

# Repaglinide Acutely Amplifies Pulsatile Insulin Secretion by Augmentation of Burst Mass With No Effect on Burst Frequency

CLAUS B. JUHL, MD  
NIELS PØRKSEN, MD  
MALENE HOLLINGDAL, MD  
JEPPE STURIS, PHD

STEVEN PINCUS  
JOHANNES D. VELDHIJS, MD  
ANDERS DEJGAARD, MD  
OLE SCHMITZ, MD

**OBJECTIVE** — Repaglinide is a new oral hypoglycemic agent that acts as a prandial glucose regulator proposed for the treatment of type 2 diabetes by stimulating insulin secretion. The aim of this study was to explore actions of repaglinide on the rapid pulsatile insulin release by high-frequency insulin sampling and analysis of insulin-concentration time series.

**RESEARCH DESIGN AND METHODS** — We examined 8 healthy lean male subjects in a single-dose double-blind placebo-controlled crossover design. After the subjects underwent an overnight fast, blood sampling was initiated and continued every minute for 120 min. After 40 min, a single dose (0.5 mg) of repaglinide or placebo was given. Serum insulin-concentration time series were assessed by deconvolution analyses and the regularity statistic by approximate entropy (ApEn).

**RESULTS** — Average insulin concentration was increased after repaglinide administration (basal vs. stimulated period,  $P$  values are placebo vs. repaglinide) ( $25.1 \pm 3.6$  vs.  $33.5 \pm 4.1$  pmol/l,  $P < 0.001$ ). Insulin secretory burst mass ( $15.8 \pm 2.2$  vs.  $19.6 \pm 2.8$  pmol  $\cdot$  l $^{-1}$   $\cdot$  pulse $^{-1}$ ,  $P = 0.02$ ) and amplitude ( $6.1 \pm 0.9$  vs.  $7.7 \pm 1.2$  pmol  $\cdot$  l $^{-1}$   $\cdot$  min $^{-1}$ ,  $P = 0.008$ ) were augmented after repaglinide administration. A concomitant trend toward an increase in basal insulin secretion was observed ( $2.5 \pm 0.3$  vs.  $3.2 \pm 0.4$  pmol  $\cdot$  l $^{-1}$   $\cdot$  min $^{-1}$ ,  $P = 0.06$ ), while the interpulse interval was unaltered ( $6.8 \pm 1.0$  vs.  $5.4 \pm 0.4$  min/pulse,  $P = 0.38$ ). ApEn increased significantly after repaglinide administration ( $0.623 \pm 0.045$  vs.  $0.670 \pm 0.034$ ,  $P = 0.04$ ), suggesting less orderly oscillatory patterns of insulin release.

**CONCLUSIONS** — In conclusion, a single dose of repaglinide amplifies insulin secretory burst mass (and basal secretion) with no change in burst frequency. The possible importance of these mechanisms in the treatment of type 2 diabetes characterized by disrupted pulsatile insulin secretion remains to be clarified.

*Diabetes Care* 23:675–681, 2000

From the Department of Medicine M (Endocrinology & Diabetes) (C.B.J., N.P., M.H., O.S.) and the Department of Clinical Pharmacology (O.S.), Århus Kommunehospital, University Hospital of Århus, Århus; Novo Nordisk A/S (J.S., A.D.), Bagsvaerd, Denmark; (S.P.) Guilford, Connecticut; and the Endocrinology Division (J.D.V.), General Clinical Research Center, National Science Foundation, Center for Biological Timing, University of Virginia, Charlottesville, Virginia.

Address correspondence and reprint requests to Claus B. Juhl, MD, Medical Department M (Endocrinology & Diabetes), Århus Kommunehospital, 8000, Århus, Denmark. E-mail: cbj@dadlnet.dk.

Received for publication 10 November 1999 and accepted in revised form 1 February 2000.

J.S. and A.D. are employed by and hold stock in Novo Nordisk A/S, which manufactures and markets products related to the treatment of diabetes.

**Abbreviations:** ANOVA, analysis of variance; ApEn, approximate entropy; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; SU, sulfonylurea.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Insulin release is characterized by a coordinated pulsatile mode of secretion with a periodicity of 5–15 min/pulse, leading to oscillations in peripheral insulin concentrations (1,2). The impact of pulsatile insulin delivery on metabolism is well established in vivo and in vitro (3–6). Compounds that regulate insulin secretion, including glucose, sulfonylurea, glucagon-like peptide 1 (GLP-1), and somatostatin, all seem to exert their actions primarily via modulation of insulin secretory burst mass rather than regulation of burst frequency (7–10). Insulin secretagogues have provided pivotal means of treating type 2 diabetes for >40 years. Treatment with insulin secretagogues is likely to influence the insulin delivery pattern qualitatively as well as quantitatively. Abnormalities in the insulin secretory pattern have been demonstrated in the diabetic state as well as in nondiabetic glucose-intolerant and glucose-tolerant relatives (11–13). It seems reasonable to assume that preservation of the normal physiological release pattern is of importance for the insulin action in peripheral tissues as well as for the acute and long-term  $\beta$ -cell performance. Despite the fact that the majority of insulin is released in a pulsatile pattern and that the use of insulin secretagogues in the treatment of diabetes is widespread, the influence of the latter on the oscillatory insulin-release pattern is only anecdotally elucidated in humans (14). Repaglinide, ((S)-(+)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl)-phenyl]-butyl]amino]-2-oxy-ethyl]benzoic acid), is a new oral insulin secretagogue belonging to a class of so-called prandial glucose regulators. To achieve further insight into the mechanistic actions of this class of insulin secretagogues, we examined the impact of an acute exposure to repaglinide on the high-frequency insulin-release pattern as measured by oscillations in peripheral insulin concentration.

## RESEARCH DESIGN AND METHODS

### Study subjects and design

The protocol was performed in accordance with the Helsinki Declaration and was

Table 1—Results from concentration measurements, deconvolution, and ApEn analysis

	Placebo		Repaglinide		P
	Basal period	Stimulated period	Basal period	Stimulated period	
Concentrations*					
Plasma glucose (mmol/l)	5.2 ± 0.2	5.2 ± 0.2	5.1 ± 0.1	5.0 ± 0.1	NS
Free fatty acids (mmol/l)	0.33 ± 0.12	0.38 ± 0.15	0.43 ± 0.13	0.47 ± 0.15	NS
Serum insulin (pmol/l)	22.0 ± 5.7	23.0 ± 6.2	25.1 ± 10.3	33.5 ± 11.7	<0.01
C-peptide (pmol/l)	367 ± 66	366 ± 70	393 ± 77	444 ± 95	<0.01
Deconvolution					
Interpulse interval (min/pulse)†‡	6.8 ± 0.5	6.6 ± 0.5	6.8 ± 1.0	5.4 ± 0.4	NS
Basal secretion (pmol/l/min)†‡	2.4 ± 0.3	2.3 ± 0.2	2.5 ± 0.3	3.2 ± 0.4	0.06
Secretory burst mass (pmol/l/pulse)†‡	14.9 ± 1.9	14.2 ± 1.3	15.8 ± 2.2	19.6 ± 2.8	0.02
Secretory burst amplitude (pmol/l/min)†‡	5.6 ± 0.6	5.5 ± 0.5	6.1 ± 0.9	7.7 ± 1.2	<0.01
Insulin secretory rate (pmol/min)*§	9.4 ± 0.2	10.3 ± 0.5	9.2 ± 0.2	13.4 ± 0.5	<0.01
ApEn					
First differencing	0.691 ± 0.030	0.636 ± 0.021	0.623 ± 0.044	0.670 ± 0.034	0.04
7-Point moving average residuals	0.703 ± 0.021	0.655 ± 0.021	0.639 ± 0.030	0.714 ± 0.017	0.02

Data are means ± SEM. \*Statistical calculations based on ANOVA (GLM); †data derived from deconvolution of insulin concentration time series; ‡statistical calculation based on ANOVA (see STATISTICAL ANALYSIS); §data derived from deconvolution of C-peptide measurements.

approved by the local Ethics Committee of Århus County. In total, 8 healthy male volunteers, mean (± SD) age 26 ± 2 years and BMI 22.1 ± 1.2 kg/m<sup>2</sup>, were studied. None took regular medication or had a family history of diabetes.

**Protocol**

The effect of repaglinide on pulsatile insulin secretion was studied in a double-blind placebo-controlled single-dose crossover trial. Subjects were studied on 2 occasions with a mean of 12 days (range 7–13) intervening. Studies were performed after a 10-h overnight fast. At 8:00 A.M., subjects were placed in a bed and intravenous catheters were placed in each antecubital vein for infusion and sampling purposes. After 30 min of rest (t = 0), blood was collected every minute for 120 min. At t = 40 min, 0.5 mg of repaglinide or placebo was administered with 150 ml of water to the study subject, who was positioned in a 45° supine angle to facilitate uptake of the study drug. Plasma glucose was allowed to decline only 0.3 mmol/l below baseline during the sampling period and otherwise maintained at this level by variable infusion with 20% glucose. To avoid inducing changes in insulin pulsatility by the glucose infusion, the infusion rate was increased only in minor steps and was never decreased. Details on the sampling procedure have been described previously (10). Additional blood samples were collected every 10 min for measurement of C-peptide and free fatty acids (FFAs). Every 5 min

from time of drug administration until 50 min, and thereafter every 10 min, blood was collected for repaglinide measurements. Blood samples were stored at –20°C and analyzed within 1 month.

**Assays**

All biochemical analyses except repaglinide measurements were performed in duplicate. Serum insulin concentration was measured by using a 2-site immunospecific enzyme-linked immunosorbent assay (ELISA) with no cross-reactivity with pro-insulin or C-peptide (15). The detection range was 5–600 pmol/l and the inter- and intra-assay coefficients of variation were 5 and 2.5%, respectively. Plasma glucose concentration was measured by the glucose oxidation method (Beckman, Palo Alto, CA), and FFA was measured by a calorimetric method (Wako, Neuss, Germany). C-peptide measurement was performed by a 2-site monoclonal-based ELISA assay (K6218; Dako, Cambridgeshire, U.K.). Serum repaglinide was analyzed by a liquid chromatography mass spectrometry assay after solid-phase extraction. The limit of detection of the assay is 0.2 ng/ml.

**Data analysis**

**Deconvolution analysis.** Serum insulin-concentration time series were analyzed in a blinded manner by deconvolution analysis to quantify basal secretion, interpulse interval, secretory burst mass, and burst amplitude (16). Deconvolution was performed with a previously validated iterative multi-

parameter technique given the following assumptions: 1) The hormone is secreted in a finite number of bursts, each with 2) an individual amplitude and 3) a common half-duration. Bursts are superimposed on a basal time-invariant secretory rate, and 4) insulin is removed according to a biexponential disappearance rate, as previously described (17). The half-lives of insulin were assumed to be 2.8 and 5 min with a fractional slow compartment of 28% (17).

**Derivation of insulin secretory rates.**

Insulin secretory rates were estimated by mathematical analysis (deconvolution) of peripheral C-peptide concentrations with use of a 2-compartment model as described by Eaton et al. (18) and Polonsky et al. (19). This model allows estimation of insulin secretory rates by standard C-peptide kinetic parameters rather than individually derived parameters without significant loss of accuracy, even under non-steady-state conditions (19,20).

**Approximate entropy.** Regularity of insulin-concentration time series was assessed by the model-independent and scale-invariant statistic approximate entropy (ApEn) (21). ApEn 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 by Pincus (21). ApEn is to be considered as a family of parameters dependent of the choice of the input parameters m and r and is to be compared

only when applied to time series of equal length. In this study, ApEn was calculated with  $r = 1 \times SD$  in the individual time series and  $m = 1$ . By application of a small  $r$  value (e.g.,  $r = 0.2 \times SD$ ), ApEn evaluates the fine (sub-) patterns in the time series, and a larger  $r$  value (e.g.,  $r = 1.0 \times SD$ ) is applied to evaluate the more coarse patterns (22). A larger absolute value of ApEn indicates a higher degree of process randomness. ApEn is rather stable to noise that lies within the tolerance width  $r$ . To obviate the effect of trends in the time series, ApEn was calculated on the first difference of data as well as on the residuals after subtraction of 7-point moving-average data.

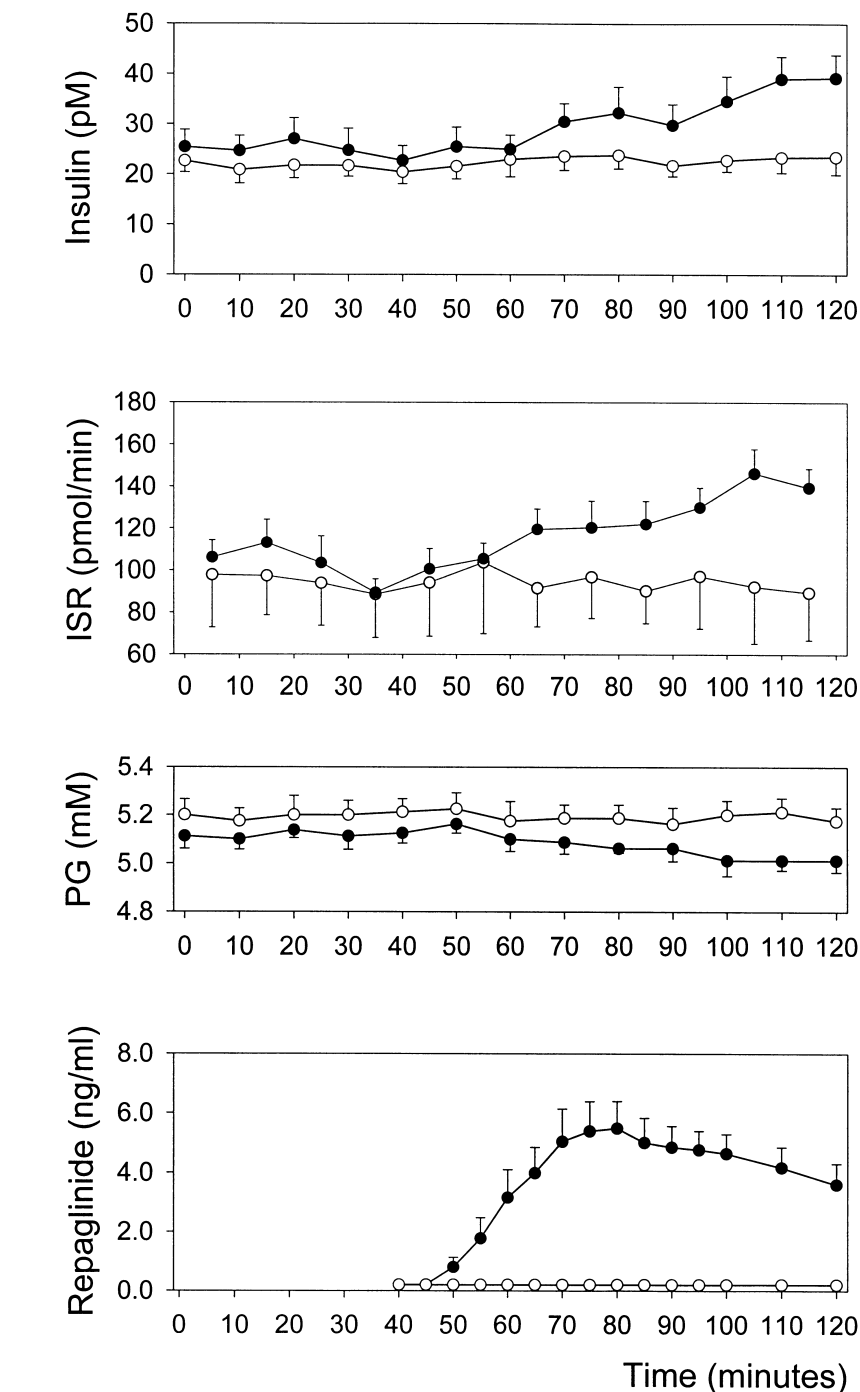
**Statistical analysis.** Data were analyzed by analysis of variance (ANOVA), adjusting for sequence, subject visit, and treatment when testing for differences in post- minus prevalues between repaglinide and placebo treatment. The significance of insulin secretory burst amplitude and burst mass was corrected for multiple testing using Bonferroni's method. General linear model ANOVA was used to examine whether insulin concentrations, insulin secretory rates, plasma glucose, and FFA concentrations changed differently over time after repaglinide and placebo administration. Significance level was set to 5%.

RESULTS

**Circulating concentrations**

Average concentrations of plasma glucose, FFA serum insulin, and C-peptide are listed in Table 1. After repaglinide administration, mean serum insulin concentration rose by 33% compared with the basal period (placebo vs. repaglinide,  $P < 0.01$ ). Serum FFA remained unaltered during placebo as well as after repaglinide administration. Mean plasma-glucose level was slightly but not significantly lower despite glucose infusion after repaglinide administration compared with placebo and remained within a range of  $\pm 0.3$  mmol/l relative to the basal period. Insulin secretory rate was increased by 30% ( $P < 0.01$ ). Mean profiles of insulin, glucose, and repaglinide concentrations and insulin secretory rates are shown in Fig. 1.

Examples of insulin-concentration profiles after administration of repaglinide and placebo are illustrated in Fig. 2. An increase in the measured insulin concentration occurred 30–40 min after the intake of repaglinide. Regular high-frequency oscillations of insulin are seen in the basal state,



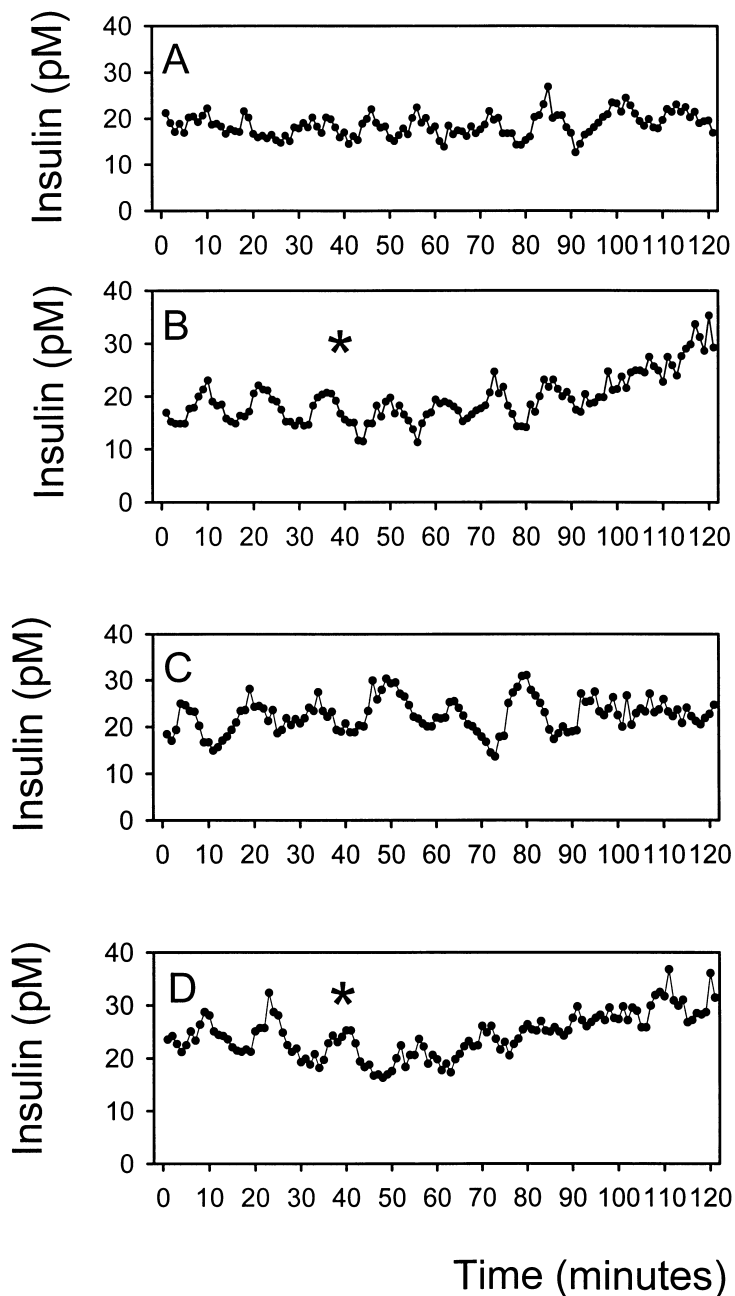
**Figure 1**—Mean concentration profiles of serum-insulin, plasma-glucose, serum repaglinide, and mean insulin secretory rates based on C-peptide deconvolution during the 120-min observation periods after placebo (○) and repaglinide (●) administration. Bars represent SEM.

and a changed dynamic is observed after repaglinide treatment.

**Insulin secretion**

High-frequency oscillations of insulin secretion were quantified by deconvolution analysis of insulin-concentration time

series. The main data are listed in Table 1. No significant change was observed in the interpulse interval. Basal secretion was increased by 28% ( $P = 0.06$ ), and pulsatile insulin secretion increased significantly by ~25% when quantified as secretory burst mass ( $P = 0.02$ ) and burst amplitude ( $P <$



**Figure 2**—Two examples of serum insulin-concentration time series at the day of placebo (A and C) and repaglinide (B and D) administration. \*Time of repaglinide administration.

0.01). The pulsatile fraction was unchanged (42 vs. 48% of total secretion,  $P > 0.5$ ), indicating proportionate increase in the basal and the pulsatile secretion during acute exposure to repaglinide. The overall insulin secretory rate as estimated by C-peptide deconvolution increased significantly by 46% after repaglinide treatment.

One representative example of deconvoluted insulin-concentration time series

during placebo and repaglinide administration is shown in Fig. 3.

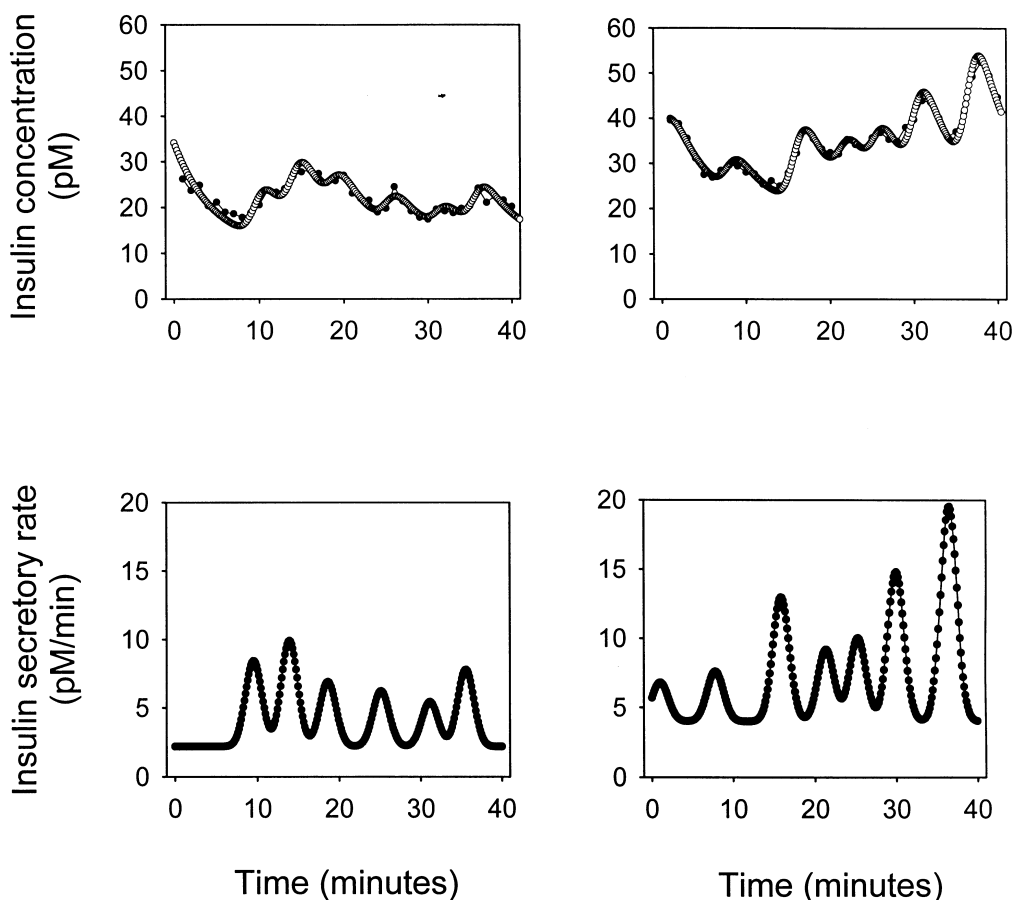
**Regularity analysis (ApEn)**

ApEn increased significantly ( $P = 0.04$ ) after acute exposure to repaglinide, indicating a more irregular insulin-release pattern (Fig. 4). Similar results were obtained when ApEn was applied to the first difference of insulin-concentration time series as well as to the residuals after subtraction of

a 7-point moving average, indicating independence of the procedure of detrending.

**CONCLUSIONS** — The present study further defines the mechanisms by which repaglinide increases total insulin secretion in healthy people. Repaglinide belongs to a new class of chemical compounds, the so-called prandial glucose regulators (23). This agent stimulates insulin secretion by binding to the sulfonylurea (SU) receptor at a binding site different from the SU-binding site. Furthermore, repaglinide, unlike SU compounds, seems to exert no direct intracellular actions on insulin secretory granules (24). The stimulatory actions of repaglinide could arise mechanistically either via an increase in the pulsatile mode of secretion (e.g., by an increase of secretory burst mass and/or burst frequency) or via an increase in basal secretion. Tolbutamide elevates the amplitude of insulin concentration profiles, with no effect on the oscillation period in people (14). In dogs, tolbutamide infusion as well as ingestion stimulates pulsatile and basal insulin secretion with no significant changes in the pulsatile fraction or pulse frequency (7).

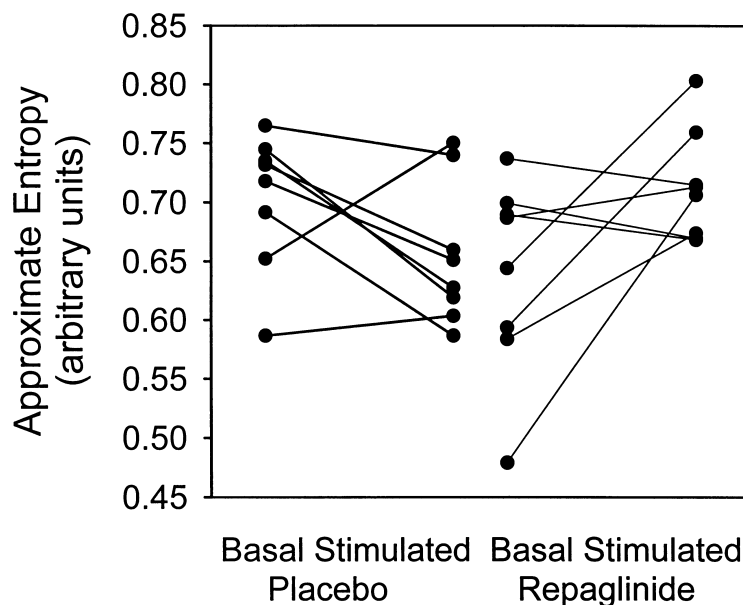
The mechanisms generating the coordinate oscillations of insulin release are still debated. It has been argued that intracellular metabolic oscillations of, for example, glycolysis are of major importance in driving the rhythmic insulin release (25). Although oscillations of intracellular  $Ca^{2+}$  can directly cause oscillatory insulin secretion (26,27),  $Ca^{2+}$  may be seen as a transducer rather than a generator of oscillations (25). The action of repaglinide, similar to that of SU agents, is mediated via the ATP-dependent potassium ion channels, where closure leads to depolarization of the  $\beta$ -cell membrane and subsequent  $Ca^{2+}$  influx. The glycolytic steps involved in the generation of pulsatile insulin release are thus bypassed. It is well established that insulin acts more efficient in lowering blood glucose, inhibiting hepatic glucose output, and lowering FFA levels when delivered in a pulsatile rather than in a constant mode (3–6). Consequently, it may be of importance that insulin secretagogues exert actions by preserving or enhancing the physiological pulsatile pattern, rather than impairing this highly coordinated system. In this study, we assessed the acute effect of a single dose of repaglinide using a rather low dose to unmask primary time-dependent effects. Our study demon-



**Figure 3**—An example of deconvolution analysis of serum insulin-concentration time series at a day of repaglinide administration. Upper panels show observed insulin concentrations (●) and the estimated best-fit curve (○). Lower panels show the calculated insulin secretory rate that results in the observed concentration profile. Leftside panels are during the basal period and the rightside panels are from 40 to 80 min relative to repaglinide administration.

strates that repaglinide in healthy male subjects preserves the physiological rhythmic release pattern by a uniform increase in basal and pulsatile insulin secretion.

Another novel observation in this study is repaglinide's induction of a significant increase in the regularity parameter ApEn, which indicates a change in the insulin-concentration profiles toward less orderliness. ApEn is able to separate hormone secretory patterns in various disease states from normal physiology and to quantify age-related hormonal changes (28,29). This is the first demonstration of significant changes in regularity as measured by ApEn after treatment with insulin secretagogues. Whether the apparent effect on orderliness would be similar after acute exposure to other  $\beta$ -cell stimulants, for example in response to acute hyperglycemia, is not known. In addition, decreased orderliness of release patterns may be part of a physiological response pattern rather than a deterioration of coordination in the secretory



**Figure 4**—Changes in ApEn after administration of placebo and repaglinide in the 8 study subjects. ApEn increased in the majority of the study subjects at the day of repaglinide administration, and no changes were observed at the day of placebo administration.

response. For example, growth-hormone disorderliness is greater under secretagogue drive (e.g., growth hormone-releasing hormone or growth hormone-releasing peptide) and in normal mid- to late puberty (30–32). GLP-1, another insulin secretagogue, has been shown to increase pulsatile insulin secretion without significant changes in ApEn (10). In the present study, we applied  $r = 1 \times SD$  rather than  $0.2 \times SD$ , which has traditionally been used as an input parameter for the ApEn analysis, thus primarily examining the coarse patterns in the time series. We have found that this input results in a better discrimination between the insulin secretory pattern in type 2 diabetic patients and healthy control subjects and may thus be preferential in unmasking defects and changes in insulin secretory patterns (unpublished data). In the GLP-1 study (10), though, the input parameter  $r = 0.2 \times SD$  was used. When applying this to the present cohort, no significant changes were detected, thus emphasizing the importance of the choice of sensitive input parameters. It is unlikely that glucose infusion induced the change in secretion dynamics. Only 4 of 8 subjects were infused with glucose on the day of active drug administration, and the infusion rate was low ( $0.4\text{--}1.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Moreover, to avoid inducing pulsatile activity by a pulsatile exposure of glucose, the infusion rate was increased only in minor steps ( $0.2\text{--}0.6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and never decreased throughout the study period. Although major trends in the data are known to be critical to the ApEn calculations, it is unlikely that the findings reflect a mathematical estimation phenomenon rather than a physiological one. Any significant trend in data would result in a decreased rather than an increased ApEn value, and 2 fundamentally different detrending procedures were applied with similar results (33).

In summary, acute exposure to repaglinide increases insulin release by maintaining the normal physiological oscillatory release pattern with a concomitant change in the regularity dynamic. The precise impact of these acute changes in type 2 diabetic patients in whom oscillatory insulin release is disrupted is not yet known. In addition, long-term effects on pulsatility and regularity might be important in the chronic actions of insulin secretagogues in type 2 diabetic patients or relatives with impaired regularity of insulin secretion (11,13,34).

**Acknowledgments**— The study was supported by the Institute of Clinical Experimental Research, University of Århus, the Danish Research Council, Foundation for Medical Research, Vejle County, the Danish Diabetes Association, Novo Nordisk A/S, and National Institutes of Health RR00437 General Clinical Research Center Grant.

We thank Anette Mengel and Elsebeth Horneman for excellent technical assistance.

**References**

1. 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
2. Goodner CJ, Walike BC, Koerker DJ, Einsack 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
3. Schmitz O, Pedersen SB, Mengel A, Porksen N, Bak J, Moller N, Richelsen B, Alberti KG, Butler PC, Orskov 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
4. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC: Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes* 32:617–621, 1983
5. Komjati M, Bratusch-Marrain P, Waldhauser W: Superior efficacy of pulsatile versus continuous hormone exposure on hepatic glucose production in vitro. *Endocrinology* 118: 312–319, 1986
6. Bratusch-Marrain PR, Komjati M, Waldhauser WK: Efficacy of pulsatile versus continuous insulin administration on hepatic glucose production and glucose utilization in type I diabetic humans. *Diabetes* 35: 922–926, 1986
7. Porksen NK, Munn SR, Steers JL, Schmitz O, Veldhuis JD, Butler PC: Mechanisms of sulfonylureas stimulation of insulin secretion in vivo: selective amplification of insulin secretory burst mass. *Diabetes* 45: 1792–1797, 1996
8. Porksen 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
9. Porksen 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
10. Porksen NK, Grøfte T, 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

11. Schmitz O, Porksen 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
12. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231–1239, 1988
13. 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
14. Matthews DR, Lang DA, Burnett MA, Turner RC: Control of pulsatile insulin secretion in man. *Diabetologia* 24:231–237, 1983
15. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME: Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 39:578–582, 1993
16. 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
17. Porksen NK, Nyholm B, Veldhuis JD, Butler PC, Schmitz O: In humans at least 75% of insulin secretion arises from punctuated secretory bursts. *Am J Physiol* 273:E908–E914, 1997
18. Eaton RP, Allen RC, Schade DS, Erickson KM, Standefer J: Prehepatic insulin production in man: kinetic analysis using peripheral connecting peptide behavior. *J Clin Endocrinol Metab* 51:520–528, 1980
19. Polonsky KS, Licinio-Paixao J, Given BD, Pugh W, Rue P, Galloway J, Karrison T, Frank B: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type I diabetic patients. *J Clin Invest* 77:98–105, 1986
20. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
21. Pincus SM: Approximate entropy as a measure of system complexity. *Proc Natl Acad Sci U S A* 88:2297–2301, 1991
22. Pincus SM, Hartman ML, Roelfsema F, Thorner MO, Veldhuis JD: Hormone pulsatility discrimination via coarse and short time-sampling. *Am J Physiol* 277:E948–957, 1999
23. Owens DR: Repaglinide—prandial glucose regulator: a new class of oral antidiabetic drugs. *Diabet Med* 15 (Suppl. 4):S28–S36, 1998

24. Fuhlerdorff J, Rorsman P, Kofod H, Brand CL, Rolin B, MacKay P, Shymko R, Carr RD: Stimulation of insulin release by repaglinide and glibenclamide involves both common and distinct processes. *Diabetes* 47:345–351, 1998
25. Tornheim K: Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes* 46:1375–1380, 1997
26. Bergsten P, Lin J, Westerlund J: Pulsatile insulin release: role of cytoplasmic  $Ca^{2+}$  oscillations. *Diabetes Metab* 24:41–45, 1998
27. Jonas J-C, Gilon P, Henquin J-C: Temporal and quantitative correlations between insulin secretion and stably elevated or oscillatory cytoplasmic  $Ca^{2+}$  in mouse pancreatic  $\beta$ -cells. *Diabetes* 47:1266–1273, 1998
28. Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD: Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. *Proc Natl Acad Sci U S A* 93:14100–14105, 1996
29. Pincus SM, Gevers EF, Robinson IC, van den Berg G, Roelfsema F, Hartman ML, Veldhuis JD: Females secrete growth hormone with more process irregularity than males in both humans and rats. *Am J Physiol* 270:E107–E115, 1996
30. Veldhuis JD, Metzger DL, Martha PM Jr, Mauras N, Kerrigan JR, Keenan B, Rogol AD, Pincus SM: Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. *J Clin Endocrinol Metab* 82:3414–3420, 1997
31. Iranmanesh A, South S, Liem AY, Clemmons D, Thorner MO, Weltman A, Veldhuis JD: Unequal impact of age, percentage body fat, and serum testosterone concentrations on the somatotrophic, IGF-I, and IGF-binding protein responses to a three-day intravenous growth hormone-releasing hormone pulsatile infusion in men. *Eur J Endocrinol* 139:59–71, 1998
32. Shah N, Evans WS, Bowers CY, Veldhuis JD: Tripartite neuroendocrine activation of the human growth hormone (GH) axis in women by continuous 24-hour GH-releasing peptide infusion: pulsatile, entropic, and nyctohemeral mechanisms. *J Clin Endocrinol Metab* 84:2140–2150, 1999
33. Diggle PJ: *Time Series: A Biostatistical Introduction*. Oxford, U.K., Oxford Science, 1990
34. 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