Intra and inter-day repeatability of peripheral arterial function: suitability and potential limitations

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Running title: Intra and inter-day RHI repeatability

ABSTRACT

The present study aimed to investigate the inter- and intraday repeatability of reactive hyperemia index (RHI) measured by Endo-PAT in healthy volunteers.

Interday RHI repeatability was tested in two consecutive days in a group of thirty-one male subjects. Intraday repeatability was investigated at baseline and after 2 and 4 h in a group of sixteen volunteers. Data were evaluated by analysis of variance. Bland–Altman plot, coefficient of variation (CV), coefficient of repeatability (CR) and intraclass correlation coefficient (ICC) were measured.

While interday RHI repeatability was found to be reliable (CV: 6.0%; CR: 0.51; ICC: 0.77), multiple evaluations within the same day significantly ($p<0.001$) affected RHI (repeatability of the measurement - CV: 18.8%; CR: 1.26; ICC: 0.48). In particular, a significant increase in RHI occurred at 4 h compared to 2 h (+16.8%; $p<0.05$) and to baseline (+30.1%; $p<0.05$).

In conclusion, RHI showed good interday but poor intraday repeatability. Multiple evaluations increased RHI especially in subjects with endothelial dysfunction who improved or reversed their impairment. These results show the potential limitations of multiple Endo-PAT measurements within the same day and the importance of standardizing the protocols before RHI evaluations.

KEY WORDS: peripheral arterial tonometry, reactive hyperemia index, augmentation index, repeatability, healthy young male

Abbreviations:

AI, augmentation index; AI@75, augmentation index normalized for heart rate of 75 bpm; ANOVA, Analysis of variance; CR, coefficient of repeatability; CV, coefficient of variation; DBP, diastolic blood pressure; F-RHI; Framingham reactive hyperemia index; HR, heart rate; ICC, intraclass correlation coefficient; LSD, Least Significant Difference; PAT, peripheral arterial tone; RHI, reactive hyperemia index; SD, standard deviation; SEM, standard error of mean SBP; systolic blood pressure.

INTRODUCTION

Endo-PAT is a novel non-invasive plethysmographic system developed to measure peripheral arterial function at the level of the fingertips through an index of reactive hyperemia (RHI). This index is a ratio of the post-to-pre occlusion PAT amplitude of the tested arm, divided by the post-to-pre occlusion PAT amplitude of the control arm [1, 2]. Simultaneously with endothelial function, Endo-PAT can also measure the peripheral augmentation index (AIx), which is an established marker of arterial wave reflection [3]. Thus, PAT technology is particularly interesting for the application in clinical research studies, since the measurement of peripheral arterial function and arterial stiffness requires separate equipments. In addition, Endo-PAT has the advantage of providing reliable and reproducible results; it is operator-independent and it records systemic changes on the contra-lateral arm, allowing for an internal control system [4, 5]. Several studies have found a significant correlation between peripheral arterial tonometry and flow-mediated dilation [6, 7], which represents the most popular clinical method to assess endothelial function by means of brachial artery ultrasound scanning [6, 7].

Endothelial nutrition is a new and innovative concept that involves the study of the role of dietary compounds on endothelial function. Preventing the endothelium from becoming dysfunctional by means of nutrients or extra-nutrients that modulate vascular tone and maintain homeostasis of the endothelium, can be of great importance to human health. Endo-PAT has been used to evaluate the effect on arterial function of both short- and long-term exposure to foods and bioactive compounds, however the results are still conflicting [8-14]. This discordance may depend on subject characteristics such as age, sex, dietary and life-style habits and physical activity, but also on the experimental protocol used. A report of the International Brachial Artery Reactivity Task Force suggested that multiple measurements performed within the day can affect vascular function due to the stimulation of endothelial dependent mechanisms [6]. Multiple evaluations could affect endothelial function and arterial stiffness measurements, which would have significant

implications for clinical practice and for trials involving PAT measurements. This information is crucial since the effects of foods and/or bioactives in the modulation of vascular function could be of lower magnitude when compared to drugs, so that the overall impact on endothelial function may be masked or overestimated following multiple measurements. Therefore, the aim of the present study was to evaluate the intra- and interday repeatability of arterial function measurements by Endo-PAT in a group of healthy male volunteers. The evaluation of intra- and interday measurement repeatability is pivotal for the interpretation of RHI values and their variations over time, as well as for designing clinical trials and determining the appropriate sample size.

Materials and Methods

Subjects recruitment

A homogeneous group of 47 healthy, male volunteers was recruited from the student population of the University of Milan. Subjects were selected according to the following criteria: 20-30 years of age, not overweight, non-smokers, moderate physical activity (25-30 min per day of brisk walk or jog) and moderate alcohol consumption (up to 10-14 drinks, expressed as red wine or beer, per week). Subjects were selected based on an interview to evaluate their dietary habits and the use of a food frequency questionnaire. Subjects declared no history of cardiovascular, coronary, diabetes, hepatic, renal, or gastrointestinal diseases, dyslipidemia, anemia, chronic asthma, allergy, traumas of the arms or hand, fingers, atopic dermatitis, thyroid disturbance, depression, anxiety, palpitations and chronic backache. Moreover, subjects did not make use of supplements, drugs or medications for at least one month before the beginning of the study.

Experimental design

The study was performed in accordance with the ethical standards established in the 2003 Declaration of Helsinki. All participants signed a written informed consent.

Subjects abstained from eating bioactive-rich foods for two days before the experimentation. Specific attention was devoted to foods with potential vasoactive properties such as chocolate, soft fruits (i.e. blueberries, strawberries), red or blue fruits, red wine, and green tea. Volunteers were asked to limit coffee intake to three espresso shots per day, as well as caffeine-rich beverages (e.g. energy drinks), to standardize their intake and reduce a factor affecting vascular function. The day before the experiment and during the trial, breakfast, lunch and dinner were standardized. Breakfast consisted of milk and biscuits, while lunch was composed of two sandwiches (one with cooked ham and cheese and one with raw ham). For dinner, subjects could eat pasta or rice with butter and cheese, a steak with potatoes, two slices of white bread and no more than one espresso coffee. Dinner was eaten by 9.00 pm. No alcoholic beverages or soft drinks were permitted. Overall, the meals were standardized in order to provide adequate energy/macronutrients intake, limiting bioactives and taking into account Italian dietary habits. Moreover, all participants were asked to refrain from physical activity from the day before the experiments.

Interday repeatability of RHI was tested in 31 subjects at the same time of the day in two consecutive days. The day of the experiment, overnight-fasted subjects came to the laboratory of the University at 7.00 a.m. Intraday repeatability of RHI was tested in 16 subjects. The measurements were performed at 7.00 a.m. (time 0 h) after an overnight fast and at 2 and 4 h from the consumption of a standard light breakfast composed of milk and biscuits (about 200 kcal). In this protocol breakfast was added since a long fasting period could cause a stress condition in the volunteers.

Evaluation of peripheral arterial function

Endothelial-dependent vasodilation in the small finger arteries was assessed by Endo-PAT2000 (Itamar Medical Ltd., Caesarea, Israel). The Endo-PAT equipment consists of two finger-mounted probes, which includes a system of inflatable latex air-cushions within a rigid external case; pulsatile volume changes of the fingertip are sensed by a pressure transducer, located at the end of

each probe, and transferred to a personal computer where the signal is band pass-filtered (0.3 to 30 Hz), amplified, displayed, and stored. For the evaluation, subjects were in the supine position with both hands on the same level in a comfortable, thermo-neutral environment. Tests were performed in a dark, noise-and climate-controlled (22-24°C) room. Arterial systolic and diastolic blood pressure and heart rate frequency were measured before starting the test. A blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm). After a 10-min equilibration period, the blood pressure cuff on the study arm was inflated to 60 mmHg above systolic pressure for 5 min. The cuff was then deflated to induce reactive hyperemia (RH) while the signals from both PAT channels (Probe 1 and Probe 2) were recorded by a computer. The technique provides values for the calculation of RHI and Framingham (F) RHI, which give an indication of the endothelial vasodilator function [4, 15]. FRHI is an alternative method of calculating RHI, developed within the Framingham Heart Study. FRHI is automatically provided by the instrument and calculated by taking into account a different post-occlusion hyperemia period (90 to 120 s) without baseline correction factor. Evidence from the Framingham Heart Study suggested a strong correlation between FRHI and cardiovascular risk [15]. Low RHI and FRHI scores indicate endothelial dysfunction. A RHI value of 1.67 provides a sensitivity of 82% and a specificity of 77% for diagnosing endothelial dysfunction [16]. The Endo-PAT device also generates the digital augmentation index (AI), a measure of pulse wave reflection and a surrogate marker for arterial stiffness. Augmentation index derived from digital pulse volume waveforms and strongly correlated to aortic AI. Peripheral AI is calculated from the shape of the pulse wave recorded by the probes during baseline [17-18]. Because digital AI is influenced in an inverse and linear manner by heart rate, the AI was automatically normalized by considering a heart rate of 75 bpm (AI@75).

146 **Statistical analysis**

147 Thirty-one subjects were enrolled for the inter-day RHI repeatability assuming a power of 80%, alfa
148 = 0.05, K measurements = 2 and width of the 95% confidence interval for s_w = 0.25. As regards the
149 intraday RHI repeatability, sixteen subjects were recruited assuming a power of 80%, alfa = 0.05, K
150 measurements = 3 and width of the 95% confidence interval for s_w = 0.25. Within subjects standard
151 deviation s_w, of repeated measurements was used to estimate sample size.

152 One way repeated-measures analysis of variance (ANOVA) was used to compare the data obtained
153 for intraday repeatability; post-hoc analysis of differences between paired data was assessed, when
154 appropriate, by the Least Significant Difference (LSD). Differences between interday
155 measurements were analyzed by paired Student t-test.

156 The agreement between paired (intraday and interday) measurements was assessed with the **Bland-**
157 **Altman method** in which the differences between two repeated measurements were plotted with
158 their mean [19]. In the plot, horizontal lines were drawn at the mean difference and the 95% limits
159 of agreement, defined as the mean difference ±1.96 times the standard deviation of the differences.

160 **Coefficient of repeatability** (CR) was calculated, as suggested by Bland and Altman, multiplying
161 the standard deviation of the differences between the two measurements for 1.96 [19]. This value
162 provides an interval, within which 95% of test-retest measurement differences lie.

163 **Coefficient of variation** (CV) was reported and expressed as a percentage. CV derives from the
164 formula (average of individual standard deviations / average of individual means) x 100; a lower
165 CV was associated with higher repeatability.

166 Test-retest reliability was assessed by **intraclass correlation coefficient** (ICC). ICC, that measures
167 the extent of absolute agreement, is defined as the ratio of the between-subjects variance to the sum
168 of the pooled within-subject variance and the between-subjects variance and it derives from two-
169 way random effects ANOVA [20]. The ICCs range from 0 to 1 and are classified as follows: ICC
170 <0.75 poor agreement, 0.75-0.90 moderate agreement, and >0.90 high agreement [21].

A two tailed P < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS for Windows, release 17.0 (SPSS, Chicago, IL). Data are presented as mean values ± standard error of the mean (SEM).

RESULTS

Characteristics of the subjects

The anthropometric and clinical characteristics of the forty-seven subjects enrolled for the inter- and intraday reproducibility study are reported in **Table 1**. Blood pressure and BMI were in the normal range. Nineteen out of 47 subjects showed endothelial dysfunction (RHI <1.67).

Interday repeatability

The interday repeatability of RHI, FRHI and AI@75 measured at day 1 and day 2 are reported in **Figure 1 (A-C)**. The Bland–Altman graphs documenting the degree of concordance between the pairs of measurements obtained on separate days for RHI, FRHI and AI@75 are shown in **Figure 2 (A-C)**. No significant difference between days has been observed for all the parameters under study. CV, CR and ICC of RHI were 5.8%, 0.47 and 0.79, respectively. FRHI showed a CV of 21.1%, a CR of 0.39 and ICC of 0.79. AI@75 showed a good level of reliability (ICC: 0.88) between days, but poor repeatability (CR: 1.74). In particular, Bland–Altman graph (**Figure 2C**) displays a clear upward slope due to a high intra-individual variability of AI@75 between days.

Intraday repeatability

The intraday repeatability of RHI, FRHI and AI@75 are reported in **Figure 3 (A-C)**. Multiple evaluations within the day significantly affected RHI ($p=0.001$), FRHI ($p<0.001$) and the repeatability of the measurements. RHI significantly increased at 4 h compared to baseline (+30.1%, $p<0.05$) and 2 h (+16.8%, $p<0.05$), while no significant effect was observed at 2 h with

196 respect to baseline (+11.2%; p>0.05). The improvement of arterial function at 4 h was particularly
197 evident in the group of subjects with endothelial dysfunction (RHI from 1.40 ± 0.08 to 2.05 ± 0.14;
198 p<0.001; n =8) compared to the group of subjects with normal endothelial function (RHI from 2.40
199 ± 0.57 to 2.63 ± 0.53 RHI; p= 0.063; n=8). On the whole, RHI repeatability was high until 2 h (CV:
200 7.71%; CR: 0.56; ICC: 0.91) but low after 4 h (CV: 18.8%; CR: 1.26; ICC: 0.53).
201 FRHI significantly increased after 2 h (+233%; p<0.01) and 4 h (+337%; p<0.001) with respect to
202 baseline, and it was also high at 4 h (+76.5%; p<0.001) compared to 2 h. These results were also
203 confirmed by the low repeatability after 2 h (CV: 34.4%; CR: 0.52; ICC: 0.83) and 4 h (CV: 58.8%;
204 CR: 1.08; ICC: 0.42) compared to baseline.
205 Multiple measurements significantly affected arterial stiffness (AI@75; p<0.01). In particular, a
206 significant reduction was observed after 2 h compared to baseline (-25.3%; p<0.01) and 4 h (-
207 17.5%; p<0.01), while no significant difference was observed between baseline and 4 h. The test-
208 retest repeatability was low both at 2 h (CV:13%; CR:13.0; ICC: 0.60) and 4 h (CV:15%; CR:11.8;
209 ICC:0.73).
210 The Bland–Altman plots of the RHI, FRHI and AI@75 measurements (time interval: 0 h *vs* 2 h, and
211 0 h *vs* 4 h) are shown in **Figure 4 A-C**. On the whole, the graph plots indicate a clear upward slope
212 and a high intra-individual variability between measures for each variable at all time points.
213 To exclude the contribution of breakfast in the modulation of RHI repeatability, a group of 8
214 volunteers repeated intraday measurement in a fasting condition (**Figure 5 A-C**). The results
215 showed that multiple evaluations within the day significantly affected RHI also in the fasting
216 condition. In particular, RHI significantly increased at 4 h compared to baseline (from 1.84±0.34 to
217 2.27±0.44 RHI; +23.4%; p<0.01) while no significant increase occurred after 2 h with respect to
218 baseline (from 1.84±0.34 to 2.08±0.38 RHI; p>0.05). RHI repeatability was high until 2 h (CV:
219 8.71%; CR: 0.62; ICC: 0.71) but low after 4 h (CV: 19.7%; CR: 1.33; ICC: 0.11).

FRHI significantly increased after 4 h (from 0.49±0.16 to 0.79±0.14 FRHI +527%; $p<0.01$) compared to baseline, while no difference was observed at 2 h ($p=0.06$), and between 2 and 4 h ($p=0.34$). However, a low repeatability after 2 h (CV: 24.1%; CR: 0.63; ICC: 0.78) and 4 h (CV: 36.6%; CR: 0.91; ICC: 0.54) was observed compared to baseline.

Regarding arterial stiffness (AI@75), no significant difference occurred between test and retest without breakfast at 2 h and 4 h compared to baseline ($p=0.52$). The test-retest repeatability was low both at 2 h (CR:26.3; ICC: 0.91) and 4 h (CR:15.2; ICC:0.96).

The Bland–Altman plots of the RHI, FRHI and AI@75 measurements (time interval: 0 h *vs* 2 h, and 0 h *vs* 4 h) documented a high intra-individual variability between measures for each variable at all time points (data not shown).

Discussion

Together with other studies, we previously reported the feasibility of using the Endo-PAT technique to measure improvements of endothelial function following dietary interventions, lifestyle modifications or pharmacological treatments [8, 18, 22-26]. However, information about the performance of this tool in intervention trials is limited. For example, we recently documented that one portion of blueberry was able to counteract an impairment of endothelial function following acute cigarette smoking evaluated through RHI measurements performed in two separate occasions (i.e. day 1: baseline; day 2: treatment) [26]. This protocol was selected since we observed an improvement in endothelial function following multiple RHI measurements in the same day. Here, we reported the inter- and intra-day repeatability of Endo-PAT in a group of volunteers with characteristics (e.g. lifestyles, dietary habits) comparable with those of the subjects involved in the previous trial. The results obtained in the present study suggest that the measurements of peripheral arterial function (RHI and FRHI), but not arterial stiffness, are reproducible when assessed in two

consecutive days. On the contrary, it was found that multiple measurements within the same day increased RHI and FRHI and temporarily reduced AI@75.

In the present study, we observed a small CV for RHI (about 6%) and a good ICC for RHI, FRHI and AI@75 (0.77, 0.79 and 0.88, respectively). These results are in accordance with data reported by others authors [7, 27-29]. Onkelinx *et al.* showed no difference in RHI index between days and a good coefficient of variation (CV: 11%) and intraclass correlation coefficient (ICC: 0.73) of the measurements in a group of male patients with coronary artery disease [7]. Sauder *et al.* showed a robust RHI repeatability (ICC: 0.74) between days in a group of subjects with metabolic syndrome [29]. Liu *et al.* reported no significant differences in the RHI values measured at the same time points on each of the 3 days tested in a group of healthy male volunteers [27]. However, the ICC for RHI measured at 2-hour intervals (from 8.00 am to 8.00 pm) among the 3 days was very low (ICC: 0.03-0.46). In addition, the authors reported that the mean intra-individual coefficient of variation for arterial stiffness was rather variable (37%) [27]. On the contrary, McCrea *et al.* reported a good ICC for RHI and arterial stiffness (0.74 and 0.83, respectively) evaluated for two measurements performed within a week in a group of healthy subjects [28]. Selamet *et al.* investigated RHI repeatability in a large cohort of healthy adolescents and documented an excellent repeatability with an ICC of 0.78 when the measurements were performed in two separate occasions [30]. Degnan *et al.* showed a low ICC for RHI but excellent for arterial stiffness (0.43 and 0.78, respectively) when the tests were performed in three separate occasions in a group of healthy female subjects [31]. Conversely, in our study a low reliability of arterial stiffness was found suggesting that the measurements between days were not reproducible. This result may be attributed to the small arteries of the fingertips that are more sensitive than brachial arteries and are more susceptible to variations in sympathetic nervous system activity.

Regarding the intraday repeatability, we previously reported that multiple measurements within the day can affect arterial function, thus a minimum time interval (at least 2 hours from our

observations) between test and retest should be recommended also in accordance to the data reported in the literature [6;27]. In addition, it is widely recognized that RHI is influenced by temperature, sympathetic nervous system activity and humoral factors [32]. In the present experiment, we tried to limit the possible confounding factors by standardizing the dietary habits and lifestyle of participants, by repeating the measurements at intervals of 2 hours, by maintaining a constant temperature in the room, and by providing a comfortable environment for all patients. We documented that multiple measurements within the same day caused a significant increase at 4 h for RHI and at 2 and 4 h for FRHI compared to baseline. In particular, the improvement of arterial function at 4 h was particularly high in the group of subjects with initial endothelial dysfunction that was reversed following the multiple measurements.

The effect of multiple measurements on RHI values was poorly investigated in the literature and the results available are quite controversial. Onkelinx *et al.* reported a significant increase in RHI values when the tests were performed at intervals of 30 minutes [7]. Similar results were also observed by Liu and coworkers that reported a significant increase in RHI when measurements were carried out at 0.5 h intervals for 6 h, hypothesizing a crossover effect [27]. However, when the authors performed the measurements at intervals of 1 and 2 h, the values were comparable and no significant changes occurred compared to baseline [27]. Forchammer *et al.* showed a good intra-day RHI reproducibility in a group of healthy subjects when endothelial function was assessed in four different occasions (in the morning, before and after lunch, in the afternoon) within the same day [33].

In the present study, subjects consumed a light breakfast early in the morning before the test in order to avoid the potential effect of long term fasting on vascular function. However, it is recognized that consumption of a meal can affect RHI; this can also depend on subjective metabolic features [27-28]. In literature, we could not find data comparing the effect of overnight fasting versus breakfast intake on RHI intraday repeatability. In order to exclude the contribution of

breakfast to the results obtained, we asked to a group of volunteers to repeat intraday measurement in a fasting condition. Only 8 out of 16 subjects previously enrolled joined the study and completed the test. Overall, data obtained on the overnight-fasted group of participants were in line with those found following the light breakfast consumption. The improvement observed could be attributed to several variables such as sympathetic nervous system activity, diurnal rythms, ischemic preconditioning mechanisms, or production of endothelial-dependent (e.g. endothelium-derived relaxing factors) or endothelial-independent (e.g. leptin, glucagon-like peptide 1) factors able to induce vasodilation [34-35]. In this regard, several studies showed that insulin induces changes in microvascular vasomotion, promotes capillary recruitment and NO synthesis [36-37]. The binding of insulin to its receptor on endothelium could lead to the activation of eNOS pathway and vasodilation [38-39]. These observations can be useful to explain the results obtained in our study after consuming breakfast. On the other hand, low blood-sugar levels in the fasting state bring to a decrease in insulin secretion and a rise in glucagon secretion. Glucagon stimulates glycogen breakdown and inhibits glycogen synthesis by triggering the cyclic adenosine monophosphate (AMP) cascade. An increase in endothelial cyclic AMP levels showed to amplify agonist-induced formation of endothelium-derived relaxing factor that plays a pivotal role in the vasodilation [40]. Moreover, we cannot exclude that the improvement of endothelial function could be somehow related to a shift in the energy fuel used during fasting condition, even if all these hypotheses remain to be ascertained.

Digital AI reflects changes in vessel diameter, blood pressure and heart rate [41]. We considered the value of digital AI standardized for the heart rate provided by the Endo-PAT system, documenting a significant decrease in AI@75 at 2 h, but not at 4 h, compared to baseline only after the consumption of breakfast. Albeit temporary, this improvement was not dependent on heart rate as no significant change occurred for this variable. Since insulin have been reported to play a

beneficial effect on arterial stiffness both in healthy and diabetic subjects [37,42], its involvement cannot be excluded.

Strengths and limitations of the study

In the present study, we tried to eliminate as many confounding factors as possible affecting the endothelial function and its variability; for example we selected a homogenous population for dietary, smoking (non-smokers) and physical activity habits. All the subjects were healthy and did not take any supplement or medication. Moreover, we standardized the meals the day before and the breakfast the day of the experiment in order to limit postprandial effects. In addition, the testing procedure was standardized for the posture of the subjects, the probe placement, the time of the day, the room temperature and the resting period to eliminate, or at least to limit, the sympathetic stimulation prior to testing. The use of a light breakfast could have had an impact on results obtained; however, the study performed on starved subjects, as control confirmed results on the time-dependent RHI intraday variability. Owing to the many variables that can affect the reliability of measurements, the use of well-described, standardized and controlled protocols is highly recommended with a particular focus on occlusion times and metabolic state of the subjects, in order to avoid crossover effects and limit as much as possible the confounding factors.

Conclusions

In conclusion, we documented a good interday repeatability for RHI measurements. Conversely, intraday repeatability was influenced by the number of measurements and generally accepted when performed up to 2 h. Subjects with endothelial dysfunction seemed to be more prone to modifications following multiple measurements causing a reversal of vascular impairment. Further studies are needed in order to elucidate the effects of multiple measurements on RHI, FRHI and AI@75, especially before performing clinical or dietary intervention studies. A specific attention should be devoted to subjects with endothelial dysfunction whose RHI improvement

merits further investigation and to the role of insulin and glucagon in the modulation of vascular function.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure 1: Interday repeatability of RHI, FRHI and AI@75 measured in two consecutive days by Endo-PAT2000

Figure legend

Subjects involved: n=31

RHI, reactive hyperemia index; FRHI, Framingham reactive hyperemia index; AI@75, arterial stiffness standardized for heart rate.

Data are expressed as mean ± standard error of the mean.

Figure 2: Bland-Altman plot: the difference between the two measurements for RHI (2A), FRHI (2B) and AI@75 (2C) is plotted. The continuous line represents the mean of differences and the broken lines the 95% limits of agreement (± 1.96 SD of the differences)

Figure legend

Subjects involved: n=31

RHI, reactive hyperemia index; FRHI, Framingham reactive hyperemia index; AI@75, arterial stiffness standardized for heart rate.

Figure 3: Intraday repeatability of RHI, FRHI and AI@75 measured at intervals of 2 hours from a light breakfast by Endo-PAT2000

Figure legend

Subjects involved: n=16

RHI, reactive hyperemia index; FRHI, Framingham reactive hyperemia index; AI@75, arterial stiffness standardized for heart rate.

Data are expressed as mean ± standard error of the mean.

[a,b,c] Data with different letters are significantly different ($p<0.05$)

Figure 4: Bland-Altman plot: the difference between two measurements after a light breakfast (time 0 h vs time 2 h, and time 0 h vs time 4 h) for RHI (4A), FRHI (4B) and AI@75 (4C) is plotted. The continuous line represents the mean of differences and the broken lines the 95% limits of agreement (± 1.96 SD of the differences)

Figure legend

Subjects involved: n=16

RHI, reactive hyperemia index; FRHI, Framingham reactive hyperemia index; AI@75, arterial stiffness standardized for heart rate.

Figure 5: Intraday repeatability of RHI, FRHI and AI@75 measured at intervals of 2 hours in fasting conditions by Endo-PAT2000

Figure legend

Subjects involved: n=8

RHI, reactive hyperemia index; FRHI, Framingham reactive hyperemia index; AI@75, arterial stiffness standardized for heart rate.