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● *Original Contribution*

DIFFERENCES IN THE ERYTHROCYTE AGGREGATION LEVEL BETWEEN VEINS AND ARTERIES OF NORMOLIPIDEMIC AND HYPERLIPIDEMIC INDIVIDUALS

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Abstract—The objectives of this study were to detect differences in the Doppler power backscattered by blood *in vivo*, and to identify factors affecting the backscattered power. The main hypothesis was that variations in the erythrocyte aggregation level between veins and arteries of normolipidemic and hyperlipidemic individuals can be detected with power Doppler ultrasound. Doppler measurements were performed at 5 MHz, with an Acuson 128 XP/10 system, over the carotid artery and jugular vein, external iliac artery and vein, common femoral artery and vein and popliteal artery and vein. Doppler signals were recorded at the center of each vessel to optimize the detection of erythrocyte aggregation, and processed off-line to obtain the backscattered power. The power of each recording was compensated for Doppler gain differences, tissue attenuation with depth and transmitted power variations occurring with pulse-repetition interval modifications. Results showed statistically stronger backscattered power in veins compared to arteries for the iliac, femoral and popliteal sites. In comparison with healthy subjects, stronger powers were observed in hyperlipidemic patients for the femoral and popliteal sites. Power differences were also found between peripheral measurements. On the other hand, no difference was observed between the power measured in the carotid artery and jugular vein for both groups of individuals. Multiple linear regression analyses were performed to identify factors affecting the backscattered power. Results showed a correlation (r) of 71.2% between the Doppler power in the femoral vein and the linear combination of two parameters: an erythrocyte aggregation index S_{10} measured with a laser scattering method, and the diameter of the vessel measured on B-mode images. Statistically significant linear correlation levels were also found between S_{10} and the Doppler power in various vessels. In conclusion, this study showed that power Doppler differences exist *in vivo* in large vessels between veins and arteries of normolipidemic and hyperlipidemic individuals. The Doppler power variations were also shown to be related to erythrocyte aggregation. © 1997 World Federation for Ultrasound in Medicine & Biology.

Key Words: Spontaneous echo contrast, Acoustic backscattering, Red cell aggregation, Power Doppler ultrasound, Hyperlipidemia, Blood flow, Hemodynamics, Biorheology.

INTRODUCTION

Although it is currently difficult to determine the exact contribution of the rouleau size, shape, number, and packing organization on ultrasound backscattering, B-mode blood echogenicity and Doppler backscattered power measurements provide very useful information on erythrocyte aggregation *in vitro*. For instance, it is now recognized that ultrasound backscattered power measurements can be used to characterize the kinetics of rouleau formation (Kim et al. 1989; Kitamura et al. 1995) and the shear rate dependence of erythrocyte aggregation (Shehada et al. 1994; Van Der Heiden et al. 1995; Cloutier et al. 1996b). However, *in vivo*, ultrasound has not been shown to be as useful. Moreover, the source of *in vivo* blood echogenicity is still controversial.

Using M-mode echocardiography, intracardiac echoes in patients with mitral prosthetic valves were first reported in 1975 (Schuchman et al. 1975). Today, using mainly transesophageal B-mode echocardiography, the etiology of similar echoes observed under stasis conditions in the left heart of patients with mitral valve disease

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and dilated cardiomyopathy is still debated. Some studies linked the smoke-like echoes to the presence of aggregated erythrocytes (Beppu et al. 1985; Wang et al. 1992; Siostrzonek et al. 1993; Briley et al. 1994), aggregated platelets (Mahony et al. 1994) or both (Erbel et al. 1986). Even though the source of such echoes has not been clearly identified, it is nevertheless recognized that intracardiac spontaneous echo contrast is a significant predictor of thrombus formation (Daniel et al. 1988; Black et al. 1991; Hwang et al. 1994; Shen et al. 1996).

In addition to the aforementioned heart studies, spontaneous echogenicity in the venous and arterial systems has been documented. For the venous system, B-mode echoes were first seen in the inferior vena cava of a 66-y-old man suffering from constrictive pericarditis (Hjemdahl-Monsen et al. 1984). Spontaneous jugular vein echoes were occasionally observed on B-mode following carotid endarterectomy; this finding, plus the early appearance of neointima, was found to be predictive of restenosis (Maiuri et al. 1995). The first study showing spontaneous echoes in the arterial system is attributed to Panidis et al. (1984), who reported echoes in the aortic arch of patients with aortic dissection. Transesophageal spontaneous echo contrast in the aorta of patients with cardiovascular disease has been observed, but only in a very limited number of cases (deFilippi et al. 1994; Finkelhor et al. 1995; Sukernik et al. 1996). In one of these studies, the echogenicity was attributed to erythrocyte aggregation (deFilippi et al. 1994).

With B-mode imaging, *in vivo* blood echogenicity is rarely seen, probably because of the limited dynamic range of the technique. Because wall filters are used to remove strong echoes from tissues in Doppler ultrasound, this approach should be more sensitive to the presence of erythrocyte aggregates, especially in deep vessels. The first objective of the study was to detect differences in the Doppler power backscattered by blood *in vivo*. Doppler backscattered power measurements were performed in various adjacent veins and arteries of patients with hyperlipidemia and of normolipidemic controls. Three hypotheses were tested: 1. The Doppler power should be higher in veins than in arteries because smaller shear rates are present in the former vessels compared to the latter; 2. the Doppler power should be higher in patients with hyperlipidemia because of the enhancement of erythrocyte aggregation in this group; and 3. the Doppler power should vary between peripheral sites (carotid, iliac, femoral and popliteal) because of changes in flow characteristics (shear rate, pulsatility, presence of a helical flow pattern), and the possibility of local release of substances promoting the aggregation. The second objective of the study was to identify factors affecting the backscattered power *in vivo*. It was hypothesized that physiological, rheological and hemodynamic parameters may contribute to the backscattered power.

METHODS

Patient population

Twenty-five patients (16 men) with primary hyperlipidemia and scheduled for their periodic exams at the Lipid Clinic of the Clinical Research Institute of Montreal were asked to participate to the research protocol. There were no pregnant women among the candidates. Following body mass index (BMI) and blood pressure determination, each participant was asked to complete a medical questionnaire inquiring about smoking habits, current medication and medical history, particularly conditions known to influence erythrocyte aggregation. These included cardiovascular disease, hypertension, diabetes mellitus, arterial and venous thromboembolic disease, plasmocytic dyscrasias, neoplasia and anemia.

The mean age of patients was 58 $\pm$ 11 y (range of 31 to 80 y). Four patients presented with a history of cardiovascular disease and one patient was fortuitously diagnosed during the ultrasonic protocol as having an asymptomatic subtotal internal carotid stenosis. Nine patients suffered from hypertension and three had a history of either glucose intolerance or noninsulin-dependent diabetes mellitus. Sixteen patients had a BMI $>$ 27 and seven were active smokers. According to the WHO/Fredrickson phenotypic classification of hyperlipidemias (Fredrickson and Lees 1965), 8 patients presented as type IIa, 12 as type IIb, 2 as type III, and 3 as type IV.

A total of 14 patients were taking medication: 9 were treated for hypertension, 1 was taking an oral hypoglycemic agent, 5 were on a lipid-lowering therapy, 1 was taking oral anticoagulants, and 1 was on aspirin antiplatelet therapy. Therapeutic agents, other than those just mentioned, were given to 6 patients for conditions such as breast cancer, depression, menopause, hypothyroidism and benign prostate hypertrophy. One patient had multiple myeloma and one had lymphoma.

Eighteen individuals (12 men), with a mean age of 30 $\pm$ 4 y (range of 22 to 39 y) and no history of lipid disorders, were selected as controls. Both normolipidemic subjects and patients were intentionally not matched for age to optimize differences in erythrocyte aggregation between populations, because the aggregation is known to increase for subjects older than 50 y (Pignon et al. 1994). Two normolipidemic subjects had a BMI $>$ 27 and none smoked. One subject was treated for asthma, one presented with hypertension 1 y before the study, and one had Raynaud's disease. All participants to this study gave their written informed consent and the research protocol was approved by the ethics committee of our institution.

Blood sample analyses

After an overnight fast, 55 mL of blood were collected in standard vacutainer tubes for both normolipidemic subjects and patients. Enzymatic methods were used to measure the total cholesterol (Chol) and triglyceride (Trig) concentrations. The high-density lipoprotein cholesterol (HDL-C) concentration was determined after precipitation of apo B-containing lipoproteins by dextran sulphate/$MgCl_2$. The low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the standard Friedewald formula. This formula is reliable if the triglyceride concentration is below 4.5 mmol/L. For patients with levels higher than 4.5 mmol/L, this parameter was not available. The fibrinogen (Fb) concentration was evaluated using the Von Clauss approach and the hematocrit measured by microcentrifugation. The Fb level was measured because this plasma protein has a major effect on erythrocyte aggregation (Rampling 1988).

To assess the aggregation properties of erythrocytes, 1.5 mL of EDTA (ethylenediamine tetra-acetic acid dipotassium salt) anticoagulated blood was rotated in a Couette flow instrument, based on laser light scattering (erythroaggregameter, Regulest, Florange, France). Blood samples were adjusted to 40% hematocrit and parameters on erythrocyte aggregation were derived from the analysis of light intensity variations of blood-scattered signals. All measurements were performed at 37°C by controlling the temperature in the erythroaggregameter with circulating water.

Two series of measurements were performed within 5 h of blood collection. In the first series, the blood sample was sheared for 10 s at 550 s^{-1} to provide rouleau disruption. After abrupt cessation of the rotation, the variation of the scattered light intensity was recorded during the process of rouleau formation. The instrument provided the following two parameters on erythrocyte aggregation kinetics: tA and S_{10}, which correspond to the primary aggregation time and a mean kinetic index at 10 s, respectively. For the second series of measurements, the blood sample was sheared at 96 different levels from 6 to 720 s^{-1}. The partial (γD) and total (γS) dissociation thresholds were extracted from a curve describing the scattered light intensity as a function of the shear rate. These last two parameters provide information on the adhesive forces between erythrocytes. The effect of the pulsatility of the flow on erythrocyte aggregation was not considered with this instrument.

Ultrasound examination

Ultrasonic duplex scans were performed immediately after blood collections with an Acuson 128 XP/10 equipped with a linear array probe (L7384). In B-mode, the transducer operated at 7 MHz, whereas it transmitted at 5 MHz in Doppler mode. For a given position of the probe, both arterial and venous recordings were performed on adjacent segments with the subject lying in supine position. The peripheral sites selected in this study were the common carotid artery (CCA) and jugular vein (JU1), the internal carotid artery (ICA) and jugular vein (JU2), the external iliac artery (ILA) and vein (ILV), the common femoral artery (FMA) and femoral vein (FMV) and the popliteal artery (POA) and vein (POV). Before recording Doppler signals for backscattered power determination, each participant underwent a duplex color Doppler examination of the arterial sites to ensure that no plaque was present. For a given segment, the right side of the individuals was usually scanned first and, if a plaque was detected, the left side was selected. In cases of significant atherosclerosis, measurements were performed proximal to the stenosis. This occurred in only 3 patients and the procedure was done to ensure that turbulence did not affect the backscattered power (Cloutier et al. 1996a). The absence of a mosaic pattern on the color-flow image helped in positioning the sample volume proximal to the stenosis.

For each participant, the forward and reverse audio Doppler signals and the electrocardiogram (ECG) were digitized for 25 s with 12-bit resolution (Data Translation Inc., Marlboro, MA, USA. DT-2828 computer board) at sampling rates of 20 kHz and 200 Hz, respectively. Doppler signals were recorded at the level of the midarterial segment in the center of each vessel to optimize the detection of erythrocyte aggregation (Cloutier et al. 1996b). For the ICA, the sample volume was located after the bulb, 2 cm downstream of the bifurcation. The wall filter was set at 125 Hz and the Doppler angle set at 60°. The sample volume had an axial dimension of 1.5 mm and the transmitted power setting (< 800 mW/cm^2) was the same for all Doppler recordings. The color-flow velocity mode was activated, the gain of the Doppler channels was optimized to avoid background noise on the spectral display of the Acuson, the pulse repetition interval (PRI) was selected to avoid frequency aliasing and the focus was located at the level of the Doppler sample volume. The gain in dB, the PRI in μs, and the depth of the sample volume were noted and used to compensate the backscattered power, as explained below. All other adjustments of the Acuson were kept constant for all recordings. The diameter (D) of the vessels was measured with calipers on the B-mode zoomed images.

Processing of the Doppler signals

Both digitized forward and reverse Doppler signals were Hilbert-transformed to obtain the in-phase (I) and quadrature (Q) components, and Fourier-transformed. Each spectrogram was ECG-gated using a cross-correlation algorithm to locate the beginning of each cardiac

cycle. Hanning windows of 8 ms were applied to the I and Q components and a Doppler spectrum (or a spectral line) was computed at each time interval of 5 ms. Each complex signal of 8 ms was zero padded to 512 samples before computing the Fourier spectrum.

To minimize artifacts in the backscattered power computation, the spectrogram of each cardiac cycle was visually selected before spectral averaging. For arterial sites, a spectrogram was rejected when a clutter signal was present or when the spectral shape was suspected to have changed due to probe or patient movement. For venous flow, only spectrograms characterized by the presence of signal all over the cycle were kept for averaging. In lower limbs, the respiration and insufficient venous return could produce a loss of signal over a portion of the spectrogram, due to the wall filter. Compression of the calf and upper inclination of the legs was occasionally attempted to increase venous flow. The signal loss could be detected either on the spectrogram or audio Doppler signals. Doppler spectrograms selected for both arterial and venous sites were averaged to obtain a mean representation of the frequency content over a cardiac cycle.

In addition to the manual selection of cardiac cycles, windowing of mean spectrograms was performed for arterial sites to eliminate the influence of the wall filter. For spectrograms recorded in lower limbs (ILA, FMA and POA), biphasic and triphasic flow patterns are typical and result in signal loss during the transition from positive to reverse flow and *vice versa.* To eliminate this artifact in the backscattered power computation, only the portion of the mean spectrogram corresponding to the systolic acceleration and early diastolic deceleration of the flow was kept. This window selection was performed according to the time-varying mean Doppler frequencies, and wall filter value. Only spectral lines with mean Doppler shifts above approximately 300 Hz were selected. The value of 300 Hz was chosen to ensure a flat response of the wall filter. Similar selections were performed for carotid artery recordings, although no reverse flow was generally observed. For venous recordings, the whole mean cardiac cycle duration was considered in the backscattered power computation. For all mean spectrograms, the backscattered power was computed as:

$$P = \frac{1}{NFFT \times NSPEC} \sum_{f_k=-SFREQ/2+\Delta F}^{+SFREQ/2} P(f_k) , \quad (1)$$

where $NFFT = 512$ is the number of samples of each spectrum, $NSPEC$ is the number of spectral lines selected within the cardiac cycle, $SFREQ = 20$ kHz is the sampling frequency, and $P(f_k)$ is the power in a bandwidth Δf of 39 Hz (20 kHz/512 samples) centered at the frequency f_k. Using the above equation, the backscattered power was independent of the number of spectral lines considered within the cardiac cycle. For each spectral line, the mean velocity was computed and the maximum of these velocities detected on each mean spectrogram (V_{max}), was also computed to evaluate its effect on the backscattered power.

Compensation of the backscattered power

Because different Doppler gains were used for each acquisition (between −36 dB and +35 dB), the backscattered power was normalized to a gain of 0 dB. In addition to this normalization, three very important factors had to be considered before comparing backscattered power results: 1. The effect of tissue attenuation with respect to depth; 2. the influence of the pulse repetition interval (PRI) of the transmitted ultrasound bursts; and 3. the effect of activating the color flow velocity mode. The attenuation of soft tissue at 5 MHz is, on the average, 3.5 dB/cm (McDicken 1991). For all Doppler measurements, the backscattered power was normalized to a tissue attenuation thickness of 1 cm, knowing the depth of the sample volume.

It was observed experimentally that PRI had a strong influence on the backscattered power.[1] *In vitro* gravity-driven steady flow experiments were performed to test the influence of PRI. Doppler recordings were performed at 60° in a 7.94-mm internal diameter wall-less agar phantom (Rickey et al. 1995). Superfine sephadex particles (Sigma Chemical, Oakville, Ontario, Canada no. G25) were suspended in a 40% glycerol 60%-saline mixture at a concentration of 1 g/L. The blood mimic was circulated in the model at a constant flow rate of 175 mL/min, as measured with an electromagnetic flowmeter (Cliniflow II, Carolina Medical Electronics, King, NC, USA Model FM701D). Careful selection of the flow rate was performed to avoid both the effect of the wall filter and the effect of frequency aliasing on the backscattered power. At 175 mL/min, the Doppler mean frequency shift at the center of the tube was 357 ± 6 Hz; this was higher than the wall filter frequency of 125 Hz and below the Nyquist frequency of 520 Hz, which corresponds to the highest PRI for the Acuson (960 μs).

Pulse repetition intervals between 960 and 60 μs were chosen to cover the range of values used in the *in vivo* study. Ten measurements were performed, in random order, for each PRI selected using the Acuson instrumental settings used *in vivo.* The strongest back-

[1] The reduction of the transmitted power level performed automatically by the instrument when reducing the pulse repetition intervals may explain these observations. For instance, the power was probably reduced to maintain the spatial peak time average intensity level selected by the operator below 800 mW/cm^2.

Table 1. Physiological parameters measured in normolipidemic subjects and patients with hyperlipidemia.

Physiological parameters	Normolipidemic subjects	Hyperlipidemic patients
BMI (kg/m^2)	23 ± 3	27 ± 3[a]
SP (mmHg)	116 ± 11	142 ± 18[a]
DP (mmHg)	72 ± 9	84 ± 9[a]
Chol (mmol/L)	4.77 ± 0.78	7.26 ± 0.94[a]
HDL-C (mmol/L)	1.27 ± 0.33	1.00 ± 0.27[a]
LDL-C (mmol/L)	2.98 ± 0.63	4.86 ± 0.80[a]
Trig (mmol/L)	1.02 ± 0.68	4.62 ± 4.46[a]

BMI = body mass index; SP = systolic pressure; DP = diastolic pressure; Chol = total cholesterol level; HDL-C = high-density lipoprotein cholesterol level; LDL-C = low-density lipoprotein cholesterol level; Trig = triglyceride level.
[a] $p < 0.01$ based on unpaired *t*-tests.

scattered power normalized to a value of 0 dB was found at the PRI of 960 μs. The power at PRIs of 960, 640, 320, 240, 160, 80 and 60 μs were 0.0 ± 1.0, −1.1 ± 1.0, −1.5 ± 0.9, −2.8 ± 0.9, −5.0 ± 0.9, −8.1 ± 0.8 and −10.5 ± 0.9 dB, respectively. For each *in vivo* Doppler recording, the backscattered power was increased by 0, 1.1, 1.5, 2.8, 5.0, 8.1 or 10.5 dB, according to the PRI selected. These power differences were measured by displaying Doppler spectra and color flow velocity images simultaneously.[2]

Reproducibility of the backscattered power, D and V_{max}

The reproducibility of the Doppler backscattered power, diameter (D), and maximum velocity (V_{max}) was evaluated in 5 normolipidemic subjects by repeating measurements over 3 consecutive days. Measurements were limited to arterial sites CCA and FMA, and venous sites JU1 and FMV. The mean absolute difference between the value of each parameter obtained on Days 1 and 2, and 1 and 3 was determined and averaged over all subjects as a measure of reproducibility.

Statistical analyses

All results were expressed as means ± 1 standard deviation (SD). Differences between groups for physiological and rheological parameters were assessed with unpaired *t*-tests. Two-way analyses of variance using the Bonferroni's method for multiple comparisons were used to confirm differences in the backscattered power measured in veins and arteries of normolipidemic and hyperlipidemic individuals. Differences between peripheral sites were assessed with 1-way Kruskal–Wallis analyses of variance using the Dunn's test for multiple comparisons. Multiple linear regression models were tested to assess the relationship between the backscattered power, physiological, rheological and hemodynamic (D and V_{max}) parameters. A significance level of 5% was used in all analyses. All statistics were computed using Sigma Stat (ver. 1.0), Jandel Scientific, San Rafael, CA, USA.

[2] These backscattered power reductions include a 0- to 2-dB variation, depending on the PRI, attributed to the simultaneous use of the color-flow velocity and pulse-wave Doppler modes. The reduction of the transmitted power to respect the spatial peak time average intensity constraint may also explain this effect on the backscattered power when using both ultrasonic modes.

RESULTS

Description of the populations

Physiological parameters measured in normolipidemic subjects and patients with hyperlipidemia are summarized in Table 1. The BMI, systolic and diastolic pressures, cholesterol, LDL-cholesterol and triglycerides were statistically higher and HDL-cholesterol lower in patients. Table 2 presents rheological parameters, such as erythrocyte aggregation indices measured with the laser light erythroaggregameter, the fibrinogen level, and the hematocrit for both groups of individuals. Statistically significantly higher erythrocyte aggregation levels were found in patients with hyperlipidemia. The erythrocyte aggregation kinetics were increased as indicated by the lower values of tA and the higher values of S_{10}. The adhesive forces between erythrocytes were also increased in patients. For instance, both γD and γS were statistically higher than the values measured in normolipidemic subjects. The level of fibrinogen in patients was also significantly higher. No difference was found between populations for the hematocrit.

Manual selection of Doppler spectrograms

Manual selections of Doppler spectrograms were performed to reduce artifactual cardiac cycles. Most cardiac cycles digitized for arterial sites were selected. The mean number of spectrograms averaged to obtain the

Table 2. Physiological parameters related to the rheology of blood and measured in normolipidemic subjects and patients with hyperlipidemia.

Rheological parameters	Normolipidemic subjects	Hyperlipidemic patients
tA (s)	2.74 ± 0.80	1.90 ± 0.46[a]
S_{10}	23.3 ± 3.4	29.5 ± 4.5[a]
γD (s^{-1})	50 ± 9	55 ± 6[b]
γS (s^{-1})	127 ± 17	170 ± 60[a]
Fb (g/L)	2.59 ± 0.38	3.25 ± 0.74[a]
H (%)	43 ± 3	42 ± 4

The parameter tA = primary aggregation time; S_{10} = mean kinetic index at 10 s; γD = partial dissociation threshold of erythrocytes; γS = total dissociation threshold; Fb = plasma fibrinogen concentration; H = hematocrit.
[a] $p < 0.01$; [b] $p < 0.05$ based on unpaired *t*-tests.

mean spectral representation over a cardiac cycle ranged between 23 and 26, depending on the arterial segment. For venous sites, many cycles were discarded because of the disappearance of the signal over a portion of the cycle. The mean number of cycles averaged to obtain mean spectrograms ranged between 11 and 21. The lowest number of cycles selected was at the popliteal vein (11 cycles for normolipidemic subjects and 12 for patients). Another particularity of this site was the rejection of all cycles or difficulties in recording satisfactorily Doppler signals in 10 normolipidemic subjects and 10 patients.

Figure 1 shows examples of Doppler mean spectrograms recorded over CCA, JU1, FMA and FMV of a patient with hyperlipidemia. Similar spectrograms were obtained for other patients and normolipidemic subjects. As expected, the flow in CCA was mainly unidirectional, whereas a triphasic flow pattern was observed for FMA. For JU1, the flow was pulsatile because of the pulse propagation from the artery to the vein. In FMV, the blood-flow velocity was almost constant on the mean spectrogram because small oscillations produced by the respiratory cycle were damped out by averaging. The mean spectrograms for ICA and JU2 were similar to CCA and JU1, respectively. The iliac and popliteal arterial and venous spectrograms were similar to those obtained in the femoral vessels.

Reproducibility of the backscattered power, D and V_{max}

Table 3 presents the results of reproducibility. With the exception of FMV, the variation of the backscattered power between Days 1 and 2, and 1 and 3 was within 2.5 dB. An unexplained high backscattered power value in one subject resulted in a mean absolute difference of 4 dB for FMV. The reproducibility of the diameters was around 0.04 cm for arteries (CCA and FMA), and 0.20 cm for veins (JU1 and FMV). With the exception of CCA, V_{max} varied by less than 8 cm/s, on the average, between measurements.

Backscattered power in veins and arteries

Based on 2-way analyses of variance, no statistically significant difference was found between the power in CCA and JU1, and ICA and JU2 for both normolipidemic subjects and patients with hyperlipidemia. The results for these sites are presented in Table 4. For the other sites located in lower limbs, statistically significant 1-way interactions were found between veins and arteries ($p < 0.0001$ for the iliac, $p < 0.05$ for the femoral and $p < 0.0001$ for the popliteal). As shown in Fig. 2, the power in ILV was statistically higher than that in ILA for both patients ($p < 0.01$) and normolipidemic subjects ($p < 0.05$). For the femoral site (Fig. 3), statistically significant differences ($p < 0.05$) were observed between FMV and FMA of patients. Between POV and POA, statistically significant differences ($p < 0.01$) were found in patients, as seen in Fig. 4.

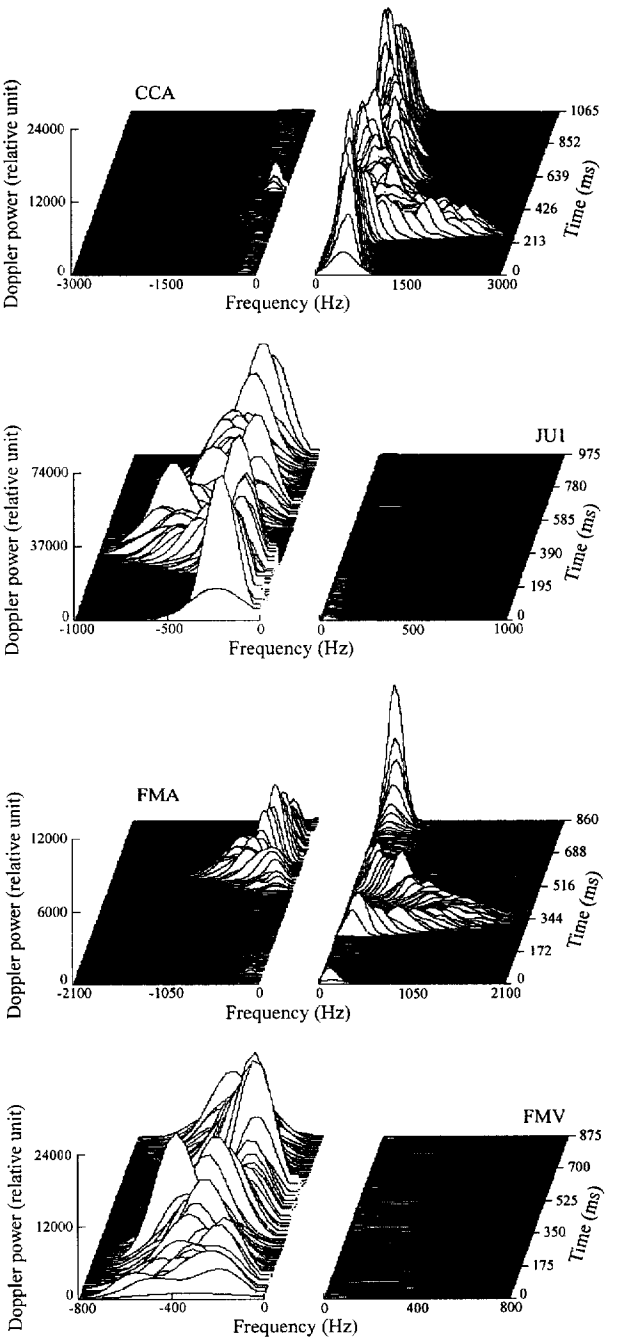

Fig. 1. Examples of mean Doppler spectrograms recorded over the common carotid artery (CCA), jugular vein (JU1), common femoral artery (FMA) and femoral vein (FMV) of a patient with hyperlipidemia. The compensation of the backscattered power for the Doppler gain, tissue attenuation, and PRI was not performed for this figure.

Table 3. Reproducibility of the Doppler backscattered power, diameter of the vessel (D) and maximum velocity (V_{max}).

Recording sites	Power difference (dB)	Diameter difference (cm)	Velocity difference (cm/s)
CCA	1.5 ± 0.4	0.04 ± 0.03	21 ± 20
JU1	1.5 ± 0.8	0.20 ± 0.16	7 ± 6
FMA	2.4 ± 1.5	0.04 ± 0.02	7 ± 4
FMV	4.0 ± 5.5	0.19 ± 0.04	8 ± 6

Measurements were performed on 5 normolipidemic subjects and repeated on 3 consecutive days. The reproducibility was assessed by measuring the mean absolute difference of each parameter between Days 1 and 2, and 1 and 3.

Backscattered power in normolipidemic and hyperlipidemic individuals

No population type interaction was found for measurements in the neck. Statistically significant 1-way interactions were found between healthy subjects and patients for the femoral ($p < 0.001$) and popliteal ($p < 0.05$) sites. For the iliac, a borderline population interaction ($p = 0.056$) was found. The contrast analyses showed a statistically significant difference ($p < 0.05$) between the power in the femoral vein of patients and normolipidemic subjects (Fig. 3).

Backscattered power in peripheral sites

The powers in healthy subjects and patients were pooled to study the effect of the recording site for both arteries and veins. Statistically significant differences ($p < 0.0001$) were found when comparing the power in CCA (60.0 ± 4.8 dB), ICA (53.7 ± 4.5 dB), ILA (54.7 ± 4.3 dB), FMA (57.3 ± 4.3 dB) and POA (55.2 ± 5.6 dB). The contrast analysis showed differences ($p < 0.05$) between the power in CCA and ICA, CCA and ILA, CCA and POA, and ICA and FMA. For venous sites, statistically significant differences ($p < 0.0001$) were also observed. The power in JU1, JU2, ILV, FMV and POV was 60.8 ± 4.4 dB, 54.2 ± 4.9 dB, 59.8 ± 6.7 dB, 60.8 ± 8.3 dB and 62.6 ± 7.4 dB, respectively. The contrast analysis showed differences ($p < 0.05$) only between JU2 and all other venous sites.

Table 4. Doppler backscattered power in the common carotid artery (CCA), internal carotid artery (ICA) and jugular vein (JU1, JU2) of normolipidemic subjects and patients with hyperlipidemia.

	Doppler backscattered power (dB)			
	CCA	JU1	ICA	JU2
Normolipidemic subjects	59.4 ± 5.5	60.5 ± 4.4	54.0 ± 3.9	55.2 ± 4.3
Hyperlipidemic patients	60.4 ± 4.3	61.1 ± 4.5	53.5 ± 4.9	53.5 ± 5.2

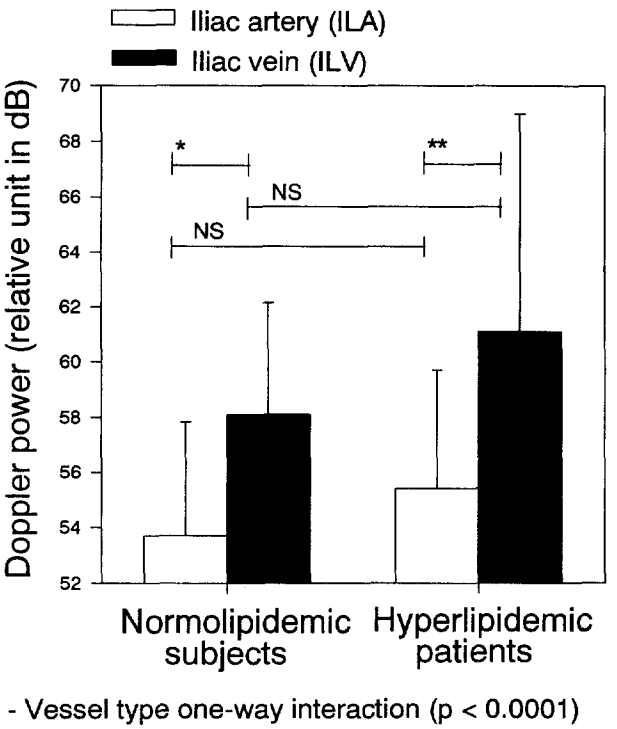

Fig. 2. Doppler backscattered power in the iliac artery and vein of normolipidemic subjects and patients with hyperlipidemia. Multiple comparisons using the Bonferroni's method showed statistically significant differences between the vein and the artery of normolipidemic subjects and patients with hyperlipidemia (**$p < 0.01$; *$p < 0.05$, NS = nonsignificant).

Effects of physiological, rheological and hemodynamic parameters on the backscattered power

Multiple linear regression analyses were used to assess the relationship between the backscattered power in different vessels, D, V_{max}, and physiological and rheological parameters described in Tables 1 and 2. For each recording site, the data of both normolipidemic subjects and patients were pooled for the statistical analyses. No multilinear relationship was found by including more than two parameters. The most statistically significant result was obtained by modeling the power in FMV with two parameters, S_{10} and D. As seen in Fig. 5, including these two parameters in the model resulted in a correlation coefficient (r) of 71.2% ($p < 0.0001$). No statistically significant result was found by combining two parameters for the other recording sites. For models including only one parameter, the best results were characterized by correlation coefficients (r) of 59.9% ($p < 0.0001$), 60.5% ($p < 0.01$), 44.8% ($p < 0.01$) and 36.2% ($p < 0.05$) between S_{10} and the power in FMV, POV,

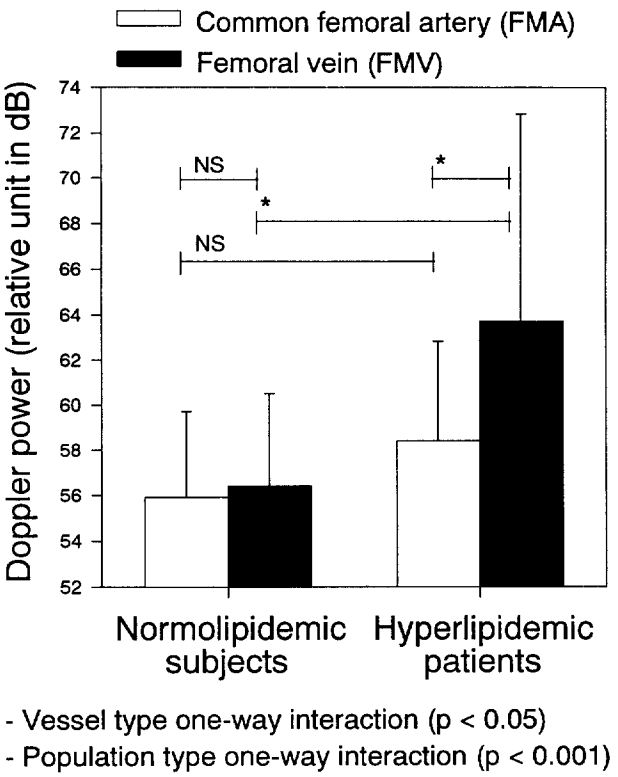

Fig. 3. Doppler backscattered power in the common femoral artery and femoral vein of normolipidemic subjects and patients with hyperlipidemia. Multiple comparisons using the Bonferroni's method showed statistically significant differences between the vein and the artery of patients with hyperlipidemia, and veins of normolipidemic subjects and patients (*$p < 0.05$, NS = nonsignificant).

ILV and FMA, respectively. No statistically significant correlation was found in the remaining vessels, between any other single parameters and the power.

DISCUSSION

Differences in the Doppler power backscattered by blood were observed in the present study. Stronger backscattered power was found in veins compared to arteries for the iliac, femoral and popliteal sites. The lower shear rate in veins may explain the stronger backscattered power obtained. Stronger backscattered power was also observed in hyperlipidemic individuals for the femoral and popliteal sites. Moreover, the differences between normolipidemic and hyperlipidemic individuals were almost significant for the iliac. These differences are explained by the enhancement of erythrocyte aggregation in these patients. In addition to these results, Doppler power differences between peripheral sites were detected. However, these results should be interpreted with caution because some variability may be attributable to the method used to compensate the backscattered power for depth. In the present study, a constant attenuation coefficient of 3.5 dB/cm was assumed, independently of the recording site. Finally, the parameters explaining the backscattered power variations for arteries and veins of healthy subjects and patients were identified. The variations were related to the aggregation kinetic index S_{10}, and the vessel diameter for the femoral vein.

Backscattered power in the carotid artery and jugular vein

As seen in Table 4, no vessel type or population type interactions were found for the carotid artery and jugular vein. For a given recording site (the level of the CCA or ICA segment), the absence of power differences can be explained by the pulsatility of the flow in the jugular vein (the pulsatility seen in Fig. 1 was present for all individuals). For instance, it was demonstrated, using the laser scattering method, that the pulsatility reduces erythrocyte aggregation (Riha and Stoltz 1996). The erythrocyte aggregates expected, especially in the veins of patients, were probably reduced in size by the pulsa-

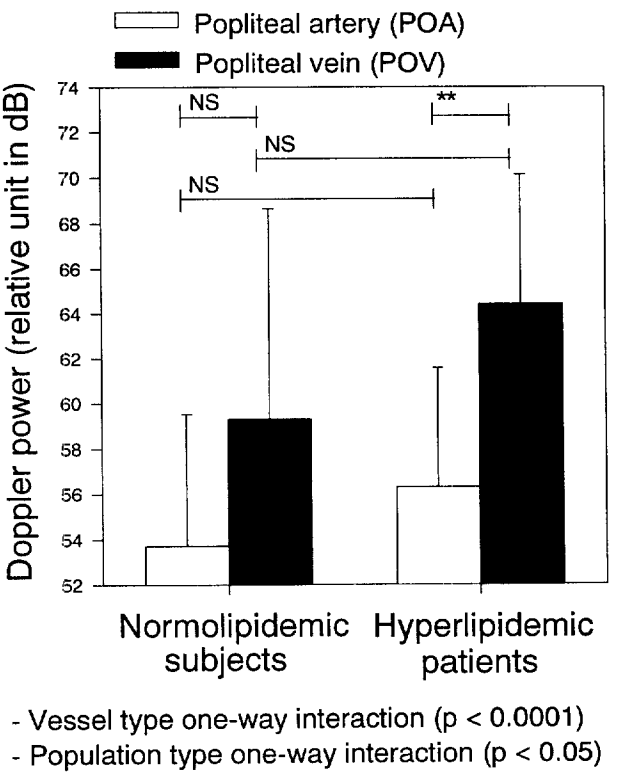

Fig. 4. Doppler backscattered power in the popliteal artery and vein of normolipidemic subjects and patients with hyperlipidemia. Multiple comparisons using the Bonferroni's method showed statistically significant differences between the vein and the artery of patients with hyperlipidemia (**$p < 0.01$, NS = nonsignificant).

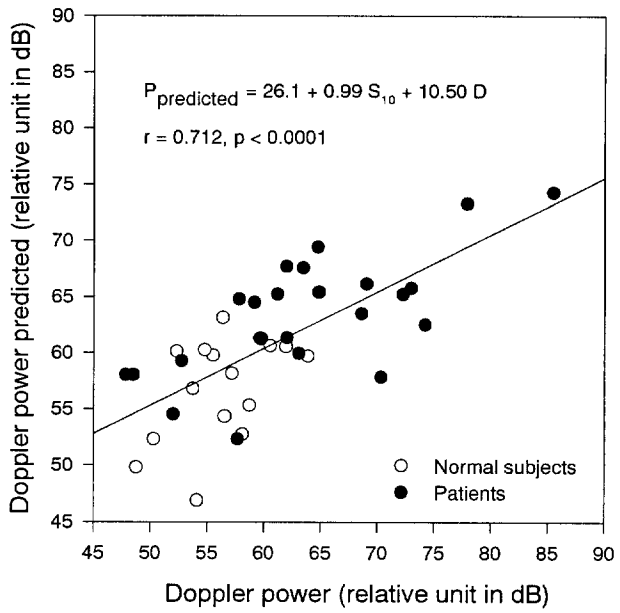

Fig. 5. Multiple linear regression model relating the Doppler backscattered power measured in the femoral veins of normolipidemic subjects and patients with hyperlipidemia to the aggregation index S_{10} and the diameter D of the vessel in cm. The parameter $P_{predicted}$ is the backscattered power predicted by the regression model.

tility of the flow in JU1 and JU2. It was also intriguing to observe large differences in power between CCA and ICA, and JU1 and JU2. As reported previously, the power in CCA was, on the average, 6.3 dB stronger than that in ICA, thus suggesting the presence of larger aggregates in CCA. It is believed that the shearing effect of the complex flow pattern in ICA (helical flow and boundary layer separation in the bulb) may have reduced the aggregation level. For JU1, the power was 6.6 dB stronger than that in JU2. The mean diameter of the vessels was 1.16 ± 0.36 cm for JU1 and 0.65 ± 0.21 cm for JU2 ($p < 0.0001$), which suggests the presence of higher shear rates within the Doppler sample volume for JU2.

The source of the Doppler backscattered power

As reported in the Introduction, the presence of spontaneous echo contrast in the human cardiovascular system has been associated with both erythrocyte and platelet aggregation. It is well recognized that platelet aggregates can be detected *in vitro* with ultrasound (Machi et al. 1984, 1987; Mahony 1987). However, the fact that platelet aggregates may be involved in the production of spontaneous echoes *in vivo* is less accepted. In a case report (Mahony et al. 1989), it was shown that trifluoperazine, an inhibitor of platelet aggregability, eliminated the presence of spontaneous echo contrast in the left ventricle of a patient. However, a similar treatment protocol could not reproduce these results in 5 patients (Hoffmann et al. 1990). Recently, Kearney and Mahony (1995) showed a significant decrease in the echo intensity measured in brachial veins of normolipidemic subjects following 7 days of aspirin administration. They concluded that a component of the spontaneous echo contrast was aspirin-sensitive and probably related to platelet aggregation. Because aspirin was recently shown to also reduce erythrocyte aggregation (Tanahashi et al. 1996), the role of platelet aggregation on *in vivo* blood echogenicity may be questioned. In the present study, erythrocyte aggregation was shown as a major determinant of the Doppler power *in vivo*.

Another factor that may affect the backscattered power between recording sites

In a study in patients with peripheral arterial occlusive disease (Forconi et al. 1979), blood viscosity, providing an indirect assessment of erythrocyte aggregation, was measured from arterial and venous blood samples. It was found that samples taken from the brachial and femoral veins had higher viscosities than samples withdrawn from the femoral artery. Little differences were found between brachial and femoral veins. Because the fibrinogen concentration was constant between samples, it was suggested that the hyperviscosity may have been locally generated in the blood flowing through ischemic tissues. Local delivery of unknown substances by ischemic tissues or aggregated platelets may trigger an enhancement of erythrocyte aggregation, as suggested by Tanahashi et al. (1989). Patients scheduled for coronary artery surgery and/or valve replacement also showed higher viscosity in venous blood compared to arterial blood collected by intravenous catheter (Mokken et al. 1996). In that study, higher concentrations of plasma proteins in venous blood was thought to be responsible for the enhanced erythrocyte aggregation. Based on these studies, the power Doppler differences between vessels may be explained by *in situ* localized changes in erythrocyte aggregation "activated" by ischemic tissues, aggregated platelets, or local changes in plasma protein concentrations.

Variability of the results

As observed in Figs. 2 to 4, the SDs of the Doppler power ranged between 3.8 and 9.3 dB, depending on the population and vessel type. Normal physiological interindividual differences in erythrocyte aggregation, shear rate variations within the Doppler sample volume, and local changes in plasma protein concentrations promoting the aggregation may explain these variations. For patients, the possible influence of medication on erythrocyte aggregation and the local release of substances by ischemic tissues may have contributed. The scattering of

the results seen in Fig. 5 is attributed, in part, to some of the above factors. Also, the duration between blood collection and the measurement of S_{10} varied between individuals, and may have contributed to the scattering of the results. The compression of the femoral vein by the transducer may have also increased the variability of D. It is expected that having access to the shear rate within the Doppler sample volume would have increased the correlation coefficient of the model. In the present study, our methodology did not provide access to the measurement of the shear rate acting on erythrocytes within the Doppler sample volume.

CONCLUSION

This study demonstrated that power Doppler ultrasound can detect erythrocyte aggregation *in vivo* in large vessels. The pathophysiological consequences of hyper-erythrocyte aggregation in large vessels are not known. The presence of aggregation in large arteries and veins may play a role in flow resistance. It may also influence thrombus formation (Daniel et al. 1988) and arterial restenosis (Maiuri et al. 1995).

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