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Lack of seasonal variation in flow-mediated dilatation of the brachial artery in women with primary Raynaud's phenomenon and healthy controls

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Summary

Background: The aim of this study was to examine endothelium function and seasonal variations of endothelium function in women with primary Raynaud's phenomenon (RP) and healthy controls.

Patients and methods: After a fast of at least 8 hours we studied 21 patients with primary RP (mean age 31.1 years, mean duration of RP 9.1 years) and 22 controls (mean age 27.8 years) by use of high resolution brachial artery sonography in winter (December/January 2000) and summer (July/August 2001). To exclude circadian variations all examinations were performed in the late afternoon only. All subjects were non-smokers. Confounding factors like serum glucose, HbA1c, and lipid concentrations were analyzed immediately before the investigations. Nicotine contamination was randomly analyzed in hair samples in 8 subjects of each study group. Flow mediated dilatation (FMD%) and nitroglycerin induced dilatation (NID%) were calculated by putting the basal vessel diameter as 100%.

Results: Basal, flow-mediated, and nitroglycerin-induced absolute diameters of the brachial artery did not differ significantly between the study groups (p = 0.85). The test conditions (basal, postocclusive, nitroglycerin-induced) always let to the same vessel response in winter and summer (p = 0.61) and there was no significant influence between these test conditions and the study groups (p = 0.07). Compared to patients FMD% was slightly reduced in controls in summer (p = 0.09). Analysis of variance excluded a significant relation between study group and season (p = 0.43). For NID% too, no statistically significant differences were found.

Conclusions: We were not able to show impaired or seasonally variant flow-mediated or nitroglycerin-induced dilatation of the brachial artery in patients with primary RP. Our results argue against the presence of a more generalized endothelium dysfunction detectable with high resolution ultrasound of the brachial artery in patients with primary RP.

Key words

Brachial artery, endothelium function, Raynaud's phenomenon, Seasonal variation

Zusammenfassung

Die flowmediierte Dilatation der Brachialarterie von Frauen mit primären Raynaud Phänomen und gesunden Probanden ist nicht jahreszeitabhängig

Hintergrund: Ziel unserer Studie war die Untersuchung der Endothelfunktion und des saisonalen Verhaltens der Endothelfunktion bei Frauen mit primärem Raynaud Phänomen (RP) und gesunden Probandinnen.

Patienten und Methoden: Nach einer achtstündigen Fastenperiode wurden 21 Patientinnen mit primärem RP (mittleres Alter 31,1 Jahre, mittlere Dauer der RP 9,1 Jahre) und 22 Probandinnen (mittleres Alter 27,8 Jahre) mittels hochauflösender Brachialissonographie im Winter und Sommer untersucht. Um zirkadiane Schwankungen auszuschließen, erfolgten alle Untersuchungen am späten Nachmittag. Die flussmediierte (FMD%) und nitroinduzierte (NID%) Dilatation wurde in Bezug auf den Ruhegefäßdurchmesser berechnet.

Ergebnisse: Der basale, flußmediierte und nitroinduzierte absolute Gefäßdurchmesser der A. brachialis unterschied sich nicht signifikant zwischen den Studiengruppen (p = 0.85). Die Testbedingungen führten im Winter und Sommer immer zur gleichen Gefäßantwort (p = 0.61) und es gab keinen signifikanten Einfluss zwischen diesen Testbedingungen und den Studiengruppen (p = 0.07). Verglichen mit den Patientinnen war die FMD% bei den Probandinnen im Sommer leicht reduziert (p = 0.09), aber die Varianzanalyse schloss eine signifikante Beziehung zwischen der Studiengruppe und dem Untersuchungszeitpunkt auf das Messergebnis aus (p = 0.43). Auch für die NID% ergab sich kein signifikanter Unterschied.

Zusammenfassung: Wir fanden keine Störung oder saisonale Änderung der flussmediierten oder nitroinduzierten Vasodilatation der A. brachialis bei Patientinnen mit primären RP. Unsere Ergebnisse sprechen gegen eine generalisierte, mittels hochauflösender Brachialissonographie detektierbare endotheliale Dysfunktion bei Patientinnen mit primärem RP.

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Introduction

Increasing knowledge of the active role of the endothelium in modifying vascular tone by nitrite oxide (NO) production let to the hypothesis that endothelium dysfunction might be the leading disorder in primary Raynaud's phenomenon (RP) [1, 2, 6].

Assessment of flow mediated dilatation (FMD) of the brachial artery by high resolution sonography has gained widely acceptance as a method of non-invasive determination of endothelium function [5, 18]. FMD is mediated mainly by release of NO from the endothelium [12, 17, 23]. Thus, impaired brachial artery dilatation under increased flow conditions reflects impaired NO release which indirectly indicates endothelium dysfunction. A dysfunction of the L-arginine-NO-cGMP-pathway in primary RP has been described using cGMP concentrations, nitrate levels, and direct vessel responses to acetylcholine as indicators [13, 15, 22, 26]. Nevertheless, Ringqvist and coworkers could not find impaired FMD of the brachial artery in primary RP [21]. This finding might be the result of a more distally located and limited endothelium dysfunction in primary RP undetectable by brachial artery sonography or it might be influenced by the neglection of chronobiological aspects of endothelium function suggested by recent publications [7, 20].

We therefore examined endothelium function and seasonal variations of endothelium function in women with primary RP and healthy controls by use of high resolution brachial artery sonography under study conditions excluding circadian variations of FMD.

Patients and methods

Between December 2000 and August 2001 we studied 51 women during two investigations performed in winter (December/January 2000) and summer (July/August 2001). To exclude circadian variations all examinations were performed in the late afternoon.

10 subjects had to be excluded from the study, leaving 21 patients and 22 controls for final evaluation. Reasons for exclusion were β-blocker-intake in one case, appearance of RP in a control, and refusal of a second examination in summer in 8 cases.

Table I summarizes the clinical characteristics of the study populations.

Patients were classified as suffering from primary Raynaud's syndrome, if they were symptomatic for more than one year with a daily number of RP ≥ 1 during winter, there was a completely reversible vasospastic reaction in the digital photo-pletysmograms, and no clinical symptoms of an underlying disease could be found. Any drugs known to evoke or increase vasospasm were not allowed. Additionally, nailfold capillary image had to be normal or at least unspecific without presence of avascular fields or megacapillaries. Further more, there had to be a normal thyroid function and antinuclear antibodies, extractable nuclear antibodies, rheumatic factor, cold agglutinins, cryoglobulin, and cryofibrinogen had to be negative.

The controls were recruited from among friends, hospital staff and students. All were in good health and underwent a clinical examination and interview were the presence of RP or other vascular diseases as well as the presence of an actual or chronic disease was excluded. Vasoactive drugs were not allowed.

All subjects gave informed consent. The study protocol was approved by the Ethical Committee of the Medical Faculty of the University of Innsbruck.

Table I: Clinical characterization of the study population

	Patients	Controls	p-value
n	21	22	–
Age (yrs)	31.1 (23–44)	27.8 (22–36)	0.07
Duration of RP (yrs)	9.1 (1–24)	0	–
RP per week in winter (n)	8.3 (1–31)	0	–
RP in summer (%)	71.4	0	–

Apparative examination

Besides clinical examination all patients underwent bilateral blood-pressure measurement and oscillographic examination of the arms (Gutmann-Medizintechnik, Germany). After acclimatization photo pletysmograms of the finger-tips D1–D5 were recorded under room temperature conditions and after bathing the hands in 15°C water for 2 min using a acal light-reflex-plethysmometer (Gutmann Medizintechnik, Germany). A vasospastic reaction to cold exposure was assumed when systolic signal amplitudes reduced > 50%. Reversibility was tested after bathing the hands in 40°C water for 5 min.

Capillary microscopy was performed with a Capiscope (KK-Research Technology LTD, UK) connected to a standard computer unit. Images were generated by a lens providing a magnification of 200 × and a CCD camera giving high resolution images of 752 × 582 pixels. Capillary morphology was assessed according to the recommendations of the Microcirculatory Section of the German Society of Angiology [24].

Brachial artery sonography was performed with a HDI 5000 and a 5–12 MHz linear ultrasound transducer (ATL Ultrasound Inc., USA) according to the protocol shown in Figure 1. During the examination the subjects lay quietly in a supine position. After acclimatization brachial artery was scanned up to 10 cm above the elbow in longitudinal sections with the focus zone set to the depth of the near wall and gain optimized for detection of the wall structures. The transducer position was marked on the skin for repeated measurements. Settings were not changed until the examination was over. For determination of the arterial width the enddiastolic endothelium-endothelium diameter was used (leading edge zone 3 to leading edge zone 5). For assessment of FMD a suprasystolic pressure (30 mmHg above systolic blood pressure) was applied for 5 min using a pneumatic arm cuff. Diameter measurements were done manually between the first and second minute after cuff release. To ensure correct time intervals of the measurements in all subjects examinations were recorded on video. Thereafter, 10 min was allowed for recovery of the vessel. 0,4 mg nitroglycerin was then administered sublingually

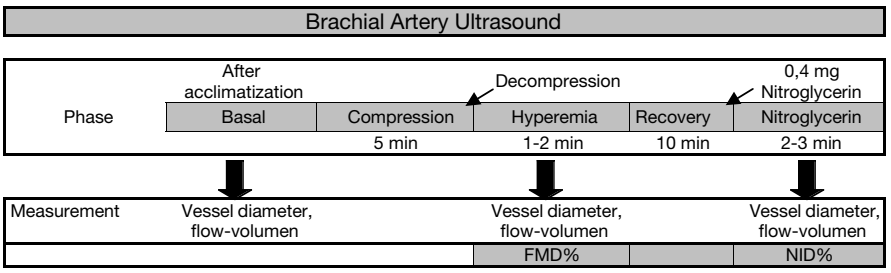

Fig. 1: Protocol of brachial artery ultrasound examination.

by a pump-spray. All vessel diameters are expressed as means calculated from three consecutive measurements by the same examiner. Flow mediated dilatation (FMD%) and nitrogycerin induced dilatation (NID%) were calculated by putting the basal diameter as 100% ($\Delta D/D_{basal} \times 100$).

All examinations were performed after a fast of at least 8 hours. To document factors potentially influencing FMD determination of serum glucose, HbA1c, and lipid concentrations were performed immediately before the two investigations in all subjects. Although all participants were non smokers nicotine contamination was randomly analyzed in 8 patients of each study group by assessment of cotinine concentrations in hair samples.

Statistics

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) for Windows 10.1.3.

For comparison of FMD, NID, FMD% and NID% analysis of variance for repeated measurements (season = innersubject factor, study group = intersubject factor) was performed. For comparison of other influencing factors the t-test was used. Normal distribution of variables was assessed by Kolmogorov-Smirnov-test. Dichotomized variables were compared using the χ^2-test. Significance niveau was defined as $\alpha < 0.05$. The number of subjects included was calculated to reach a statistical power of at least 80%.

Results

Table II summarizes the body-mass-indices, pulse rates, blood pressure measurements, and the laboratory data of the two study groups in winter and summer. In winter, there was a marginally significant difference in systolic blood pressure and a significant difference in diastolic blood pressure between the groups. In summer pulse rate was significantly lower in patients. Nevertheless, over the whole study period a significant influence of the season or the study group on these parameters could not be shown by analysis of variance ($RR_{systolic}$ p = 0.24, $RR_{diastolic}$ p = 0.14, HR p = 0,20). Laboratory data was not different between the groups or seasons.

The results of the vessel diameter measurements and the FMD% and NID% is shown in Table III.

As expected, the basal, flow-mediated, and nitroglycerin-induced absolute diameters of the brachial artery dif-

Table II: BMI, blood pressure, pulsrate and laboratory data of the study groups in winter and summer

Winter	Patients	Controls	p-value
n	21	22	
BMI (kg/m²)	20.2 ±1.6	21.5 ± 2.5	0.07
RR_{sys}	116 ± 15	123 ±10	0.06
RR_{dia}	68 ± 11	77 ± 8	0.004
Pulsrate	67 ± 13	73 ± 13	0.15
Cholesterol (mg/dl)	189 ± 30	194 ± 30	0.63
HDL (mg/dl)	67 ± 13	69 ± 13	0.63
LDL (mg/dl)	112 ± 28	115 ± 29	0.73
Triglycerides (mg/dl)	85 ± 46	81 ± 30	0.73
Glucose (mg/dl)	68 ± 8	68 ± 13	0.93
Hb A1C (%)	5.7 ± 0.4	5.6 ± 0.4	0.43
Cotinin (ng/mg) (n = 8)	1.6 ± 2.4	1.7 ± 2.9	0.99

Summer	Patients	Controls	p-value
n	21	22	
BMI (kg/m²)	20,2 ±1,6	21,5 ± 2,5	0.07
RR_{sys}	117 ±13	117 ± 12	0.93
RR_{dia}	77 ± 8	75 ± 8	0.32
Pulsrate	64 ± 9	72 ± 14	0.045
Cholesterol (mg/dl)	194 ± 37	194 ± 39	0.95
HDL (mg/dl)	70 ± 13	68 ± 15	0.71
LDL (mg/dl)	117 ± 34	120 ± 35	0.80
Triglycerides (mg/dl)	74 ± 26	84 ± 35	0.32
Glucose (mg/dl)	84 ± 16	86 ± 21	0.71
Hb A1C (%)	5.4 ± 0,3	5.3 ± 0.4	0.70
Cotinin (ng/mg) (n = 8)	1.0 ± 1.2	1.6 ± 3.1	0.65

Table III: Results of brachial artery ultrasound in winter and summer

Winter	Patients	Controls	p-value
n	21	22	
Basal diameter (1/10 mm)	29.5 ± 3.6	29.5 ± 2.6	0.99
Flow-mediated diameter (1/10 mm)	33.8 ± 4.3	33.2 ± 3.5	0.62
Nitroglyerin-induced diameter (1/10 mm)	35.0 ± 3.5	35.70 ± 3.25	0.50
Flow-mediated dilatation %	14.7 ± 9.0	12.5 ± 8.2	*
Nitroglycerin-induced dilatation %	18.9 ± 7.7	21.0 ± 6.7	*

Summer	Patients	Controls	p-value
n	21	22	
Basal diameter (1/10 mm)	30.0 ± 3.4	30.8 ± 2,2	0.35
Flow-mediated diameter (1/10 mm)	34.5 ± 3.5	34.0 ± 3.4	0.69
Nitroglyerin-induced diameter (1/10 mm)	36.3 ± 3.7	36.9 ± 3.0	0.58
Flow-mediated dilatation %	15.2 ± 6,2	10.6 ± 9.0	*
Nitroglycerin-induced dilatation %	21.4 ± 8.8	19. 8 ± 8.7	*

* differences between groups and season not significant (p = 0.43 for FMD, p = 0.24 for NID).

fered significantly ($p < 0.001$). There was a significant seasonal influence ($p = 0.002$), but absolute diameters did not differ significantly between the study groups ($p = 0.845$). The test conditions (basal, postocclusive, nitroglycerin-induced) always let to the same vessel response in winter and summer ($p = 0.61$) and there was no significant influence between the test conditions and the study groups ($p = 0.07$).

Figure 2 graphically illustrates the results of FMD% and NID% in winter and summer of both groups. Compared to patients FMD% was reduced in controls in summer, but the difference did not reach statistical significance ($p = 0.09$). Analysis of variance also excluded a significant relation between study group and season ($p = 0.43$). For NID% too, no statistically significant differences between the groups ($p = 0.89$) or season ($p = 0.69$) was found.

Discussion

The aim of our study was to examine endothelial function and possible seasonal variations of endothelial function in healthy controls and patients with primary RP. Circadian influences on FMD have already been described, but possible seasonal variations of FMD were not examined yet.

Using FMD as a widely accepted indirect determinant of endothelial function we could not supply evidence for an endothelial dysfunction in patients with primary RP, nor could we verify a seasonal influence on flow-mediated dilatation in healthy controls and patients. These result were obtained by exclusion of circadian variations.

The finding of FMD undistinguishable from healthy controls is in accordance with data published by Rinqvist et al., who also failed to show impairment of FMD of the brachial artery in patients with primary RP during a single examination in winter [21]. On the other hand, Singh and coworkers demonstrated a dysfunction of endothelium-dependent vasodilatation of the digital arteries after infusion of acetylcholine chloride using a high frequency A-mode ultrasound scanner for determination of digital artery diameter [26].

Kahn et al. also reported a reduced vasodilatating capacity in patients suffering from primary RP after acetylcholine infusion [13]. They also found a reduced vasodilatation after application of nitroprussid. Interestingly, L-arginine supplementation failed to restore endothelial responses in their study.

In contrast to our results seasonal variations of L-Arg-NO-cGMP pathway in primary RP were reported using different methods of evaluation. Rinqvist et al. described increased nitrate concentrations in winter indicating an upregulation of NO production during the cold season [22]. The same group also published seasonally different cGMP-responses to whole body cooling, arguing for a disturbance of L-Arg-NO-cGMP-pathway-adaption to cold exposure in patients with primary RP in winter [15]. Finally, Gardner-Medwin et al. found exaggerated seasonal differences in finger skin temperature and microvascular blood flow assessed by red blood cell flux in women with primary RP compared to healthy controls [8].

The conflicting reports on seasonal variation of endothelium function rises the question of reliability of the different methods used for assessment of endothelial function in Raynaud's phenomenon. Determination of FMD by high resolution sonography of the brachial artery might not be able to detect localized endothelium dysfunction in small digital arteries, as is indicated by the results of Singh et al. [26]. On the other hand, the method used for stimulation of a dilatory response might be decisive, as is suggested by the divergent results depending on the use of acetylcholine infusions and postocclusive flow enhancement [8, 21, 26]. It also must be taken into account that postischemic flow enhancement induced by forearm compression does not reflect flow conditions under "normal circumstances" in patients with RP. Thus, vasodilatation in this experimental setting might not allow direct conclusions on the functional state of the L-Arg-NO-cGMP-pathway in patients with primary RP in "normal life".

Inclusion and exclusion criteria in our study ensured strict diagnosis of primary RP and homogenous study conditions between the two groups. We also determined influencing factors like blood glucose, serum lipids and nicotine exposure immediately before the examinations, which did not differ in both collectives [3–6]. Nevertheless, it can't be totally excluded that some other confounding factors FMD might have influenced our results [9,–11, 19, 25, 27].

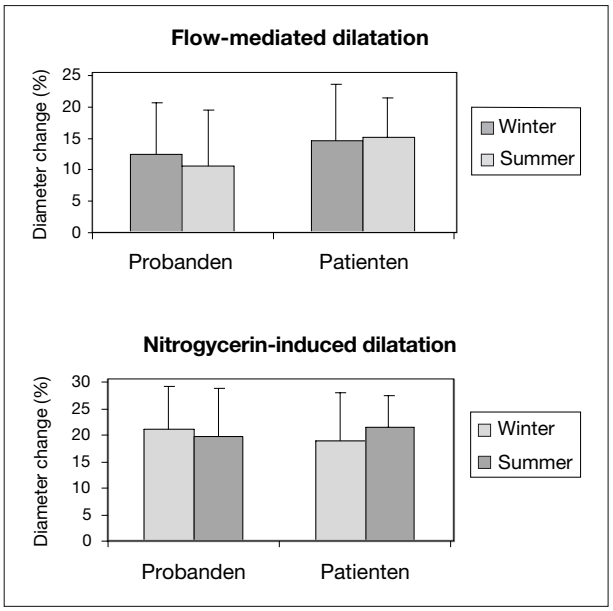

Fig. 2: Results of flow-mediated dilatation (FMD (%)) and nitroglycerin-induced dilatation (NTG (%)).

In conclusion, we were not able to show impaired or seasonally variant flow-mediated or nitroglycerin-induced dilatation of the brachial artery in patients with primary RP and healthy controls. Our results strongly argue against the presence of a more generalized endothelium dysfunction detectable with high resolution ultrasound of the brachial artery in patients with primary RP.

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