

Instantaneous Time Course of the Autonomic Cardiovascular and Respiratory Response of Healthy Subjects to Hypoglycemic Stimulus

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Abstract

In 13 healthy subjects we assessed the effect of hypoglycemia (HG) provoked by insulin on: R-R intervals (RR), systolic pressure (SP), diastolic pressure (DP), pulse pressure (PP), respiratory frequency (RF) and tidal volume (V_T) 5-min time series; the instantaneous time courses of their low-frequency (LF_{RR} , LF_{SP} , LF_{DP} , LF_{PP}), high-frequency (HF_{RR} , HF_{Res}) powers and their respective central frequencies ($cfLF_{RR}$, $cfLF_{SP}$, $cfLF_{DP}$, $cfLF_{PP}$), computed by a time-frequency distribution; instantaneous baroreflex (BRS) and respiratory sinus arrhythmia sensitivities (RSAS), obtained by alpha index, and their coherences (cBRS and cRSAS) by cross time-frequency analysis. Peak HG (2.7 ± 0.3 mmol/l) induced: 1) decreases ($p < 0.03$) in five 1-min epoch means (EM) of HF_{RR} , LF_{RR} , BRS and RSAS dynamics, three EM of $cfLF_{PP}$ and cBRS, two EM of $cfLF_{RR}$ and $cfLF_{SP}$; 2) increases ($p < 0.02$) in five EM of SP, DP, PP, V_T and RF, three EM of HF_{Res} , two EM of LF_{SP} and LF_{DP} , one EM of LF_{PP} ; 3) no change in $cfLF_{DP}$, RR and cRSAS. In healthy subjects, insulin-provoked HG elicits changes in the fluctuating time courses of all measures studied, integrating a counterregulatory response of autonomic control mechanisms and vagal depression associated with sympathetic, cardiovascular and respiratory activation.

1. Introduction

In diabetic patients, the rigorous glycemic control to reduce the progression of microvascular complications increases the incidence of hypoglycemia (HG) events [1]. This condition has been associated to increased mortality [2], mainly provoked by fatal cardiac arrhythmias [3], possibly promoted by autonomic-cardiovascular (AC) function impairment [1,3]. Hence the relevance of studies aimed at establishing the physiopathological mechanisms of HG. The AC responses of healthy and diabetic subjects to clamped HG have been studied with spectral analysis techniques for stationary signals, although restricted to HRV analysis, i.e., there are hardly any studies that employ spectral analysis of systolic pressure variability

[4]. Moreover, the reported findings in HG condition are fragmentary and contradictory [5]. The dynamic study using a time-based analysis method of the AC and respiratory (ACR) response of healthy subjects to experimentally provoked HG -condition that impairs the neuronal energy production-, may clarify the mechanism of the counterregulatory response it elicits. Therefore, our aim was to characterize the dynamic and integrative ACR response to the peak uncontrolled HG induced by the injection of insulin, assessed by time-frequency analysis, time courses of baroreflex (BRS) and respiratory sinus arrhythmia (RSAS) sensitivities (computed by alpha index) and their respective instantaneous coherences (cBRS and cRSAS, estimated by a cross-time-frequency distribution).

2. Methods

2.1. Subjects

Thirteen euglycemic, normotensive and sedentary subjects, 6 men and 7 women, were studied. Their mean age, height and weight were 22.2 ± 2.0 years, 162.5 ± 5.2 cm and 61.5 ± 6.4 kg, respectively. Their written informed consent was requested to participate.

2.2. Protocol

On a first visit, the health status and anthropometric variables of the volunteers were evaluated, and on the second, the experimental stage was carried out. After an overnight fast, subjects were injected with a single dose of 0.2 U/kg of rapid-acting insulin. A blood sample of 35 μ l was collected from a fingertip every 10 minutes for the next 180 min to measure glycemic level by enzymatic method (Boehringer). 5-min recordings of ECG, arterial pressure (AP) and respiration (Res) signals were obtained before insulin injection (control), during peak HG, and when glucose returned to baseline (recovery).

2.3. Signal recording and acquisition

ECG was detected at the CM5 bipolar lead using a

bioelectric amplifier (Biopac Systems). Noninvasive AP was measured by Finapres (Ohmeda). Res was detected by a pneumograph (Nihon Kohden). All recorded signals were digitized at a sampling rate of 1 kHz via an acquisition system (Biopac Systems).

2.4. Data processing

Fiducial points were detected in ECG, AP and Res signals to generate time series of RR intervals (RR), systolic pressure (SP), diastolic pressure (DP) and pulse pressure (PP, as SP-DP difference), tidal volume (V_T) and respiratory frequency (RF). All series were cubic-spline interpolated, detrended and resampled at 4 Hz. Time-frequency spectra of the series were estimated with the smoothed pseudo-Wigner-Ville distribution and integrated in the standard frequency bands of HRV analysis to compute the instantaneous time courses of their low-frequency powers (LF_{RR} , LF_{SP} , LF_{DP} , LF_{PP}), their respective central frequencies ($CF_{LF_{RR}}$, $CF_{LF_{SP}}$, $CF_{LF_{DP}}$, $CF_{LF_{PP}}$), and high-frequency powers (HF_{RR} , HF_{Res}). Instantaneous BRS and RSAS were computed by alpha index, using LF_{RR}/LF_{SP} and HF_{RR}/HF_{Res} ratios respectively, and their instantaneous coherences (cBRS and cRSAS) were estimated by cross time-frequency analysis. Measures dynamics were expressed as changes from their mean baseline and ensemble-averaged for visualization.

2.5. Statistical analysis

Data were expressed as mean \pm SD. 1-min epoch means (EM) of measures dynamics were obtained and their differences between baseline, HG and recovery stages were tested by ANOVA for repeated measures. Post-hoc pairwise comparisons were performed by the Tukey test. Linear correlations between the indexes were computed for each subject. Statistical significance was set at $p < 0.05$.

3. Results

From a control value of 4.7 ± 0.5 mmol/l, after about 43 min, insulin induced a peak HG of 2.7 ± 0.3 mmol/l that recovered to control values after around 180 min.

HG modified the baseline fluctuations of the spectral components of RR and SP series, evident in the representative example of time-frequency spectra (Fig. 1) as reduction of LF_{RR} and HF_{RR} fluctuations (Fig. 1B) and increment of LF_{SP} fluctuations (Fig. 1D).

HG increased ($p < 0.02$) the five EM of SP, DP (to a lesser degree), PP, V_T and RF. Though the subtle fall of RR EM was not significant, its variability was notably reduced (Fig. 2A). In recovery, all EM of cardiovascular measures were similar to their control mean, except for SP and RF, that remained elevated (Fig. 2B,E).

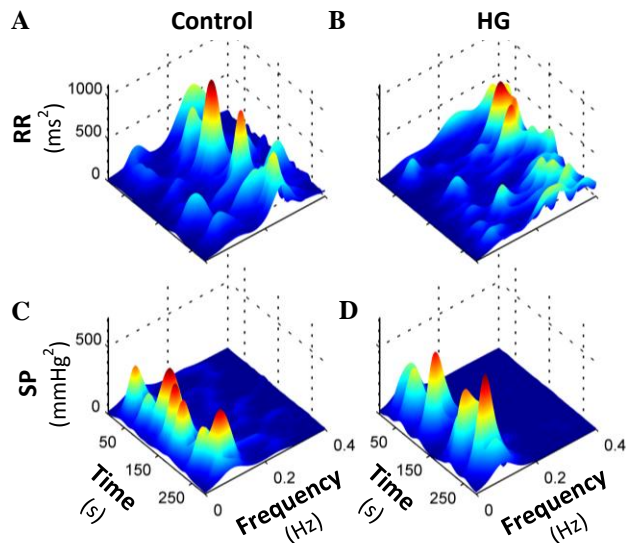


Fig. 1. Representative time-frequency spectra of RR and SP 5-min time series during control (A and C) and HG (B and D) respectively.

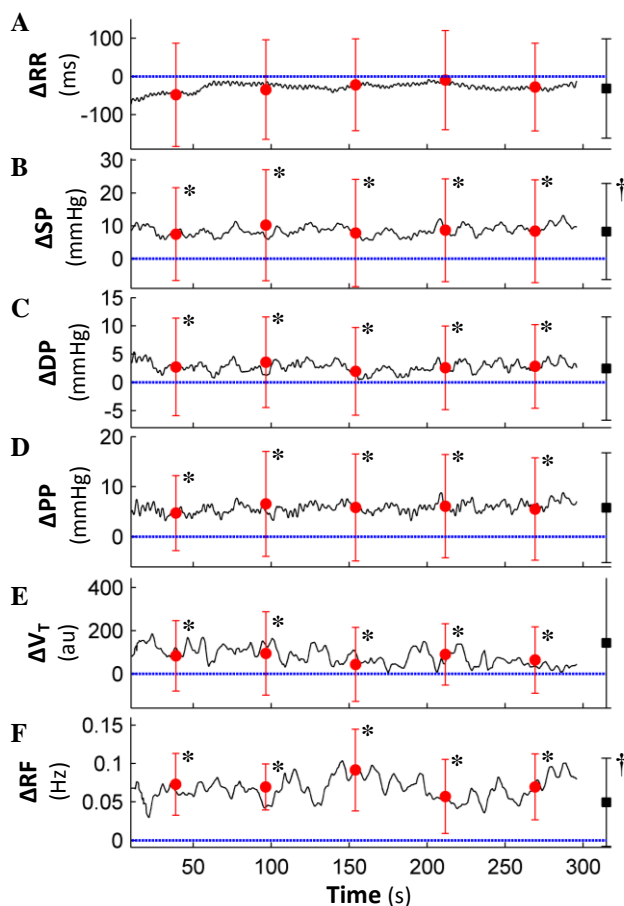


Fig. 2. Ensemble averages and 1-min EM \pm SD of the dynamics of cardiovascular respiratory variables: A)RR, B)SP, C)DP, D)PP, E) V_T and F)RF. ■=mean recovery. * $p < 0.02$ HG vs. control. † $p < 0.03$ recovery vs. control.

In the autonomic activity measures, HG provoked: enhancement ($p<0.03$) of three EM of HF_{Res} , two EM of LF_{SP} and LF_{DP} dynamics, one EM of LF_{PP} ; and decrease ($p<0.02$) of five EM of HF_{RR} and LF_{RR} , three EM of $CF_{LF_{PP}}$, two EM of $CF_{LF_{RR}}$ and $CF_{LF_{SP}}$ dynamics, with no change in EM of $CF_{LF_{DP}}$ (Fig. 3). In recovery, EM of all measures were similar to their respective control mean.

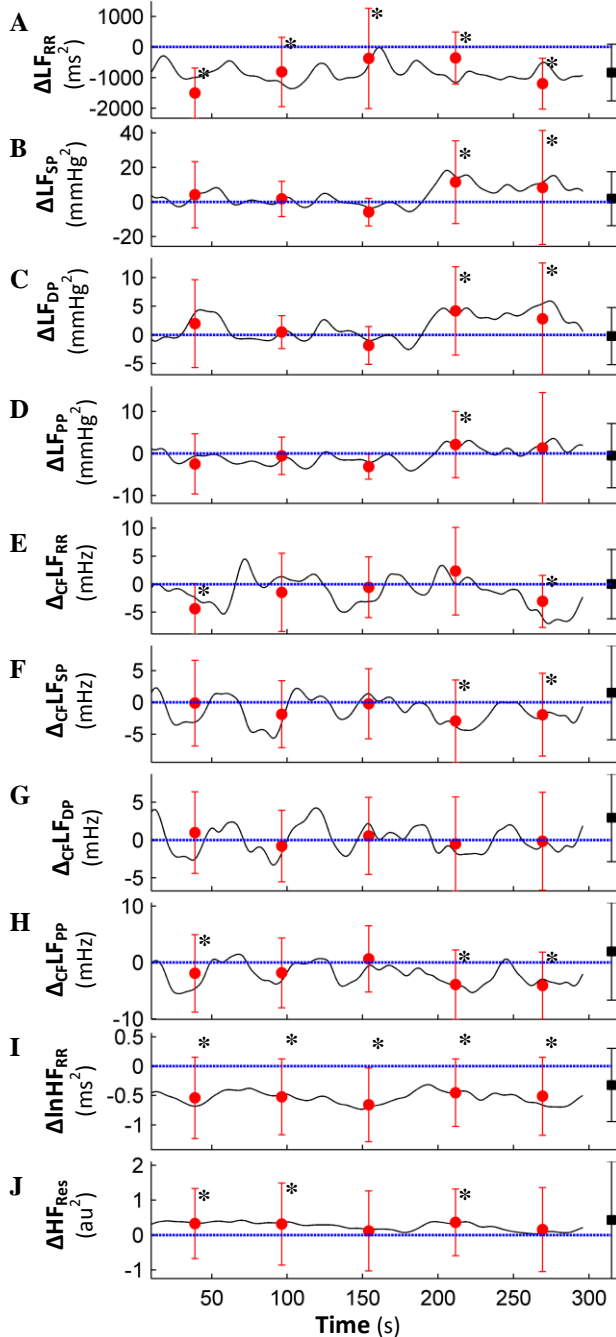


Fig. 3. Ensemble averages and 1-min $EM \pm SD$ of spectral autonomic measures: A) LF_{RR} , B) LF_{SP} , C) LF_{DP} , D) LF_{PP} , E) $CF_{LF_{RR}}$, F) $CF_{LF_{SP}}$, G) $CF_{LF_{DP}}$, H) $CF_{LF_{PP}}$, I) $\ln HF_{RR}$ and J) HF_{Res} . ■=mean recovery. * $p<0.03$ HG vs. control.

In control measures, HG decreased ($p<0.02$) five EM of BRS and RSAS dynamics and three EM of cBRS, but did not change cRSAS (Fig. 4). In recovery, all EM of control measures were similar to their baseline value.

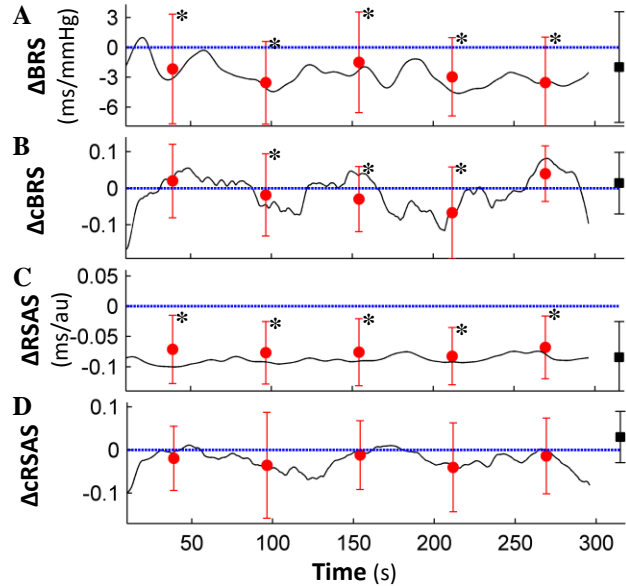


Fig. 4. Ensemble averages and 1-min $EM \pm SD$ of the time courses of autonomic control estimators: A) BRS, B) cBRS, C) RSAS, D) cRSAS. ■=mean recovery. * $p<0.02$ HG vs. control.

Mean $CF_{LF_{SP}}-LF_{SP}$ and $CF_{LF_{DP}}-LF_{DP}$ correlations were -0.33 ± 0.11 and -0.31 ± 0.09 ($p<0.001$) respectively.

4. Discussion

Our robust time-based analysis methodology revealed an integrative functional picture of the time course of the ACR response of healthy subjects to insulin-provoked HG. The measures show fluctuations in the control period that HG changes either in sustained form, such as those of the cardiovascular, respiratory, vagal and autonomic control function, or in transitory form, in which the changes are significant only for some periods, i.e., sympathetic activity indexes. Our main findings on the changes of four types of measures are: *Cardiovascular-respiratory function*, increase in SP, DP, PP, V_T and RF; *sympathetic activity*, fluctuating increments in LF_{SP} , LF_{DP} , LF_{PP} and fluctuating decreases in $CF_{LF_{SP}}$, $CF_{LF_{PP}}$ and $CF_{LF_{RR}}$; *vagal activity*, decline of HF_{RR} ; and *autonomic control mechanisms*, reductions of BRS, cBRS and RSAS. Additionally, $CF_{LF_{SP}}-LF_{SP}$ and $CF_{LF_{DP}}-LF_{DP}$ correlations were significant and negative.

The study of AC function of healthy subjects and diabetic patients under clamped HG condition using HRV spectral analysis has yielded contradictory results [5]. Thus, it has been reported that, in healthy subjects: LF_{RR}

increases [6], does not change [4] or decreases [5]; HF_{RR} increases [7], does not change [4,5] or reduces [6]; heart rate raises [8] or is unchanged [4,5,7]; and that the effect of HG on AC measures is time-dependent [3].

The static and fragmentary functional picture provided by previous studies of the changes induced in AC activity by HG, which have not considered the time course of AC variables and the effects on pulmonary ventilation, cBRS and RSAS, contrasts with the functional motion picture provided by the robust time-based methodology we used: 1) fluctuating sympathetic activation, indicated by the increased power and reduced central frequencies of low-frequency components derived from AP (Fig. 3 B,C,D,E,F,H), contrary to the reported no change in LF_{SP} and LF_{DP} [4], associated to unchanged RR (Fig. 2A) in accordance with previous results [4, 7], SP increase (Fig. 2B), in agreement with the elevation reported [8], slight DP increase (Fig. 2C), in disagreement with the reported reduction [4,8], and PP rise (Fig. 2D), which roughly indicate the elevation of stroke volume, effect previously documented [4,8]; 2) BRS reduction (Fig. 4A), having a permissive effect on AP rise, opposite to the no change reported [4], and with cBRS decrease (Fig. 4B); 3) vagal activity decrease (Fig. 3I) and RSAS reductions (Fig. 4C) that lead to greater RR regularity (Fig. 2A), in disagreement with the HF_{RR} rise reported [7]; 4) respiratory activity increase (Fig. 2E,F, Fig. 3J) with augmented pulmonary ventilation. The aforementioned findings integrate a dynamic counterregulatory mechanism of vagal and control mechanisms activities depression that permits sympathetic, cardiovascular and respiratory activation, which lead to increased cardiac output, specifically cerebral blood flow, possibly with greater blood O_2 content.

To the best of our knowledge, this study is the first to report the fluctuating sympathetic activation, associated to AP increase, reduced vagal activity, BRS and RSAS and hyperventilation as the counterregulatory response of healthy subjects to an insulin-provoked HG of similar level of previous studies [7].

The sympathetic activity markers we used, LF_{SP} , LF_{DP} , LF_{PP} , and LF_{RR} (which decreases, and is considered an ambiguous index), show bursts of increasing sympathetic outflow. Moreover, their central frequencies display possible capability as sympathetic activity markers, supported by their reduction in response to HG (Fig. 3E,F,H) and the significant correlation with their powers.

The reduction of cBRS, which indicates altered input-output coupling of the baroreflex control system, is consistent with the observed reduction of BRS. The decrease of RSAS results from the reduction of vagal activity (Fig. 3I) and the HF_{Res} (Fig. 3J) increase, thus attenuating the modulatory effect of Res on RR dynamics, therefore producing greater regularity in the tachogram (Fig. 2A). Given its relevant modulatory effect on the high frequency components of the spectral autonomic

indexes, it is necessary to record and analyze respiratory activity when studying the effect of physiological or clinical conditions on cardiovascular variability.

In conclusion, in healthy subjects, HG modifies the basal fluctuating time courses of ACR measures, specifically eliciting: sympathetic measures powers increase (except LF_{RR}) associated with central frequencies reductions (except $CF_{LF_{DP}}$); SP and PP increments, indicating stroke volume elevation; BRS and cBRS decreases allowing AP to rise; vagal activity index and RSAS reductions determining greater RR regularity; and increased pulmonary ventilation. These effects integrate a dynamic mechanism of autonomic control systems and vagal activity depression that permits sympathetic, cardiovascular and respiratory activation, whose outcome is increased cardiac output with greater oxygen content to ameliorate the harmful effects of neuroglycopenia.

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