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ORIGINAL ARTICLES

Validation of a Deconvolution Procedure (AutoDecon) for Identification and Characterization of Fasting Insulin Secretory Bursts

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Abstract

Background:

Insulin secretion is pulsatile, and has been shown to be altered in both physiologic and pathophysiologic conditions. The identification and characterization of such pulses have been challenging, partially because of the low concentrations of insulin during fasting and its short half-life. Existing pulse detection algorithms used to identify insulin pulses either cannot separate hormone pulses into their secretory burst and clearance components, or have been limited by both the subjective nature of initial peak selection and a lack of statistical verification of bursts.

Methods:

To address these concerns, we have developed AutoDecon, a novel deconvolution computer program.

Results:

AutoDecon was applied to synthetic insulin concentration-time series modeled on data derived from normal fasting subjects and simulated to reflect several sampling frequencies, sampling durations, and assay replicates. The operating characteristics of AutoDecon were compared to those obtained with Cluster, a standard pulse detection algorithm. AutoDecon performed considerably better than Cluster with regard to sensitivity and secretory burst detection rates for true positives, false positives, and false negatives. As expected, given the short half-life of insulin, sampling at 30-second intervals is required for optimal analytical results. The choice of sampling duration is more flexible and relates to the number of replicates assayed.

Conclusion:

AutoDecon represents a viable alternative to standard pulse detection algorithms for the appraisal of fasting insulin pulsatility.

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Abbreviations: (CV) coefficient of variation, (ELISA) enzyme-linked immunosorbent assay, (MDC) minimal detectable concentration, (SD) standard deviation, (SEM) standard error of the mean

Keywords: deconvolution, hormone pulsatility, insulin, software

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Introduction

Insulin secretion is pulsatile and has been detected *in vivo*[1,2] in perfused pancreas[3] and in isolated islets.[4–7] Such pulsatility typically evolves as rapid spikes with a duration of tens of seconds[1,5,8] superimposed on one slower component exhibiting a duration of about 5 minutes or more[1,2,9] and a second even slower and variable component with a greater than 60-minute duration.[10,11] The slower components are typically detected *in vivo,* depending on the specific sampling frequency. This pulsatility may play an important physiological role in insulin action[12] and is altered in patients with type 2 diabetes and their near relatives[13–16] as well as under other pathophysiological conditions.[17] However, in part because of the very low concentrations of insulin during fasting, the quantification of pulsatile insulin secretion has been a challenging undertaking.

A number of computer-assisted algorithms created to define hormone pulses, including those of insulin, have sought to identify and subsequently characterize perturbations within hormone concentration-time series.[18] However, such methods fail to provide information about the secretory event and subsequent elimination functions known to underlie hormone pulses. To address this issue, a mathematical approach known as deconvolution has been employed to both identify and characterize hormone secretory burst activity, and simultaneously provide information about elimination.[19,20] Such deconvolution procedures have been used to characterize the secretion of a number of hormones including insulin.[7,18,21–25] However, despite their advantages over standard pulse detection techniques, these deconvolution procedures have inherent limitations, including the subjective nature of the choice of candidate secretory bursts, the lack of robust statistical verification of resolved pulses, and the user-unfriendly interface of the programs.

To address these concerns, we developed an automated deconvolution procedure known as AutoDecon.[26,27] In this study, we analyzed and validated the performance of AutoDecon using synthetic fasting serum insulin concentration-time series, which were created by mimicking serum insulin concentration-time series obtained from normal fasting subjects. The synthetic time series were designed to reflect differing sampling intensities and sampling intervals, to simulate differing numbers of replicates within the assay system and measurement uncertainties of common assay systems.[26,27] The operating characteristics of AutoDecon were then compared to those of Cluster,[28] a standard pulse detection algorithm widely applied to analyze insulin pulsatility.

Methods

Description of AutoDecon

Building upon our original multiparameter deconvolution approach, Deconv,[19] a novel and statistically valid automatic algorithm, AutoDecon, was developed.[26,27] Previously published hormone pulsatility analysis approaches[19,20,29] necessitate prior knowledge or presumption of the number of secretion events, and initial estimates of the secretion event positions and amplitudes are required to be provided by the user. Many require a fixed predetermined estimate of the elimination half-life(s). Finding the number of secretion events and initial parameter estimates is a system identification problem. In this regard, a quintessential data analysis dilemma is the identification and subsequent characterization of small pulsatile events within a concentration-time series where the amplitudes of these events are comparable to the magnitude of the experimental measurement errors. Event identification is further complicated when the pulsatile events are aperiodic (i.e., they occur at apparently random intervals) and/or when the experimental measurement uncertainties are heteroscedastic (i.e., the measurement uncertainties are variable). These issues are addressed via a fully automated and statistically based algorithmic approach, AutoDecon, which finds the optimal number of secretion events and initial parameter estimates while simultaneously performing deconvolution.

AutoDecon implements a rigorous statistical test for the existence of secretion events. In addition, the subjective nature defining the earlier deconvolution procedures is eliminated by the ability of the program to insert automatically and subsequently test the significance of presumed secretion events. No user intervention is required subsequent to the initialization of the algorithm. This automatic algorithm combines three modules: a maximum likelihood parameter fitting module,[19,20,29] a new insertion module that automatically adds presumed secretion events, and a new triage module that automatically removes secretion events deemed to be statistically non-significant. The AutoDecon algorithm iteratively adds presumed secretion events, tests the significance of all events, and removes nonsignificant secretion events.

The procedure is repeated until no additional secretion events are added. The specific details of this algorithm are outlined elsewhere.[26,27]

Description of Cluster

The Cluster[28] pulse detection algorithm performs a sliding student's *t*-test to identify significant increases and decreases within the hormone concentration-time series. Nadirs are identified as a significant decrease followed by a significant increase, and peaks are identified as the regions between the nadirs.

Validation of AutoDecon

Collection of Serum Insulin Concentration-Time Series from Normal Women

Subjects. Seven healthy women (mean age 30.2 years) were studied at the Clinical Research Unit at the Institute of Endocrinology, Prague, Czech Republic. No subject had a history of diabetes, impaired glucose tolerance, thyroid dysfunction, or hyperprolactinemia. All women had normal thyroid function tests, serum prolactin, and serum testosterone concentrations. The mean body mass index was 20.1 (range 18.9–21.2 kg/m^2). All women reviewed and signed consent forms approved by the Institutional Ethical Committee at the Institute of Endocrinology.

Protocol. After an overnight fast, an indwelling cannula was placed into a forearm vein and blood samples were obtained at 30-second intervals for 60 minutes. After each sample was obtained, 0.6 ml of saline was administered into the dead space, withdrawn, and discarded 10 seconds prior to the next sample collection.

Assays. Serum insulin was measured using an enzyme-linked immunosorbent assay (ELISA) (Roche Diagnostics GmbH, Mannheim, Germany). The sensitivity [minimal detectable concentration (MDC)] of the ELISA was 0.2 μU/ml and the intra- and interassay coefficients of variation (CV) were 1.5 and 4.9%, respectively, for a concentration of 5.93 ± 0.09 μU/ml.

Creation of Synthetic Serum Insulin Concentration-Time Series

Synthetic serum insulin concentration-time series were created to mimic the experimentally observed serum insulin concentration-time series and measurement uncertainties.[26,27] The series were constructed in a manner such that the locations and sizes of the synthetic secretion events as well as the half-lives and the secretion standard deviations (SD) were known *a priori*.[26,27] When these synthetic time series were analyzed with AutoDecon[26,27] and Cluster,[28] comparisons between the correct answers and those produced by the algorithms could be made, thus yielding true-positive and false-positive rates for each algorithm and for the specific hormone concentration-time series being considered.

Although the most common method for constructing synthetic concentration-time series involves simulating a series of secretion events based upon the average values, this approach incorrectly assumes that the parameters are not dependent upon one another (i.e., it assumes that they are orthogonal to each other) and that the parameters are normally distributed with known distribution characteristics. In fact, the size of a secretion event (modeled as a convolution integral) may depend on the size of the previous secretion event, the time elapsed since the previous event, and the elimination half-life, or may contain a circadian rhythm. In this study, the simulation of the synthetic series took these into account and included a description of the covariance between the hormone pulsatility parameters. It also assumed that distributions of the estimated parameters were log-normal.[26,27]

One thousand synthetic data sets (i.e., concentration-time series) were constructed to simulate each different sample collection protocol (i.e., samples obtained every 0.5, 1, and 2 minutes for 30, 60, and 120 minutes) and assay replicates (i.e., singlicates, duplicates, and triplicates). A realistic amount of Gaussian-distributed pseudo-random experimental observational error based upon the assay MDC and percentage CV was added to the simulated data.[26,27] All combinations were subsequently analyzed with AutoDecon[26,27] and Cluster.[28]

Data Analysis

The performance of each analytical method was determined by comparing method-identified peak locations with known locations for each peak in synthetic data. Identified secretion event locations within a ±3-minute time window of a simulated event were counted as true positives, but if more than one peak determined by the program fell into this window, only the first one of them was considered to be a true positive. Peaks identified that were outside the ±3-minute positive identification window of simulated events were counted as false positives. The true-positive detection rate was determined as the ratio of the true-positive count by each method to the total peaks found by the method. The false-positive detection rate was computed as the ratio of the false-

positive count to total peaks found by the program. The true positive detection rate was equal to 100% minus the false positive detection rate. The sensitivity detection rate was determined as the ratio of the true-positive count by each method to the total number of simulated peaks within the synthetic data set. Finally, the false-negative detection rate was determined as the number of simulated secretory bursts that the program failed to identify divided by the number of simulated secretory bursts. The sensitivity detection rate was equal to 100% minus the false positive detection rate.

Results

Identification of Insulin Secretory Bursts/Pulses by AutoDecon and Cluster

The upper panel of **Figure 1** shows a representative experimentally measured serum insulin profile obtained in a normal woman sampled at 1-minute intervals for 60 minutes and assayed in duplicate. The lower panel of **Figure 1** depicts a representative synthetic serum insulin profile created to mimic the 1-minute sampling for the 60-minute protocol and assayed in duplicate. Synthetic insulin data sets were created to simulate concentration-time series sampled at 30-second, 1-, and 2-minute intervals for 30-, 60-, and 120-minute intervals and assayed as singlicates, duplicates, or triplicates. One thousand synthetic data sets were formulated for each of the 27 combinations of sampling intensities, intervals, and measurement replicates.

To assess whether the specific pulses identified with AutoDecon[26,27] and Cluster[28] were identical to those created within the synthetic data series, four performance characteristics were appraised. Results are shown in **Tables 1–4** as follows: **Table 1**, sensitivity rates (number of secretory bursts found by the program and known to be correct/number of secretory bursts simulated) for each of the sampling protocols; **Table 2,** true positive detection rates (number of secretory bursts found by the program and known to be correct/number of secretory bursts found by the program); **Table 3,** false positive detection rates (number of secretory bursts found by the program that were not correct/number of secretory bursts found by the program); and **Table 4,** false negative detection rates (number of secretory bursts known to be correct that the program failed to identify/number of secretory bursts known to be correct).

Of interest, **Tables 2** and **3** depict an interesting property of both AutoDecon and Cluster: a decrease of the sampling frequency typically led to an increase of the true-positive

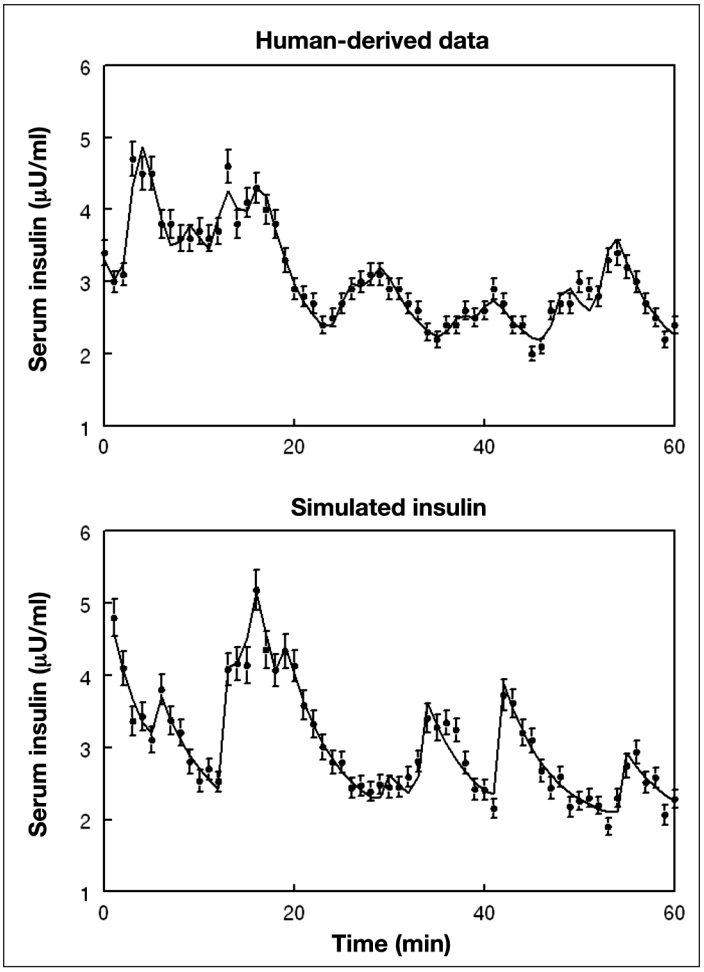

Figure 1. In the upper panel is shown a representative serum insulin concentration-time series obtained from a normal woman sampled at 1-minute intervals for 60 minutes. In the lower panel is depicted a synthetic insulin concentration-time series also representing 1-minute sampling for 60 minutes. The solid lines correspond to the calculated concentration based upon the analyses of the data sets by the AutoDecon algorithm. Intrasample SDs are shown as vertical lines through each data value. These SDs were calculated from the assay CVs and MDCs as the square root of the sum of squared contributions of the individual terms as described previously.[26-27]

detection rate and a decrease of the false-positive detection rate. This reflects the fact that at lower sampling frequencies, the programs missed many peaks (lower sensitivity detection rate, **Table 1**), but most of those identified were true-positives.

In order to determine the optimal combination of operating characteristics for a given sampling protocol (i.e., where both sensitivity and true positive detection rates approach 100%, and false positive and false negative detection rates approach 0%), the absolute values representing the differences between actual values and these target values were calculated and expressed as the mean of each of the four operating characteristics (**Table 5**). Inspection of these values confirmed (1) that

the choice of sampling protocol exerts a major effect on information that can be gleaned from the insulin concentration-time series and (2) that AutoDecon performs considerably better than Cluster in terms of maximizing both the sensitivity and true-positive detection rates and minimizing both false-positive and false-negative rates of detection. From these 54 protocols, the 6 that represent the top 10% exhibiting the smallest mean absolute values (and thus the best combination of the four operating characteristics) are listed in **Table 6**. All were analyzed with AutoDecon as opposed to Cluster. Although all 6 required 30-second sampling, the choice of sampling duration and the number of assay replicates were interdependent, an observation with potential implications on protocol cost, as discussed later.

Characterization of Insulin Secretory Burst Properties by AutoDecon

In addition to identifying hormone secretory bursts, AutoDecon provides information about certain burst characteristics including mass and half-duration, and provides an estimate of the half-life of the hormone. **Figure 2** shows the mean [± standard error of the mean (SEM)] attributes for mass (μU/ml), half-duration (minutes), and half-life (minutes) utilized to simulate

Table 1.
Median (± SEM) Percent Sensitivity Detection Rates for the Identification of Insulin Secretory Bursts Using the Two Analytical Methods AutoDecon and Cluster

Sampling duration (min):		30		60		120	
Analytical method:		AutoDecon	Cluster	AutoDecon	Cluster	AutoDecon	Cluster
Sampling frequency	Replicates						
Every 30 seconds	Singlicates	89.9 ± 0.52	57.7 ± 0.69	92.7 ± 0.35	60.7 ± 0.50	92.8 ± 0.34	60.9 ± 0.38
	Duplicates	94.0 ± 0.90	63.3 ± 0.68	96.7 ± 0.24	66.7 ± 0.50	96.5 ± 0.21	65.7 ± 0.40
	Triplicates	96.2 ± 0.30	76.2 ± 0.58	97.7 ± 0.20	79.8 ± 0.43	98.1 ± 0.15	81.2 ± 0.33
Every 1 minute	Singlicates	76.2 ± 0.85	47.4 ± 0.72	79.7 ± 0.65	50.1 ± 0.51	77.2 ± 0.69	48.6 ± 0.42
	Duplicates	84.2 ± 0.75	51.9 ± 0.69	88.0 ± 0.55	76.6 ± 0.43	86.1 ± 0.58	56.8 ± 0.46
	Triplicates	87.5 ± 0.40	63.3 ± 0.68	91.2 ± 0.48	68.2 ± 0.49	90.4 ± 0.50	70.0 ± 0.41
Every 2 minutes	Singlicates	34.1 ± 0.88	35.4 ± 0.69	41.9 ± 0.48	39.1 ± 0.47	44.5 ± 0.66	38.5 ± 0.38
	Duplicates	36.3 ± 0.91	39.9 ± 0.71	52.4 ± 0.83	45.8 ± 0.49	52.9 ± 0.72	46.3 ± 0.41
	Triplicates	40.9 ± 1.10	45.4 ± 0.69	58.3 ± 0.30	52.5 ± 0.48	58.0 ± 0.74	54.6 ± 0.38

Table 2.
Median (± SEM) Percent True-Positive Detection Rates for the Identification of Insulin Secretory Bursts Using the Two Analytical Methods AutoDecon and Cluster

Sampling duration (min):		30		60		120	
Analytical method:		AutoDecon	Cluster	AutoDecon	Cluster	AutoDecon	Cluster
Sampling frequency	Replicates						
Every 30 seconds	Singlicates	91.9 ± 0.45	79.4 ± 0.71	93.4 ± 0.33	80.6 ± 0.47	96.2 ± 0.18	80.1 ± 0.32
	Duplicates	91.9 ± 0.48	84.9 ± 0.61	93.6 ± 0.33	83.9 ± 0.43	95.8 ± 0.20	89.0 ± 0.33
	Triplicates	91.4 ± 0.48	78.3 ± 0.61	93.8 ± 0.36	76.9 ± 0.45	96.5 ± 0.18	74.9 ± 0.31
Every 1 minute	Singlicates	95.5 ± 0.46	89.9 ± 0.76	97.2 ± 0.22	93.5 ± 0.38	97.5 ± 0.16	90.8 ± 0.30
	Duplicates	96.2 ± 0.33	94.4 ± 0.55	96.6 ± 0.24	83.7 ± 0.42	97.4 ± 0.15	93.8 ± 0.25
	Triplicates	95.3 ± 0.40	93.5 ± 0.46	95.6 ± 0.26	94.0 ± 0.30	97.0 ± 0.16	92.9 ± 0.24
Every 2 minutes	Singlicates	80.7 ± 1.30	89.5 ± 0.95	97.2 ± 0.48	97.4 ± 0.30	99.4 ± 0.18	96.6 ± 0.26
	Duplicates	80.0 ± 1.30	92.7 ± 0.80	98.7 ± 0.29	98.7 ± 0.24	99.5 ± 0.07	98.0 ± 0.17
	Triplicates	84.4 ± 1.10	96.9 ± 0.51	98.5 ± 0.30	99.0 ± 0.20	99.4 ± 0.01	98.6 ± 0.13

the synthetic insulin secretory bursts compared to those recovered by analysis with AutoDecon. For both burst mass and half-duration and for the estimate of half-life, those recovered by AutoDecon were statistically indistinguishable from those parameters known to define synthetic insulin bursts.

Discussion and Conclusion:

Alterations in the pulsatile secretion of insulin have been reported in a number of clinical situations including in patients with type 2 diabetes,[12,13,16] in the relatives of such patients,[14] in obese children,[10] and in patients with insulinoma.[17] A variety of mathematical techniques have been applied to identify and characterize insulin pulses including several of the original computer-assisted pulse detection techniques such as Ultra,[15,30] Pulsar,[10] Detect,[11,17] and Cluster.[24,25] However, these earlier approaches to pulse detection are limited in that they characterize perturbations in hormone concentration-time series, but do not provide information about the secretory burst itself or about the elimination of the hormone. The more recent application of deconvolution procedures has allowed for the separation of insulin pulses into their constituent secretory burst and elimination components and has provided insights into insulin secretion not achieved previously.[7,21–25] However, although providing significantly more information about the dynamics of insulin secretion than do the standard pulse detection methods, it has become increasingly recognized that

Table 3.
Median (± SEM) Percent False-Positive Detection Rates for the Identification of Insulin Secretory Bursts Using the Two Analytical Methods AutoDecon and Cluster

Sampling duration (min):		30		60		120	
Analytical method:		AutoDecon	Cluster	AutoDecon	Cluster	AutoDecon	Cluster
Sampling frequency	Replicates						
Every 30 seconds	Singlicates	8.9 ± 0.43	19.8 ± 0.68	6.6 ± 0.33	19.4 ± 0.47	3.9 ± 0.18	19.9 ± 032
	Duplicates	8.9 ± 0.48	15.0 ± 0.61	6.4 ± 0.33	16.1 ± 0.43	4.2 ± 0.20	18.1 ± 0.33
	Triplicates	8.6 ± 0.48	21.7 ± 0.61	6.2 ± 0.33	23.1 ± 0.45	3.5 ± 0.18	25.1 ± 0.31
Every 1 minute	Singlicates	3.2 ± 0.31	7.1 ± 0.58	2.7 ± 0.20	6.5 ± 0.38	2.5 ± 0.16	9.2 ± 0.30
	Duplicates	3.6 ± 0.30	4.3 ± 0.43	3.3 ± 0.22	16.3 ± 0.42	2.6 ± 0.15	6.2 ± 0.25
	Triplicates	4.1 ± 0.32	6.2 ± 0.44	4.3 ± 0.26	6.0 ± 0.30	3.0 ± 0.16	7.1 ± 0.24
Every 2 minutes	Singlicates	0.38 ± 0.19	1.3 ± 0.31	0.55 ± 0.10	2.6 ± 0.30	0.33 ± 0.06	3.4 ± 0.26
	Duplicates	0.40 ± 0.16	1.2 ± 0.29	0.46 ± 0.08	1.2 ± 0.22	0.51 ± 0.01	2.0 ± 0.18
	Triplicates	0.18 ± 0.11	0.92 ± 0.20	0.72 ± 0.11	1.0 ± 0.18	0.59 ± 0.01	1.4 ± 0.13

Table 4.
Median (± SEM) Percent False Negative Detection Rates for the Identification of Insulin Secretory Bursts Using the Two Analytical Methods AutoDecon and Cluster.

Sampling duration (min):		30		60		120	
Analytical method:		AutoDecon	Cluster	AutoDecon	Cluster	AutoDecon	Cluster
Sampling frequency	Replicates						
Every 30 seconds	Singlicates	10.0 ± 0.52	42.2 ± 0.69	7.3 ± 0.35	39.3 ± 0.50	7.1 ± 0.34	39.0 ± 0.38
	Duplicates	5.9 ± 0.39	36.6 ± 0.68	3.5 ± 0.24	33.2 ± 0.50	3.4 ± 0.21	34.2 ± 0.40
	Triplicates	3.7 ± 0.30	23.8 ± 0.58	2.3 ± 0.20	20.2 ± 0.43	1.9 ± 0.15	18.8 ± 0.33
Every 1 minute	Singlicates	25.8 ± 0.85	52.6 ± 0.72	19.8 ± 0.65	49.6 ± 0.51	22.5 ± 0.67	51.2 ± 0.42
	Duplicates	16.6 ± 0.75	47.5 ± 0.70	11.4 ± 0.54	22.9 ± 0.42	12.5 ± 0.55	43.0 ± 0.46
	Triplicates	11.2 ± 0.63	35.4 ± 0.68	8.1 ± 0.48	31.2 ± 0.49	9.3 ± 0.50	29.8 ± 0.41
Every 2 minutes	Singlicates	64.4 ± 0.91	63.0 ± 0.71	57.2 ± 0.74	59.9 ± 0.48	55.1 ± 0.66	61.1 ± 0.38
	Duplicates	63.7 ± 0.91	58.2 ± 0.72	46.8 ± 0.84	53.2 ± 0.49	46.7 ± 0.72	53.2 ± 0.42
	Triplicates	57.6 ± 1.00	52.6 ± 0.69	40.8 ± 0.85	46.3 ± 0.48	41.6 ± 0.74	44.8 ± 0.38

Table 5.
Mean Absolute Values Representing the Differences between % Sensitivity and % True-Positive Secretory Burst Detection Rates Compared to 100% and between % False-Positive and % False-Negative Detection Rates Compared to 0% for Each of the 27 Sampling Protocols with Data Subsequently Analyzed with AutoDecon and Cluster

Sampling duration (min):		30		60		120	
Analytical method:		AutoDecon	Cluster	AutoDecon	Cluster	AutoDecon	Cluster
Sampling frequency	Replicates						
Every 30 seconds	Singlicates	9.03	31.2	6.95	29.4	5.50	29.5
	Duplicates	7.73	25.9	4.90	24.7	3.83	44.4
	Triplicates	6.18	22.8	4.25	21.7	2.75	22.0
Every 1 minute	Singlicates	14.3	30.6	11.4	28.1	12.6	30.3
	Duplicates	9.95	26.4	7.53	19.7	7.90	24.6
	Triplicates	8.13	21.2	6.40	18.8	6.23	18.5
Every 2 minutes	Singlicates	37.5	34.9	29.6	31.5	27.9	32.3
	Duplicates	37.0	31.7	24.0	23.9	23.7	29.4
	Triplicates	29.3	27.8	21.2	24.0	21.2	23.3

Table 6.
Rank Order of the Upper 10% of Sampling Protocols Exhibiting the Smallest Mean Absolute Values for % Sensitivity and True-Positive Secretory Burst Detection Rates Compared to 100% and for % False Positives and False Negatives Compared to 0%

Rank order	Protocol			Mean absolute value	Mean absolute value
	Sampling frequency (s)	Sampling duration (min)	Assay replicates	AutoDecon	Cluster
1	Every 30	120	Triplicates	2.75	22.0
2	Every 30	120	Duplicates	3.83	44.4
3	Every 30	60	Triplicates	4.25	21.7
4	Every 30	60	Duplicates	4.90	24.7
5	Every 30	120	Singlicates	5.50	29.5
6	Every 30	30	Triplicates	6.18	22.8

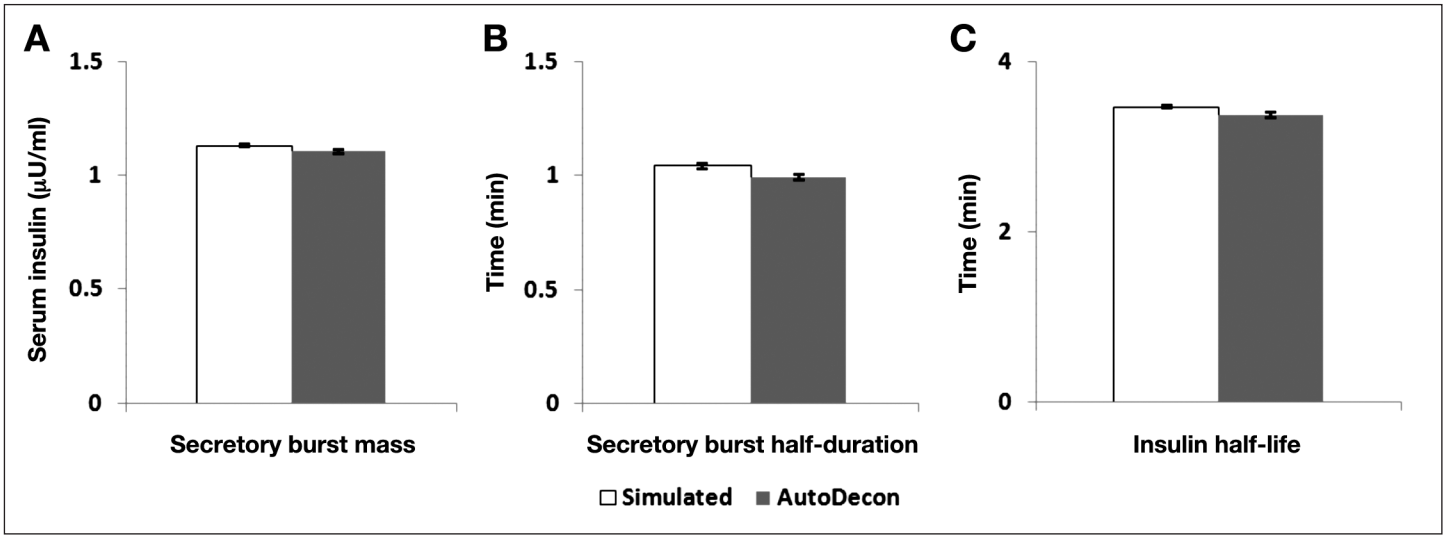

Figure 2. Mean (± SEM) insulin secretory burst mass **(A)**, half-duration **(B)**, and half-life **(C)** resolved with AutoDecon from synthetic insulin concentration-time series (n = 1000) in comparison to known values. No differences between the known values and those recovered by AutoDecon were detected ($p > .05$).

the deconvolution techniques used[19,20,29] have several limitations that have implications for both its application and its interpretation of results. In addition, the use of such analysis techniques has raised questions as to what constitutes optimal sampling and assay techniques. Thus, this study attempted to address concerns both about the deconvolution technique itself and about sampling/assay protocols employed to acquire appropriate data for analysis.

Previous multiparameter deconvolution procedures[19,20,29] have significant limitations including the fact that Deconv requires the user to determine where potential secretory bursts may be located; does not have a rigorous, statistically-based process for retaining or eliminating prospective secretory bursts; and is inherently user-unfriendly in nature. To address these limitations, we developed a deconvolution procedure known as AutoDecon.[26,27] This procedure is fully automated, thus eliminating the need to choose potential secretory bursts for consideration. Moreover, *AutoDecon* uses a statistical approach to test the significance of secretion events and is considerably more user-friendly than the previous algorithm. AutoDecon has been validated previously for the identification and characterization of both luteinizing hormone[26] and growth hormone[27] secretory bursts.

The second issue mentioned earlier, i.e., the need to define an optimal sampling and assay replicate protocol for insulin, is of both significant analytical and practical importance. Using a canine model, Pørksen and colleagues concluded that, in terms of secretory burst identification, sampling at 1-minute intervals was better than at 2-minute intervals, and that the sampling duration needed to be at least 40 minutes.[24] However, no data were presented using a sampling frequency less than 30 seconds, an issue that assumes increased importance when it is recalled that the resolution of pulses into their constituent secretory burst and elimination components with deconvolution requires a sampling frequency of at least five times the estimated half-life of the hormone. Thus, if insulin has a half-life approaching 3 minutes, then an optimal sampling frequency could approach 30–60 seconds. To confound the issue further, although technically possible, such a sampling frequency would, in turn, place limitations on the duration of the sampling process given appropriate concerns about the amount of blood being taken during a clinical study. Moreover, the number of samples obtained raises assay cost issues, which relate directly to the number of replicates performed for a given sample. Thus, in addition to validating AutoDecon for use with insulin concentration-time series, we felt it prudent to obtain information about the operating characteristics of AutoDecon when applied to synthetic data series representing three sampling frequencies (30, 60, and 120 seconds), three sampling intervals (30, 60, and 120 minutes) and three assay replicates (singlicates, duplicates, and triplicates).

Having created the synthetic insulin concentration-time series to reflect the sampling frequency, duration, and assay replicate conditions described earlier, we assessed the operating characteristics of both AutoDecon and Cluster when used to analyze these data. Results clearly demonstrate that AutoDecon performs significantly better than Cluster in identifying secretory bursts. This was assessed on the synthetic data series by estimating four performance characteristics: sensitivity rate, true-positive detection rate, false-positive detection rate, and false-negative detection rate. The choice of sampling protocol is critical with regard to optimizing one or more of the four operating characteristics related to pulse detection and also undertaking clinical studies in a cost-effective manner. In terms of the operating characteristics, there may be situations where, for example, maximizing the sensitivity and true positive detection rates may be more desirable than minimizing the false-positive and false-negative rates of detection or vice versa. Data presented in **Tables 1–4** provide guidance for these decisions to be made. For other studies, striking the best balance among all four operating characteristics may be the objective. As shown in **Table 6**, several of the sampling protocols that exhibited the best overall set of operating characteristics are presented. Of interest, the best performers all involved sampling at 30-second intervals and required analysis with AutoDecon rather than Cluster. However, these data suggest that there is some flexibility with regard to sampling duration and assay replicates, which may be important in terms of cost. For example, under one set of circumstances, it may be more cost effective to constrain the sampling duration to 30 minutes and run the samples as triplicates, whereas under different circumstances the cost of the assay may be substantially more than that related to the nursing/technical personnel who obtain the samples.

In summary, AutoDecon is a multiparameter deconvolution method that addresses a number of concerns raised about earlier deconvolution procedures. With regard to insulin secretion, it appears to perform considerably better than Cluster. Based on its operating characteristics, cost-effective insulin sampling protocols can be designed that will provide adequate information to address the particular issue of interest in a clinical protocol.

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