Background: The skin microcirculation may be evaluated noninvasively by laser Doppler flowmetry and iontophoresis with acetylcholine and sodium nitroprusside. Wavelet transform of the perfusion signal shows periodic oscillations of five characteristic frequencies in the interval 0.0095–1.6 Hz. The aim of the current study was to investigate alterations in skin microcirculation induced by brachial plexus block, with emphasis on the periodic oscillations.

Methods: Healthy nonsmokers undergoing hand surgery (n = 15) were anesthetized with brachial plexus block, using bupivacaine, lidocaine, and epinephrine. Skin microcirculation was evaluated by laser Doppler flowmetry and iontophoresis with acetylcholine and sodium nitroprusside before and after brachial plexus block. Wavelet transform of the perfusion signal was performed. As a control group, 10 healthy nonsmokers were included.

Results: In the anesthetized arm, skin perfusion after brachial plexus block increased from 19 (12–30) to 24 (14–39) arbitrary units (P < 0.01). A significant increase was also seen in the contralateral arm from 17 (14–32) to 20 (14–42) arbitrary units (P < 0.01). After brachial plexus block, spectral analysis revealed a significant reduction in relative amplitude of the oscillatory components within the 0.0095–0.021- (P < 0.001) and 0.021- to 0.052-Hz (P < 0.001) intervals in the anesthetized arm.

Conclusion: Alterations in skin microcirculation induced by brachial plexus block can be evaluated by wavelet transform of the laser Doppler flowmetry signal. Brachial plexus block reduces the oscillatory components within the 0.0095–0.021- and 0.021- to 0.052-Hz intervals of the perfusion signal. These alterations are related to inhibition of sympathetic activity and a possible impairment of endothelial function.

THE human microcirculation shows rhythmic variations produced by central and local oscillators. The most obvious are the heart and respiration, which produce rhythmical variations in blood flow around 1.0 and 0.3 Hz, respectively. Guyton and Harris in 1951 proposed the term vasomotor waves for slower oscillations than those related to respiration. Studies performed on humans and animals have concluded that these slow oscillations are influenced by the sympathetic nervous system and the microvascular wall. However, there have been conflicting results regarding the mechanisms for the different frequency intervals.

A relatively new approach to the analysis of nonstationary signals, such as human biologic signals, is wavelet analysis. Wavelet analysis of laser Doppler flowmetry (LDF) signals from human cutaneous circulation has revealed five oscillations, including those from the heart and the respiration. Data indicate that the 0.1-, 0.03-, and 0.01-Hz oscillations are related to intrinsic myogenic activity of vascular smooth muscle cells, neurogenic activity on the vessel wall, and the vascular endothelium, respectively.

Brachial plexus block induces changes in peripheral hemodynamics. In addition, sympathetic impairment is a key finding and has been measured by stimulation of cutaneous vasoconstrictor reflexes, which are attenuated after brachial plexus block. It has also been reported that local anesthetics impair endothelium-mediated vasodilatation.

Materials and Methods

Subjects
The study was approved by the local ethics committee of Oslo, Norway. We included 13 previously healthy patients undergoing hand surgery (aged 22–60 yr). They were all nonsmokers without regular medication, classified as American Society of Anesthesiologists physical status I. For anthropometric data, see table 1. As a control group, we included 10 healthy nonsmokers. Written informed consent was obtained from all patients (table 1).

Protocol
Recordings were performed with subjects in the supine position in a quiet room. The temperature was kept...
at 23°C. The experimental procedure started after an acclimatization period of 20 min. Two probes for basal LDF recordings and temperature measurement (MP1 probes; Moor Instruments, Axminster, Devon, United Kingdom) were positioned with double-sided adhesive tape on the volar aspect of the lower arm. One probe was placed on the arm subjected to anesthesia, and one was placed on the contralateral arm. The probes were located in the same positions throughout the entire experiment. Skin temperature was measured continuously during the two registration periods. Two probes for combined iontophoresis and LDF recordings (DPIT probes; Moor Instruments) were positioned on the arm subjected to anesthesia, at least 5 cm apart, avoiding superficial veins and damaged skin areas. To avoid residual effects of the drugs, the position of the probes for combined iontophoresis and LDF recordings were changed to untreated skin after each of the measurements. The first recording was obtained for an interval of 30 min. Brachial plexus block was then performed. After the procedure was complete, there was an interval of 50 min. The second recording was then obtained for an interval of 30 min. Blood pressure was measured before and after the registrations. Respiration frequency, heart rate, and skin temperature were measured during both recordings. The control group followed an identical protocol with two recordings lasting for 30 min. The statistical analysis is based on the mean value of the total period (fig. 1).

**Brachial Plexus Block**

Brachial plexus block was performed by transarterial technique combined with a bolus injection in a permanent catheter, guided by a nerve stimulator. The same anesthesiologist (T.K.) performed all of the procedures. Each patient received a total of 0.75 ml/kg of a 50:50 mixture of 20 mg/ml lidocaine with 12.5 μg/ml epinephrine and 5.0 mg/ml bupivacaine, giving a concentration of 2.5 mg/ml bupivacaine, 10 mg/ml lidocaine, and 6.25 μg/ml epinephrine.

Complete block was obtained in all cases before the second registration. Supplemental ulnar block was performed in one patient before surgery, but after our measurements were obtained. No complications of the procedure were registered.

### Laser Doppler Flowmetry

The principles of LDF have previously been described thoroughly elsewhere. LDF gives a semiquantitative measurement of microvascular blood perfusion, expressed in arbitrary units (AU). LDF measurements from the skin reflect perfusion in capillaries, arterioles, venules, and dermal vascular plexus. A major part of the signal reflects thermoregulatory perfusion. The LDF measurements were obtained with a two-channel flowmeter (DR 4; Moor Instruments) for recordings during iontophoresis with acetylcholine and sodium nitroprusside (SNP). A sampling frequency of 40 Hz and a time constant of 0.1 s were selected.

### Iontophoresis

Iontophoresis allows transdermal delivery of polar drugs by means of a small electrical current. It is then possible to assess microvascular reactivity by measuring blood perfusion simultaneously in the same area with LDF. Two combined probe holders for iontophoresis and perfusion measurement (MIC 1-ION; Moor Instruments) of opposite polarity were attached to the volar side of the forearm. The probe holder has a small chamber for deposition of the test substance which is in direct contact with the laser Doppler probe. A constant current stimulator (MIC 1; Moor Instruments) was used to provide a direct current for the drug iontophoresis. Acetylcholine and SNP were used to produce an endothelium-dependent and -independent increase in flow. We used a charge of 2 mC (100 μA for 20 s) followed by a 240-s response measurement period after each iontophoresis. The iontophoresis was repeated seven times, giving a total registration period of 30 min. The statistical analysis is based on the mean value of the total period (fig. 1).

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**Table 1. Anthropometric Data in the Two Groups**

| Age, yr | 38 ± 13 | 33 ± 9 |
| Height, cm | 177 ± 10 | 176 ± 11 |
| Body mass, kg | 78 ± 12 | 70 ± 14 |
| ASA physical status | 1 | 1 |
| Male:female | 9:4 | 6:4 |

Data are presented as mean ± SD.

ASA = American Society of Anesthesiologists.
Spectral Analysis and Wavelet Transform

Most biologic signals, such as LDF signals from human skin, are nonlinear. The characteristic frequencies of the signal continuously change in time, like variability in heart rate and respiration. The traditional way of analyzing such signals, the Fourier transform, cannot detect these changes in time, although a variation of this transform, short-time Fourier transform, can do so. However, the ratio between lower and higher basic frequencies in the blood perfusion signal (1:100) also implies the necessity for good low-frequency resolution and good time resolution for higher-frequency components. This is difficult to detect by any of the Fourier transforms. The wavelet transform offers optimal frequency resolution and time localization, and an adjustable window length. Slow events are analyzed with a long window, whereas faster events are analyzed with a shorter window. This results in logarithmic frequency resolution.

The wavelet transform used in this study is a modification of the continuous wavelet transform, based on the Morlet mother waves. A frequency interval between 0.0095 and 1.6 Hz is calculated. In this interval, periodic oscillations with five different characteristic frequencies are observed. The mean amplitude of oscillations of the total spectrum from 0.0095 to 1.6 Hz (mean amplitude), and the amplitude of each particular frequency interval (spectral amplitude) can be calculated. We will then define the relative amplitude for each frequency interval as the ratio between the spectral amplitude at a particular frequency interval and the mean amplitude of the entire spectrum. The relative amplitude gives information about the contribution of each component to the total variability observed. The effects of acetylcholine and SNP on the 0.0095- to 0.02-Hz frequency interval have previously been described only in the “relative amplitudes.” The relative amplitude is synonym with normalized amplitude used in some articles. Therefore, data presented in the figures are relative amplitude (fig. 2).

Statistics

Based on sample size calculations, we found that with $\alpha = 0.05$ and a desired power of 80%, we would need a minimum number of 10 patients to show a 50% decrease of the amplitude in the 0.021- to 0.052-Hz frequency interval. Major parts of the flow and wavelet data were not normally distributed. Therefore, the data are presented as median and total range, or as box plots. Each box plot contains five horizontal lines representing the 10th, 25th, 50th, 75th, and 90th percentiles. Anthropometric data and hemodynamic data were normally distributed and are presented as mean $\pm$ SD. The Wilcoxon signed rank test for comparison of dependent samples was used to evaluate differences in skin perfusion and wavelet transform in basal probes. Differences in the wavelet transform between acetylcholine and SNP probes were evaluated by the Mann-Whitney rank sum test. A $P$ value of 0.05 was considered statistically significant. Data were analyzed in Sigmastat (Systat Software Inc., Richmond, CA).

Results

Effects of Brachial Plexus Block on Hemodynamics, Respiration Frequency, and Skin Temperature

Brachial plexus block increased heart rate by 20 ± 2% ($P < 0.001$) and systolic blood pressure by 7 ± 1% ($P = 0.046$). No significant changes were seen in mean arterial blood pressure, diastolic blood pressure, or respiration frequency (table 2). After brachial plexus block, skin temperature increased by 1.5° ± 0.9°C ($P < 0.001$) in the anesthetized arm and by 1.1° ± 1.5°C in the contralateral arm ($P = 0.03$). No difference was detected between the two arms ($P = 0.53$). In the control group, there were no significant differences between the two registrations in blood pressure, respiration frequency, or skin temperature (table 2).

Effects of Brachial Plexus Block on Basal Skin Perfusion and on Skin Perfusion in Response to Iontophoresis with Acetylcholine and SNP

After brachial plexus block, the basal skin perfusion, measured by LDF, increased from 19 (12–30) to 24 (14–39) AU ($P = 0.003$) in the anesthetized arm (fig. 3). A significant increase from 17 (14–32) to 20 (14–42) AU ($P = 0.006$) was also seen in the contralateral arm (fig. 3A). There was no significant difference between these increments ($P = 0.55$). Average blood flows in response to iontophoresis with acetylcholine and SNP were not significantly altered by brachial plexus block (fig. 3B).
the control group, there was no significant difference in average blood flow between the two registrations (probe 1, \( P = 0.43 \); probe 2, \( P = 0.56 \); acetylcholine, \( P = 0.71 \); and SNP, \( P = 0.16 \); fig. 3).

Effects of Brachial Plexus Block on Relative Amplitudes within Each of the Five Frequency Intervals during Basal perfusion

In the anesthetized arm, there were highly significant reductions in relative amplitudes in the 0.021- to 0.052- (\( P < 0.001 \)) and the 0.0095- to 0.02-Hz frequency intervals (\( P < 0.001 \)) (fig. 4A). There was also a slight increase in the 0.15- to 0.6-Hz frequency interval (\( P = 0.002 \)). In the contralateral arm, there were no significant changes in the frequency intervals, except for a reduction in the 0.6- to 2-Hz frequency interval (\( P = 0.006 \)) (fig. 4B). In the control group, there were no significant differences in relative amplitude between the two registrations (figs. 4C and D).

Discussion

The current study shows that changes in human skin microcirculations during brachial plexus block, such as sympathetic impairment, can be evaluated by wavelet transform of the LDF signal. These changes in the anesthetized arm are illustrated in figure 6, which three-dimensionally illustrates the major reductions in the two lower frequency intervals, whereas the three higher frequency intervals are unaltered. In the following discussion, we will relate these alterations to inhibition of local physiologic processes in the skin microcirculation.

We found a significant increase in skin perfusion and skin temperature, both in the anesthetized and contralateral arms, with no significant difference between the increments. Several aspects might contribute to this finding. Sympathetic tone in nerves supplying the forearm skin is low, and skin perfusion correlates with changes in systemic blood pressure.21 This is in contrast to skin areas containing arteriovenous anastomoses, such as hands and feet, and in skeletal muscles, where sympathetic tone is high. Accordingly, measurements obtained by venous occlusion plethysmography after brachial

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Table 2. Hemodynamic Data in the Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Patient Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Block</td>
<td>After Block</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>100 ± 10</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>139 ± 13</td>
<td>150 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>79 ± 11</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>67 ± 2.4</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>Respiration rate, breaths/min</td>
<td>12.4 ± 1.4</td>
<td>12.9 ± 1.5</td>
</tr>
<tr>
<td>Skin temperature, °C</td>
<td>29.7 ± 1.1</td>
<td>31.1 ± 1.3</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Reg = registration.
Plexus block have shown a four-times increase in total blood flow to the arm. Increased blood pressure as a result of anxiety, the result of a systemic effect of epinephrine supplementing the local anesthetics, or the combination of the two may increase skin blood flow and temperature in both arms. A systemic effect of local anesthetics could also contribute to the increase in perfusion and temperature. Both lidocaine and bupivacaine have a biphasic effect on peripheral vasculature. Low concentrations induce vasoconstriction, and high concentrations induce vasodilation. Therefore, inhibition of sympathetic activity may only partly contribute to the increased flow after brachial plexus block in our study.

Although there were no significant differences in perfusion between the two arms after brachial plexus block, wavelet transform of the perfusion signal revealed major differences between the two arms. After brachial plexus block, the oscillation within the 0.021- to 0.052-Hz interval was reduced in the anesthetized arm. Several studies suggest that this oscillation is related to sympathetic activity. In human skin, Kastrup et al. demonstrated that rhythmical variations in blood flow, with a peak amplitude around 0.025 Hz, disappeared completely af-

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**Fig. 4.** Relative amplitudes for the five frequency intervals within the 0.0095- to 1.6-Hz interval of the laser Doppler perfusion signal. Data are shown as box plots. (A) Before and after brachial plexus block in the anesthetized arm. (B) Before and after brachial plexus block in the contralateral arm. (C) Control group, probe 1, registrations 1 and 2. (D) Control group, probe 2, registrations 1 and 2.

**Fig. 5.** Relative amplitudes for the five frequency intervals within the 0.0095- to 1.6-Hz interval of the laser Doppler perfusion signal during iontophoresis with acetylcholine (ACh) and sodium nitroprusside (SNP). Data are shown as box plots. (A) Before brachial plexus block. (B) After brachial plexus block. (C) Control group, probe 1, registrations 1 and 2. (D) Control group, probe 2, registrations 1 and 2.
Fig. 6. A three-dimensional wavelet transform of a laser Doppler flowmetry signal, from a patient before and after brachial plexus block. This illustration shows the amplitudes for the different frequencies over time. The amplitudes for the lower frequencies are clearly reduced.

ter denervation, both after local and ganglionic nerve block and after sympathectomy. Söderström et al. examined LDF signals in human free flaps and found that the relative amplitude of the 0.021- to 0.052-Hz frequency interval was significantly reduced. Others have found evidence for a sympathetic oscillation around 0.1 Hz. However, most of these studies are performed with other spectral analysis techniques, such as the fast Fourier transform, by which slow and fast oscillations are difficult to detect at the same time. In many cases, the registration period is also too short to detect the slowest oscillations. In our study, there were no significant changes in oscillations around 0.1 Hz. The reduced oscillation in the 0.021- to 0.052-Hz frequency interval after brachial plexus block strongly indicates inhibition of sympathetic activity.

Brachial plexus block also reduced the relative amplitude of the oscillation within the 0.0095- to 0.021-Hz interval in the anesthetized arm, whereas it was unaltered in the contralateral arm. We have previously demonstrated that this oscillation (around 0.01 Hz) is modulated by the vascular endothelium, because iontophoresis with acetylcholine increased the normalized amplitude to a greater extent than SNP selectively in this frequency interval. In a later study, we showed that this effect was inhibited by \( \text{L-arginine} \), an inhibitor of nitric oxide synthesis, and reversed by \( \text{N}^\text{G} \)-monomethyl-L-arginine, the substrate for nitric oxide synthesis. In accord with this interpretation, the difference between acetylcholine and SNP in this frequency interval was abolished after brachial plexus block. We therefore interpret the reduced amplitude of the oscillation within the 0.0095- to 0.021-Hz interval as an effect on the vascular endothelium activity induced by the brachial plexus block. This reduction of the endothelial-related oscillations could be a direct effect of the local anesthetic on the endothelium, as has been described by several investigators. Another explanation could be that alterations in sympathetic activity during brachial plexus block have a direct influence on endothelial function. Accordingly, epinephrine, supplementing the local anesthetics, could contribute to the increased amplitudes in both sympathetic and endothelial-related oscillations in the contralateral arm.

After brachial plexus block, there was also a slight increase in the 0.15- to 0.6-Hz frequency interval in the anesthetized arm and a trend toward increase in the contralateral arm (\( P = 0.068 \)). A possible explanation for this could be an altered respiratory pattern due to anxiety or epinephrine after anesthesia. A reduction in the 0.6- to 2-Hz frequency interval in the contralateral arm was also found. The changes of the 0.15- to 0.6- and the 0.6- to 2-Hz frequency intervals were not detected in the spectral amplitude.

Deschamps et al. have previously shown that data on sympathetic and parasympathetic activity induced by epidural anesthesia can be obtained by analyzing heart rate variability and blood pressure variability using wavelet transform of the signals. The results from our study extend data from Deschamps et al. showing that wavelet transform of human biologic signals may give important hemodynamic information during interventions. However, there are important differences between the two studies. In the current study, we focused on slower oscillations (0.04 and 0.01 Hz), whereas Deschamps et al. focused on faster oscillations (1.0 Hz). We investigated the consequences of a different kind of regional anesthesia, brachial plexus versus epidural anesthesia, and the variables analyzed by Deschamps et al. were related to central hemodynamic (heart rate and blood pressure), whereas we focused on the skin microcirculation.

Today, variation of the respiratory-related oscillation of the blood pressure signal, as an indication of variation of stroke volume, is a useful diagnostic tool in hemodynamic evaluation of critically ill patients. The method of decomposing noninvasive hemodynamic signals, such as the LDF signal, into oscillatory components using the wavelet transform can be useful in understanding changes both in central and peripheral circulations during different forms of anesthesia and in pathophysiologic conditions. In the future, this could be helpful in developing new noninvasive monitoring equipment.
Limitations

In our study, we included 13 patients. However, because of larger variations in some of our results and the number of variables calculated, we cannot exclude the possibility of having type II errors in our statistical calculations.

The effects of epinephrine could be a confounding factor in our study. Based on previous studies, the sympathetic effect of epinephrine is difficult to predict with the high doses used in our study. Performing the study without epinephrine, or measuring the concentrations of epinephrine in blood during the experiment, could add further knowledge to this aspect.

In our study, relative amplitudes are presented and not absolute amplitude or power calculations (spectral power). The relative amplitude gives information about the contribution of each component to the total variability observed. In the literature, there are no standards in calculation, or in presentation of wavelet analysis from hemodynamic signals. This represents a challenge in future research in this field.

Conclusion

Wavelet transform of the skin microcirculation after brachial plexus block demonstrates a reduced oscillatory component within the 0.0095- to 0.021- and 0.021- to 0.052-Hz intervals of the perfusion signal. These frequency intervals represent neurogenic and endothelial activity, respectively, and our findings indicate an inhibitory effect on the sympathetic and endothelial activity. 

References

8. Johns RA: Local anesthetics inhibit endothelium-dependent vasodilation. ANESTHESIOLOGY 1990; 70:805–11