Glucagon-Like Peptide 1 Increases Secretory Burst Mass of Pulsatile Insulin Secretion in Patients With Type 2 Diabetes and Impaired Glucose Tolerance

Robert Ritzel,[1] Miriam Schulte,[1] Niels Pørksen,[2] Markus S. Nauck,[3] Jens J. Holst,[4] Claus Juhl,[2] Winfried März,[3] Ole Schmitz,[2] Wolff H. Schmiegel,[1] and Michael A. Nauck[1]

The insulinotropic gut hormone glucagon-like peptide (GLP)-1 increases secretory burst mass and the amplitude of pulsatile insulin secretion in healthy volunteers without affecting burst frequency. Effects of GLP-1 on secretory mechanisms in type 2 diabetic patients and subjects with impaired glucose tolerance (IGT) known to have impaired pulsatile release of insulin have not yet been studied. Eight type 2 diabetic patients (64 ± 9 years, BMI 28.9 ± 7.2 kg/m^2, HbA_{1c} 7.7 ± 1.3%) and eight subjects with IGT (63 ± 10 years, BMI 31.7 ± 6.4 kg/m^2, HbA_{1c} 5.7 ± 0.4) were studied on separate occasions in the fasting state during the continued administration of exogenous GLP-1 (1.2 pmol · kg^{-1} · min^{-1}, started at 10:00 P.M. the evening before) or placebo. For comparison, eight healthy volunteers (62 ± 7 years, BMI 27.7 ± 4.8 kg/m^2, HbA_{1c} 5.4 ± 0.5) were studied only with placebo. Blood was sampled continuously over 60 min (roller-pump) in 1-min fractions for the measurement of plasma glucose and insulin. Pulsatile insulin secretion was characterized by deconvolution, autocorrelation, and spectral analysis and by estimating the degree of randomness (approximate entropy). In type 2 diabetic patients, exogenous GLP-1 at ~90 pmol/l improved plasma glucose concentrations (6.4 ± 2.1 mmol/l vs. placebo 9.8 ± 4.1 mmol/l, $P = 0.0005$) and significantly increased mean insulin burst mass (by 68%, $P = 0.007$) and amplitude (by 59%, $P = 0.006$; deconvolution analysis). In IGT subjects, burst mass was increased by 45% ($P = 0.019$) and amplitude by 38% ($P = 0.02$). By deconvolution analysis, insulin secretory burst frequency was not affected by GLP-1 in either type 2 diabetic patients ($P = 0.15$) or IGT subjects ($P = 0.76$). However, by both autocorrelation and spectral analysis, GLP-1 prolonged the period (lag time) between subsequent maxima of insulin concentrations significantly from ~9 to ~13 min in both type 2 diabetic patients and IGT subjects. Under placebo conditions, parameters of pulsatile insulin secretion were similar in normal subjects, type 2 diabetic patients, and IGT subjects based on all methodological approaches ($P > 0.05$). In conclusion, intravenous GLP-1 reduces plasma glucose in type 2 diabetic patients and improves the oscillatory secretion pattern by amplifying insulin secretory burst mass, whereas the oscillatory period determined by autocorrelation and spectral analysis is significantly prolonged. This was not the case for the interpulse interval determined by deconvolution. Together, these results suggest a normalization of the pulsatile pattern of insulin secretion by GLP-1, which supports the future therapeutic use of GLP-1–derived agents. *Diabetes* 50:776–784, 2001

In normal subjects, insulin in the basal state and after stimulation is secreted in a pulsatile manner (1–4). This leads to oscillations of insulin concentrations, especially in the portal vein (5,6), that are much smaller but also detectable in the systemic circulation. According to a popular hypothesis, intrapancreatic ganglia serve as a pacemaker to generate these oscillations, whereas nerve fibers also belonging to the intrinsic pancreatic nervous system coordinate the secretory activity of multiple islets (7). In the fasting state, these insulin secretory bursts occur at intervals of 5–15 min (1–6). The length of the interval, as determined by frequent sampling, depends, among other factors, on the sampling frequency (5). The amount of insulin secreted is greatly influenced by modifications primarily of the mass of insulin secretory bursts. Modulations of the frequency appears to be of lesser importance (8). Agents that stimulate insulin secretion (glucose [9], sulfonylureas [10], and incretin hormones [9,11]) increase burst mass, whereas inhibitory factors (like somatostatin [12]) lead to a decrement.

In patients with type 2 diabetes, abnormalities in pulsatile insulin secretion have been described using the method of "autocorrelation" (13) or "approximate entropy" (a measure of the orderliness) (14), which appear to be already present at early stages of the development of type 2 diabetes, i.e., in first-degree relatives (15,16).

Glucagon-like peptide (GLP)-1 is an insulinotropic gut hormone that has received attention as a potential new therapeutic agent for type 2 diabetic patients (17,18). GLP-1 is able to render B-cells "glucose-competent" (19), i.e., able to enhance the glucose responsiveness of pancreatic B-cells. Of particular interest is the glucose dependence of the stimulation of insulin secretion by GLP-1 (20,21), because this limits the risk of hypoglycemia. In

From the [1]Department of Internal Medicine, Ruhr-University, Knappschafts-KH, Bochum, Germany; the [2]Department of Endocrinology and Metabolism, University of Aarhus, Aarhus, Denmark; the [3]Department of Clinical Chemistry, University of Freiburg, Freiburg, Germany; and the [4]Department of Medical Physiology, Panum Institute, University of Copenhagen, Copenhagen, Denmark.

Address correspondence and reprint requests to Prof. Dr. med. Michael Nauck, Diabeteszentrum Bad Lauterberg, Kirchberg 21, 37431 Bad Lauterberg im Harz, Germany. E-mail: m.nauck@diabeteszentrum.de.

Received for publication 11 May 1999 and accepted in revised form 12 December 2000.

ELISA, enzyme-linked immunosorbent assay; GIP, gastric inhibitory polypeptide; GLP, glucagon-like peptide; IGT, impaired glucose tolerance.

TABLE 1
Patients' characteristics

Parameter (unit)	Patients with type 2 diabetes	Subjects with IGT	Healthy control subjects	P*
Sex (M/F)	5/3	1/7	3/5	—
Age (years)	64 ± 9	63 ± 10	62 ± 7	0.89
Height (cm)	166 ± 5	161 ± 6	167 ± 8	0.48
Weight (kg)	80.4 ± 21.8	81.9 ± 13.2	77.5 ± 14.7	0.87
BMI (kg/m^2)	28.9 ± 7.2	31.7 ± 6.4	27.7 ± 4.8	0.43
HbA_{1c} (%)	7.7 ± 1.3	5.7 ± 0.4	5.4 ± 0.5	0.001
Diabetes duration (years)	10 ± 9	—	—	—
Antidiabetic therapy (n)				
Diet alone	3	—	—	—
Sulfonylureas	5	—	—	—
Metformin	0	—	—	—
Acarbose	0	—	—	—
Fasting glucose (mg/dl)	187 ± 74	99 ± 9	99 ± 9	0.0014
120-min glucose (mg/dl)	NA	165 ± 18	123 ± 7	<0.001
Total cholesterol (mg/dl)	211 ± 42	267 ± 65	226 ± 35	0.092
Triglycerides (mg/dl)	160 ± 69	266 ± 216	140 ± 52	0.17
Systolic blood pressure (mmHg)	149 ± 20	129 ± 14	135 ± 18	0.083
Diastolic blood pressure (mmHg)	78 ± 7	75 ± 8	79 ± 11	0.70
Creatinine (mg/dl)	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.1	0.71

Data are means ± SD unless otherwise indicated. *Determined by analysis of variance. NA, not applicable.

healthy subjects receiving a continuous glucose infusion, GLP-1 specifically increased the secretory burst mass and amplitude of pulsatile insulin secretion, whereas burst frequency was not affected (11). The influence of exogenous GLP-1 on pulsatile insulin secretion in type 2 diabetic patients and subjects with impaired glucose tolerance (IGT) has not yet been studied. Therefore, it was the aim of the present study to determine effects of an intravenous infusion of GLP-1 at a "therapeutic" dose on parameters of pulsatile insulin secretion in fasting type 2 diabetic patients and IGT subjects. Preliminary data have been published in abstract form (22).

RESEARCH DESIGN AND METHODS

Study protocol. The study was approved by the ethics committee of the medical faculty of the Ruhr University Bochum on 4 December 1995 (registration number 717). Written informed consent was obtained from all participants.

Subjects and patients. Eight patients with type 2 diabetes and eight subjects with impaired oral glucose tolerance (World Health Organization criteria, 75-g O.G.T. Boehringer; Boehringer Mannheim, Mannheim, Germany) with moderate obesity (BMI <35 kg/m^2) aged between 40 and 80 years (Table 1) were studied on two occasions with exogenous GLP-1 and placebo in random order. Type 2 diabetic patients were selected to be in poor metabolic control while on therapy with diet alone and/or sulfonylureas (fasting plasma glucose >8.9 mmol/l on two occasions). None of them had been on metformin or insulin treatment.

Eight healthy subjects with a normal oral glucose tolerance test were studied only with placebo for comparison and to assure that under our experimental conditions, normal oscillatory insulin secretion could be detected.

Peptides. Synthetic GLP-1[7-36] amide was purchased from Saxon Biochemicals (Hannover, Germany; lot number FGLP 7369301 A, net peptide content 88.1%). The peptide was dissolved, filtered through 0.2-μm nitrocellulose filters (Millipore, Bedford, MA), and stored frozen at –30°C. Net peptide content rather than gross weight was used for dose calculations. High-performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was >99% pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and for pyrogens (Limulus amebocyte lysate endo-LAL; Chromogenix AB, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1[7-36] amide stock solutions were always <0.03 EU/ml.

Study design. Type 2 diabetic patients and subjects with IGT were studied twice in random sequenced order (GLP-1 or placebo infusions on days 1 and 3) and came to the metabolic ward in the evening of days 0 and 2. All antidiabetic medication was discontinued, and patients did not receive any antidiabetic medication before or during the experiments on days 1 and 3. A day with regular meal and drug schedules was allowed for day 2 between the experiments with GLP-1 and placebo. The intravenous infusion of GLP-1 or placebo was started at 10:00 P.M. and was continued until the end of the 60-min experimental period (from ~8:00 to 9:00 A.M. of days 1 and 3). Medications were withheld until the end of the experiments. Control subjects came to the metabolic ward on the morning of their study.

Experimental procedures and continuous blood sampling. The patients were in a semi-recumbent position throughout the tests. A distal forearm vein (infusion arm) was punctured with a Teflon cannula (Vasofix Braunüle, 18 G; Braun Melsungen, Melsungen, Germany). At 10:00 P.M. the evening before, an intravenous infusion of GLP-1[7-36] amide at a rate of 1.2 pmol · kg^{-1} · min^{-1} or placebo (0.9% NaCl containing 1% human serum albumin; Merieux, Norderstedt, Germany) was started and continued until the end of each experiment. At ~8:00 A.M. the next morning, a cubital vein (sampling arm) was punctured with a Teflon cannula (Braunüle MT, 16 G, effective length 5 cm; Braun Melsungen). A tube system (described below) was inserted for continuous blood sampling and connected to a roller pump and fraction collector. During 60 min, blood was sampled in 1-min fractions for determination of plasma glucose, insulin, and C-peptide, as previously described by Sir-Petermann et al. (23), using a 75 cm long silastic tube (inner diameter 1.0 mm, outer diameter 3.0 mm; shore hardness A60 according to DIN 53505, Thomafluid-SI-Chemical-tubing, Nr. 92012, Reichelt Chemietechnik, Heidelberg, Germany) and a peristaltic roller pump (BVP-pump, type ISM 736-0240; Ismatec, Glattbrugg-Zürich, Switzerland). Fractions were collected (automatic Neolab three fraction collector; Neolab, Heidelberg, Germany). Modifications of the original procedures (23) included the omission of T-extensions and internal metal connectors. External silastic cuffs were used instead to connect tubings. At 0, 15, 30, 45, and 60 min, blood was sampled for determination of GLP-1 and glucagon.

Blood specimens. Blood (~2 ml) was collected in 1-min fractions into tubes containing EDTA (1.6 mg/ml blood) and aprotinin (40 μl Trasylol, 20,000 kallikrein inhibitor units/ml; Bayer, Leverkusen, Germany) for the determination of plasma glucose and insulin. A sample (~100–200 μl) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the measurement of plasma glucose. For determination of GLP-1 and glucagon, blood (~9 ml) was drawn into tubes containing EDTA and aprotinin (180 μl as described above). After centrifugation, plasma for hormone analyses was kept frozen at –30°C.

Laboratory determinations. Glucose was measured using the glucose oxidase method with a Glucose Analyser 2 (Beckmann Instruments, Munich, Germany). Insulin was measured using insulin antibody–coated microtiter wells (insulin enzyme-linked immunosorbent assay [ELISA]) from Dako Diagnostics (Cambridgeshire, U.K.). Assay sensitivity was <6 pmol/l with an assay range from 0 to 1,080 pmol/l. The intra-assay coefficient of variation was

TABLE 2
Influence of intravenous GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) on mean concentrations of GLP-1, plasma glucose, insulin, C-peptide, and glucagon in patients with type 2 diabetes, subjects with IGT, and healthy control subjects (placebo conditions only)

	Patients with type 2 diabetes		Subjects with IGT			
Parameter (unit)	Placebo conditions	GLP-1 conditions	Placebo conditions	GLP-1 conditions	Healthy control subjects (placebo)	*P**
GLP-1 (pmol/l)	12 ± 6	89 ± 12†	4 ± 1	91 ± 20†	7 ± 1	0.31
Glucose (mg/dl)	179 ± 27	113 ± 11†	88 ± 3	82 ± 3†	89 ± 3	0.0005
Insulin (pmol/l)	55 ± 12	70 ± 13†	52 ± 10	71 ± 18	46 ± 5	0.82
C-peptide (nmol/l)	0.76 ± 0.12	1.04 ± 0.15	0.75 ± 0.10	0.90 ± 0.18	0.62 ± 0.07	0.56
Glucagon (pmol/l)	11 ± 2	10 ± 2	8 ± 2	8 ± 2	8 ± 2	0.24

Data are means ± SE. Average values of blood samples were drawn at 0, 15, 30, 45, and 60 min (GLP-1 and glucagon) or every minute (glucose, insulin, and C-peptide) of the 60-min sampling period. *Comparison of placebo experiments only; determined by analysis of variance; †significant difference between placebo and GLP-1 treatment in one patient group ($P < 0.05$, t test).

3.6 ± 0.3%. The interassay coefficient of variation was 7.4%. In this insulin assay, cross-reactivity was 0.3% with intact human proinsulin, 0.3% with 32,33 split proinsulin, and 0.5% with des 31,32 split proinsulin, but 45% with 65,66 split proinsulin and 66% with des 64,65 split proinsulin. There was no cross-reactivity with C-peptide. Human insulin was used as standard.

C-peptide was measured using C-peptide antibody–coated microtiter wells (C-peptide ELISA) from Dako Diagnostics. The detection limit was 0.017 nmol/l, and the working range was up to 4.0 nmol/l. Human C-peptide was used as standard.

Immunoreactive GLP-1 was determined in ethanol-extracted plasma, as previously described (24), using antiserum 89 390 (final dilution 1:150,000) and synthetic GLP-1[7–36] amide for tracer preparation and as standard. Recovery of GLP-1[7–36] amide standards after alcohol extraction was 75 ± 8%. The experimental detection limit (2 SDs over samples not containing GLP-1[7–36] amide) was <5 pmol/l. Antiserum 89 390 binds to the amidated COOH-terminus of GLP-1[7–36] amide.

Pancreatic glucagon was assayed in ethanol extracted plasma using antibody 4305 as previously described (25). Each patient's set of plasma samples was assayed at the same time to avoid errors due to interassay variation.

Deconvolution analysis. The venous plasma insulin concentration time series were analyzed by deconvolution (multiparameter technique [26]) to detect and quantify insulin secretory bursts as described previously (5). Deconvolution analysis depends on the following assumptions. The venous plasma insulin concentrations measured in samples collected at 1-min fractions were assumed to result from five determinable and correlated parameters: *1*) a finite number of discrete insulin secretory bursts occurring at either regular or randomly dispersed times and having *2*) individual amplitudes (maximal rate of secretion attained within a burst) and mass (integral of the calculated secretory event) and *3*) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude) that are superimposed on *4*) a basal time-invariant insulin secretory rate, and *5*) a bi-exponential insulin disappearance model from the systemic circulation with half-lives of 2.8 and 5.0 min, respectively, and a fractional slow compartment of 0.28 as determined in healthy fasting humans (8). All secretory rates were expressed as mass units of insulin (picomoles) released per unit distribution volume (liters) per unit time (minutes), where the volume of distribution was assumed to be 0.206 l/kg as determined by Pørksen et al. (8). The calculated values represent hepatic vein insulin appearance rather than total insulin secretion. All data analyses were performed in a blinded manner on a personal computer using software specifically developed for purposes of hormone serial data deconvolution. A waveform-independent program screening for significant secretory bursts (PULSE2) provided estimates of secretory burst amplitude and mass (5,26).

Spectral analysis. To eliminate nonstationarity, spectral analysis was performed on residuals after subtraction of a fitted line calculated as nine-point centered equal-weighted moving average. Spectral analysis was performed using noncommercial software. A Turkey window of 25 data points was used, and the spectra were normalized using the assumption that the total variance in each time series was 100%. This enables comparison of spectral estimates despite the different absolute values of insulin. The size of the dominant peaks during basal and stimulated conditions were compared statistically.

Autocorrelation analysis. The analysis was performed on unsmoothed data stationarized as described for spectral analysis. Autocorrelation analysis was performed using the statistical software package SPSS version 9.0 (SPSS, Chicago, IL). The correlation coefficients of the first nonnegative peak of the autocorrelogram were compared statistically (27).

Approximate entropy. The regularity of the insulin concentration time series was assessed by the model-independent and scale-invariant statistic approximate entropy (28), which measures the logarithmic likelihood that runs of patterns that are close (within r) for m contiguous observations remain close (within the tolerance width r) on subsequent incremental comparisons. A precise mathematical definition is given in the study by Pincus (28). Approximate entropy is to be considered a family of parameters dependent on the choice of the input parameters m and r and is to be compared only when applied to time series of equal length, as we do here. It was calculated with $r = 0.2 \times$ SD in the individual time series and $m = 1$, which are standard input parameters (14,16). To obviate the effect of trends in the time series, approximate entropy was calculated on the first differences of data. A larger absolute value indicates a higher degree of process randomness. Approximate entropy is rather stable to noise that lies within the tolerance width r.

Statistics. Patient characteristics are presented as mean ± SD, and results are reported as mean ± SE. The significance of differences between control subjects, IGT subjects, and type 2 diabetic patients (placebo experiments) was tested using one-way analysis of variance. Student's two-tailed paired t test was used to test for statistical significance between placebo and GLP-1 experiments. A P value <0.05 was taken to indicate significant differences. All statistical analyses were performed using Number Crunchers Statistical Software version 5.0 (Jerry Hintze, Keysville, UT).

RESULTS

Effects of GLP-1 on mean glucose and hormone concentrations. During the infusion of GLP-1 (1.2 pmol · kg^{-1} · min^{-1}), mean GLP-1 plasma concentrations were significantly increased (to ~90 pmol/l) compared with placebo experiments (Table 2). The mean fasting plasma glucose concentrations during the placebo experiments were significantly different between the three patient groups (Table 2). Intravenous GLP-1 significantly lowered plasma glucose concentrations in type 2 diabetic patients and IGT subjects compared with placebo experiments (Table 2). In type 2 diabetic patients, exogenous GLP-1 reduced plasma glucose to 4.9 ± 0.6 mmol/l (vs. 10.7 ± 2.4 mmol/l; $P = 0.04$) at midnight, to 4.8 ± 0.4 mmol/l (vs. 9.4 ± 1.4 mmol/l; $P = 0.02$) at 3:00 A.M., and to 5.9 ± 0.6 mmol/l (vs. 10.5 ± 1.5 mmol/l; $P = 0.006$) at 7:00 A.M.

Mean insulin concentrations during placebo experiments were not significantly different between the three subject/patient groups (Table 2). Insulin was significantly increased by GLP-1 in type 2 diabetic patients (placebo vs. GLP-1; $P = 0.02$) but only slightly in IGT patients ($P = 0.12$). C-peptide increments were similarly influenced by GLP-1. There were no significant differences in glucagon concentrations between subject/patient groups or between placebo and GLP-1 experiments in any group.

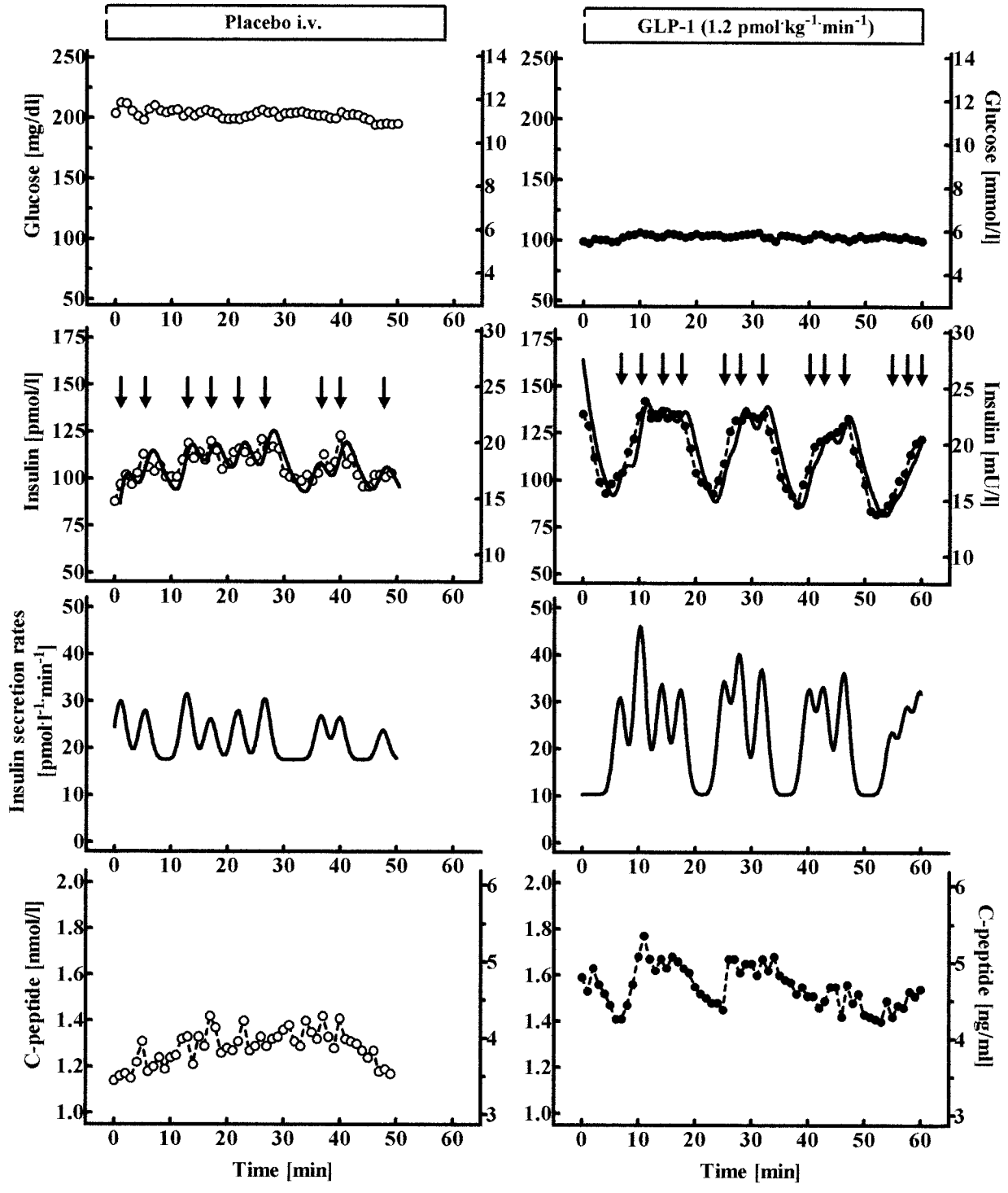

FIG. 1. An example of plasma glucose (upper panels), serum insulin concentrations and calculated "best-fit" insulin concentrations (second row of panels), insulin secretions rates (third row of panels), and C-peptide concentrations (lower panels) of one patient with type 2 diabetes sampled over 60 min in the morning during intravenous (i.v.) GLP-1 (1.2 pmol · kg^{-1} · min^{-1}, started at 10:00 P.M. the evening before) or placebo. The "best fit" is the predicted concentration profile that would occur based on calculated insulin secretory profiles shown in the third row of panels. Arrows indicate time points of insulin pulses detected by deconvolution analysis.

Deconvolution analysis. Deconvolution analysis of insulin concentrations revealed distinct and punctuated insulin secretory bursts in each data set analyzed (examples of type 2 diabetic patients, subjects with IGT, and normal control subjects are shown in Figs. 1-3).

There was an underlying basal insulin secretion that was not quantitatively affected significantly by intravenous GLP-1 (Table 3). In both type 2 diabetic patients and subjects with IGT, exogenous GLP-1 significantly increased the individual mean secretory burst mass (Fig. 4). Likewise, the mean amplitudes of insulin secretory pulses were stimulated by GLP-1 (type 2 diabetic patients: 11.1 ± 1.8 vs. 17.6 ± 2.7 pmol · l^{-1} · min^{-1}, P = 0.006; IGT subjects: 13.3 ± 2.1 vs. 18.4 ± 3.2 pmol · l^{-1} · min^{-1}, P = 0.02). The burst

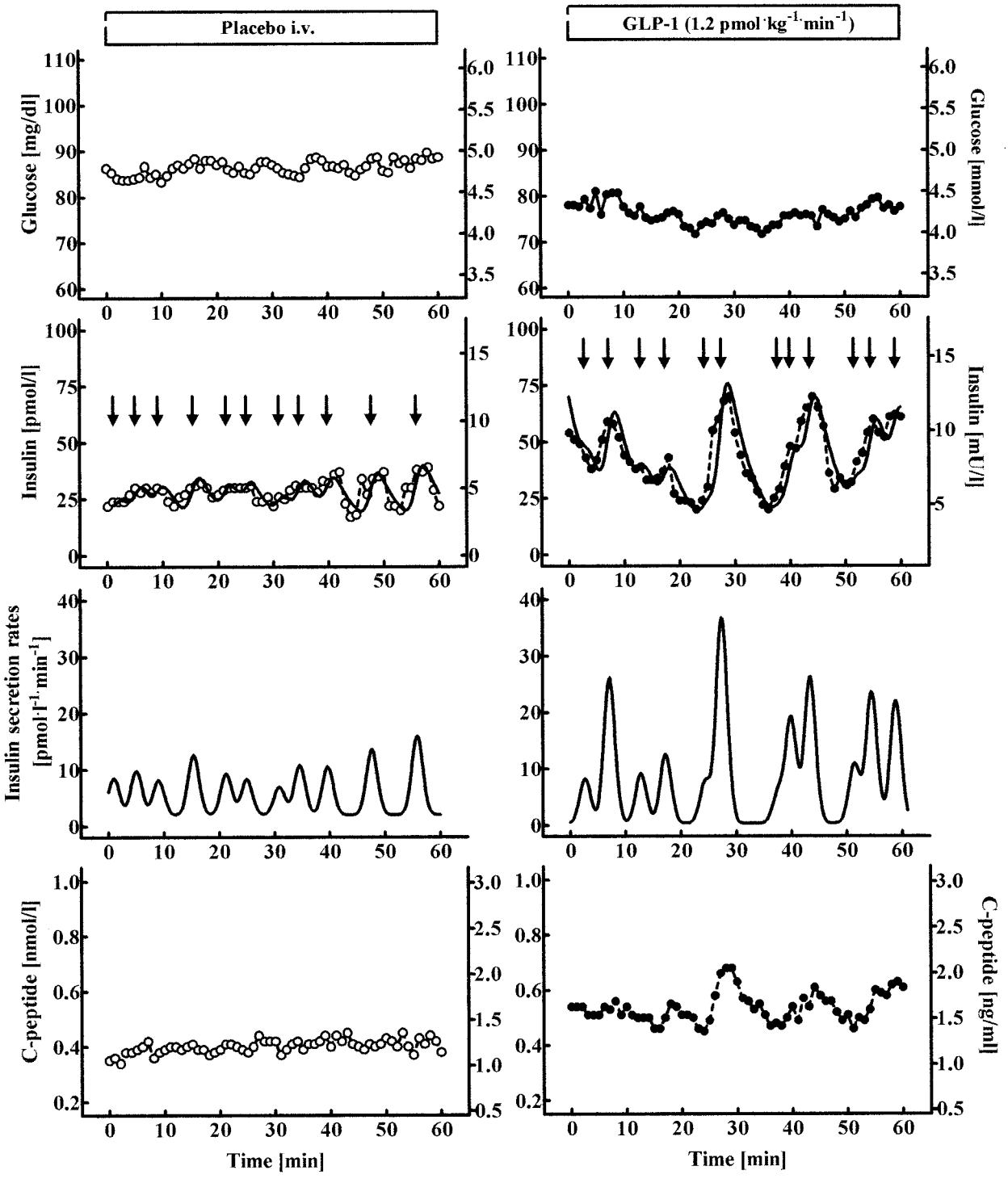

FIG. 2. An example of plasma glucose (upper panels), serum insulin concentrations and calculated "best-fit" insulin concentrations (second row of panels), insulin secretions rates (third row of panels), and C-peptide concentrations (lower panels) of one subject with impaired oral glucose tolerance sampled over 60 min in the morning during intravenous (i.v.) GLP-1 (1.2 pmol · kg^{-1} · min^{-1}, started at 10:00 P.M. the evening before) or placebo. Arrows indicate time points of insulin pulses detected by deconvolution analysis.

frequency was not changed significantly (Fig. 4). The proportion of the pulsatile component of insulin secretion was slightly, but not significantly, stimulated by GLP-1 if type 2 diabetic patients and subjects with IGT were looked at separately. For the combined group, the effect of GLP-1 was significant (51.9 ± 5.5 vs. 65.5 ± 5.5%, $P = 0.048$; Table 3).

Spectral analysis. There was a trend toward a reduced maximum spectral power in type 2 diabetic patients under placebo conditions, but this was not significant. GLP-1 did not enhance maximum spectral power significantly. However, the mean interpulse periods were prolonged significantly by 5.9 and 4.3 min by exogenous GLP-1 in type 2 diabetic patients and in IGT subjects, respectively (Table 3). This was also apparent when the type 2 diabetic and IGT groups were combined (8.5 ± 0.3 vs. 13.3 ± 0.8 min, $P < 0.0001$).

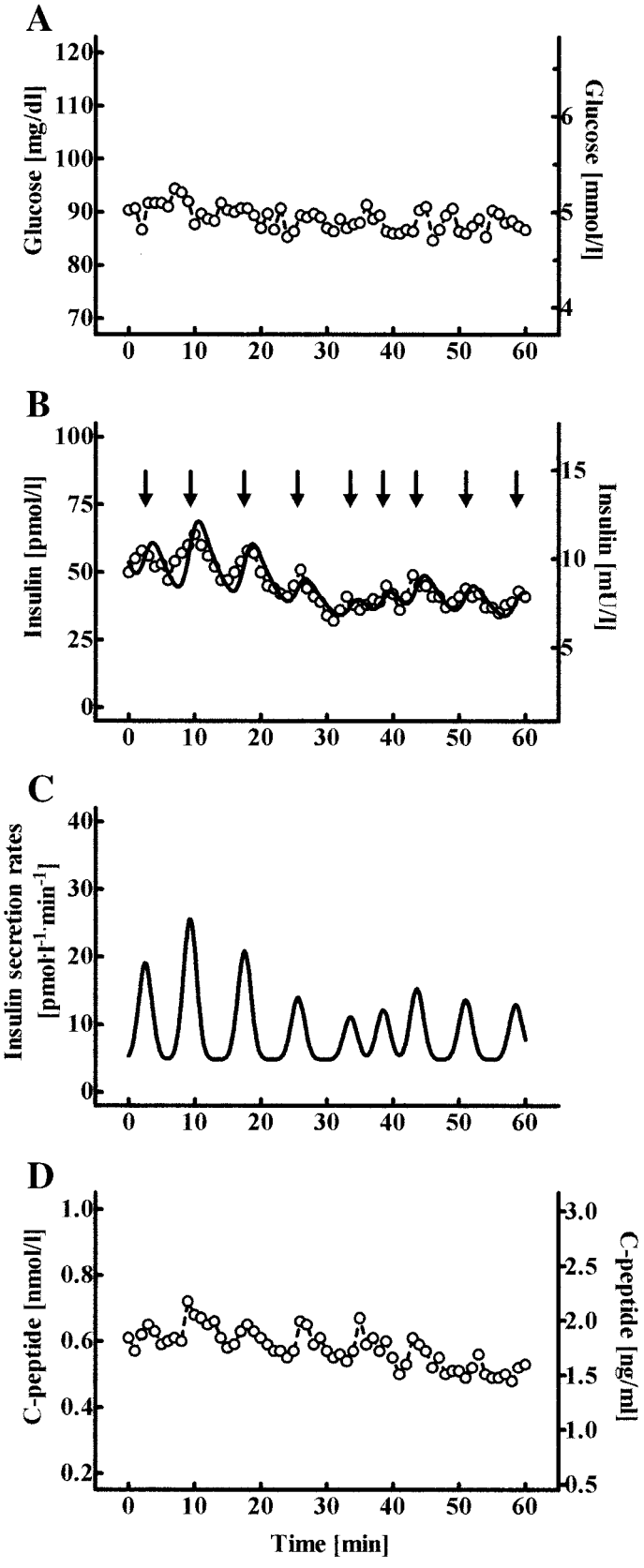

FIG. 3. An example of plasma glucose (*A*), serum insulin concentrations and calculated "best-fit" insulin concentrations (*B*), insulin secretion rates (*C*), and C-peptide concentrations (*D*) of one healthy control subject sampled over 60 min in the morning under placebo conditions. Arrows indicate time points of insulin pulses detected by deconvolution analysis.

Autocorrelation analysis. There was a trend toward a reduced maximum correlation in type 2 diabetic patients in comparison to normal subjects ($P = 0.08$; Table 3) under placebo conditions. Exogenous GLP-1 did not change maximum correlation in any group but prolonged the lag time (corresponding to the interpulse interval) in a similar fashion as described for the period calculated by spectral analysis (Table 3). This was also apparent when the type 2 diabetic and IGT groups were combined (8.0 ± 0.3 vs. 13.1 ± 0.7 min, $P < 0.0001$).

Approximate entropy. There was no characteristic difference in the estimate of approximate entropy between the three groups studied under placebo conditions, and exogenous GLP-1 had no significant effect on this parameter. The reduction in approximate entropy also fell short of statistical significance for the combined group of type 2 diabetic and IGT patients ($P = 0.052$ in comparison to healthy control subjects; $P = 0.08$ for experiments with GLP-1 vs. placebo).

DISCUSSION

In healthy men, the gut hormone GLP-1 increased secretory burst mass but did not affect frequency or orderliness of pulsatile insulin secretion (11). In the current study, we demonstrate by deconvolution analysis that in patients with type 2 diabetes or subjects with IGT, GLP-1 similarly augmented pulsatile insulin secretion by amplification of the mean insulin burst mass. Evaluation of plasma insulin concentration profiles using autocorrelation and spectral analysis revealed a decreased frequency (or a longer interpulse interval) of plasma insulin oscillations in the systemic circulation with GLP-1 compared with placebo (Table 3). Deconvolution analysis, which takes into account parameters of insulin appearance in (secretion) and disappearance from (distribution and metabolic clearance) systemic circulation, did not confirm changes in intervals between pulsatile insulin secretion with GLP-1 treatment (Table 3 and Fig. 4). An explanation for this difference may be an increased overlap of plasma insulin oscillations in the systemic circulation because the troughs between insulin secretory bursts become less apparent because of the elimination kinetics of insulin, whereas burst mass and pulse amplitude increase in response to GLP-1 treatment. This may lead to a reduced detectability of single secretion events in peripheral plasma insulin concentration profiles. Spectral analysis and autocorrelation analysis do not depend on the same assumptions, and their results better correspond to the insulin concentration time series (see examples presented in Figs. 1–3). Therefore, our results may be interpreted to indicate a prolonged periodicity of pulsatile insulin secretion under the influence of exogenous GLP-1. The enhanced pulse amplitude (Table 3) or burst mass (Fig. 4) described by deconvolution analysis may, along the line of this argument, even underestimate the influence of GLP-1 on the magnitude of the secretory events that correspond to the broad insulin peaks shown in Figs. 1 and 2. No matter which method is used to analyze this secretory behavior, exogenous GLP-1 was found to profoundly influence parameters of pulsatile or oscillatory insulin secretion, even in type 2 diabetic patients and in subjects with impaired oral glucose tolerance (Fig. 4 and Table 3).

TABLE 3
Influence of intravenous GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) on parameters of pulsatility analysis based on deconvolution analysis, spectral analysis, and autocorrelation analysis and on the estimation of approximate entropy of individual insulin concentration time series in type 2 diabetic patients and subjects with IGT

Parameter (unit)	Patients with type 2 diabetes		Subjects with IGT		Healthy control subjects (placebo)	*P**
	Placebo conditions	GLP-1 conditions	Placebo conditions	GLP-1 conditions		
Deconvolution analysis						
Mean pulse interval (min/pulse)	5.9 ± 0.3	5.5 ± 0.4	6.0 ± 0.4	5.6 ± 0.3	6.0 ± 0.2	0.95
Pulse amplitude (pmol · l^{-1} · min^{-1})	11.1 ± 1.8	17.6 ± 2.7†	13.3 ± 2.1	18.4 ± 3.2†	11.5 ± 1.4	0.65
Basal secretion (pmol · l^{-1} · min^{-1})	6.7 ± 2.0	5.4 ± 1.4	5.6 ± 1.5	6.6 ± 2.7	4.8 ± 0.6	0.68
Proportion of insulin released in a pulsatile manner (%)	48.8 ± 8.5	66.7 ± 7.2	55.0 ± 7.4	64.3 ± 8.90	48.8 ± 2.9	0.33
Spectral analysis						
Maximum spectral power (%)	8.6 ± 1.3	11.5 ± 1.2	11.5 ± 1.0	12.3 ± 0.8	11.9 ± 1.2	0.12
Period (min)	8.1 ± 0.3	14.0 ± 1.1†	8.3 ± 0.5	12.6 ± 1.1†	9.7 ± 1.1	0.24
Autocorrelation						
Maximum correlation	0.21 ± 0.06	0.36 ± 0.05	0.40 ± 0.08	0.28 ± 0.04	0.39 ± 0.03	0.08
Lag time (min)	8.0 ± 0.4	13.6 ± 0.9†	8.0 ± 0.4	12.6 ± 1.0†	9.5 ± 0.8	0.10
Approximate entropy (arbitrary units)	1.382 ± 0.018	1.309 ± 0.044	1.404 ± 0.031	1.356 ± 0.044	1.322 ± 0.035	0.14

Data are means ± SE. Healthy control subjects (placebo conditions) are shown for comparison. *Comparison of placebo experiments only; determined by analysis of variance; †significant difference between placebo and GLP-1 treatment in the same subject/patient group ($P < 0.05$, paired t test).

This could not generally be expected because the pulsatility of insulin secretion has been found impaired in previous studies in type 2 diabetic patients (2,4,13,16) and even in nondiabetic first-degree relatives of type 2 diabetic patients (14,15). Given the impaired insulin secretory capability of B-cells in type 2 diabetic patients (29) and the reduced effectiveness of insulin secretagogues like glucose (30) and sulfonylureas (31), it may even be considered remarkable that GLP-1 is able to elicit a similar effect on insulin secretory burst mass as has previously been demonstrated in healthy subjects (11). A difference to this previous study is, however, that a fourfold increment in secretory burst mass was described in healthy subjects (11), whereas in the present study, GLP-1 led to an increment of only 68 and 45% in type 2 diabetic patients and subjects with IGT, respectively (Fig. 4 and Table 3). Another difference is that Pørksen et al. (11) infused glucose to raise plasma concentrations to ~6 mmol/l, whereas in the present study, glucose levels were influenced only by the action of exogenous GLP-1 (Table 2). This means, for example, that in type 2 diabetic patients, plasma glucose concentrations with placebo were higher (9.9 ± 1.5 mmol/l) than those with GLP-1 (6.3 ± 0.6 mmol/l). Given the glucose dependence of the insulinotropic activity of GLP-1 (20,21,32,33), this may have led to an underestimation of GLP-1 effects on insulin secretion in general and on augmenting insulin secretory burst mass in particular. This has led to a nonsignificant increment in the proportion of insulin released in a pulsatile manner under the influence of GLP-1 in the present study, both in type 2 diabetic patients and in subjects with IGT (Table 3). This proportion had increased significantly in Pørksen et al.'s previous study (11). We also noted this significant difference only when results from type 2 diabetic and IGT subjects/patients were combined.

A recent study analyzed the immediate effects of a GLP-1 infusion on parameters of pulsatile insulin secretion under hyperglycemic clamp conditions in patients with type 2 diabetes and supports the current results that GLP-1 exerts its insulinotropic effect primarily by stimulation of insulin secretory burst mass (34). Similar to the present study, insulin burst frequency calculated by deconvolution analysis and the orderliness of oscillatory insulin concentrations were not affected. Spectral analysis showed no change in pulse frequency with GLP-1 treatment in this study. This difference may be explained by the fact that, unlike in the present study, sampling from the systemic circulation was started from the beginning of a GLP-1 infusion, and the resulting insulin concentration profiles did not show similar increments as observed in the present study, both in type 2 diabetic patients and subjects with IGT. This characteristic may be an exclusive finding after a long-term GLP-1 infusion. The overnight normalization of glycemia may have reduced glucose toxicity (35), thereby allowing a more physiological response to GLP-1.

Given the preserved insulinotropic action of GLP-1 in type 2 diabetic patients but the reduced effectiveness of gastric inhibitory polypeptide (GIP) reported previously (36,37), it would also be of interest whether GIP is able to augment pulsatile insulin release in patients with type 2 diabetes, in subjects with IGT, and in normal subjects.

Previous studies have found differences in the pulsatile pattern of insulin release among normal subjects (1–4), type 2 diabetic patients (13,14), and first-degree relatives of type 2 diabetic patients (15,16). Based on the present analysis, the number of pulses per hour (burst frequency, period, and lag time, as defined by the different methods to analyze pulsatility) and the mean pulse amplitude (Table 3) or burst mass (Fig. 4) have not been found different among the three groups of subjects/patients studied under placebo conditions. The previous studies primarily used autocorrelation (2,13,15) and approximate entropy (14,16) analyses—basically the same procedures used in the present study. Deconvolution analysis has not been used to characterize pulsatile (oscillatory) insulin secretion in patients with type 2 diabetes or impaired oral glucose tolerance previously. The main differences between Lang et al.'s study (13) and our study are that their patients were

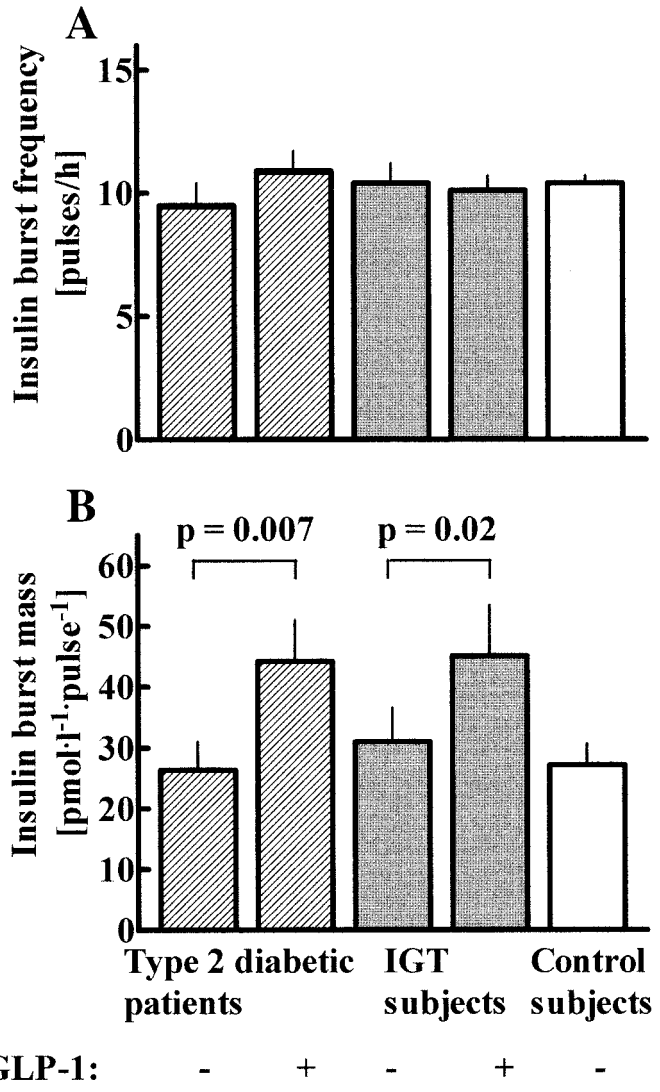

FIG. 4. *A*: Insulin secretory burst frequency of patients with type 2 diabetes (P = 0.15, placebo vs. GLP-1; t test), subjects with IGT (P = 0.76), and healthy control subjects during infusion of GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) or placebo (P = 0.59 for comparison of placebo experiments only; analysis of variance). *B*: Insulin secretory burst mass of patients with type 2 diabetes, subjects with IGT, and healthy control subjects during infusion of GLP-1 (1.2 pmol · kg^{-1} · min^{-1}) or placebo (P = 0.75 for comparison of placebo experiments only; analysis of variance). Data are means ± SE.

all treated by diet alone (five with oral antidiabetic medication in our group) and that they were slightly younger (mean 60 vs. 64 ± 9 years) and less obese (106% ideal body weight vs. BMI 28.9 ± 7.2 kg/m^2). The insulin assay used (13) probably was less precise, as judged from our coefficient of variation and their "mean precision" of 0.8 mU/l. Moreover, the insulin concentration profiles had been smoothed using a 3-min moving average, which suppresses rapid changes in the insulin concentration time series. The preliminary finding by Schmitz et al. (14) of a reduced orderliness of pulsatility in patients with type 2 diabetes relied on exactly the same methodology as that used in our present study. Overall, the information on pulsatile insulin secretion in patients with type 2 diabetes is sparse. Whereas the available literature supports some abnormalities (13,14), our results show that these abnormalities may be less apparent, even when using several different methods to describe pulsatile/oscillatory secretory behavior, and that larger patient groups need to be examined to clarify this point.

The present findings partly explain the exquisite "antidiabetogenic" activity of GLP-1 (38). The augmentation of insulin secretory burst amplitude and mass may play an important role in the normalization of plasma glucose concentrations by exogenous GLP-1 in type 2 diabetic patients (39,40) because pulsatile insulin (in comparison to continuously administered insulin) has been found to be more effective (41–43).

In conclusion, exogenous GLP-1 is able to augment pulsatile secretory burst mass and amplitude in type 2 diabetic patients and subjects with impaired oral glucose tolerance, as has previously been demonstrated in healthy subjects. These results encourage the future therapeutic use of GLP-1 or GLP-1 analogs in patients with type 2 diabetes.

ACKNOWLEDGMENTS

The excellent technical assistance of S. Richter, Th. Gottschling, K. Faust, and L. Albæk is gratefully acknowledged. We thank H. Achner and L. Faber for secreterial assistance.

REFERENCES

1. Goodner CJ, Walike BC, Koerker DJ, Ensinck JW, Brown AC, Chideckel EW, Palmer J, Kalnasy L: Insulin, glucagon, and glucose exhibit synchronous, sustained oscillations in fasting monkeys. *Science* 195:177–179, 1977
2. Lang DA, Matthews DR, Peto J, Turner RC: Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. *N Engl J Med* 301:1023–1027, 1979
3. Hansen BC, Jen KC, Belbez Pek S, Wolfe RA: Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. *J Clin Endocrinol Metab* 54:785–792, 1982
4. Matthews DR, Lang DA, Burnett MA, Turner RC: Control of pulsatile insulin secretion in man. *Diabetologia* 24:231–237, 1983
5. Pørksen N, Munn S, Steers J, Veldhuis JD, Butler PC: Impact of sampling technique on appraisal of pulsatile insulin secretion by deconvolution and cluster analysis. *Am J Physiol* 269:E1106–E1114, 1995
6. Pørksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P: Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. *Am J Physiol* 269:E478–E488, 1995
7. Stagner JI, Samols E, Weir GC: Sustained oscillations of insulin, glucagon, and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration. *J Clin Invest* 65:939–942, 1980
8. Pørksen N, Nyholm B, Veldhuis JD, Butler PC, Schmitz O: In humans at least 75% of insulin secretion arises from punctuated insulin secretory bursts. *Am J Physiol* 273:E908–E914, 1997
9. Pørksen N, Munn S, Steers J, Veldhuis JD, Butler PC: Effects of glucose ingestion versus infusion on pulsatile insulin secretion: the incretin effect is achieved by amplification of insulin secretory burst mass. *Diabetes* 45:1317–1323, 1996
10. Porksen NK, Munn SR, Steers JL, Schmitz O, Veldhuis JD, Butler PC: Mechanisms of sulfonylurea's stimulation of insulin secretion in vivo: selective amplification of insulin secretory burst mass. *Diabetes* 45:1792–1797, 1996
11. Pørksen N, Grofte B, Nyholm B, Holst JJ, Pincus SM, Veldhuis JD, Schmitz O, Butler PC: Glucagon-like peptide 1 increases mass but not frequency or orderliness of pulsatile insulin secretion. *Diabetes* 47:45–49, 1998
12. Pørksen N, Munn SR, Steers JL, Veldhuis JD, Butler PC: Effects of somatostatin on pulsatile insulin secretion: elective inhibition of insulin burst mass. *Am J Physiol* 270:E1043–E1049, 1996
13. Lang DA, Matthews DR, Burnett M, Turner RC: Brief, irregular oscillations of basal plasma insulin and glucose concentrations in diabetic man. *Diabetes* 30:435–439, 1981
14. Schmitz O, Pørksen N, Juhl C, Veldhuis JD, Butler PC, Pincus SM: Disorderly insulin release processing in NIDDM assessed by approximate entropy (Abstract). *Diabetologia* 41 (Suppl. 1):S84, 1998
15. O'Rahilly S, Turner RC, Matthews DR: Impaired pulsatile secretion of

insulin in relatives of patients with non-insulin-dependent diabetes. *N Engl J Med* 318:1225–1230, 1988
16. Schmitz O, Pørksen N, Nyholm B, Skjaerbaek C, Butler PC, Veldhuis JD, Pincus SM: Disorderly and nonstationary insulin secretion in relatives of patients with NIDDM. *Am J Physiol* 272:E218–E226, 1997
17. Nauck MA, Holst JJ, Willms B, Schmiegel W: Glucagon-like peptide 1 (GLP-1) as a new therapeutic approach for type 2-diabetes. *Exp Clin Endocrinol Diabetes* 105:187–195, 1997
18. Nauck MA, Holst JJ, Willms B: Glucagon-like peptide 1 and its potential in the treatment of non-insulin-dependent diabetes mellitus. *Horm Metab Res* 29:411–416, 1997
19. Holz GG, Kuhtreiber WM, Habener JF: Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7-37). *Nature* 361:362–365, 1993
20. Göke R, Wagner B, Fehmann HC, Göke B: Glucose-dependency of the insulin stimulatory effect of glucagon-like peptide-1 (7-36) amide on the rat pancreas. *Res Exp Med* 193:97–103, 1993
21. Qualmann C, Nauck MA, Holst JJ, Ørskov C, Creutzfeldt W: Insulinotropic actions of intravenous glucagon-like peptide-1 (GLP-1) [7-36 amide] in the fasting state in healthy subjects. *Acta Diabetol* 32:13–16, 1995
22. Ritzel R, Schulte M, Nauck M, Marz W, Perksen N, Schmiegel W, Nauck MA: GLP-1 increases secretory burst mass of pulsatile insulin secretion in patients with impaired glucose tolerance and type 2 diabetes (Abstract). *Diabetologia* 41 (Suppl. 1):A182, 1998
23. Sir-Petermann T, Recabarren SE, Bittl A, Jager W, Zimmermann U, Wildt L: A simple device for serial blood collection in human subjects and animals. *Exp Clin Endocrinol Diabetes* 103:398–401, 1995
24. Ørskov C, Holst JJ: Radio-immunoassays for glucagon-like peptides 1 and 2 (GLP-1 and GLP-2). *Scand J Clin Lab Invest* 47:165–174, 1987
25. Holst JJ, Bersani M, Johnsen AH, Kofod H, Hartmann B, Ørskov C: Proglucagon processing in porcine and human pancreas. *J Biol Chem* 269:18827–18833, 1994
26. Veldhuis JD, Carlson ML, Johnson ML: The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci U S A* 84:7686–7690, 1987
27. Chatfield C: *The Analysis of Time Series: an Introduction.* London, Chapmann and Hall, 1996
28. Pincus SM: Approxiamte entropy as a measure of system complexity. *Proc Natl Acad Sci U S A* 88:2297–2301, 1991
29. Nauck MA: Physiology and pathophysiology of endocrine pancreatic secretion. In *The Pancreas.* Vol. 1. Beger HG, Warshaw AL, Buechler MW, Carr-Locke DL, Neoptolemos JP, Russell C, Sarr MG, Eds. Oxford, U.K., Blackwell Science, 1998, p. 101–137
30. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D Jr: Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest* 74:1318–1328, 1984
31. Shapiro ET, Van Cauter E, Tillil H, Given BD, Hirsch L, Beebe C, Rubenstein A, Polonsky K: Glyburide enhances the responsiveness of the beta-cell to glucose but does not correct the abnormal patterns of insulin secretion in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 69:571–576, 1989
32. Kreymann B, Williams G, Ghatei MA, Bloom SR: Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* ii:1300–1304, 1987
33. Nauck MA, Bartels E, Ørskov C, Ebert R, Creutzfeldt W: Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. *J Clin Endocrinol Metab* 76:912–917, 1993
34. Juhl CB, Schmitz O, Pincus S, Veldhuis J, Pørksen N: Short-term treatment with GLP-1 increases pulsatile insulin secretion in type II diabetes with no effect on orderliness. *Diabetologia* 43:583–588, 2000
35. Robertson RP: Type II diabetes, glucose "non-sense," and islet desensitization. *Diabetes* 38:1501–1505, 1989
36. Jones IR, Owens DR, Moody AJ, Luzio SD, Morris T, Hayes TM: The effects of glucose-dependent insulinotropic polypeptide infused at physiological concentrations in normal subjects and type 2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion. *Diabetologia* 30:707–712, 1987
37. Nauck MA, Heimesaat MM, Ørskov C, Holst JJ, Ebert R, Creutzfeldt W: Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91:301–307, 1993
38. Gutniak M, Ørskov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* 326:1316–1322, 1992
39. Nauck MA, Kleine N, Ørskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744, 1993
40. Nauck MA, Wollschläger D, Werner J, Holst JJ, Ørskov C, Creutzfeldt W, Willms B: Effects of subcutaneous glucagon-like peptide 1 (GLP-1 [7-36 amide]) in patients with NIDDM. *Diabetologia* 39:1546–1553, 1996
41. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC: Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes* 32:617–621, 1983
42. Paolisso G, Scheen AJ, Giugliano D, Sgambato S, Albert A, Varricchio M, D'Onofrio F, LeFèbvre PJ: Pulsatile insulin delivery has greater metabolic effects than continuous hormone administration in man: importance of pulse frequency. *J Clin Endocrinol Metab* 72:607–615, 1991
43. Schmitz O, Pedersen SB, Mengel A, Pørksen N, Bak J, Møller N, Richelsen B, Alberti KGMM, Butler PC, Ørskov H: Augmented effect of short-term pulsatile versus continuous insulin delivery on lipid metabolism but similar effect on whole-body glucose metabolism in obese subjects. *Metabolism* 43:842–846, 1994