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Changes in pulse pressure, heart rate and double product during squatting in type 1 diabetes according to age

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Short title : Pulse pressure and heart rate in type 1 diabetes

Abstract

Aims We assessed changes in pulse pressure (PP) and heart rate (HR) during a squatting test, as indirect markers of arterial stiffness and cardiovascular autonomic neuropathy (CAN), respectively, according to age and gender in patients with type 1 diabetes mellitus.

Methods We evaluated 160 diabetic patients, divided in four groups of 20 men/20 women according to age (G1: 20–30 years; G2: 31–40 years; G3: 41–50 years; G4: 51–60 years), and 160 non-diabetic matched controls. Each subject underwent a 3-min posture test (standing-squatting-standing) with continuous measurement of arterial blood pressure and HR by a Finapres® device. Overall values throughout the test, baseline levels in initial standing position and squatting-induced changes in PP, HR and PPxHR double product were compared between diabetic patients and healthy controls.

Results In standing position, greater increase in PP and lower reduction in HR with age led to significantly higher PPxHR double product in diabetic patients compared with controls. In squatting position, a more marked PP increase in absence of appropriate reduction in HR resulted in a greater rise in PPxHR double product in diabetic patients than in healthy subjects. No major gender differences were noted, with the exception of a stronger relationship between PP and age in the female population with diabetes. Squatting-derived indices of CAN were also noted with increasing age in diabetic patients.

Conclusions The marked increase in PPxHR double product ("pulsatile stress") according to age, combined with CAN, may contribute to the higher cardiovascular risk of patients with type 1 diabetes.

Keywords finapres, heart rate, pulse pressure, squatting, Type 1 diabetes

Abbreviations ANOVA : ANalysis Of VAriance ; BMI : Body Mass Index; BP : Blood Pressure ; CAN : Cardiovascular Autonomic Neuropathy ; CVD : CardioVascular Disease ; Finndiane : Finnish Diabetes Nephropathy ; HR : Heart Rate ; NS : non-significant ; NA : not applicable. PP : Pulse Pressure ; PPxHR : Pulse Pressure x Heart Rate double product ; SqTs : Squatting sympathetic index ; SqTv : Squatting vagal index.

Introduction

There appears to be an association between type 1 diabetes mellitus and early vascular ageing and a more pronounced increase in arterial stiffness [1]. Arterial pulse pressure (PP) is considered as an indirect marker of arterial stiffness [2,3]. In the Finnish Diabetic Nephropathy (FinnDiane) study [4], a higher systolic blood pressure (BP) and an earlier decrease in diastolic BP resulted in a higher and more rapid increase in PP in patients with type 1 diabetes as compared with non-diabetic control subjects. PP is a risk factor for cardiovascular disease (CVD) and total mortality in patients with type 1 patients (as shown in the EURODIAB study [5,6]) and in patients with type 2 diabetes [7]. Thus, the increased PP reflecting accelerated arterial ageing may contribute to the higher CVD morbidity and mortality in patients with type 1 diabetes.

We recently reported that PP increases according to the duration of type 1 diabetes in an age range (20–60 years), whereas PP remains relatively stable in healthy subjects [8]. Furthermore, we showed that squatting position amplifies PP increase with diabetes duration, and this amplification appears to be higher in women with diabetes than in men with diabetes [9]. Some relationships between PP changes and indices of cardiovascular autonomic neuropathy (CAN) were also described in this population with type 1 diabetes [8]. Because an increase in heart rate (HR) can reduce PP [10,11], and because the presence of diabetic CAN may markedly influence changes in HR [8,12], the relationships between these two parameters warrant further evaluation, particularly during a dynamic posture test such as squatting.

The aim of the present study was to investigate changes in PP, HR and PPxHR double product (“pulsatile stress”), according to age and gender, in a large population of patients with type 1 diabetes and in a non-diabetic control group.

Patients and methods

Patients

A total of 160 patients with type 1 diabetes under follow-up in our department were evaluated. Inclusion criteria were: age 20–60 years; body mass index (BMI) < 30 kg/m²; duration of diabetes > 1 year; HbA_{1c} < 12%. Exclusion criteria were: CVD history (including hypertension); any severe illness during the previous 3 months; renal insufficiency defined as an estimated creatinine clearance < 45 mL/min; medications that may influence

cardiovascular reflexes [8](including beta blockers). All patients with diabetes were treated with multiple insulin injections or continuous subcutaneous insulin infusion.

Subjects were divided according to age and gender into four groups of 40 subjects (20 men and 20 women): G1: 20–30 years; G2: 31–40 years; G3: 41–50 years; and G4: 51–60 years.

In order to differentiate between the effects of duration of diabetes and those of normal ageing [13], 160 healthy subjects were used as controls and matched for age, sex and BMI (20 men and 20 women in each subgroup) (Table 1). The study was accepted by the ethical committee of our institution.

Orthostatic test

The squatting test (successively 1 min standing, 1 min squatting, 1 min standing) is an original active orthostatic test that leads to the most important and fast variations of the hydrostatic level with posture [14]. Squatting produces a prompt increase in cardiac output and arterial BP, essentially attributed to augmented venous return from compression of leg veins. These changes are accompanied by an immediate decrease in HR and forearm vascular resistance, probably due to activation of cardiopulmonary and arterial baroreflexes that implicate the autonomic nervous system. The active transition from squatting to standing results in a greater initial decrease in BP compared with the passive head-up tilt and this makes the squatting test a greater challenge for the cardiovascular reflexes [15]. Therefore, the squatting test has been proposed to derive both parasympathetic and sympathetic indices that are able to detect CAN [16,17] and assess baroreflex sensitivity [18] in patients with diabetes.

Measurements

Changes in systolic/diastolic BP and HR were measured continuously with a Finapres® (from FINger Arterial PRESsure) instrument (Ohmeda, USA) that allows the careful study of cardiovascular reflexes, especially during an orthostatic manoeuvre [19,20]. The Finapres® is based on servoplethysmomanometry and employs the volume clamp technique at the finger level. PP (i.e. systolic BP minus diastolic BP) was calculated automatically throughout the test. As previously described, we calculated mean levels in each subject during the whole period of the test, during initial standing position and during squatting position, after exclusion of the initial transition phase [8,9].

Tachycardia reduces PP, whereas relative bradycardia increases it [10,11]. Diabetic patients with CAN may have fixed tachycardia due to parasympathetic defect [12]. Consequently, the reduction in HR normally observed in the squatting position may be dampened in presence of CAN in patients with diabetes[16–18]. One may hypothesize that higher HR levels may contribute to reduce peripheral PP and thus underestimate arterial stiffness [21]. In an attempt to minimize this interference, we calculated the PPxHR double product in each position in the various groups of subjects described above. Such PPxHR product, which represents an alternative to the classical systolic BPxHR double product used to evaluate cardiac load, is recognized to be associated with an increased CVD risk [22]. Furthermore, a recent study demonstrated that PPxHR product (representing "pulsatile stress") also correlates with (micro)albuminuria [23].

In order to evaluate the presence of CAN, we calculated both a vagal index (SqTv : ratio between the baseline cardiac R-R interval and the longest R-R interval in the first 15 sec of squatting) and a sympathetic index (SqTs : ratio between the baseline cardiac R-R interval and the shortest R-R interval in the first 10–20 seconds of standing after squatting), as previously described [16,17]. These indices, based on HR reduction during squatting and reflex tachycardia during standing, were almost similar to those used recently by a Japanese group in a similar squatting test [18].

Concomitant HbA_{1c} levels (normal values 4–6%) were measured in order to assess recent blood glucose control in patients with type 1 diabetes.

Statistical analysis

A sample size of 32 individuals was required to provide an 80% power to detect a significant (at the two sided 5% level) difference of 10 mm Hg PP between two subgroups, with an assumed standard deviation of PP of 14 mmHg. A difference of 10 mm Hg was chosen as clinically significant based on its association with increased cardiovascular mortality in type 2 diabetes [7] and total mortality in the large EURODIAB cohort of patients with type 1 diabetes [6].

The normality of data distribution was assessed in each of the four subgroups of both diabetic and non-diabetic populations. Normality was confirmed for most variables, including the two key-parameters of PP and PPxHR product. Therefore, age-related changes in all initial parameters and subsequent calculated indices were compared between groups using one-way analysis of variance (ANOVA) and, where overall differences existed, *post-hoc* analyses were conducted with correction for multiple unpaired t-tests. Results were expressed as mean ± SD

values for continuous variables. Nevertheless, overall HR and changes from standing to squatting in PP and PPxHR product did not meet the normality criteria in some subgroups. Therefore, non-parametric ANOVA was also used to assess trends across the four groups in the diabetic and non-diabetic populations and Mann-Whitney calculations were performed to evaluate any possible differences between each diabetic subgroup and its corresponding control subgroup for the main three parameters of PP, HR and PPxHR product (both overall values measured throughout the test and squatting-induced changes) and also the HR-derived SqTv and SqTs CAN indices calculated during squatting. Corresponding data are also presented as median values (with 25% and 75% interquartiles). Multivariate regressions were applied to compare PP between patients with diabetes and controls when adjusting for HR and age effect on PP. The correlation between two variables was assessed with the Spearman correlation coefficient. All results were considered to be significant at the 5% critical level ($p<0.05$). Statistical analyses were carried out using SAS (version 9.1 for Windows) statistical packages.

RESULTS

HR and PP during continuous monitoring

HR and PP profiles throughout the three periods of the squatting test were comparable in patients with type 1 diabetes and in matched healthy subjects in the age group 20–30 years (G1). However, significant differences between the two populations became apparent with increasing age for the two parameters as shown graphically in Fig. 1 and numerically in Table 1.

For patients > 30 years of age, HR was significantly higher in diabetic than in non-diabetic patients when measured throughout the whole test (Table 1) and in the baseline standing position (Fig. 1). There was a progressive reduction in HR with increasing age in the non-diabetic subjects ($P = 0.0004$ for overall HR; $P = 0.0001$ for HR in standing position). This decline of HR with increasing age was less marked in patients with diabetes. While the trend remained significant in initial standing position ($P = 0.011$) (Fig. 2), there was no statistical significance when the overall period of measurement was taken into account ($P =$ NS) (Table 1). This trend became borderline significant when a non-parametric analysis was performed ($P = 0.0307$), but was highly significantly less than in the non-diabetic population ($P = 0.0009$) (Table 2).

PP was slightly higher in diabetic than in non-diabetic patients from G2 to G4, although the difference did not reach the level of statistical significance (except for G3 : $P =$

0.0118) (Fig. 1, Fig. 2). There was a progressive rise in PP with increasing age in the patients with diabetes, whereas such an increase in PP was not observed in control subjects (Fig. 2). These differences between diabetic and non-diabetic subjects were observed with both the parametric (P = 0.0004 in patients with diabetes vs. NS in controls) (Table 1) and non-parametric analyses (P = 0.0005 in patients with diabetes vs. NS in controls) (Table 2).

When adjusting for HR and age effect on PP using a multivariate regression analysis, significantly higher PP levels were observed in patients with diabetes than in non-diabetic controls (P = 0.034).

Baseline PPxHR product calculated during initial standing position showed a trend to opposite changes between the two populations: a progressive increase in patients with diabetes (P = 0.057) compared with a progressive reduction in controls (P = 0.042) (Fig. 2). These opposing patterns resulted in significantly higher PPxHR values in patients with diabetes (G2–G4) than in controls when double product was measured either during initial standing position (Fig. 2) or throughout the test (Table 1). A significant increase in PPxHR product measured during the whole test was observed from G1 to G4 in the diabetic population (p=0.01639), but not in controls (Table1). These results were confirmed and reinforced when using a non-parametric statistical analysis. The trend for increased PPxHR product across the four diabetic groups according to age was significant (P = 0.0175), whereas it remained non-significant in non-diabetic subjects (Table 2).

Quantitative changes in PP, HR and double product during squatting

Squatting resulted in a reduction in HR, but an increase in PP in all groups (Fig. 1, Fig. 2). With increasing age, squatting-associated reduction in HR decreased progressively in both diabetic (P = 0.00008) and non-diabetic (P = 0.0004) individuals (Fig. 2, Table 3). Despite higher baseline HR levels, the decrease in HR during squatting was similar in diabetic and in non-diabetic subjects in all G1–G4 groups (Fig. 2, Table 3). Conversely, with increasing age, the elevation of PP during squatting became progressively more marked. The age-related change was not significant in non-diabetic subjects, whereas it was highly significant in patients with type 1 diabetes in the age range 20–60 years (P = 0.0001) (Fig. 2, Table 3). Consequently, whereas squatting-induced PP increase was similar in diabetic and non-diabetic subjects in G1 and G2, it was significantly greater in diabetic vs. non-diabetic subjects in G3 (P = 0.0449) and highly significantly greater in G4 (P = 0.0084) (Figure 2). Similar results were obtained with the non-parametric analysis, which showed a highly significant trend for

PP increase according to age in patients with type 1 diabetes (P = 0.0004), but not in control subjects (P = NS) (Table 2).

When adjusting for HR and age effect on squatting-induced PP changes, a significant difference was observed between patients with diabetes and non-diabetic subjects (P = 0.012). Patients with type 1 diabetes showed a significantly greater PP increase than controls. Remarkable squatting-induced changes were observed in PPxHR double product according to age. Changes in PPxHR product increased progressively with age in both non-diabetic and diabetic subjects (age-effect: $P < 0.00001$ in both populations using the parametric statistical analysis). Nevertheless, the increase tended to be more pronounced in diabetic than in non-diabetic individuals (mean values G4: 915 vs. 519 mmHg*min^{-1}, respectively; P = 0.0399) (Fig. 2; Table 3). Using the non-parametric analysis, the trend remained highly significant in both the diabetic (P = 0.00001) and non-diabetic populations (P = 0.0001). The difference also persisted between diabetic and non-diabetic subjects in G4 groups (median values G4: 719 vs. 500 mmHg*min^{-1}, respectively; P = 0.0311).

Effects of diabetes duration on CAN indices

Changes in HR-derived indices during posture showed a progressive increase in SqTv from G1 to G4 in patients with type 1 diabetes (P = 0.0001) and in non-diabetic individuals (P = 0.0001), reflecting the dampened bradycardia during squatting according to increasing age (see above; Fig. 1). In contrast, SqTs index (reflecting post-squatting tachycardia), significantly decreased with increasing age across the four diabetic subgroups (P = 0.0018), but remained almost stable in the non-diabetic subjects within the same age range (P = NS) (Table 2). No significant differences in SqTv were observed between patients with diabetes and non-diabetic patients in any of the four groups, except for G3 in which SqTv index was higher in patients with diabetes than in controls (P = 0.0245). In contrast, SqTs was higher in patients with type 1 diabetes than in healthy subjects in G2 (P = 0.0193), G3 (P = 0.0022) and G4 (P = 0.0647) (Table 3). These results were confirmed by the non-parametric analysis (Table 2).

Changes in PP with age according to gender

In general, the aforementioned changes associated to squatting in HR, PP and PPxHR product, as well as SqTv and SqTs were not significantly different between men and women in any subgroup of either non-diabetic or diabetic individuals (data not shown).

However, the correlation of overall PP with age showed some important gender-related differences. Indeed, the correlations were similar in diabetic (r = 0.2188; P = 0.0512) and non-diabetic (r = 0.1833; P = 0.1036) men. In contrast, in the female population, the correlation between PP and age was only observed in presence of type 1 diabetes and was highly significant (r = 0.4151; P = 0.0001), but was not observed in the absence of diabetes (r = 0.0763; P = 0.5009). The correlations between squatting-induced changes in PP and age were not significant in healthy men (r = 0.1742; P = 0.1222) or women (r = 0.1170; P = 0.3014), but were significant in diabetic patients, both in males (r = 0.2518; P = 0.0242) and to an even greater extent in females (r = 0.3724; P = 0.0007).

Discussion

The main findings of the present study are: 1) higher HR, PP and HRxPP product after the age of 30 years in patients with type 1 diabetes and poor glycaemic control as compared to non-diabetic subjects; 2) higher overall PP levels and greater squatting-induced PP increases in patients with diabetes than in non-diabetic controls when adjusting for HR and age effect on PP using a multivariate regression analysis; 3) a greater difference between diabetic and non-diabetic women than between diabetic and non-diabetic men in the correlation between PP and age; and 4) altered parasympathetic and sympathetic indices measured during squatting with increasing age in patients with poorly controlled type 1 diabetes .

The earlier and greater PP increase observed in patients with type 1 diabetes as compared to controls in the age range 20–60 years is in agreement with previous studies from our group [8,9] and with the observational data from the large cross-sectional, case-control FinnDiane study [4]. As PP is considered an indirect marker of arterial stiffness [2,3], these PP results are in agreement with accelerated vascular ageing in the population with type 1 diabetes [1], especially when patients also have poor glycaemic control [24,25]. This earlier and greater PP increase is probably clinically relevant. Indeed, in the EURODIAB Prospective Complications Study, a 12 mmHg higher PP was observed in the patients with type 1 diabetes who died than those who survived, and PP was significantly associated with all-cause mortality [6]. These data confirm similar observations reported from a population with type 2 diabetes [7].

CAN incidence increases with diabetes duration [12] and also exposes patients with diabetes to an increased mortality risk [26]. When evaluating HR variations during and after squatting, we showed that the two indices reflecting parasympathetic (SqTv) and sympathetic

activity (SqTs) [16,17] were significantly affected by age. There may be correlations between PP and CAN, as previously shown and discussed by our group [8]. Increased systolic BP was identified as a factor associated with an increased risk of developing CAN in the cohort of patients with type 1 diabetes of the EURODIAB Prospective Complications study [27].

HR is known to influence PP directly: reduction in HR leading to higher stroke volume and prolonged diastole period increases PP, and *vice versa* [10,11]. Therefore, the higher HR in initial standing position and the progressive loss of reduction in HR during squatting seen in patients with long-standing diabetes may contribute to diminishing the increase in PP due to arterial stiffness and thus to underestimate the impact of diabetes on the arterial wall [24]. A way to integrate these two opposing factors is to calculate the PPxHR product [22,23]. This is in line with the more classical systolic BPxHR double product, used as a surrogate measure of cardiac workload and myocardial oxygen demand [28]. Our results showed a progressive increase in PPxHR according to age, in both non-diabetic and diabetic patients. However, the more marked increase in patients with type 1 diabetes may be associated with a higher CVD risk [22].

The influence of age on stiffening of arterial wall appears to be more marked in women with type 1 diabetes than in men [4,29]. We previously reported that the amplification of PP increase with squatting according to diabetes duration was more pronounced in women than in men with diabetes [9]. In the present study, the correlation between overall PP and age was much stronger in diabetic than in non-diabetic women, whereas this correlation was similar in men with diabetes and male healthy subjects. The correlation between squatting-induced PP rise and age was also stronger in diabetic women than in men with diabetes. This may be of importance in explaining the increased susceptibility to CVD in women with diabetes [9,29]. Plasma free fatty acids were recently shown to be associated with PP in women with type 1 diabetes, but not in men with type 1 diabetes, and their effect appeared to vary by abdominal adiposity, particularly subcutaneous adipose tissue [30]. All of these findings may help to explain the loss of the sex difference in CVD in type 1 diabetes.

There are some limitations of our study. Firstly, finger is an unusual site to measure PP. For example, the brachial site was used in the FinnDiane study [4]. Several studies have demonstrated that absolute brachial and finger PP measurements are not identical with larger differences in systolic BP at the finger site. However, the differences were generally small as compared to the magnitude of the responses during dynamic tests and therefore not considered of clinical relevance [31,32]. Furthermore, because of the amplification of the PP between central (aorta) and periphery arteries, PP measured at the finger site may not reflect

central BP. For instance, peripheral PP does not provide an accurate assessment of changes in central haemodynamics in relation to acute changes in HR in healthy subjects [21]. This may be an important issue as central BP is more closely related to organ damage than periphery BP. However, in a study evaluating the accuracy of continuous BP measurement at the finger site, similar changes in BP and PP were observed as compared with intra-aortic pressure measurement in healthy subjects, both at rest and during low-to-moderate levels of exercise-induced stress [33]. Diabetes has been shown to be an independent risk factor for higher central/periphery PP ratio in patients with type 2 diabetes and this difference may be associated with a higher CVD risk in this population [34]. In patients with type 1 diabetes, relatively higher PP value at the central site for a given PP level at the periphery was observed compared to healthy controls, and this phenomenon increased according to duration of the disease [35]. Thus, the differences observed between patients with type 1 diabetes and healthy controls in the present study where PP was recorded at the finger site would have been even greater if central (rather than periphery) BP had been measured. Thirdly, PPxHR product is a relatively complex parameter as PP reflects not only total distensibility of the arterial tree, but also stroke volume output of the heart [10], and HR is under control of complex neural and hormonal regulation. However, this product, known as ("pulsatile stress") [23], has been proposed as an independent marker of CVD, in a similar manner to the more classical systolic BPxHR double product [21,26], and was recently shown to be correlated with (micro)albuminuria [23]. The reproducibility and prognostic value of PPxHR double product, especially in relation to the squatting test, do deserve, further investigation. Fourthly, glucose control of patients with type 1 diabetes evaluated in the present study was far from optimal, despite intensified insulin therapy. Therefore, these results may not necessarily be extrapolated to patients with near normoglycaemia for many years [24]. In the FinnDiane, the ambient level of glycaemic control was not associated with increased PP, but the time of exposure to hyperglycaemia appeared to play a fundamental role in the process of premature arterial stiffening in patients with type 1 diabetes [4].

Conclusion

The original squatting test with continuous monitoring of BP and HR allows simultaneous assessment of PP, as an indirect measure of arterial stiffness, and HR changes, as a marker of CAN. Type 1 diabetes was associated with a progressive increase in PP in the 20–60 year age range where no significant influence of ageing was observed in a non-diabetic population. When taking into account the relative tachycardia seen in patients with diabetes,

the differences in PPxHR double product (“pulsatile stress”) were even more impressive between diabetic and non-diabetic individuals. The combination of both haemodynamic and autonomic disturbances may explain the higher CVD risk and the increased total mortality of patients with type 1 diabetes and long-standing poor glucose control.

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Figures

Figures

Figure 1 : Changes in pulse pressure (PP) (left panels) and heart rate (HR) (right panels) during a posture test [1 min standing (ST) – 1 min squatting (SQ) – 1 min standing (ST)] in four groups of diabetic (full circles) versus non-diabetic (open circles) subjects separated according to age (top-down : from G1 to G4). Results correspond to mean values (40 subjects in each group, 20 men and 20 women). The grey zone corresponds to the 1-min squatting period preceded and followed by a 1-min standing period.

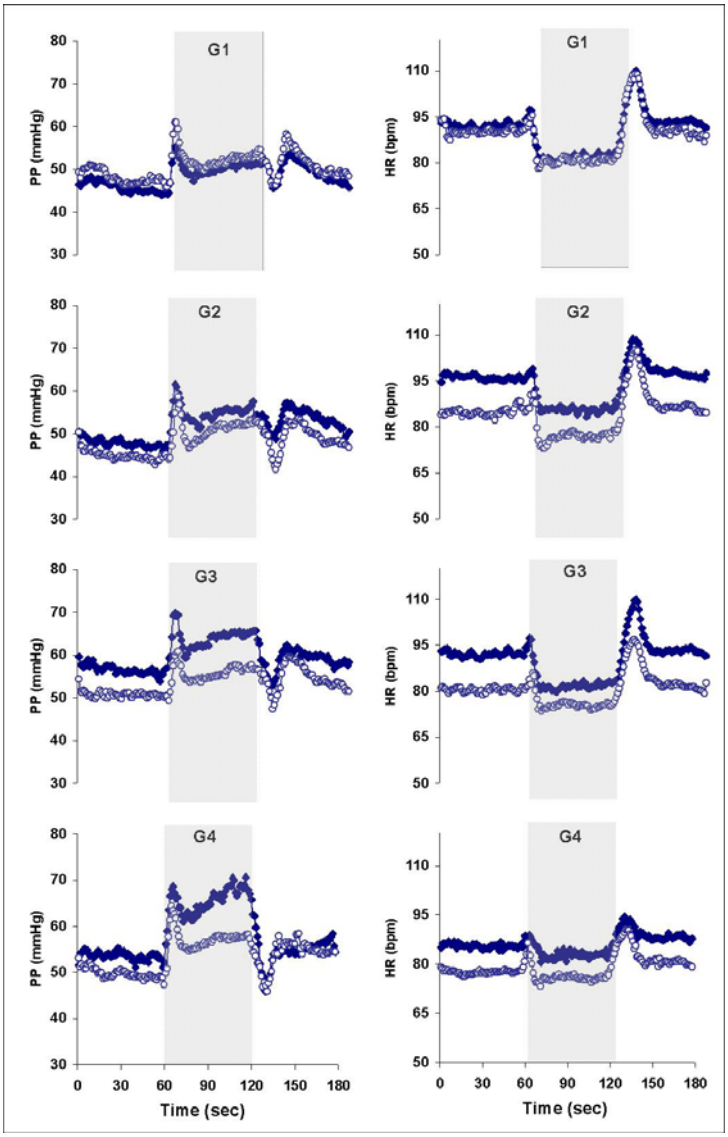
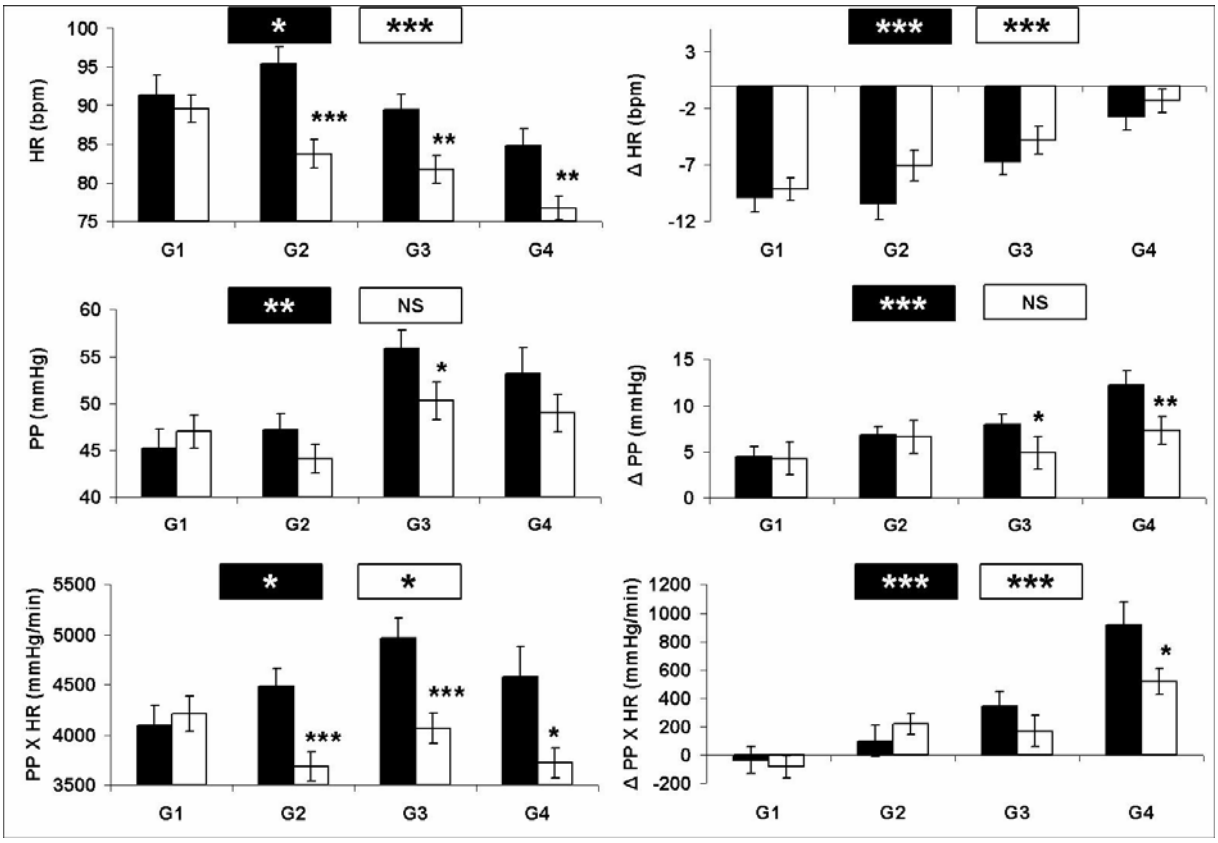


Figure 2 : Mean values of heart rate (HR), pulse pressure (PP) and PP*HR double product in the four groups of diabetic (black bars) versus non-diabetic (white bars) individuals separated according to age (from G1 to G4; n = 40 in each group). Left panels correspond to baseline values in initial standing position while right panels correspond to changes in mean values induced by squatting. Statistical analysis used ANOVA for trends with age in each population and unpaired t test for differences between diabetic and non-diabetic subjects in each group.
* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$



Tables

Table 1: Characteristics of the patients with type 1 diabetes (D) and non-diabetic subjects (ND) used as controls in the four subgroups of 40 individuals (20 men, 20 women) divided according to age, and average values of mean blood pressure (BP), systolic BP, diastolic BP, pulse pressure (PP), heart rate (HR) and PPxHR double product recorded during the whole 3-min squatting test. Results are expressed as mean ± SD. Anova: analysis of variance. NS : non significant. NA : not applicable. BMI : body mass index.

	GROUP 1 20 - 30 years		GROUP 2 31 – 40 years		GROUP 3 41 – 50 years		GROUP 4 51 - 60 years		P value Anova G1-G4	
	D	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**
N (Male/Female)	40 (20/20)	40 (20/20)	40 (20 /20)	40 (20/20)	40 (20/20)	40 (20/20)	40 (20/20)	40 (20/20)	NA	NA
Age (yrs)	25 ± 3	25 ± 3	35 ±3	36 ± 3	45 ± 3	45 ± 3	55 ± 3	56 ± 3	0.00001	0.00001
Diabetes duration (yrs)	12 ± 7	NA	17 ± 8	NA	23 ± 10	NA	26 ± 12	NA	0.00001	NA
BMI (kg/m^2)	23.2 ± 3.1	22.3 ± 2.6	24.4 ± 2.7	23.3 ± 2.7	23.8 ± 2.9	24.0 ± 3.1	23.5 ± 2.9 [a]	25.2 ± 3.8	NS	0.0004
HbA1c (%)	9.1 ± 2.1	NA	8.3 ± 1.2	NA	8.6 ± 1.1	NA	8.5 ± 1.3	NA	NS	NA
Mean BP (mm Hg)	83 ± 12	79 ± 10	88 ± 21	85 ± 15	86 ± 15	84 ± 12	83 ± 13	88 ± 14	NS	0.032
Systolic BP(mm Hg)	117 ± 18	115 ± 15	124 ± 24	118 ± 17	128 ± 20	121 ± 19	124 ± 22	124 ± 19	NS	NS
Diastolic BP (mm Hg)	69 ± 10	65 ± 10	72 ± 20	69 ± 14	68 ± 13	67 ± 11	66 ± 10	70 ± 14	NS	NS
PP (mm Hg)	48 ± 13	50 ± 11	52 ± 10	48 ± 9	60 ± 12 [a]	54 ± 13	58 ± 17	53 ± 14	0.0004	NS
HR (bpm)	89 ± 14	86 ± 9	93 ± 12 [c]	81 ± 10	88 ± 11 [b]	82 ± 10	85 ± 13 [b]	79 ± 10	NS	0.0004
PP x HR product ($mm\ Hg*min^{-1}$)	4293 ± 1316	4405 ± 1095	4798 [c] ± 1033	4014 ± 890	5286 [c] ± 1260	4351 ± 996	5024 [a] ± 1916	4146 ± 1170	0.01639	NS

Unpaired t test D (patients with type 1 diabetes) vs ND (non-diabetic controls) : a : $P < 0.05$ b : $P < 0.01$ c : $P < 0.001$

Table 2: Non parametric analysis of overall pulse pressure (PP), heart rate (HR) and PPxHR double product, and of squatting-induced changes (delta) in PP, HR and PPxHR double product in the patients with type 1 diabetes (D) and non-diabetic subjects (ND) used as controls in the four subgroups divided according to age. Values of two indices of cardiovascular autonomic neuropathy (CAN), derived from changes in HR during (SqTv) and after (SqTs) squatting, are also given for the various subgroups. Results are expressed as median (25% ; 75% interquartiles). Non parametric one-way Anova (analysis of variance).

	GROUP 1 20 - 30 years		GROUP 2 31 – 40 years		GROUP 3 41 – 50 years		GROUP 4 51 - 60 years		P value Anova G1-G4	
	D	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**
Overall PP (mm Hg)	49 (40 ; 58)	51 (43 ; 57)	52 (45 ; 59)	46 (43 ; 53)	60 (52 ; 66)[a]	52 (44 ; 60)	57 (46 ; 68)	57 (42 ; 63)	0.0005	NS
Overall HR (bpm)	91 (82 ; 98)	87 (82 ; 96)	92 [b] (84 ; 101)	83 (78 ; 89)	89 [b] (82 ; 98)	82 (76 ; 87)	83 [a] (79 ; 93)	79 (70 ; 84)	0.0307	0.0009
Overall PPxHR	4266 (3420 ; 5009)	4243 (3773 ; 5037)	4728 [b] (4068 ; 5568)	3952 (3438 ;4604)	5118 [b] (4253 ; 6164)	4226 (3766 ; 4941)	4703 [a] (3915 ; 5769)	4164 (3316 ; 4940)	0.0175	NS
Delta PP (mm Hg)	4 (1 ; 8)	4 (1 ; 8)	7 (4 ; 9)	6 (4 ; 10)	8 [a] (5 ; 10)	6 (1 ; 9)	11 [a] (5 ; 20)	7 (5 ; 11)	0.0004	NS
Delta HR (bpm)	-10 (- 15 ; -4)	-10 (- 14 ;- 6)	-11 (- 14 ;- 6)	- 5 (- 11 ; - 2)	- 7 (- 11 ; - 2)	- 5 (- 12 ; -1)	- 4 (- 7 ; 2)	-1 (- 5 ; 3)	0.0001	0.0001
Delta PPxHR	- 55 (- 219 ; 380)	- 164 (- 393 ; 231)	38 (- 245 ; 387)	179 (- 34 ; 550)	344 (- 4 ; 741)	147 (- 200 ; 546)	719 [a] (386 ; 1369)	500 (94 ; 679)	0.00001	0.0001
SqTv index	0.78 (0.72 ; 0.86)	0.77 (0.69 ; 0.84)	0.81 (0.76 ; 0.90)	0.78 (0.71 ; 0.87)	0.90 (0.83 ; 0.95)[a]	0.81 (0.75 ; 0.91)	0.89 (0.85 ; 0.95)	0.91 (0.86 ; 0.97)	0.00001	0.00001
SqTs index	1.21 (1.18 ; 1.28)	1.24 (1.18 ; 1.30)	1.19 (1,13 ; 1,24)[a]	1.26 (1.18 ; 1.34)	1.14 (1,09 ; 1,20)[b]	1.22 (1.16 ; 1.28)	1.13 (1.05 ; 1.25)	1.17 (1.12 ; 1.22)	0.0011	0.0241

Mann-Whitney for D vs ND comparison in each age group. a : $P < 0.05$ b: $P < 0.01$.

Table 3: Changes in systolic blood pressure (BP), diastolic BP, mean BP, pulse pressure (PP), heart rate (HR), and PP x HR product from the initial standing position to the squatting position in the patients with type 1 diabetes (D) and non-diabetic subjects (ND) used as controls in the four subgroups divided according to age. Values of two indices of cardiovascular autonomic neuropathy (CAN), SqTv and SqTs, derived from changes in HR during and after squatting, respectively, are also given for the various subgroups. Results are expressed as mean ± SD. Anova: analysis of variance. NS : non significant.

	GROUP 1 20 - 30 years		**GROUP 2 31 – 40 years**		**GROUP 3 41 – 50 years**		**GROUP 4 51 - 60 years**		**P value Anova G1-G4**	
	D	**ND**	D	**ND**	D	**ND**	D	**ND**	**D**	**ND**
n	40	40	40	40	40	40	40	40	NA	NA
Changes in mean BP (mm Hg)	5 ± 9	5 ± 6	7 ± 7	5 ± 7	7 ± 7	5 ± 5	14 ± 13	9 ± 9	0.00026	0.0087
Changes in systolic BP (mm Hg)	6 ± 13	6 ± 10	9 ± 10	8 ± 10	11 ± 9 [a]	6 ± 9	20 ± 16 [a]	13 ± 11	0.00001	0.0093
Changes in diastolic BP (mm Hg)	2 ±8	2 ± 5	3 ± 7	1 ± 7	3 ± 6	1 ± 5	8 ± 11	5 ± 9	0.0017	0.007
Changes in PP (mmHg)	5 ± 7	4 ± 7	7 ± 6	7 ± 6	8 ± 7 [a]	5 ± 7	12 ± 10 [b]	7 ± 6	0.0001	NS
Changes in HR (/min)	-10 ± 8	-9 ± 6	-10 ± 9	-7 ± 9	-7 ± 7	-5 ± 8	-3 ± 8	-1 ± 7	0.00008	0.0004
Changes in PPxHR product ($mmHg*min^{-1}$)	-35 ± 597	-82 ± 488	101 ± 688	221 ± 456	348 ± 634	170 ± 694	915 [a] ± 1043	519 ± 579	0.00001	0.00001
SqTv index	0.79 ± 0.10	0.76 ± 0.14	0.82 ± 0.10	0.79 ± 0.10	0.88 ± 0.09 [a]	0.83 ± 0.13	0.88 ± 0.09	0.89 ± 0.12	0.00001	0.00001
SqTs index	1.24 ± 0.12	1.24 ± 0.11	1.20 ± 0.11 [a]	1.26 ± 0.11	1.16 ± 0.10 [b]	1.23 ± 0.11	1.15 ± 0.13	1.21 ± 0.15	0.0018	NS

Unpaired t test D (patients with type 1 diabetes) vs ND (non-diabetic controls) : a : $P < 0.05$ b : $P < 0.01$