 2.4*†‡§‖	0.0166
$bsAUC_{0–120\ min}$ (nmol/L × min)	0.2 ± 0.1	5.8 ± 1.3	7.7 ± 2.1	4.4 ± 1.3	12 ± 2.4*†	21 ± 3.2*†‡§‖	0.0148

Continued on p. 1996

Table 3—Continued

Intervention	PLA+NaCl	PLA+GIP	PLA+GLP-1	SU+NaCl	SU+GIP	SU+GLP-1	Interaction *P* value
Glucagon							
HNF1A mutation carriers							
Baseline (pmol/L)	13 ± 1	12 ± 1	11 ± 1	11 ± 2	13 ± 1	12 ± 2	
$bsAUC_{0–60\ min}$ (pmol/L × min)	−306 ± 59	−182 ± 59	−381 ± 59	−186 ± 59	−152 ± 59	−255 ± 59	0.5167
$bsAUC_{60–120\ min}$ (pmol/L × min)	−463 ± 57	−435 ± 57	−488 ± 57	−351 ± 60	−353 ± 57	−420 ± 57	0.9094
$bsAUC_{0–120\ min}$ (pmol/L × min)	−770 ± 106	−618 ± 106	−869 ± 106	−560 ± 111	−505 ± 106	−675 ± 106	0.8320
Control subjects without diabetes							
Baseline (pmol/L)	8 ± 2	9 ± 2	9 ± 2	10 ± 2	8 ± 2	13 ± 2	
$bsAUC_{0–60\ min}$ (nmol/L × min)	−93 ± 76	−163 ± 76	−248 ± 76	−148 ± 76	−52.7 ± 76	−321 ± 81	0.2472
$bsAUC_{60–120\ min}$ (nmol/L × min)	−325 ± 100	−388 ± 100	−408 ± 100	−374 ± 100	−274 ± 100	−538 ± 105	0.2707
$bsAUC_{0–120\ min}$ (nmol/L × min)	−419 ± 171	−605 ± 177	−655 ± 171	−523 ± 171	−325 ± 171	−857 ± 179	0.1979

Data are mean ± SEM. A significant interaction describes a supra-additive of combining SU+GIP and/or SU+GLP-1. FPG, fasting plasma glucose; PG, plasma glucose; PLA, placebo. Symbols show significant differences ($P < 0.05$) between interventions: *significantly greater than PLA+NaCl; †significantly greater SU+NaCl; ‡significantly greater than PLA+GIP; §significantly greater than PLA+GLP-1; ||significantly greater than SU+GIP.

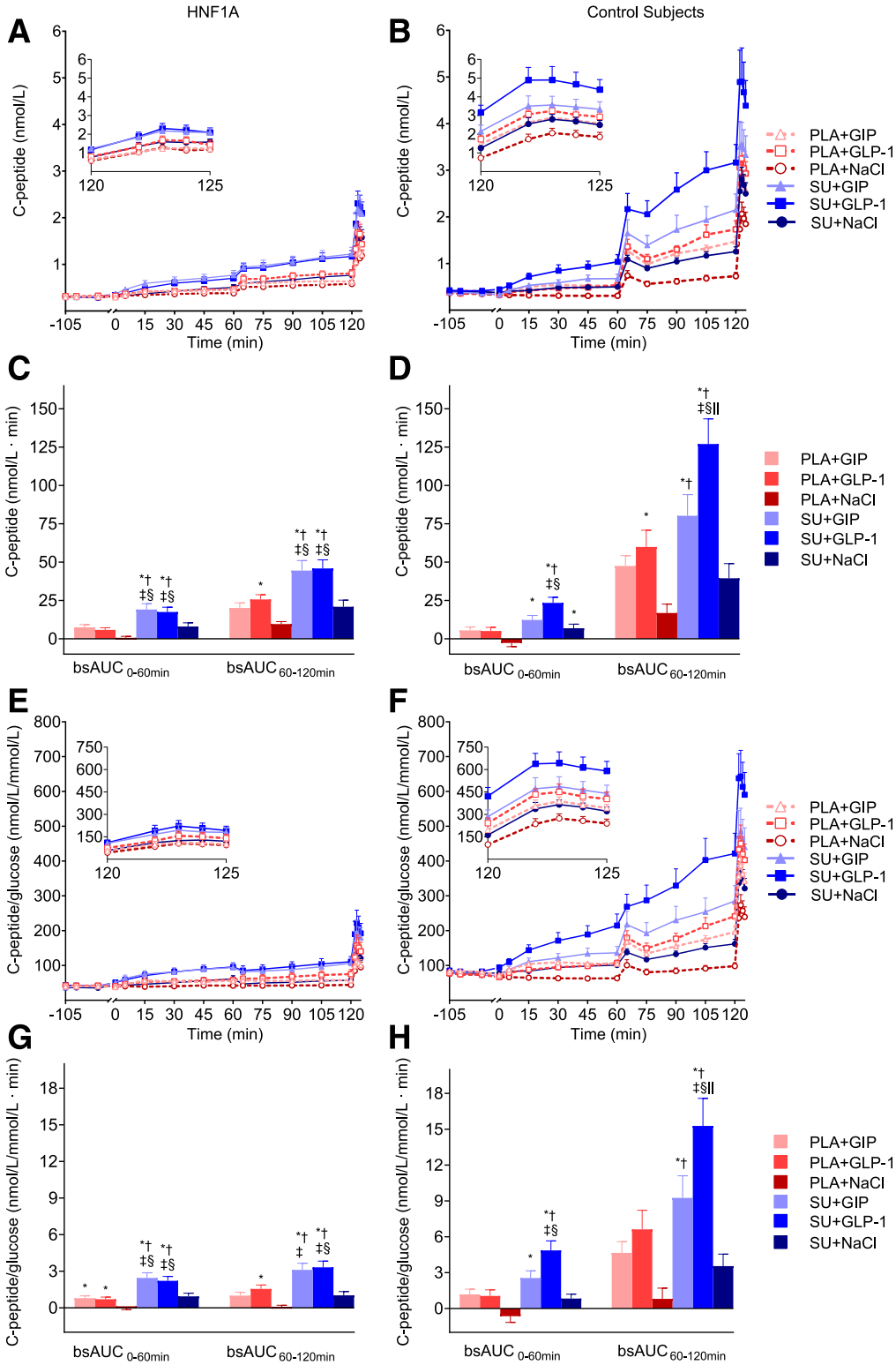

Figure 2—C-peptide and C-peptide/glucose. Serum C-peptide (*A* and *B*) and C-peptide/glucose (*E* and *F*) vs. time during the 2-h two-step glucose clamp in 10 *HNF1A* mutation carriers and 10 control subjects without diabetes. Corresponding $bsAUC_{0–60\ min}$ and $bsAUC_{60–120\ min}$ are presented in *C*, *D*, *G*, and *H*. Continuous infusions of saline (NaCl), GIP, or GLP-1 were started at time 0 min preceded by either single-dose SU 1 mg glimepiride or placebo (PLA) at time −90 min. Data from time 120 min to 125 min is magnified in insets in *A*, *B*, *E*, and *F*. Data are presented as mean ± SEM. Symbols show significant differences ($P < 0.05$) between interventions: *significantly greater than PLA+NaCl; †significantly greater than SU+NaCl; ‡significantly greater than PLA+GIP; §significantly greater than PLA+GLP-1; ||significantly greater than SU+GIP.

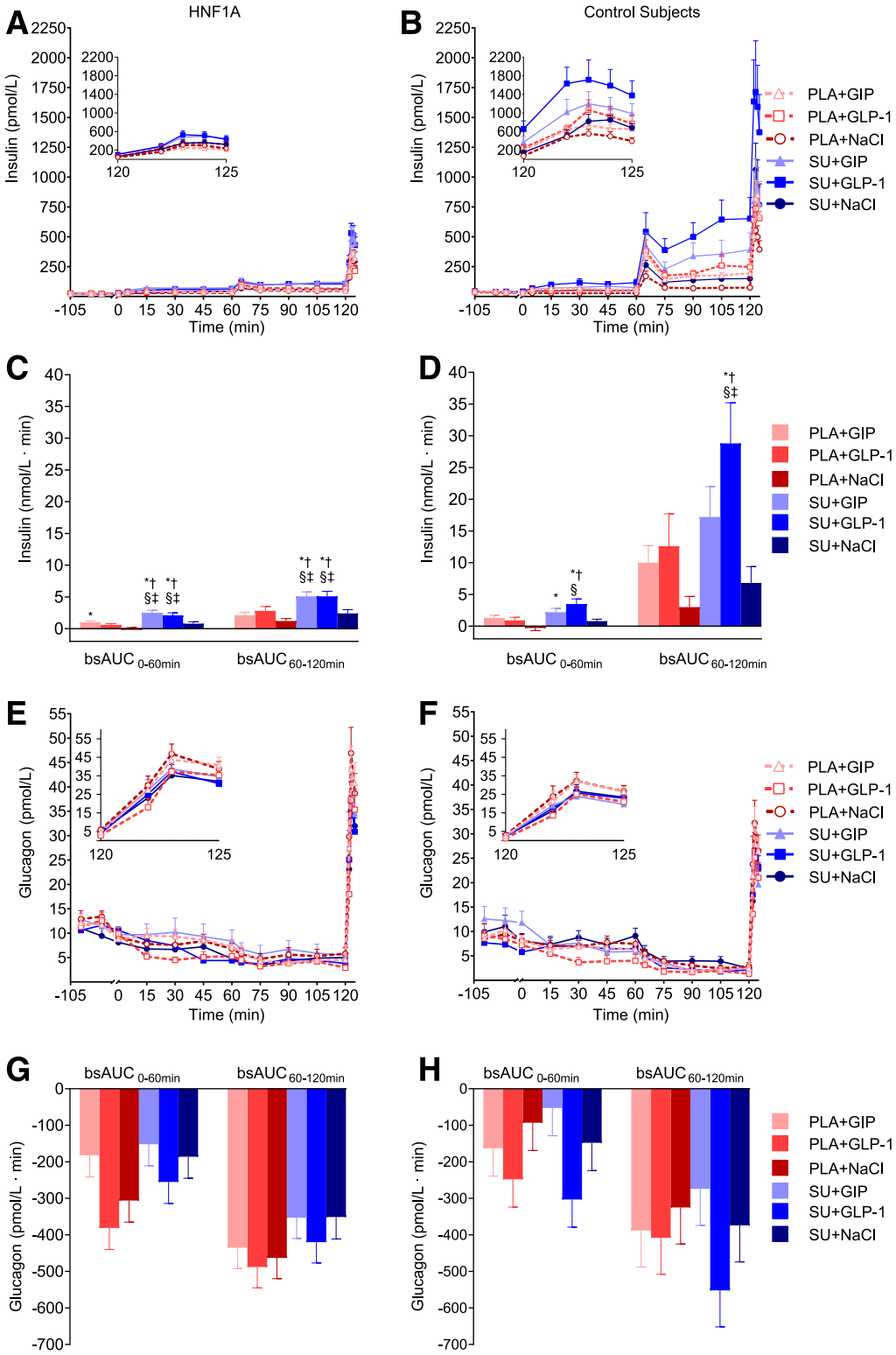

Figure 3—Insulin and glucagon. Serum insulin (*A* and *B*) and plasma glucagon (*E* and *F*) vs. time in 10 *HNF1A* mutation carriers and 10 control subjects without diabetes. Corresponding $bsAUC_{0–60\ min}$ and $bsAUC_{60–120\ min}$ are presented in *C*, *D*, *G*, and *H*. Continuous infusions of saline (NaCl), GIP, or GLP-1 were started at time 0 min preceded by either a single-dose SU 1 mg glimepiride or placebo (PLA) at time −90 min. Data from time 120 min to 125 min is magnified in insets in *A*, *B*, *E*, and *F*. Data are presented as mean ± SEM. Symbols show significant differences ($P < 0.05$) between interventions: *significantly greater than PLA+NaCl; †significantly greater than SU+NaCl; ‡significantly greater than PLA+GIP; §significantly greater than PLA+GLP-1.

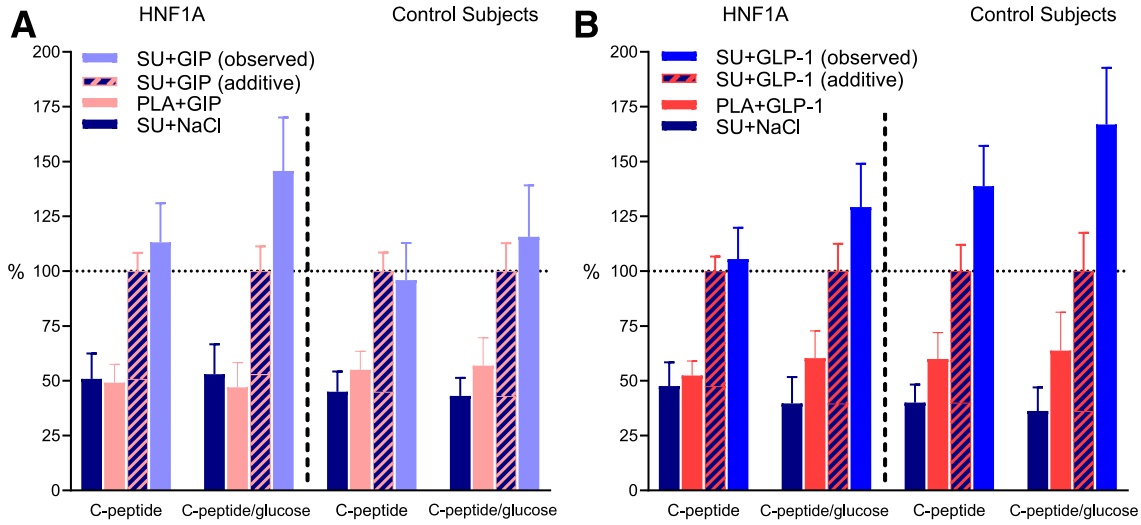

Figure 4—Interaction between SU and the incretin hormones. $bsAUC_{0–120\ min}$ for C-peptide and C-peptide/glucose for 10 *HNF1A* mutation carriers and 10 control subjects without diabetes during the 2-h two-step glucose clamp. Continuous infusions of saline (NaCl), GIP, or GLP-1 were started at time 0 min preceded by either single-dose SU 1 mg glimepiride or placebo (PLA) at time −90 min. Data are shown as mean percentage ± SEM. *A*: The reference value (100%) is the sum of $bsAUC_{0–120\ min,\ SU+NaCl}$ + $bsAUC_{0–120\ min,\ PLA+GIP}$, which on the graphs is shown as SU+GIP (additive). SU+GIP (observed) is the observed $bsAUC_{0–120\ min,\ SU+GIP}$ during our study. If SU+GIP (observed) is greater than SU+GIP (additive), this is indicative of an interaction and thus a supra-additive effect. *B*: The calculations are identical for *A*, but with GLP-1 instead of GIP.

observed with SU+GIP in both groups (Fig. 3*G* and *H* and Table 3).

Arginine-Induced Glucagon Secretion

The arginine-induced glucagon levels were significantly higher in *HNF1A* mutation carriers compared with control subjects without diabetes evaluated as peak (P = 0.0215), $AUC_{120–125\ min}$ (P = 0.0093), and $iAUC_{120–125\ min}$ (P = 0.0332) for glucagon (Table 4 and insets in Fig. 3*E* and *F*). In both groups, there was no difference between experimental days.

DISCUSSION

This study investigates the insulinotropic properties of a combination of SU with infusions of either GIP or GLP-1 in *HNF1A* mutation carriers. The primary finding is that SU combined with GIP or GLP-1 increases C-peptide concentrations in an additive to supra-additive fashion in *HNF1A* mutation carriers, indicating that a combination of SU and incretin-based therapy may have synergistic effects in the treatment of patients with HNF1A diabetes.

Despite the fact that most patients with HNF1A-diabetes eventually need additional treatment on top of SU, no study has evaluated potential second-line glucose-lowering agents (4,10,14). In the current study, infusion rates of exogenous GIP and GLP-1 were chosen to result in plasma levels seen during treatment with a DPP-4i. A considerable strength of this study is the design, in which we isolate the effects of GIP, GLP-1, and SU on the endocrine pancreas from that of glucose using a two-step glucose clamp. Another strength is the placebo-controlled crossover design, which reduces the intraindividual differences. Considering the rarity of *HNF1A* mutations, it is also a strength that none of the *HNF1A* mutation carriers were related. A limitation to our study is the heterogeneity of the *HNF1A* mutation carriers regarding their diabetes status, fasting plasma glucose, and oral glucose-lowering treatment, which included incretin-based treatment. Our study was powered to detect changes in C-peptide levels but may not be powered adequately to detect changes in glucagon.

We demonstrate a significant insulinotropic effect evaluated as C-peptide $bsAUC_{0–120\ min}$ in the current study when using supraphysiological doses of both GIP (1.5 pmol/kg/min) and GLP-1 (0.5 pmol/kg/min). This is in line with a previous study by Vilsbøll et al. (22) that found a significant insulinotropic effect of exogenous infusions of GIP (4 pmol/kg/min) and GLP-1 (1 pmol/kg/min), respectively, compared with saline during a 2-h hyperglycemic clamp (15 mmol/L) in patients with HNF1A diabetes. Together, GIP and GLP-1 are responsible for the incretin effect (i.e., the amplification of insulin secretion with an oral glucose challenge compared with isoglycemic intravenous glucose infusion). Østoft et al. (9,14) described impaired incretin effect in *HNF1A* mutation carriers and that a GLP-1 receptor agonist has glucose-lowering actions with low risk of hypoglycemia in patients with HNF1A diabetes. Taken together, studies investigating the effect of incretins in *HNF1A* mutation carriers indicate that a diminished activation of both GIP and GLP-1 receptors contribute to impaired insulin responses and thus hyperglycemia, but that GIP and GLP-1 receptors may constitute viable treatment targets during elevated plasma levels of the peptides

Table 4—Arginine-induced maximal secretion test

Intervention	PLA+NaCl	PLA+GIP	PLA+GLP-1	SU+NaCl	SU+GIP	SU+GLP-1	Interaction *P* value
C-peptide							
HNF1A mutation carriers							
Peak (nmol/L)	1.2 ± 0.1	1.4 ± 0.2	1.8 ± 0.2*‡	1.8 ± 0.2	2.2 ± 0.3*†‡	2.5 ± 0.3*†‡§	0.1963
$AUC_{120–125\ min}$ (nmol/L × min)	4.9 ± 0.4	5.4 ± 0.7	7.0 ± 0.7*	6.8 ± 0.8	9.3 ± 1.1*†‡	10.0 ± 1.0*†‡§	0.1193
$iAUC_{120–125\ min}$ (nmol/L × min)	2.2 ± 0.3	2.1 ± 0.4	2.8 ± 0.4	3.1 ± 0.4	3.2 ± 0.5	3.9 ± 0.6‡	0.9680
Control subjects without diabetes							
Peak (nmol/L)	2.2 ± 0.2	3.0 ± 0.3	3.4 ± 0.4*	2.9 ± 0.4	3.7 ± 0.5	5.0 ± 0.6*†‡§	0.1801
$AUC_{120–125\ min}$ (nmol/L × min)	8.3 ± 0.9	12.3 ± 1.1	14.1 ± 1.7*	11.8 ± 1.7	16.1 ± 2.1*	22.0 ± 2.6*†‡§	0.1385
$iAUC_{120–125\ min}$ (nmol/L × min)	4.8 ± 0.6	5.3 ± 0.7	5.7 ± 0.6	5.8 ± 0.9	5.9 ± 0.9	6.9 ± 1.0	0.8340
Glucagon							
HNF1A mutation carriers							
Peak (nmol/L)	41 ± 5	44 ± 4	36 ± 4	32 ± 4	35 ± 3	34 ± 4	0.1797
$AUC_{120–125\ min}$ (nmol/L × min)	508 ± 71	428 ± 88	293 ± 52	414 ± 69	467 ± 89	356 ± 55	0.2300
$iAUC_{120–125\ min}$ (nmol/L × min)	128 ± 16	129 ± 13	104 ± 15	95 ± 17	112 ± 11	109 ± 17	0.2671
Control subjects without diabetes							
Peak (nmol/L)	30 ± 4	31 ± 3	25 ± 3	24 ± 3	26 ± 3	20 ± 3†	0.9993
$AUC_{120–125\ min}$ (nmol/L × min)	287 ± 50	256 ± 41	160 ± 39*	237 ± 41	217 ± 32	212 ± 54	0.2801
$iAUC_{120–125\ min}$ (nmol/L × min)	99 ± 14	97 ± 13	73 ± 16	78 ± 15	79 ± 12	79 ± 15	0.5468

Data are mean ± SEM. $iAUC_{120–125\ min}$ is the incremental values from time 120 min, when 5 g arginine was given as a bolus. PLA, placebo. Symbols show significant differences ($P < 0.05$) between interventions: *significantly greater than PLA+NaCl; †significantly greater SU+NaCl; ‡significantly greater than PLA+GIP; §significantly greater than PLA+GLP-1.

seen with incretin-based therapies such as DPP-4i (GIP and GLP-1) and GLP-1 receptor agonist (GLP-1).

Interestingly, during the hyperglycemic part of our clamp study, we found a supra-additive effect on insulin secretion with the combination of SU+GIP and SU+GLP-1 in both *HNF1A* mutation carriers and control subjects without diabetes. This observation could be explained by a combined effect on the K_{ATP} channel by SU, GIP, and GLP-1. SU binds directly to the K_{ATP} channel, while GIP and GLP-1 receptor activation increase levels of cAMP, in turn activating protein kinase A, which increases the sensitivity of the K_{ATP} channel to ATP (23). The combined actions increase the likelihood of K_{ATP} channel closure and depolarization and subsequently increased insulin release. In addition, acute GIP and GLP-1 receptor activation also increase insulin secretion via several other mechanisms than the K_{ATP} channel (23), and chronic GLP-1 receptor stimulation has been shown to increase glycolysis and ATP production in β-cells through transcriptional activation and expression of glycolytic genes (24). Whether this is the case in *HNF1A* mutation carriers is unknown; however, case series have indicated remarkable HbA_{1c} reduction and increase peak insulin levels during an intravenous glucose tolerance test in patients with HNF1A-diabetes when adding a DPP-4i to SU (25). In patients with type 2 diabetes, the glucose-lowering effect of DPP-4i is mainly attributed to the increase of GLP-1 levels because the insulinotropic effect of GIP is severely diminished (26); however, our study indicates that GIP could mediate the glucose-lowering effects of DPP-4i to a greater extent in patients with HNF1A diabetes.

Unexpectedly, we observed a greater mean fasting and arginine-induced glucagon concentration in *HNF1A* mutation carriers compared with control subjects without diabetes. Our study is the first to indicate increased fasting glucagon concentrations in *HNF1A* mutation carriers compared with control subjects without diabetes, to our knowledge. In addition, postprandial hyperglucagonemic responses during meal tests have also been observed in *HNF1A* mutation carriers (9,10,14). The observation of hyperglucagonemia in fasting, postprandial, and after arginine infusion could indicate an altered secretion pattern of glucagon in *HNF1A* mutation carriers.

To our knowledge, the effect of an *HNF1A* mutation on α-cell functions has not been investigated in either animals or cell lines. The nature of postprandial hyperglucagonemia could theoretically be due to insufficient glucose sensing in the glucagon-producing α-cells (like that observed in β-cells); however, the normal suppression of glucagon during intravenous glucose infusion in our and other studies contradicts this (14,22). Insulin is also known as an inhibitor of glucagon secretion (27), which is why hypoinsulinemia could disrupt the normal paracrine signaling between β- and α-cells, resulting in increased glucagon concentrations. Finally, an explanation could be that the total α-cell mass or α- to β-cell ratio could be increased. A study of a single pancreatic human islet from a 33-year-old diseased donor with HNF1A diabetes displayed elevated α-cell mass and increased α/β-cell ratio compared with seven control subjects without diabetes (28). Arginine induces maximal glucagon release from α-cells via a mechanism independent of both glucose metabolism and K_{ATP} channel (29,30) and is thought to be correlated with total α-cell mass (31). Thus, our study could potentially be in line with an increased α-cell mass; however, our data could just as well indicate increased glucagon secretory capacity of α-cells.

In patients with type 2 diabetes, both exogenous GLP-1 infusions and GLP-1 receptor agonists show glucagonostatic properties alleviating postprandial hyperglucagonemia (32). On the contrary, the hyperglucagonemia of *HNF1A* mutation carriers does not seem to respond to GLP-1, as we did not see a decrease in glucagon with GLP-1 infusion, nor did 6 weeks of GLP-1 receptor agonism change fasting or postprandial glucagon concentrations (9). The elevated plasma glucagon concentrations in *HNF1A* mutation carriers most likely add to their state of diabetes, and future studies should investigate the role of glucagon in more detail.

Conclusions

We investigated the insulinotropic properties of SU in combination with incretin hormones in *HNF1A* mutation carriers and report additive to supra-additive effects on insulin secretion with the combination of a low-dose SU with either GIP or GLP-1. We also report increased fasting and arginine-induced levels of glucagon in *HNF1A* mutation carriers. Our results and previous work indicate that targeting the GIP and/or GLP-1 receptors in combination with SU therapy may constitute a viable strategy for the management of hyperglycemia in patients with HNF1A diabetes. An ongoing clinical trial (EudraCT no. 2017-000204-15) is investigating the efficacy and safety of combined SU and DPP-4i therapy (33).

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Duality of Interest. H.S. has served on scientific advisory panels for Boehringer Ingelheim and Novo Nordisk. J.J.H. has served on advisory boards for Novo Nordisk. F.K.K. has served on scientific advisory panels and/or been part of speakers bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Carmot Therapeutics, Inc., Eli Lilly and Company, Gubra, Lupin Limited, MedImmune, LLC, Merck Sharp & Dohme/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi, and Zealand Pharma. T.V. has served on scientific advisory panels and/or speakers bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Mundipharma, Novo Nordisk, Sanofi, and Sun Pharmaceutical Industries Ltd. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. A.S.C., S.H., H.S., T.H., F.K.K., and T.V. designed the study. A.S.C. wrote the study protocol. A.S.C., K.R., and N.L.H. performed the study. J.J.H. generated data. A.S.C. and T.V. performed the data analysis and wrote the manuscript. All authors critically edited the manuscript and approved the final version. A.S.C. and T.V. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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