Effects of Fasting on Physiologically Pulsatile Insulin Release in Healthy Humans

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Insulin is released as secretory bursts superimposed on basal release. The overall contribution of secretory bursts was recently quantified as at least 75%, and the main regulation of insulin secretion is through perturbation of the amount of insulin released and the frequency of these secretory bursts. The mode of delivery of insulin into the circulation seems important for insulin action, and therefore physiological conditions that alter the pattern of insulin release may affect insulin action through this mechanism. To assess the mechanisms by which fasting changes the amount of insulin released and the frequency, amplitude, and overall contribution of pulsatile insulin secretion, we used a validated deconvolution model to examine pulsatile insulin secretion during 10 and 58 h of fasting in seven healthy subjects. The subjects were studied for 75 min before (0–75 min) and 75 min during (115–190 min) a glucose infusion (2.5 mg · kg^{-1} · min^{-1}). We found that the pulsatile insulin release pattern was preserved and that, at fasting, overall insulin release is adjusted to needs by a reduced amount of insulin released (10.1 ± 1.7 vs. 16.0 ± 3.2 pmol/l/pulse, $P < 0.05$) but similar frequency (6.3 ± 0.4 vs. 6.1 ± 0.4 min/pulse) of the insulin secretory bursts. In both states, glucose infusion caused an increase ($P < 0.05$) in amount (100–200%) and frequency (~20%). The impact of increased glucose concentration on pulse frequency seems distinct for in vivo versus in vitro pulsatile insulin secretion and may indicate the presence of a glucose-sensitive pacemaker, which initiates the coordinated secretory bursts. Increased insulin/C-peptide ratio at long-term fasting (6.0 vs. 9.1%, $P < 0.01$) indicates that the changes in insulin release patterns may be accompanied by changes in hepatic insulin extraction. *Diabetes* 51 (Suppl. 1): S255–S257, 2002

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ELISA, enzyme-linked immunosorbent assay.

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Insulin is secreted in a pulsatile manner resulting in detection of high-frequency insulin concentration oscillations in the peripheral circulation (1), together with ultradian oscillations (2,3). These high-frequency oscillations are caused by interislet coordinated insulin secretory bursts, at a frequency of 5–15 min per pulse (1,4,5). The contribution of these insulin secretory bursts to overall insulin secretion has been quantified in a canine model by direct sampling across the pancreas (5) and in a human model employing high-frequency sampling, a highly specific insulin assay, and validated deconvolution analysis (6). In both species, the contribution of pulsatile insulin secretion is at least 70–75%, and changes in overall insulin secretion after stimulation (7) and inhibition (8) are primarily through modulations in the pulsatile component of insulin secretion via changes in the amount of insulin released and/or the frequency of insulin secretory bursts.

Increased actions of insulin on muscle (9), adipose (10), and liver (11) tissues have been reported when the hormone is delivered in a pulsatile versus a constant manner, indicating a possible role of the insulin release pattern in the modulation of insulin action at circumstances with special needs in terms of glucose homeostasis. At long-term fasting, the glucose concentration needs to be within a normal range in order to ensure normal glucose delivery to the brain and other (mainly) glucose-dependent tissues, but glucose-sparing metabolism is needed in other tissues. Therefore, by using a validated deconvolution analysis, we sought to examine the mechanisms by which the physiologically pulsatile insulin release is modulated to meet the special needs for the insulinization of insulin-sensitive tissues when glucose metabolism is brought to a minimum.

RESEARCH DESIGN AND METHODS

Subjects and design

The protocol was approved by the Ethical Committee of Aarhus County and was performed in accordance with the Helsinki Declaration. A total of seven healthy volunteers (mean age 24 ± 2 years, two women, five men, mean BMI 23 ± 2 kg/m^2) were studied. They had no family history of diabetes and were not on any medication. The volunteers were on a weight-maintaining diet for at least 3 days before the beginning of each study day. The volunteers were not allowed to perform exercise during same time period. In a randomly assigned order and with at least 4 weeks of washout, they were studied after either an overnight fast (10 h), designated as control, or a long-term fast (58 h), referred to as fasting. After the fasting period (10 vs. 58 h) they were brought to the Research Unit, Institute of Experimental Clinical Research, Aarhus University Hospital, placed in bed, and a 14-gauge catheter was placed in each antecubital vein for infusion and sampling purposes. After catheter placement, we

TABLE 1
Glucose and hormonal concentrations in control and fasting states

	Fasting		Control	
	PRE	POST	PRE	POST
Glucose (mmol/l)	4.05 ± 0.13*	6.18 ± 0.11†	5.02 ± 0.07	6.10 ± 0.19†
C-peptide (pmol/l)	262 ± 26*	768 ± 65†	450 ± 32	941 ± 109†
Insulin (pmol/l)	31.7 ± 4.1	48.0 ± 5.3†	27.7 ± 3.6	63.9 ± 9.1†
Glucagon (pmol/l)	129 ± 16*	53 ± 4*†	71 ± 5	45 ± 2†
Growth hormone (μg/l)	5.8 ± 2.2*	3.1 ± 1.5*†	2.6 ± 1.3	1.0 ± 0.4†
Insulin:C-peptide ratio (%)	9.1 ± 2.1*	11.3 ± 2.3†	6.0 ± 1.2	8.6 ± 1.4†

*Data are means ± SE. PRE, pre–glucose infusion; POST, post–glucose infusion. *$P < 0.05$ for fasting vs. control, †$P < 0.05$ for pre– vs. post–glucose infusion.

started blood sampling from a peripheral vein every minute for 75 min. At 75 min, glucose (2.5 mg · kg^{-1} · min^{-1}) was commenced and samples were collected every minute from time 115–190 min. Sampling procedures have been validated (6).

Assays

Glucose. Plasma glucose concentrations were measured by the glucose oxidation method (Beckman Instruments, Palo Alto, CA).

Insulin. Serum insulin concentrations were measured in triplicate by a two-site immunospecific insulin enzyme-linked immunosorbent assay (ELISA) (12). In brief, the assay uses two monoclonal murine antibodies (Novo Nordisk, Bagsvaerd, Denmark) specific for insulin. The detection range of this insulin ELISA was 5–600 pmol/l, and intra- and interassay variability was 3 and 4%, respectively. C-peptide was measured using a similar ELISA.

Data analysis

Detection and quantification of pulsatile insulin secretion by deconvolution. The plasma insulin concentration time series were analyzed by deconvolution for the purpose of detection and quantification of insulin secretory bursts. Deconvolution of venous insulin concentration data were performed with a multiparameter technique (13) that requires the following assumptions. The venous plasma insulin concentrations measured in samples collected at 1-min intervals were assumed to result from five determinable and correlated parameters: (*1*) a finite number of discrete insulin secretory bursts occurring at specific times and having (*2*) individual amplitudes (maximal rate of secretion attained within a burst) and (*3*) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), which are superimposed upon a (*4*) basal time-invariant insulin secretory rate and (*5*) a biexponential insulin disappearance model in the systemic circulation consisting of estimated half-lives of 2.8 and 5.0 min and a fractional slow compartment of 28%, as previously measured (6). All data analyses were performed in a blinded manner.

Statistics

The statistics dealing with data analysis are described above. All data in the text and tables are given as means ± SE. The data during overnight and long-term fasting were compared, as were the data before versus during glucose infusion in both overnight and long-term–fasted subjects. Student's two-tailed paired *t* test was used to examine for statistical significance.

RESULTS

Concentrations

Data on the concentration of glucose, C-peptide, insulin, glucagon, and growth hormone are given in Table 1. As expected, glucose concentrations were lower at fasting, resulting in lower insulin secretion reflected in lower C-peptide concentrations. Despite this, insulin concentrations were similar, C-peptide concentrations were lower, and, as a result, insulin/C-peptide ratios were significantly higher at fasting, even during hyperglycemia, possibly reflecting the fact that hepatic insulin extraction is reduced at fasting. The possible impact of differences in glycemia was abolished by glucose infusion resulting in very similar glucose concentrations, allowing the study of the impact of higher counterregulatory hormones and preceding long-term fasting. The insulin concentration time series showed dynamic high frequency changes before glucose infusion at both fasting and control study, and these oscillations were retained or increased by the constant glucose infusion. At long-term fasting, the concentration of free fatty acids increased tremendously from 485 to 2,110 nmol/l ($P < 0.01$), indicating a physiological state of hyperlipidemia during fasting.

Secretion

Data from deconvolution on overall, basal, and pulsatile insulin secretion are given in Table 2. The insulin secretion was assessed by deconvolution of serial serum insulin concentration measurements and revealed the presence of discrete punctual insulin secretory bursts that were superimposed on a basal insulin secretion in all studies. The overall insulin secretion was reduced by ~50% before glucose infusion in the fasting versus the control study, due to decreased basal and pulsatile secretion. Glucose infusion resulted in very similar insulin secretion patterns on the two study days. It is of interest that the frequency on both occasions increased with glucose infusion, indicating a glucose-sensitive pacemaker that triggers the pulsatile insulin release. It is also important to notice that the pulsatile pattern was altered via marked attenuation of

TABLE 2
Basal and pusatile insulin secretion

	Fasting		Control	
	PRE	POST	PRE	POST
Total secretion (pmol/l/min)	2.9 ± 0.4*	11.1 ± 0.9†	5.4 ± 0.9	12.7 ± 2.3†
Frequency (min/pulse)	6.3 ± 0.4	5.1 ± 0.2†	6.1 ± 0.4	5.4 ± 0.3†
Mass (pmol/l/pulse)	10.1 ± 1.7*	28.5 ± 3.8†	16.0 ± 3.2	30.9 ± 5.1†
Amplitude (pmol/l/min)	4.0 ± 0.7*	11.4 ± 0.7†	6.4 ± 1.3	12.5 ± 2.0†
Basal secretion (pmol/l/min)	1.2 ± 0.3*	5.5 ± 0.4†	2.7 ± 0.3	6.6 ± 1.4†
Pulsatile secretion (pmol/l/min)	1.6 ± 0.2*	5.6 ± 0.9†	2.7 ± 0.6	6.1 ± 1.2†

Data are means ± SE. The deconvoluted characteristics of pulsatile insulin secretion is given. PRE, pre–glucose infusion; POST, post–glucose infusion. *$P < 0.05$ for fasting vs. control, †$P < 0.05$ for pre– vs. post–glucose infusion.

the pulse mass and amplitude, which will be relevant to the understanding of the hepatic insulin exposure.

DISCUSSION

In the present study, the insulin release process was assessed in seven healthy humans at basal and fasting conditions using previously validated methods (6). The insulin concentration time series were analyzed using of deconvolution in order to assess the changes in the insulin-release process after long-term fasting. In addition, the impact of acute modest hyperglycemia was tested at each of the above conditions.

The deconvoluted insulin secretory patterns show that the release of insulin at fasting is adapted to needs by reduction in the amplitude and frequency of the secretory burst mass. The preserved release pattern as dominant common secretory bursts, rather than time-invariant basal secretion, indicate that the controlling mechanisms for coordinated (physiological) release are intact, even at this state of low glucose and high free fatty acid concentrations, as well as (physiological) insulin resistance. Free fatty acids are known to stimulate the release of insulin, but the release pattern was still pulsatile in accordance with in vitro data suggesting cyclic glycolysis driving the oscillatory insulin release pattern (14). Also, this may indicate that short-term (approximate 48 h) exposure to high concentrations of free fatty acids does not perturb the β-cell oscillatory release process, suggesting that hyperlipidemia is not causing impaired pulsatile insulin secretion, as observed in type 2 diabetes.

The pulsatile release of insulin from the β-cell population likely reflects the result of simultaneous metabolic processes in the β-cell population. These metabolic processes are known to (at least) involve cyclic glycolysis, with subsequent changes in intracellular ATP concentrations, causing closure of ATP-dependent potassium channels, depolarization of the β-cell membrane, a rise in intracellular calcium (through one or more mechanisms), and exocytosis of insulin-containing granules. Because in vivo pulsatile insulin release frequency is influenced by plasma glucose concentrations, as observed here and reported previously (7), glucose concentration changes may affect the metabolic processes in vivo, either directly or through nerves and/or other metabolites and hormones. The change in insulin-C/peptide ratio reported herein may reflect that attenuated insulin secretory burst mass is accompanied by reduced hepatic insulin extraction, as has previously been reported for somatostatin-induced inhibition of insulin pulse amplitude (8). The reduced glucose concentration could be expected to influence the insulin glucose feedback system (15,16), but, in this case, pulsatile insulin secretion was still preserved.

We conclude that at long-term fasting, insulin secretion is adjusted by a reduction in pulse mass and amplitude, accompanied by increased insulin-C/peptide ratio. The pulsatile release is preserved despite very high concentrations of free fatty acids, glucagon, and growth hormone, despite insulin resistance.

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