Impaired Incretin-Induced Amplification of Insulin Secretion after Glucose Homeostatic Dysregulation in Healthy Subjects

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Objective: The insulinotropic effect of the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) is impaired in patients with type 2 diabetes. It remains unclear whether this impairment is a primary pathophysiological trait or a consequence of developing diabetes. Therefore, we aimed to investigate the insulinotropic effect of GIP and GLP-1 compared with placebo before and after 12 d of glucose homeostatic dysregulation in healthy subjects.

Research Design and Methods: The insulinotropic effect was measured using hyperglycemic clamps and infusion of physiological doses of GIP, GLP-1, or saline in 10 healthy Caucasian males before and after intervention using a high-calorie diet, sedentary lifestyle, and administration of prednisolone (37.5 mg once daily) for 12 d.

Results: The intervention resulted in increased insulin resistance according to the homeostatic model assessment (1.2 ± 0.2 *vs.* 2.6 ± 0.5, $P = 0.01$), and glucose tolerance deteriorated as assessed by the area under curve for plasma glucose during a 75-g oral glucose tolerance test (730 ± 30 *vs.* 846 ± 57 mM for 2 h, $P = 0.021$). The subjects compensated for the change in insulin resistance by significantly increasing their postintervention insulin responses during saline infusion by 2.9 ± 0.5-fold ($P = 0.001$) but were unable to do so in response to incretin hormones (which caused insignificant increases of only 1.78 ± 0.3 and 1.38 ± 0.3-fold, *P* value not significant).

Conclusions: These data show that impairment of the insulinotropic effect of both GIP and GLP-1 can be induced in healthy male subjects without risk factors for type 2 diabetes, indicating that the reduced insulinotropic effect of the incretin hormones observed in type 2 diabetes most likely is a consequence of insulin resistance and glucose intolerance rather than a primary event causing the disease. (***J Clin Endocrinol Metab*** **97: 1363–1370, 2012)**

Maintenance of glucose homeostasis depends critically on pancreatic insulin secretion, which, apart from glucose itself, is regulated by numerous neural and hormonal mechanisms (1). The gut-derived incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) play a major role in the postprandial regulation of insulin secretion. Both hormones are secreted from the intestine in response to the intraluminal presence of nutrients and possess strong insulinotropic properties amplifying postprandial insulin secretion from pancreatic β-cells severalfold (2, 3). This amplification is designated the incretin effect and accounts for up to 70% of insulin secretion after ingestion of glucose in healthy individuals (2). Impaired insulinotropic

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Abbreviations: AUC, Area under the curve; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HOMA, homeostatic model assessment; iAUC, incremental AUC; IR, insulin resistance; ISR, insulin secretion rate; NS, not significant; OGTT, oral glucose tolerance test; PG, plasma glucose; T2DM, type 2 diabetes mellitus.

effect of the incretin hormones is an important element of the pathophysiology of type 2 diabetes mellitus (T2DM) (4–6). The insulinotropic effect of GIP has been shown to be severely compromised in patients with T2DM (7–9), even during administration of highly supraphysiological doses of GIP (9). Also, the potency of GLP-1 is reduced in these patients (10–12), but iv administration of GLP-1 in slightly supraphysiological doses restores the ability of β-cells to sense and respond to glucose (9, 12). These perturbations are thought to contribute significantly to postprandial glucose intolerance of T2DM. However, the mechanisms behind the loss of β-cell sensitivity to GIP and GLP-1 in patients with T2DM is far from clear. Therefore we induced insulin resistance (IR) and mild glucose intolerance in perfectly healthy young men without any risk factors for developing T2DM to see whether this would impair the capability of the incretin hormones to amplify glucose-stimulated insulin secretion.

In a previous study using this experimentally induced IR and glucose intolerance, a marked reduction of the incretin effect (from 70 to 40%, similar to the loss observed in patients with type 2 diabetes) was observed (13). The reduction occurred without attenuation of GLP-1 or GIP secretion. In fact, postprandial GIP responses may actually increase after similar intervention (14). Therefore, we hypothesized that reduced insulinotropic effects of the incretin hormones could be responsible for the observed reduction in incretin effect. In the present study, we tested this hypothesis by subjecting perfectly healthy young men (without any risk factors of or predisposition to diabetes) to hyperglycemic clamps with continuous infusions of GLP-1, GIP, and saline, respectively, before and after the period of induced IR and mild glucose intolerance to see whether this would impair the capability of the incretin hormones to amplify glucose-stimulated insulin secretion.

Subjects and Methods

Subjects

Ten healthy young Caucasian men (age above 18 yr, body mass index between 18.5 and 30.0 kg/m^2) without any family history of diabetes were studied (anthropometric data in Table 1). All had normal glucose tolerance according to 75-g oral glucose tolerance test (OGTT) [2-h plasma glucose (PG) value $<$7.8 mM] performed within 2 wk of inclusion in the study. All participants were negative with regard to islet cell autoantibodies and glutamate decarboxylase-65 autoantibodies. All subjects had normal clinical and biochemical parameters including blood pressure, liver enzymes, cholesterol, electrolytes, creatinine, and no albuminuria. None of the subjects took any drugs on a daily basis. All subjects agreed to participate after receiving oral and written information. The study was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (Registration Number in the committee: H-A-2008-049) and conducted according to the principles of the Helsinki Declaration II.

TABLE 1. Anthropometric data

	Preintervention	Postintervention	*P* value
N (male/female)	0/10		
Age (yr)	26 (21–30)		
Body weight (kg)	75.5 (64–97)	76.2 (63–98)	0.045
BMI (kg/m^2)	22.6 (20–27)	22.9 (20–28)	0.047
Mean FPG	4.9 (4.6–5.1)	5.3 (4.2–6.8)	0.012
2-h PG	4.2 (2.7–5.5)	5.5 (4.5–8.0)	0.023
HbA_1c (%)	5.3 (5.1–5.9)	5.6 (5.4–6.0)	0.006
$HOMA_{IR}$	1.2 (0.3–1.9)	2.6 (1.1–5.6)	0.011
Matsuda index	24.9 (11–50)	10.2 (3–15)	0.002

Data are mean values (ranges). BMI, Body mass index; FPG, fasting plasma glucose; HbA_1c, glycated hemoglobin.

Study protocol

In randomized order, the subjects underwent a 75-g OGTT, a 1-mg glucagon test and three 10 mM hyperglycemic clamps (with concomitant infusion of GLP-1, GIP, or saline) on five separate days immediately before a 12-d intervention period (described below). During the last 5 d of the intervention period, the experimental days were repeated in randomized order.

On each experimental day, the subjects were studied in the recumbent position after an overnight (10 h) fast including liquids (and water) and tobacco. For blood sampling, a cannula was inserted into a cubital vein (the cannulated arm was wrapped in a heat pad throughout each experiment for collection of arterialized blood). The cannula was kept patent by infusing small amounts of saline (~2 ml). The saline was removed again before drawing new blood samples.

OGTT

The subjects ingested 75 g water-free glucose dissolved in 300 ml water over 5 min (0–5 min). Blood was sampled at time −15, 0, 30, 60, 90, and 120 min for analysis of PG, insulin, and C-peptide.

Glucagon test

A cannula in the contralateral cubital vein (for glucagon injection) was inserted. At time 0 min, 1 mg glucagon (GlucaGen; Novo Nordisk, Bagsvaerd, Denmark) was injected. Blood was sampled at time −15, −10, 0, 2, 3, 4, 6, 8, 10, 15, and 20 min for analysis of PG, insulin, and C-peptide.

Hyperglycemic clamps

A cannula in the contralateral cubital vein (for peptide/placebo infusions) was inserted. At time 0 min, a patient-blinded continuous infusion (25 ml/h) of GLP-1 (0.5 pmol/kg·min), GIP (1.5 pmol/kg·min), or saline was initiated. After 2 min, 50% glucose (wt/vol) was infused for 1 min to increase PG to 10 mM. The amount of glucose given was calculated as follows: milliliters of 50% glucose (wt/vol) = elevation in fasting PG needed (millimolar) × 35 (milligrams glucose × 1 mM^{-1} × kilograms body weight^{-1}) × body weight (kilograms). PG was kept at 10 mM by continuous infusion of 20% glucose (wt/vol) adjusted according to bedside PG (measured every 5 min). Blood was sampled at

time −15, −10, 0, 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min for analysis of insulin, C-peptide, GLP-1, GIP, and glucagon as described below.

During the experiments, blood for plasma analyses of glucagon, GLP-1, and GIP was distributed into chilled tubes containing EDTA plus aprotinin (500 kIU/ml blood; Trasylol; Bayer Corp., Leverkusen, Germany) and a specific dipeptidyl peptidase 4 inhibitor (valine-pyrrolidide, final concentration = 0.01 mM; a gift from Novo Nordisk). For insulin and C-peptide analyses, blood was distributed into chilled tubes containing heparin. All tubes were kept on ice before and after blood sampling and centrifuged (within 25 min after sampling) for 20 min at 1500 × *g* and 4 C. Plasma for glucagon, GLP-1, and GIP analyses was stored at −20 C, and plasma for insulin and C-peptide analyses was stored at −80 C until analysis. For bedside measurement of PG, 0.2 ml blood was added to fluoride tubes and centrifuged immediately for 2 min at 7400 × *g* and room temperature.

Intervention

The 12-d intervention period consisted of orally administered prednisolone (37.5 mg once daily), relative physical inactivity (rest for at least 8 h/d and no strenuous exercise) and a diet containing 130% of daily recommended energy intake (30% fat, 10% protein, and 60% carbohydrate). The dose of prednisolone equals the dose recommended for patients with exacerbation of chronic obstructive pulmonary disease. At this dose, the most relevant adverse event reported is hyperglycemia (15). To ensure adherence to the recommended caloric intake, the participants were given specific dietary instructions. The participants were told to record any violation of the dietary or physical activity prescriptions. Additionally, each participant was contacted by telephone once during the intervention period to ensure compliance.

Peptides

Synthetic GLP-1 (7–36) amide and human GIP 1–42 were purchased from PolyPeptide Laboratories (Strasbourg, France). The peptides were dissolved in sterilized water containing 2% (wt/vol) human serum albumin (Statens Serum Institute, Copenhagen, Denmark; guaranteed to be free of hepatitis B surface antigen, hepatitis C virus antibodies, and HIV antibodies) and subjected to sterile filtration. Appropriate amounts of peptide for each experimental participant were dispensed into glass ampoules and stored frozen (−20 C) under sterile conditions until the day of the experiment.

Analyses

PG concentrations were measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Model 2300 STAT plus analyzer; YSI Inc., Yellow Springs, OH).

Plasma insulin and C-peptide concentrations were measured using two-sided assays, ElectroChemiLuminescens ImmunoAssays (Roche/Hitachi Modular analytics; Roche Diagnostic GmbH, Mannheim, Germany). The detection limit is below 2 pM for both assays, and intraassay coefficients of variation are 1.9% for the insulin assay and 4.6% for the C-peptide assay (16).

Plasma samples were assayed for total GLP-1 immunoreactivity, as previously described (17) using antiserum no. 89390, which is specific for the C terminus of the GLP-1 molecule.

Intact, biologically active, GIP was measured using antiserum no. 98171 (18).

The glucagon assay is directed against the C terminus of the glucagon molecule (antibody code no. 4305) and, therefore, measures glucagon of mainly pancreatic origin (19). Neither glicentin nor oxyntomodulin cross-react, but proglucagon (1–61), which is mainly formed in the pancreas, does react fully in this assay (20, 21).

Calculations and statistical analyses

All results are expressed as mean ± SEM. Area under the curve (AUC) values were calculated using the trapezoidal rule and are presented as incremental AUC (iAUC) values (*i.e.* baseline levels subtracted) if nothing else is stated. The homeostatic model assessment (HOMA) based on fasting plasma concentrations of insulin and glucose was used to assess IR, predominantly reflecting hepatic insulin resistance (22). Insulin sensitivity was estimated by the Matsuda insulin sensitivity index, which takes into account mean insulin and mean glucose levels during oral glucose stimulation (23). Insulin secretion rate (ISR) was calculated by deconvolution of measured C-peptide concentrations in plasma and expressed as picomoles insulin secreted per kilogram body weight per minute (24–26). Insulinogenic index based on ISR and PG [insulinogenic index = $(ISR_{t=30} - ISR_{t=0})/(PG_{t=30} - PG_{t=0})$] (27), where t is time, and disposition index (insulinogenic index × $HOMA_{IR}^{-1}$) were calculated (28). Early-phase insulin responses (0–10 min) and late-phase insulin responses (10–120 min) were evaluated from the respective AUC values. Comparisons of experiments in which the data were distributed normally were made with paired two-tailed *t* test. For data that did not follow a normal distribution, the significance of differences was tested using the Wilcoxon test for paired differences. Poststimulus changes were evaluated using repeated-measures ANOVA, and two-factor repeated-measures ANOVA (calculated using SPSS Statistics version 17.0; IBM, Armonk, NY) was used to evaluate differences between time courses before and after intervention. *P* values <0.05 were considered statistically significant.

Results

During the intervention period, the subjects reported increased appetite. Glucagon testing resulted in transient nausea in 60% of the subjects. Otherwise, no side effects were reported. The impact of the intervention on anthropometric data are shown in Table 1.

Glucose

There was no difference in fasting PG between the five experimental days, before or after the intervention, but mean fasting PG increased significantly after the intervention (4.9 ± 0.1 *vs.* 5.3 ± 0.1 mM, $P = 0.012$). The 2-h PG (4.2 ± 0.3 *vs.* 5.5 ± 0.5 mM, $P = 0.023$) and AUC_{PG} (730 ± 30 *vs.* 846 ± 57 mM × 2 h, $P = 0.021$) during the 75-g OGTT also increased (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). During the hyperglycemic clamps, PG concentrations increased immediately af-

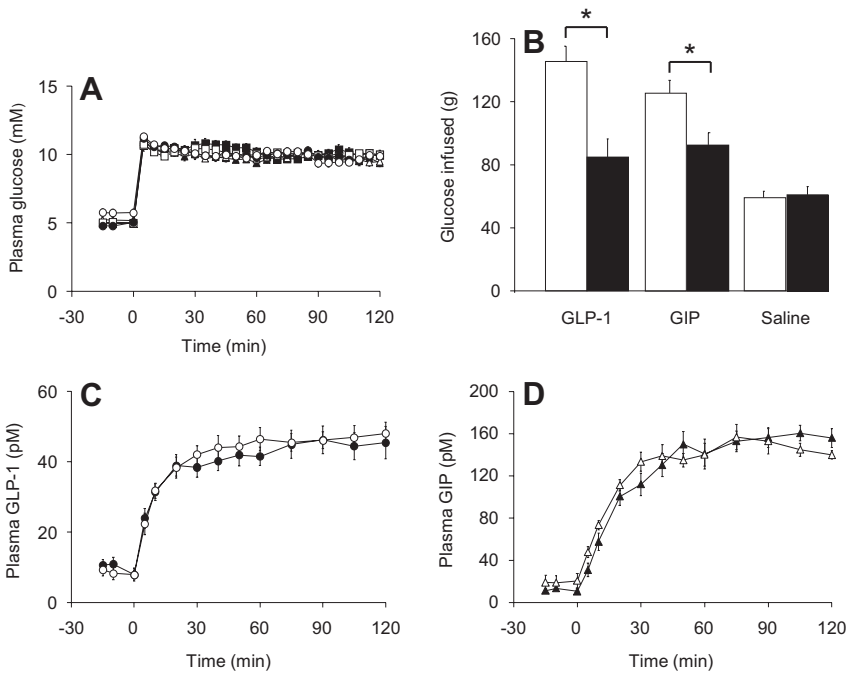

FIG. 1. A, Plasma glucose during preintervention (*solid symbols*) and postintervention (*open symbols*) hyperglycemic clamps (10 mM) with GLP-1 infusions (*circles*), GIP infusions (*triangles*), and saline infusions (*squares*), respectively. B, Total amount of glucose infused during preintervention and postintervention hyperglycemic clamps, respectively. *, Significant difference ($P < 0.05$). C and D, Plasma GLP-1 (C) and GIP (D) during preintervention (*solid symbols*) and postintervention (*open symbols*) hyperglycemic clamps with GLP-1 infusions (*circles*) and GIP infusions (*triangles*), respectively.

ter the bolus glucose injection and reached stable plateaus (Fig. 1) with no significant differences between the three different clamps or between clamps before and after intervention (mean plateau PG = 10.0 ± 0.1 mM). Although the amount of glucose infused during the saline day was unchanged (59 ± 4 *vs.* 61 ± 5 g, $P = 0.738$), the amounts of glucose infused during the GIP clamp day (125 ± 8 *vs.* 93 ± 8 g, $P = 0.008$) and GLP-1 clamp day (146 ± 9 *vs.* 85 ± 11 g, $P = 0.0005$) were significantly lower after intervention and only slightly higher than those infused during saline (Fig. 1).

GLP-1 and GIP

The intervention had no impact on mean basal plasma levels of GLP-1 [10 ± 2 *vs.* 9 ± 2 pM, *P* value not significant (NS)]. During the GLP-1 infusions (before and after intervention), plasma GLP-1 concentrations increased and reached stable plateaus of 42 ± 3 and 45 ± 2 pM (*P* value NS) after 20 min (Fig. 1).

The intervention had no impact on mean basal plasma levels of GIP (11 ± 2 *vs.* 11 ± 2 pM, *P* value NS). During the GIP infusions (before and after intervention) plasma GIP concentrations increased and reached stable plateaus of 140 ± 8 and 139 ± 7 pM (*P* value NS) after 20 min (Fig. 1).

Insulin, C-peptide, ISR, and insulin sensitivity indices

The intervention resulted in increased $HOMA_{IR}$ and reduced Matsuda insulin sensitivity index (Table 1) and increased plasma insulin responses (AUC) after OGTT (27 ± 4 *vs.* 69 ± 14 nM × 120 min, $P = 0.003$) and glucagon test (90 ± 27 *vs.* 424 ± 157 nM × 20 min, $P = 0.04$). Plasma C-peptide and ISR showed a similar increased response during OGTT (data not shown). Fasting plasma insulin, C-peptide, and ISR, respectively, were similar on the 5 d before (mean fasting insulin, 38 ± 2 pM; mean fasting C-peptide, 581 ± 47 pM; mean fasting ISR, 1.9 ± 0.2 pmol/kg·min) as well as after intervention (mean fasting insulin, 88 ± 6 pM; mean fasting C-peptide, 926 ± 39 pM; mean fasting ISR, 3.0 ± 0.1 pmol/kg·min) which brought about significant elevations ($P = 1.0 \times 10^{-9}$, $P = 1.2 \times 10^{-7}$, and $P = 3.7 \times 10^{-7}$, respectively) in all three parameters. Insulin response (AUC) during hyperglycemic clamp before intervention was augmented 7.0- and 4.7-fold by GLP-1 and GIP, respectively (compared with saline), but the fold augmentation fell to 3.4 ($P = 0.03$) and 2.7 ($P = 0.09$) after the intervention. The C-peptide AUC during hyperglycemic clamp before intervention was augmented 2.5- and 2.3-fold by GLP-1 and GIP, respectively (compared with saline), falling significantly to 1.5-fold ($P = 0.003$) and 1.6-fold ($P = 0.04$) after the intervention. Likewise, ISR responses during hyperglycemic clamp before intervention were augmented to 2.7- and 2.4-fold by GLP-1 and GIP, respectively (compared with saline), falling significantly to 1.7-fold ($P = 0.005$) and 1.7-fold ($P = 0.04$) (Fig. 2 and Table 2). When investigating the relative increase in insulin response after intervention compared with before intervention ($iAUC_{postintervention}/iAUC_{preintervention}$) on the three different paired clamp days (Fig. 3), we observed that the subjects compensated for the increased IR by significantly increasing their postintervention insulin responses during saline infusion by 2.9 ± 0.5-fold, significantly ($P = 0.001$) more than during GIP and GLP-1 infusions, respectively (1.8 ± 0.3- and 1.4 ± 0.3-fold, *P* value NS). As illustrated in Fig. 3, the same pattern was evident for C-peptide and ISR responses. These differences were not evident during the early phase (0–10 min) of the infusions

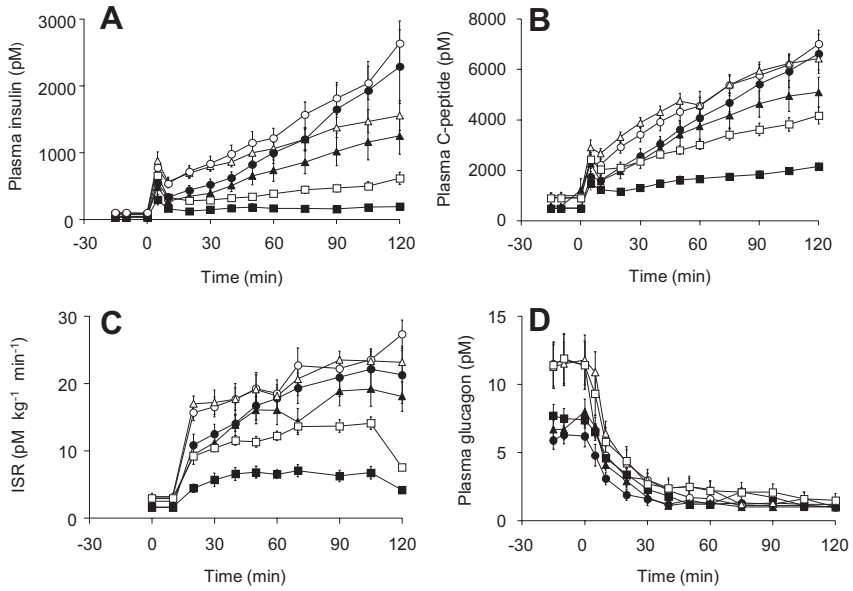

FIG. 2. Plasma insulin (A), C-peptide (B), ISR (C), and glucagon (D) responses during 12 mM hyperglycemic clamps with GLP-1 (*circles*), GIP (*triangles*), or saline infusion (*squares*) before (*solid symbols*) and after (*open symbol*) intervention with high-calorie diet, sedentary lifestyle, and administration of prednisolone (37.5 mg once daily) for 12 d.

but occurred during the late phase (10–120 min) of the infusions (Fig. 2 and Table 3).

Insulinogenic index and disposition index

Intervention-induced changes in insulinogenic index (126 ± 38 *vs.* 206 ± 37, $P = 0.09$) or disposition index (119 ± 37 *vs.* 98 ± 17, $P = 0.50$) were not significantly different.

Glucagon

There were no differences in fasting plasma glucagon during the experimental days before (mean fasting plasma glucagon, 6.9 ± 0.4 pM) or after the intervention (mean fasting plasma glucagon, 11.6 ± 1.0 pM), which, however, resulted in significantly increased fasting glucagon levels ($P = 0.033$). Interestingly, glucagon levels decreased to very low levels shortly after the hyperglycemic iv glucose stimulus independent of the reference fasting value and the type of hormone (or saline) infusion (Fig. 2).

Discussion

The present study shows that healthy subjects are able to produce a 3-fold amplification of glucose-induced (10 mM hyperglycemic clamp) insulin secretion after a 12-d intervention period aimed at inducing insulin resistance and attenuated glucose tolerance (with prednisolone administration, high-energy diet, and relative physical inactivity), whereby they were able to retain their disposition index, their β-cell function corrected for insulin sensitivity. In contrast, there was little intervention-induced amplification of glucose-stimulated insulin secretion during concomitant infusions of GIP and GLP-1, respectively, amounting to only 1.8- and 1.4-fold, suggesting that these relatively mild disturbances of glucose metabolism severely compromised the ability of the incretin hormones to amplify glucose-induced insulin secretion.

Our intervention was based on high-energy diet, reduced physical activity, and insulin resistance normally characterizing patients with prediabetes or T2DM (29). Thus, our design provides a model for investigations of pathogenetic mechanisms presumably involved in the development of prediabetes or early diabetes (whereas the design does not allow us to evaluate the individual contributions of the three components in the intervention). The order of the testing days was randomized, but this had little effect on the results; thus, participants who underwent the OGTT during the first day after intervention and participants who had the OGTT performed during the last experimental day after intervention had similar insulin responses (62 ± 15 *vs.* 57 ± 12 nM × 120 min, $P = 0.881$). The intervention brought about insulin resistance, as illustrated by HOMA and Matsuda indices.

To mimic physiological conditions, we clamped the subjects at a PG level of only 10 mM, and we infused GLP-1 and GIP in physiological doses resulting in plasma concentrations similar to those obtained after a meal in healthy individuals (30). Before the intervention, the GIP and GLP-1 clamps elicited markedly higher insulin responses compared with the saline clamp, reflecting the potent insulinotropic effects of the incretin hormones, with GLP-1 producing the greatest insulin responses. Ac-

TABLE 2. Fold increases

	GLP-1			GIP		
	Preintervention	Postintervention	*P* value	Preintervention	Postintervention	*P* value
Insulin	7.0 ± 1.2	3.4 ± 0.5	0.03	4.7 ± 1.0	2.7 ± 0.2	0.09
C-peptide	2.5 ± 0.2	1.5 ± 0.1	0.003	2.3 ± 0.5	1.6 ± 0.1	0.04
ISR	2.7 ± 0.3	1.7 ± 0.1	0.005	2.4 ± 0.3	1.7 ± 0.1	0.04

Data are mean values ± SEM. Fold increases are calculated as responses (AUC) of insulin, C-peptide, or ISR compared with saline (AUC).

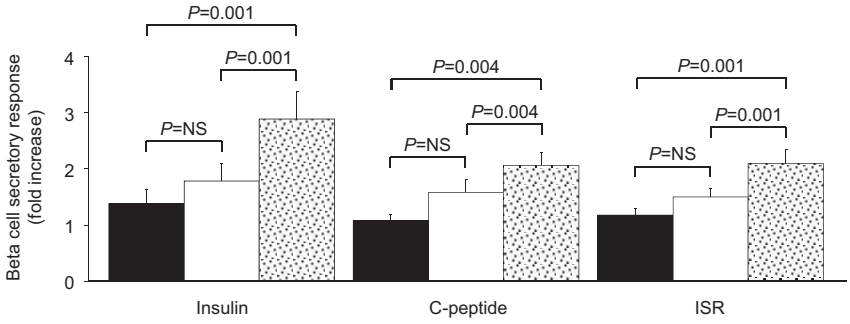

FIG. 3. Fold increases in β-cell secretory responses (insulin, C-peptide, and ISR) during 10 mM hyperglycemic clamps after intervention with high-calorie diet, sedentary lifestyle, and administration of prednisolone (37.5 mg once daily) for 12 d during GLP-1 (*black bar*), GIP (*white bar*), and saline (*dotted bar*) infusion, respectively.

cordingly, the glucose infusion rates needed to maintain PG at 10 mM were significantly higher during the GIP and GLP-1 clamps compared with the saline clamp. After the intervention, significantly less glucose had to be infused with GLP-1 and GIP, both absolutely and relative to the amount infused with saline. The compensating β-cell responses were significant for all five β-cell secretory stimuli and amounted to as much as an absolute 3-fold amplification of insulin secretion in response to the saline clamp. In contrast, the relative changes in insulin secretion were significantly lower during the GLP-1 and GIP clamps, respectively, so that there was little difference between clamp responses to glucose alone and those with additional incretin hormones. Examining the insulin response in more detail, we found differences between the early-phase (0–10 min) and late-phase (10–120 min) responses. The early-phase insulin responses appeared independent of the intervention, suggesting that the incretin hormones retain their early-phase insulinotropic properties despite disruption of glucose homeostasis. In contrast, neither GIP nor GLP-1 was able to amplify the late-phase insulin response to the same extent as observed during saline infusion. Thus, the glucose homeostatic dysregulation particularly disrupted the late-phase insulinotropic effect of the incretin hormones. This observation is in accordance with the notion that the early-phase insulin response is thought to involve release of immediately available insulin from granules close to the β-cell plasma membrane, whereas during the late-phase insulin response, release of insulin from stored granules, and *de novo* insulin synthesis in the β-cells predominates (31). The observations are also in accordance with the finding that both GIP and GLP-1 retain a small early-phase insulin response in T2DM patients, whereas GIP has lost the entire late-phase response and GLP-1 needs supraphysiological concentrations to elicit a significant late-phase insulin response (9).

The molecular mechanisms underlying the impaired insulinotropic effect of the incretin hormones remain unknown. Meier *et al.* (32) demonstrated a reduced insulinotropic effect of GIP in first-degree relatives of patients with T2DM, suggesting this deficiency to be a primary pathogenetic factor in the development of T2DM. However, the same group observed a normal insulin secretory response to GIP in women with a history of gestational diabetes and therefore at high risk of developing T2DM (33). Vilsbøll *et al.* (34) demonstrated reduced insulinotropic effect of GIP in patients with other types of diabetes (newly diagnosed type 1 diabetes, latent autoimmune diabetes of adults, maturity-onset diabetes of the young, and diabetes secondary to chronic pancreatitis), suggesting that the impaired insulinotropic effect of GIP occurs as a

TABLE 3. Insulin, C-peptide, and ISR responses during hyperglycemic clamps before and after intervention

	GLP-1		GIP		Saline	
	Preintervention	Postintervention	Preintervention	Postintervention	Preintervention	Postintervention
Insulin						
Total response (nM × 120 min)	126 ± 24[a]	149 ± 19[b,c]	83 ± 17[a]	117 ± 14[b,c]	16 ± 2[a]	41 ± 4.8[b,c]
Early phase (nM × 10 min)	3.3 ± 0.5	4.5 ± 0.8[c]	2.8 ± 0.7	5.1 ± 0.8[c]	1.6 ± 0.4	3.6 ± 0.6[c]
Late phase (nM × 110 min)	122 ± 23[a]	145 ± 19[b,c]	80 ± 16[a]	112 ± 13[b,c]	14 ± 2[a]	37 ± 5[b,c]
C-peptide						
Total response (nM × 120 min)	412 ± 45[a]	432 ± 39[b,c]	328 ± 48[a]	449 ± 34[b,c]	108 ± 20[a]	252 ± 20[b,c]
Early phase (nM × 10 min)	9.0 ± 1.2	10.4 ± 1.7[c]	9.4 ± 3.9	13.7 ± 1.7[c]	5.5 ± 1.5	10.5 ± 1.4[c]
Late phase (nM × 110 min)	403 ± 44[a]	421 ± 37[b,c]	319 ± 46[a]	435 ± 33[b,c]	102 ± 198[a]	241 ± 19[b,c]
ISR						
Total response (pmol/kg)	1,787 ± 231[a]	1,966 ± 173[b]	1,437 ± 191[a]	1,944 ± 151[b,c]	469 ± 87[a]	1,048 ± 76[b,c]
Early phase (pmol/kg)	47 ± 7	58 ± 8	36 ± 10	73 ± 7[c]	26 ± 7	50 ± 4[c]
Late phase (pmol/kg)	1,723 ± 225[a]	1,877 ± 166[b]	1,375 ± 183[a]	1,838 ± 149[c]	420 ± 86[a]	969 ± 76[b,c]

Data are mean values ± SEM. Total response is iAUC from 0–120 min; early phase is AUC from 0–10 min; late phase is AUC from 10–120 min; intervention is 12 d of prednisolone administration (37.5 mg once daily), abstinence from strenuous physical exercise, and high-energy diet.

[a] Significant difference ($P < 0.05$) when comparing preintervention GLP-1 or GIP infusions with preintervention saline infusion.

[b] Postintervention significant differences ($P < 0.05$).

[c] Significant differences ($P < 0.05$) when comparing pre- and postintervention responses to the same infusion.

consequence of any diabetic state rather than being exclusively associated with T2DM (35). Interestingly, Højberg *et al.* (10) showed that 4 wk of near-normalization of PG using intensified insulin treatment in patients with T2DM improved the potentiating effect of both GIP and GLP-1, respectively, on glucose-induced insulin secretion, suggesting that the deficiencies are reversible (11). Taken together, these results indicate that impaired action of GLP-1 and GIP are secondary to lack of metabolic control in patients with T2DM.

It has been suggested that the observed impaired insulinotropic effect of the incretin hormones is related to a general impairment of β-cell dysfunction of these patients (36), and studies of healthy offspring of patients with T2DM demonstrate a characteristic phenotype characterized by defects in insulin sensitivity and β-cell glucose sensitivity (37, 38). Exclusion of individuals with a family history of diabetes is therefore mandatory in studies like the present one. Our healthy participants compensated for the intervention-induced insulin resistance by increasing insulin and C-peptide responses to oral (2-fold) as well as iv glucose (3-fold) and iv glucagon (4-fold). A glucagon test is often used to evaluate β-cell capacity (39, 40). Thus, the β-cell reserve was large enough to compensate for the insulin resistance and prevented severe deterioration of glucose tolerance. Importantly, an accurate test of β-cell function; *i.e.* disposition index did not change after intervention indicating preserved β-cell capacity. These findings suggest that the loss of insulinotropic effect of the incretin hormones in the diabetic or prediabetic state is an early and very sensitive (possibly specific?) sign of β-cell dysfunction. Other investigators found that glucocorticoid-induced deterioration of glucose homeostasis in first-degree relatives to patients with T2DM did impair disposition index (41). This could be explained by differences in the experimental protocol or, as concluded by the authors, by a genetic predisposition to impaired disposition index already before exposition of glucocorticoids.

A positive correlation between insulin resistance and hyperglucagonemia has been observed in subjects with impaired glucose tolerance (42). However, whether inappropriate glucagon suppression in relation to the prediabetic insulin-resistant condition is a primary event is unknown. In the present study, we found significant increases in fasting plasma glucagon secondary to the intervention. This points to hyperglucagonemia as a similarly early and specific factor in relative glucose intolerance as well as possibly being an early factor in the pathogenesis and development of T2DM. The ability to suppress glucagon to very low levels during all three hyperglycemic clamps was sustained, however, indicating that this important feature of α-cell function is lost only in more advanced stages of T2DM.

In conclusion, we show that 12-d intervention (administration of prednisolone, reduced physical activity, and high-energy diet) aiming at disrupting the glucose homeostasis of perfectly healthy subjects without any disposition to develop diabetes induces impaired amplification of the insulin responses to GIP and GLP-1, respectively, relative to saline, indicating that the reduced β-cell sensitivity to the incretin hormones characterizing glucose-intolerant states like T2DM, occurs early as a consequence of an insulin-resistant and/or glucose-intolerant state.

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