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Disruption of the Pulsatile and Entropic Modes of Insulin Release during an Unvarying Glucose Stimulus in Elderly Individuals*

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ABSTRACT

Insulin is secreted in a pulsatile fashion with measurable orderliness (low entropy). Aging is characterized by alterations in pulsatile insulin release in the fasting state. We undertook the current studies to determine whether disruptions in pulsatile insulin release in response to sustained glucose infusion also accompany the age-related changes in carbohydrate metabolism. Healthy young (n = 10; body mass index, 23 ± 1 kg/m^2; age, 23 ± 1 yr) and old (n = 10; body mass index, 24 ± 1 kg/m^2; age, 80 ± 2 yr) volunteers underwent a 600-min hyperglycemic glucose clamp. During the entire 600 min, insulin was sampled every 10 min, and insulin release was evaluated by Cluster analysis. From 240–360 min, insulin was sampled every 1 min, and secretory pulse analysis was conducted using a multiparameter deconvolution technique. During the 1-min sampling interval, basal insulin secretion ($P < 0.01$), insulin production rate ($P < 0.01$), pulsatile mean and integrated insulin concentration ($P < 0.01$), insulin secretory burst mass ($P < 0.01$), and burst amplitude ($P < 0.05$) were reduced in the elderly. In addition, interpulse interval was increased in the aged ($P < 0.05$). In the 600-min studies, interpulse interval was greater in the aged ($P < 0.01$) and burst number ($P < 0.01$), basal concentration ($P < 0.01$), and burst increment ($P < 0.05$) were less. Approximate entropy, a measure of irregularity of insulin release, was increased in the aged, signifying the loss of orderliness of insulin secretion ($P < 0.05$).

We conclude that in response to a sustained (10-h) glucose infusion, normal aging is characterized by a reduction in mass and amplitude of rapid insulin pulses and a decrease in the frequency, amplitude, and regularity of ultradian pulses. Whether these changes in insulin pulsatility contribute directly to the age-related changes in carbohydrate metabolism will require further clinical studies. (*J Clin Endocrinol Metab* **84:** 1938–1943, 1999)

NORMAL aging is characterized by a progressive impairment in carbohydrate tolerance. Two major contributing factors to the age-related impairment in glucose tolerance are altered regulation of hepatic glucose output and resistance to insulin-mediated glucose disposal (1–3).

Insulin is secreted in a pulsatile and orderly fashion. There are both rapid, low amplitude pulses, which occur every 8–15 min, and ultradian pulses, which have a larger amplitude and a periodicity of 60–140 min (4, 5). Rapid pulses are important in inhibiting hepatic glucose production (6–8), whereas ultradian pulses may be important in stimulating peripheral glucose disposal (9). Both types of pulses as well as the orderliness of the insulin release process (entropy), show disruption in disease states characterized by altered glucose metabolism, including impaired glucose tolerance, obesity, and pre- or overt type 2 diabetes mellitus (10–13).

Here, we tested the hypothesis that the impairment in carbohydrate metabolism with age is accompanied by alterations in glucose-induced pulsatile and entropic (orderly) insulin secretion and/or the regularity of the insulin release process.

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Subjects and Methods

Experimental subjects

These studies were performed in healthy nonobese young and elderly subjects (Table 1). Volunteers had a normal history and physical examination, screening laboratory tests, electrocardiogram, and oral glucose tolerance test (glucose dose, 40 g/m^2; National Diabetes Data Group criteria). None of the subjects had a family history of diabetes or was taking medication. This study was approved by the committee on human investigation at the University of British Columbia (Vancouver, Canada). All subjects provided written informed consent before participation.

Experimental protocol

Studies were conducted at the Clinical Research Center at the University of British Columbia. Subjects were weight stable and ate a diet containing at least 150 g carbohydrate/day for 3 days before testing. All volunteers underwent a 600-min hyperglycemic glucose clamp study (increment above basal, 5.4 mmol/L) according to the method of Andres *et al.* (14). Studies commenced at 0730 h after an overnight fast. In each

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TABLE 1. Subject characteristics

	Young (n = 10)	Old (n = 10)
Age (yr)	23 ± 1	80 ± 2[a]
M/F	5/5	5/5
BMI (kg/m^2)	23.1 ± 0.6	24.4 ± 0.5
WHR	0.80 ± 0.03	0.89 ± 0.04
VO_2max (mL/kg·min)	44.5 ± 3.6	20.7 ± 1.3[a]

Data are the mean ± SEM.
[a] $P < 0.0001$, young *vs.* old.

study, an iv catheter was inserted into a hand vein for sampling of arterialized venous blood (15). Insulin and glucose were sampled every 10 min for 10 h. From 240–360 min, insulin was sampled every 1 min, and glucose was sampled every 2 min. VO_2 max (maximum oxygen consumption in response to exercise) was determined in all subjects using a bicycle ergometer (16). The waist to hip ratio (WHR) was determined by dividing the abdominal girth at the greatest protuberance by the hip circumference at the greater trochanter (centimeter).

Analytic methods

An aliquot of the blood sample was used to measure plasma glucose by the glucose oxidase method using a YSI glucose analyzer (YSI, Inc., Yellow Springs, OH). Blood was placed in prechilled test tubes containing aprotonin (400 kallikrein inhibitor units/mL) and ethylenediamine tetraacetate (1.5 mg/mL) and was centrifuged at 4 C. The plasma was promptly stored at −70 C until assay. All samples from each subject were analyzed in the same RIA. For the insulin assays, equal numbers of young and old subjects were included in each assay. Assays were performed in duplicate using a human insulin RIA kit from Linco Research, Inc. (St. Louis, MO), which is specific and sensitive. There is less than 1% cross-reactivity with proinsulin. The interassay coefficient of variation was 11%, and the mean intraassay coefficient of variation was 6%. The sensitivity was 10 pmol/L.

Pulse analysis

Insulin pulse profiles were analyzed for rapid insulin pulsatility with a multiparameter deconvolution technique (17, 18). This technique quantitatively describes insulin profiles as a collection of the following inputs: 1) a finite number of discrete insulin secretory bursts occur at specific times; 2) individual secretory burst amplitudes (maximal rates of secretion in a burst); and 3) a common burst half-duration (duration of an algebraically aussian secretory pulse at half-maximal amplitude), with secretory bursts superimposed on 4) a basal time-invariant insulin secretory rate, assuming a nominal insulin half-life of 2.5 min. Parameters were estimated by nonlinear least squares fitting of the multiparameter convolution integral for each insulin time series. A modified Gauss-Newton quadratically convergent iterative technique was employed with an inverse (sample variance) weighting function. Parameters were estimated until their values and the total fitted variance varied by less than 1 part in 100,000. Asymmetric, highly correlated variance spaces were calculated for each parameter by the Monte Carlo support plane procedure. Optimal peak detection was defined as less than 1 false positive error/10 true pulses and 0 false negative errors/10 true pulses. Optimal peak detection was achieved by use of 95% joint confidence intervals. The following parameters were calculated: secretory burst number (the number of significant secretory pulses/120 min), interpulse interval (time in minutes separating successive pulses), burst mass (the mass or area of the calculated secretory bursts), amplitude (maximal secretory rate within a pulse), basal (constitutive) insulin secretion rate, insulin production rate, and mean and integrated insulin concentration.

Cluster analysis was used to quantify the longer ultradian insulin rhythms, assuming that significant up-strokes and down-strokes in plasma insulin concentrations denote peaks (19). Incremental peak height, peak frequency, interpeak interval, basal (interpeak nadir) insulin concentration, and peak area above interpeak valley insulin concentrations were computed using this program. Threshold criteria included a *t* statistic of 2.0 and test clusters of 1 with dose-dependent within-assay variance.

To confirm that the pulse detection algorithms were detecting true insulin pulses, the data were subjected to Fourier time-series analysis, as previously described (20).

In addition to Cluster and deconvolution analysis, the data were evaluated by a recently developed scale- and model-independent statistic, approximate entropy (ApEn) (21). ApEn provides a measure of regularity (orderliness) of insulin release that can be compared between groups. This estimate is complementary to pulse and deconvolution analysis. ApEn assigns a single nonnegative number to a time series, in which larger values correspond to greater apparent process randomness, and smaller values correspond to more instances of recognizable patterns or consistent features in the data. ApEn measures the logarithmic likelihood that runs of patterns that are similar (within a certain distance r) for n consecutive observations remain similar on the next incremental comparisons. A more complete definition of ApEn was provided in recent reports (21–23).

Data analysis

All data are presented as the mean ± SEM. Differences between young and old subjects were determined by two-sided Student's *t* test for unpaired samples and repeated measures ANOVA, as appropriate. Correlation coefficients were calculated by the method of least squares. $P < 0.05$ was considered significant in all analyses.

Results

Subject characteristics are shown in Table 1. The older volunteers tended to have higher body mass index (BMI) and WHR, but the differences were not statistically significant. The VO_2 max was significantly lower in the aged.

Rapid insulin release

Representative individual insulin profiles for one young and one old subject are shown in Fig. 1. Mean glucose and insulin concentrations in the young and old volunteers during rapid sampling are shown in Fig. 2. Glucose values were similar in the two groups. Insulin concentrations were significantly higher in the young ($P < 0.01$, by ANOVA).

Rapid insulin pulse parameters are summarized in Table 2 and Fig. 3. Basal insulin secretion ($P < 0.01$), burst mass ($P < 0.01$), burst amplitude ($P < 0.05$), pulsatile insulin production rate ($P < 0.01$), and mean and integrated insulin concentrations (both $P < 0.01$) were less in the elderly. Interpulse interval was greater in the aged ($P < 0.05$). Burst number and approximate entropy were similar in the two age groups at this sampling rate. There was no significant correlation between BMI, WHR, or VO_2 max and any of the pulse parameters.

When the insulin data were subjected to Fourier time-series analysis, insulin pulse amplitude was less in the elderly (62 ± 17 *vs.* 20 ± 3 pmol/L; $P < 0.05$), but periodicity was similar in the two age groups (young, 28 ± 6 min; old, 32 ± 9; P = NS). Glucose values were also subjected to Fourier analysis. Periodicity and amplitude were similar in the two groups (data not shown).

Ultradian insulin release

Representative individual insulin profiles in one young and one old subject are shown in Fig. 4. Mean glucose and insulin concentrations in young and old for the entire 10-h clamp are shown in Fig. 5. Glucose values were not significantly different. Insulin values were significantly higher in the young ($P < 0.05$, by ANOVA).

Cluster analysis was used to quantify ultradian insulin

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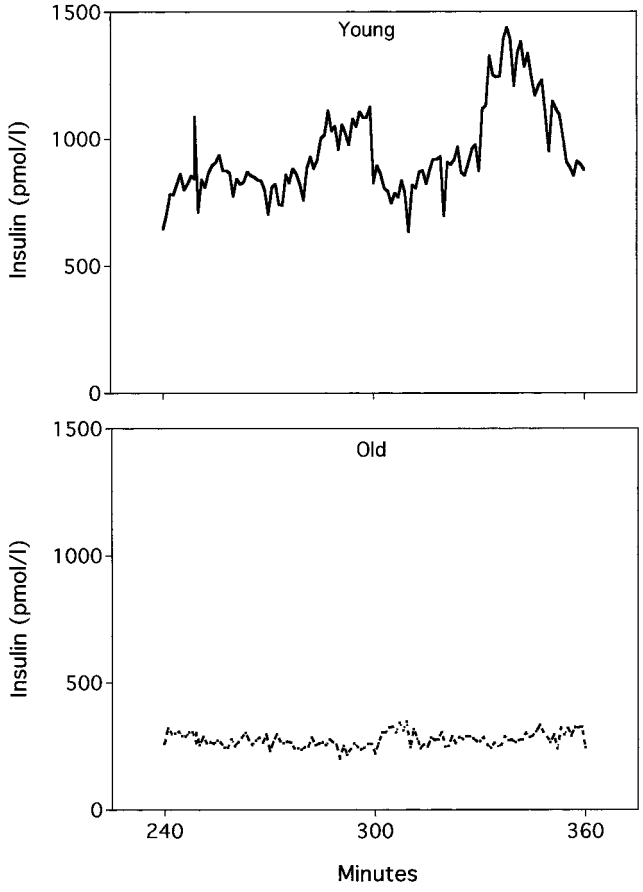

FIG. 1. Individual insulin profiles in one young and one old subject from the 240- to 360-min study.

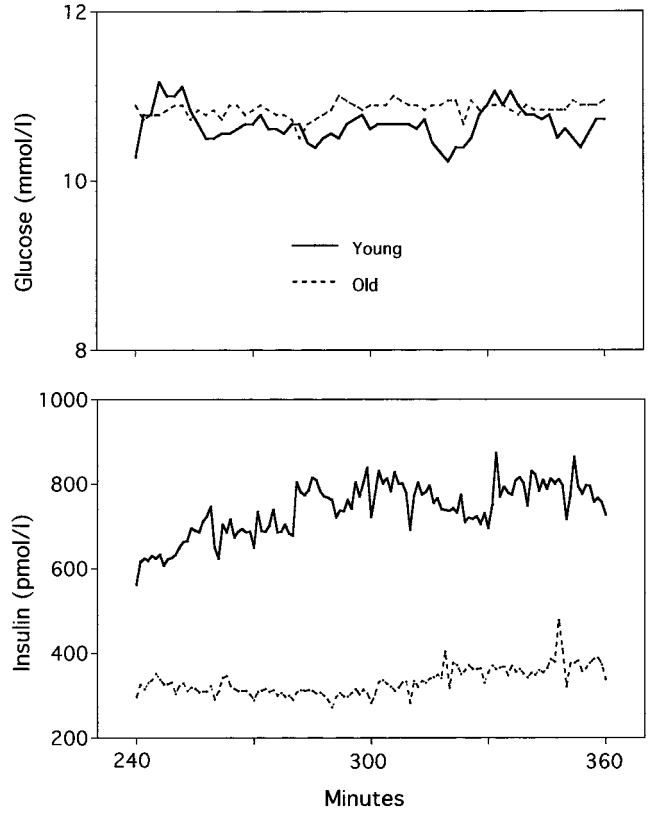

FIG. 2. Mean plasma insulin and glucose concentrations from the 240- to 360-min study.

pulsatility as shown in Table 3 and Fig. 6. Basal insulin concentration ($P < 0.01$), peak number ($P < 0.01$), and incremental peak height were less, and the interpulse interval was greater ($P < 0.01$) in the aged. ApEn was also greater in the elderly ($P < 0.05$). There was no correlation between pulse parameters and BMI, WHR, or VO_2 max.

When the insulin data were subjected to Fourier time-series analysis, insulin pulse amplitude was less in the elderly (50 ± 8 *vs.* 27 ± 3 pmol/L; $P < 0.01$), but periodicity was not different (young, 132 ± 17 min; old, 102 ± 12 min; P = NS). The periodicity and amplitude of glucose values were similar in young and old subjects (data not shown).

Discussion

In the current study, we evaluated for the first time rapid insulin pulse pulses in response to stable hyperglycemia in healthy elderly and compared their responses to those in young volunteers. We demonstrated that normal aging is characterized by an increase in interpulse interval and a reduction in the pulsatile insulin production rate and in the mass and amplitude of rapid insulin secretory pulses. Two previous studies have evaluated rapid insulin pulses during fasting in the elderly (24, 25). Matthews *et al.* reported that the apparent regularity of pulses was reduced, and interpulse interval was increased in the aged compared to those in healthy young subjects (24). We found that fasting insulin burst mass and amplitude were reduced in the elderly (25). The basal insulin concentration and insulin production rate appeared to be reduced, and the interpulse interval appeared to be increased, but the differences did not reach statistical significance. We also found that the regularity of rapid insulin pulses during fasting was reduced with age. Interestingly, hyperglycemia seems to overcome this defect and "regularize" rapid insulin pulse profiles in the elderly.

Circa-sesquihoral (ultradian) pulses of insulin release are present during fasting, hyperglycemia, and nutrient ingestion (5, 26–33). We previously reported that the frequency of ultradian insulin pulses was reduced in the aged during fasting, but peak amplitude was preserved. In the current study, we observed that in addition to a reduction in insulin peak frequency, there was a reduction in peak amplitude, and hyperglycemia elicited a more disorderly pattern of insulin secretion in the elderly. Thus, hyperglycemia unveiled age-related defects in ultradian pulses parameters that were not evident during fasting.

Scheen *et al.* administered glucose by continuous infusion for 53 h to eight moderately obese elderly subjects and compared the results to those in 8 weight-matched young controls (34). They found no differences in insulin pulse frequency, but observed a decreased pulse amplitude and decreased responsiveness of insulin secretion to ultradian oscillations in plasma glucose in the elderly. Potential plausible explanations for the differing results in relation to pulse frequency are that their elderly subjects were younger than ours and relatively more obese, their samples were obtained

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TABLE 2. Deconvolution analysis of rapid insulin pulses

Interpulse interval (min)	11.0 ± 0.5	13.1 ± 0.8[a]
Amplitude (pmol/L·min)	76 ± 18	31 ± 4[a]
Pulsatile production rate (pmol/120 min)	6,353 ± 1,542	1,916 ± 203[b]
Mean insulin conc. (pmol/L)	738 ± 130	306 ± 35[b]
Integrated insulin conc.	85,301 ± 16,461	36,749 ± 4,150[b]
Entropy	0.98 ± 0.04	1.08 ± 0.05

Data are the mean ± SEM.
[a] $P < 0.05$, young *vs.* old.
[b] $P < 0.01$, young *vs.* old.

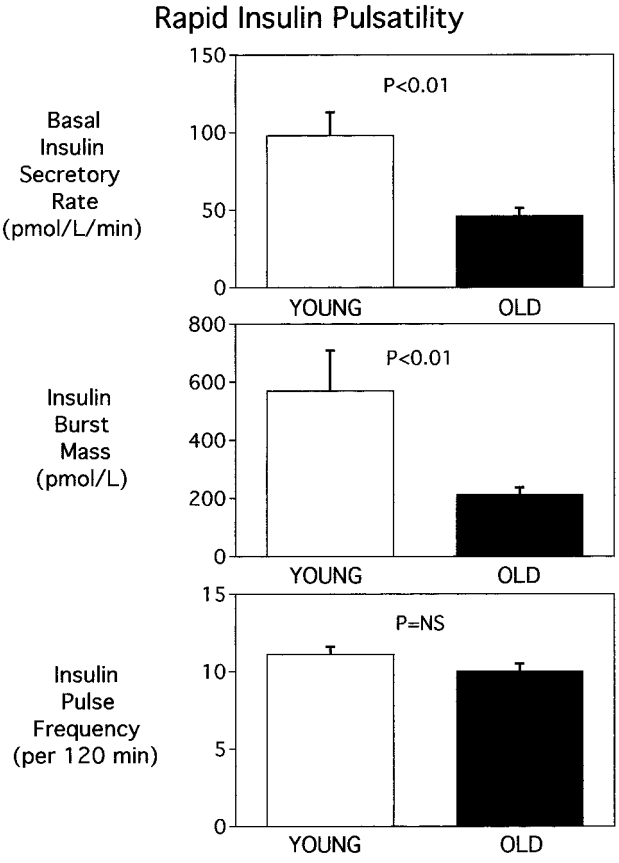

FIG. 3. Rapid pulse parameters from the 120-min study.

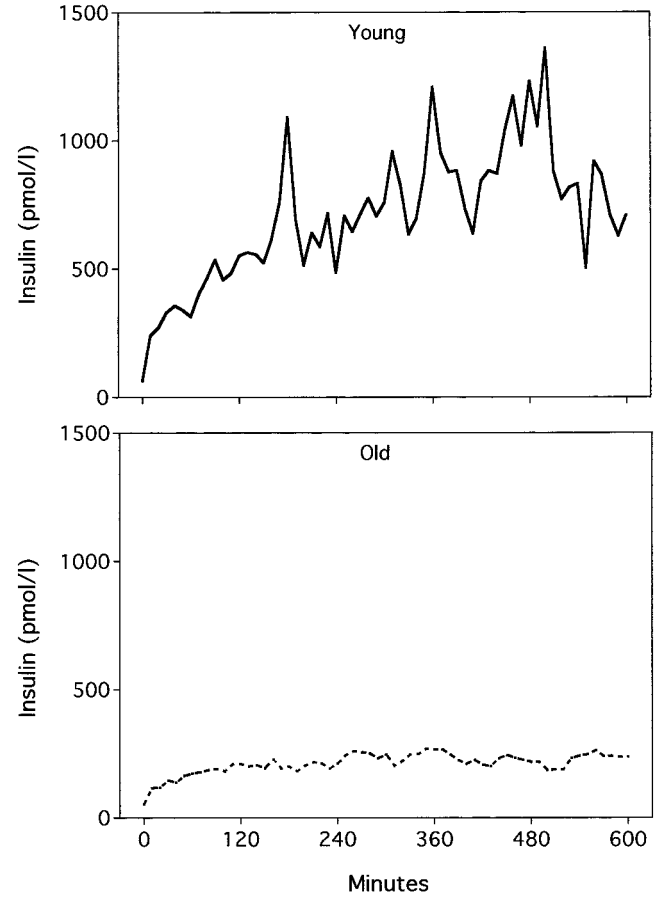

FIG. 4. Individual insulin profiles in one young and one old subject from the 0- to 600-min study.

less frequently (every 20 min *vs.* every 10 min), glucose levels were higher in their elderly subjects), and a different methodology was used to analyze insulin pulses.

We observed several differences in the age-related changes in rapid *vs.* ultradian insulin pulses in response to hyperglycemia. This should not be surprising, because rapid pulses are regulated putatively by a local neural activity within the pancreas, whereas insulin/glucose feedback probably plays an important role in regulating ultradian insulin oscillations (26).

Disrupted rapid and ultradian pulsatility of insulin release is probably due to aging rather than differences in body composition between the two age groups, as in the present study there was no correlation between BMI or WHR and insulin pulse parameters. Differences in physical fitness between age groups also are unlikely to explain our finding, because there was no correlation between insulin pulse parameters and VO_2 max.

Several methodological concerns should be addressed. It is unlikely that our results are an artifact of our pulse detection algorithm, because our findings were confirmed by Fourier analysis. Our calculations assumed that insulin half-life, volume of distribution, and clearance are unchanged with age an assumption that is supported by the literature (34–36). We did not compare insulin secretion rates calculated from C peptide values with insulin parameters calculated by deconvolution. We did not think that this significantly affects our findings, because previous studies have found that temporal variations in insulin levels closely parallel changes in insulin secretion rates in young and old (35). Finally, it has previously been demonstrated that peripheral sampling fails to detect a significant portion of high frequency pulses detected by portal sampling (37). We believe we optimized other factors known to affect pulse detection

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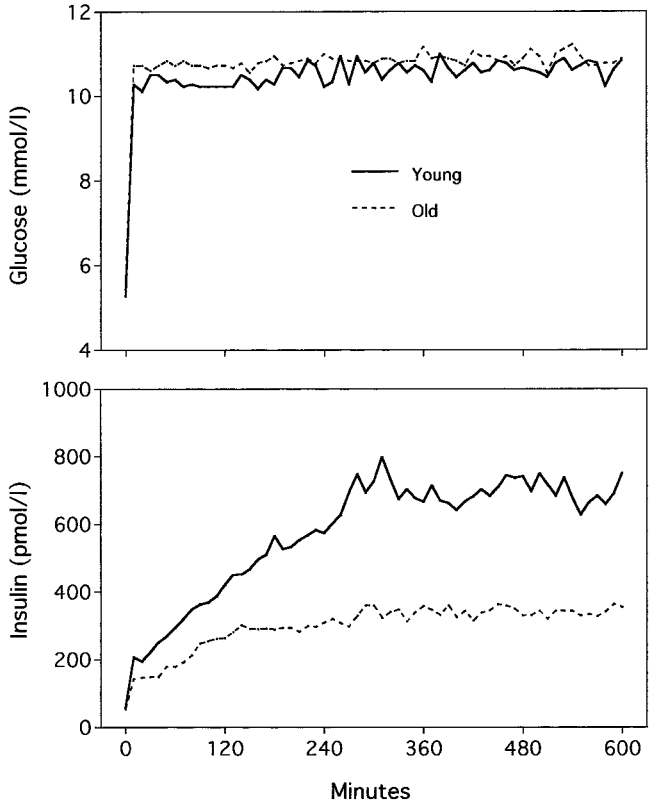

FIG. 5. Mean plasma insulin and glucose concentrations from the 0- to 600-min study.

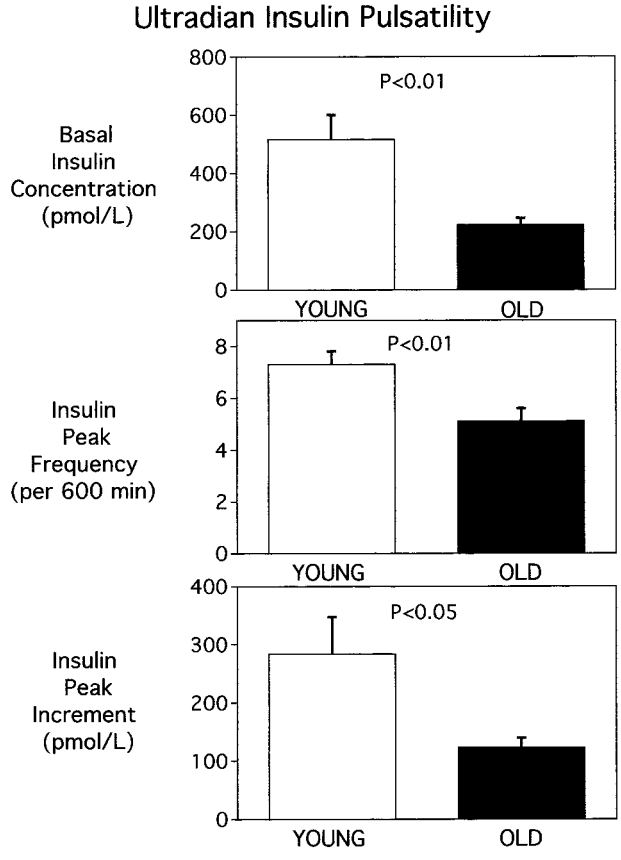

FIG. 6. Ultradian pulse parameters from the 600-min study.

TABLE 3. Cluster analysis of pulse parameters

	Young (n = 10)	Old (n = 10)
Interval (min)	64 ± 3	89 ± 8[a]
Peak area (pmol/L·min)	13,310 ± 4,069	6,365 ± 984
Entropy	1.33 ± 0.07	1.52 ± 0.04[b]

Data are the mean ± SEM.
[a] $P < 0.01$, young *vs.* old.
[b] $P < 0.05$, young *vs.* old.

in our study (frequency and duration of sampling as well as type of pulse detection algorithm) (37). In addition, portal sampling is not feasible in the elderly, and there is strong correlation between pulse parameters detected by portal *vs.* peripheral sampling (37). Thus, as insulin clearance is unchanged with age, we believe that even though we may have failed to detect the absolute number of pulses in all age groups, relative changes in young and old subjects should be similar.

Previous studies that evaluated insulin responses during hyperglycemia in young and older individuals found no important age-related difference in mean insulin levels (34, 35, 38, 39). In the current studies, prolonged hyperglycemia unmasked a defect in glucose-induced insulin release in the elderly volunteers. To our knowledge, this is the first study to evaluate insulin levels in response to prolonged hyperglycemia with matched glucose levels in young and older subjects.

In conclusion, rapid and ultradian insulin pulses in response to hyperglycemia are altered with normal aging. The pathophysiological relationship of these disturbances in the dynamics of insulin release in elderly subjects to the known alterations in carbohydrate metabolism in older individuals will ultimately require further investigation.

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