Peripheral artery disease (PAD) of the lower extremities is caused by arterial obstruction that leads to reduced arterial flow during exercise or at rest. The disease is present in approximately 4% of persons older than 40 years but in 15% of those >65 years of age.1,2 Revascularization therapy fails or is not applicable, which makes alternative approaches necessary.3,4 Thus, promotion of arteriogenesis, which refers to positive remodeling of preformed collateral arterioles, that is, collateral growth, should be induced in these patients.4,6

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Despite the fact that numerous studies pursued the important therapeutic strategy of improving collateral function in patients with PAD, there is no method available to quantify collateral arterial function of the lower limb and, thus, to determine therapeutic effects.3,7,8 In contrast to peripheral arteries, collaterals of the heart can be assessed quantitatively by coronary flow velocity or pressure measurements, which have become the gold standard.9–11 The theoretical basis of this method relates to the fact that perfusion pressure (minus the central venous back pressure) or flow velocity signals obtained distal to an occluded stenosis originate from collaterals (during a brief coronary balloon occlusion or in case of a chronic total occlusion).9 The measurement of aortic and coronary pressure or flow velocity provides the basis for the calculation of a pressure- or velocity-derived collateral flow index (CFI, or CFIp), both of which express the amount of flow via collaterals to the vascular region of interest as a fraction of the flow via the normally patent vessel. CFI measurements have been documented to be very accurate with regard to ECG-derived dichotomous collateral assessment, with
regard to each other, but also to quantitative myocardial perfusion imaging by contrast echocardiography during balloon occlusion. Pressure- and velocity-derived coronary collateral measurements are accepted as the reference method for quantitative clinical assessment of coronary collateral flow. Concerning the promotion of peripheral collateral growth, clinical studies investigating new therapeutic strategies have determined rather weak endpoints obtained during vessel patency, such as ankle-brachial index or walking distance. In contrast, to the coronary circulation, there is currently no reference method available to document successful promotion of collateral growth in patients with PAD. Therefore, the purpose of the present study was to evaluate a new invasive method to quantify arterial collateral function in the lower extremity in patients undergoing elective coronary angiography.

Methods

Patients and Study Design

Thirty patients electively referred for a diagnostic coronary angiography were included in the study. Criteria for study exclusion were as follows: (1) Rest pain, ulceration, or gangrene caused by PAD (Rutherford categories 4–6); (2) known significant stenoses (>50%) or occlusion of superficial femoral, profunda femoral, or aortoiliac arteries; (3) known aneurysm of abdominal aorta or iliac arteries; (4) acute coronary syndrome; (5) congestive heart failure (New York Heart Association functional class III–IV); (6) severe pulmonary hypertension; and (7) severe hepatic or renal failure. This was a prospective exploratory study. As the primary study endpoint, pressure-derived collateral flow index (CFIp) was determined for the calculation of pressure-derived collateral flow index [CFIp = (Poccl − CVP)/(Pao − CVP); pressure values in mm Hg]. CFIp has been documented to reflect collateral relative to normal antegrade flow as quantified by contrast echocardiography very closely and precisely. For the calculation of velocity-derived collateral flow index, CFIv, the nonocclusive baseline flow velocity (Vnonoccl) and the collateral-supplying artery and the profunda femoral artery was the collateral-receiving (ipsilateral) artery and the profunda femoral artery was the collateral-supplying (contralateral) artery (Figure 1).

Study Protocol

After diagnostic coronary angiography (and percutaneous coronary intervention if indicated), lower-extremity angiography was performed by retrograde femoral access. At the start of the protocol, 5000 U of heparin were given intravenously. The angioplasty pressure/Doppler sensor guidewire was positioned distal to the site of the imminent balloon occlusion. During the entire protocol, peripheral oxygen saturation (SaO2) was monitored by pulse oximetry of the ipsilateral left toe. Simultaneous recording of Pao, distal pressure (becoming femoral occlusive pressure, Poccl), CVP, flow velocity, and SaO2 was started before and continued throughout the balloon occlusion for a maximum of 10 minutes. An adequately sized angioplasty balloon was selected (Fox plus 7.0–9.0×20 mm; Avion plus 7.0×20 mm) and balloon inflation at low inflation pressure was performed (1–6 atm). Complete occlusion of the vessel was confirmed by contrast injection. After the first 5 minutes of distal occlusive pressure and velocity measurement under resting conditions, patients performed repetitive plantar-flexion movements in a supine position for another 5 minutes or until pain occurred (exercise protocol was performed in 28 patients). Subsequently, the angioplasty catheter and pressure/Doppler guidewire were removed, and a final angiography was performed to ensure unaltered vascular anatomy.

Statistical Analysis

Data are given as mean±SD unless stated otherwise. Categorical data are given as number (%). ANOVA for repeated measures followed by Bonferroni-Dunn post hoc test was used for analyses when appropriate. Linear regression analysis was performed between CFI as the independent variable and SaO2, changes or pain-free time span, respectively, as the dependent variable. Probability values <0.05 were considered statistically significant.
Results

Patient Characteristics and Clinical Data

Characteristics and clinical data of study patients are presented in Table 1. Study patients were predominantly men, and 57% of them had angina pectoris. Systemic arterial hypertension and dyslipidemia were the cardiovascular risk factors present in most patients, which were usually treated with acetylsalicylic acid, statins, and inhibitors of the renin-angiotensin system.

Angiographic, Hemodynamic, and Collateral Circulation Data

Angiographic and hemodynamic data taken before the start of the study protocol are given in Table 2. Coronary artery disease was observed by coronary angiography in 90% of study patients. In 12 patients (40%), angiography of the left lower limb revealed wall irregularities or nonsignificant stenoses of the SFA, respectively, and in 1 patient, a nonsignificant stenosis of the left iliac artery. Initially, 1 patient was excluded from the study because of an occluded left popliteal artery (59-year-old male patient without coronary artery disease).

Under resting conditions, balloon occlusion of the SFA over 5 minutes was painless in all patients. Consistently, angiography demonstrated 1 to 4 sites of collateral inflow during balloon occlusion (Figure 2). CFIp increased during the first 3 minutes of occlusion under resting conditions, from 0.451±0.168 to 0.551±0.172 (P=0.0003; Figure 3), whereas $\text{Sa}_{\text{O}_2}$ decreased from 98±2% to 93±7% (P=0.004). In the patient excluded from the analysis because of popliteal artery occlusion, CFIp was 0.563 after 1 minute and therefore higher than in the absence of significant stenoses (ie, 0.451±0.168 after 1 minute).

During exercise, CFIp decreased within 1 minute (ie, after 6 minutes of the study protocol) from 0.560±0.178 to 0.393±0.168 (P<0.0001; Figure 3) and reached its minimum after 2 minutes of exercise (0.347±0.176), whereas $\text{Sa}_{\text{O}_2}$ further declined to a minimum of 86±6% (P=0.002). This consistent CFIp course during rest and exercise is also illustrated by an individual recording shown in Figure 4. Both $P_{\text{o}}$ and CVP remained stable during resting conditions and increased with exercise ($P<0.0001$ and $P=0.007$, respectively; probability values are for overall trends over time; Table 2).

Figure 2. Determination of the peripheral pressure–derived collateral flow index (CFIp) in a 66-year-old man without peripheral artery disease. Top left. Collateral inflow (arrow) during balloon occlusion of the left superficial femoral artery (balloon size 8.0×20 mm, 4 bar). Bottom left. Original simultaneous recording of mean and phasic aortic pressure ($P_{\text{a}}$; scale, 120 mm Hg), distal femoral artery occlusive pressure ($P_{\text{ocl}}$; scale, 120 mm Hg), and central venous pressure (CVP; scale, 30 mm Hg) during balloon occlusion resulting in a CFIp of 0.418. Right. Doppler-derived superficial femoral artery flow velocity spectra (right vertical scale in cm/s) of the same patient as obtained during vessel occlusion ($V_{\text{ocl}}$; simultaneous to CFIp; top) and before occlusion at baseline ($V_{\text{non-oocl}}$; bottom). CFIp amounted to 0.551.
There was a direct correlation between CFIp and CFIv both at rest and during exercise (first minute and sixth minute; Figure 5). During vessel occlusion, Doppler flow velocity spectra were often difficult to obtain in analyzable quality for technical reasons and thus could be used for CFIv calculation in only 16 of 30 patients. The main reason for poor Doppler flow velocity signals was wall-motion artifacts in the context of relatively low flow velocities that resulted in a low signal-to-noise ratio. There was a direct relation between the minimal and maximal CFIp values under exercise versus those under resting conditions ($r^2=0.652$, $P<0.0001$ and $r^2=0.527$, $P<0.0001$, respectively; data not shown).

Regarding symptoms during the exercise protocol, 16 patients (57%) experienced pain after a mean occlusion time of 473±74 seconds, and 12 (43%) experienced cramps or tired muscles after 491±71 seconds (3 patients had both pain and cramps/tired muscles). Among all patients, 3 (11%) remained symptom-free for an occlusion time of 10 minutes. The small group of patients without symptoms until the end of SFA occlusion of 10 minutes showed a significantly higher maximal CFI (0.642±0.157) than the group with symptoms (0.437±0.210; $P=0.0048$, unpaired $t$ test for subgroup analysis). The mean total occlusion time was 534±82 seconds. CFIp values were directly related to the pain-free time span during exercise (eg, for minimal CFI under exercise: $r^2=0.50$, $P=0.002$; Figure 6A) but not to other symptoms (cramps/tired muscles; data not shown). Maximal changes of $Sao_2$ (ie, maximal $Sao_2$−minimal $Sao_2$) were given as mean±SEM. *$P<0.01$ vs 5 minutes; †$P<0.001$ vs 1 minute; ‡$P<0.01$ vs 0 minutes.
Sao2) at rest were inversely associated with the maximal CFIp values ($r^2=0.33, P=0.003$; Figure 6B).

**Discussion**

The present study demonstrates for the first time a quantitatively assessed functional and clinically relevant collateral circulation in the lower limb. Using direct invasive pressure measurements in humans, we show that collateral function as determined by CFIp of the SFA amounts to more than half the mean aortic perfusion pressure at rest. Importantly, this pre-existent collateral blood supply in the absence of significant stenoses is sufficient to prevent symptoms during 5 minutes of acute ischemia at rest. Measurements of CFIp as taken in the present study can be performed in every stenotic artery that is suitable for percutaneous recanalization with a guidewire. Pressure measurements can be performed simultaneously with therapeutic dilation or stenting of a stenosis or immediately thereafter during a second diagnostic balloon occlusion. Therefore, CFIp measurements are even feasible in chronic occlusions. Importantly, this method has been used successfully in stenotic and chronically occluded coronary arteries for many years.9,11

To increase collateral blood vessel development, capillary number, and blood flow, many clinical studies have used the administration of growth factors (mostly basic fibroblast growth factor or vascular endothelial growth factor, either as protein or gene therapy) or stem and progenitor cells.3,4,8,13,14 In addition, supervised exercise rehabilitation programs have been shown to improve symptoms of claudication, and meta-analyses have found an increase in the average distance walked to the onset of claudication.15,16 Usual yet imprecise measures of therapeutic effects in clinical studies are absolute walking distance, ulcer healing/decrease of ulcer size or amputation rates, rest pain, and various questionnaires that assess walking impairment or quality of life.3 Moreover, clinical studies have investigated various end points such as changes in ankle-brachial index, digital subtraction angiography, flow reserve, endothelial function, or transcutaneous oxygen pressure.3

In contrast to the numerous investigations in the field, current diagnostic methods for the assessment of PAD are limited because of their inability to directly and quantitatively measure collateral function. Only in experimental animal angiography studies, but not in their clinical counterparts, is an angiographic classification for assessing peripheral collaterals currently used (0=no filling of collaterals, 1=filling of collaterals only, 2=partial filling of distal femoral artery, and 3=complete filling of distal femoral artery).17 Alternatively, a transverse line marking the inferior border of the obliteration can be drawn, and all vessels that cross the line at the inferior border can be counted as collaterals.7 Although this method has been used successfully in a clinical study identifying risk factors for poor collateral development in PAD,7 its use may lead to a rather arbitrary classification of collaterals. Importantly, digital angiography is limited by a low resolution (>0.2 mm),9,18 and therefore, it lacks the precision and reproducibility needed to study small collaterals.3 Moreover, functional assessment is...
not feasible when angiography alone is used for follow-up; therefore, the actual relevance of newly developed collaterals may be missed entirely. Because of the intravenous injection of contrast, CT angiography holds the advantage that collaterals are filled, and arteries distal to the occlusion are opacified that otherwise may be occult to angiography. Alternatively, duplex ultrasound or pulse volume recording may be used to assess both primary and collateral flow. However, angiography, CT angiography, duplex ultrasound, and semiquantitative pulse volume recordings miss not only the functional aspects of collaterals but also the role of the microcirculation in peripheral collateral blood flow and muscle perfusion. In this context, laser Doppler flowmetry can be used to assess the local collateral blood flow and muscle perfusion. In this context, it is reasonable to speculate that CFI would be even higher in the presence of a significant SFA stenosis. Coronary collateral function was sufficient in all patients to completely prevent ischemia-related symptoms at rest, which is not the case for the coronary collateral circulation, because only 25% of patients with normal coronary arteries have no symptoms during a 1-minute balloon occlusion. With foot exercise, pressure-derived collateral function decreased significantly. It is obvious from the original recording shown in Figure 4 that this effect was caused by a drop in the distal occlusive pressure and not a relevant change in CVP or Pao. Collateral supply to the ischemic region may have (only insufficiently) increased in response to the exercise-induced demand, and the occlusive pressure drop during exercise reflects heightened viscous energy loss across inadequately dilated collaterals. Alternatively, an exercise-induced steal phenomenon via well-developed collaterals may be responsible for this observation, because of peripheral hyperemia with a more pronounced decrease in microvascular resistance in the collateral-supplying than the collateral-receiving region, thus leading to a relative flow diversion away from the occluded vascular territory. Considering pure foot dorsif plantar movement, such a mechanism of steal would seem unlikely because of the inactive thigh musculature. However, a combined knee and foot movement could be observed in practically all patients despite the fact that they had been instructed otherwise. To test this hypothesis, simultaneous flow measurements in the thigh and calf supply region would have been needed, which were not performed in the present study. The decrease in collateral function was clinically relevant, because the majority of patients (89%) had ischemia-related symptoms during exercise, and there was a positive correlation between CFI and time until the onset of pain.
Study Limitations
In the above context of potential mechanisms of the observed exercise-induced drop in CFIp, it is obvious that actual flow measurements would have provided an answer on whether a supply-demand mismatch or collateral steal is responsible. Thus, the lack of direct tissue perfusion data can be regarded as the major study limitation. Ultimately, contrast-enhanced ultrasound should have been performed simultaneously, and optimally in different supply regions during balloon occlusion. However, it would not be technically feasible to obtain these additional measurements while the patient is in the supine position during angiography and especially while performing leg movements.

In addition to CFI measurements, other markers of collateral function that are easier to obtain should be evaluated with regard to CFIp as a potential reference method. Although the time until the onset of pain and the drop in SAo2 during occlusion correlated well with the amount of collateral function, other possible methods could have been tested during the present study, such as toe pressure, ankle-brachial index, or washout angiography. However, it would have been too demanding to perform several different measurements during the same study protocol.

In conclusion, the present study not only assessed collateral function of the lower limb using pressure measurements during SFA occlusion but also demonstrated the feasibility and short-term safety of this method. Because only indirect and weak end points have been used in past studies to evaluate collateral growth, we propose that our method may possibly be used as a reference method in future clinical studies. CFIp determined in the SFA after a 5-minute occlusion under resting conditions could serve as a reliable and reproducible end point, and its use should not be withheld despite higher equipment costs or possible concerns regarding the long-term safety of diagnostic balloon occlusions.

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None.

References
Peripheral artery disease of the lower extremities is a common disease and present in 15% to 20% of persons older than 65 years. Endovascular or surgical therapy fails or is not applicable in approximately one fourth of patients who would need revascularization therapies, which makes alternative approaches such as arteriogenesis (the positive remodeling of preformed collateral arterioles) necessary. Despite the fact that numerous studies have pursued the important therapeutic strategy of improving collateral function, there is no method available to quantify collateral arterial function of the lower limb and thus to determine therapeutic effects. The present study demonstrates for the first time a quantitatively assessed functional and clinically relevant collateral circulation in the lower limb. Using direct invasive pressure measurements in humans, we show that collateral flow index of the superficial femoral artery in the absence of any significant stenosis amounts to more than half the normal antegrade flow at rest (45±17% after 1 minute, 55±17% after 3 minutes). The amount of collateral flow observed in the present study is remarkably high, especially compared with what has been described previously for normal (18±8%) and stenotic (22±15%) coronary arteries. Importantly, this preexistent collateral blood supply in the absence of significant stenoses is sufficient to completely prevent symptoms during 5 minutes of acute ischemia at rest. Because only indirect and weak end points have been used in past studies to evaluate collateral growth, we propose that the method described in the present study may possibly be used as a gold standard in future clinical studies.

**CLINICAL PERSPECTIVE**

Peripheral artery disease of the lower extremities is a common disease and present in 15% to 20% of persons older than 65 years. Endovascular or surgical therapy fails or is not applicable in approximately one fourth of patients who would need revascularization therapies, which makes alternative approaches such as arteriogenesis (the positive remodeling of preformed collateral arterioles) necessary. Despite the fact that numerous studies have pursued the important therapeutic strategy of improving collateral function, there is no method available to quantify collateral arterial function of the lower limb and thus to determine therapeutic effects. The present study demonstrates for the first time a quantitatively assessed functional and clinically relevant collateral circulation in the lower limb. Using direct invasive pressure measurements in humans, we show that collateral flow index of the superficial femoral artery in the absence of any significant stenosis amounts to more than half the normal antegrade flow at rest (45±17% after 1 minute, 55±17% after 3 minutes). The amount of collateral flow observed in the present study is remarkably high, especially compared with what has been described previously for normal (18±8%) and stenotic (22±15%) coronary arteries. Importantly, this preexistent collateral blood supply in the absence of significant stenoses is sufficient to completely prevent symptoms during 5 minutes of acute ischemia at rest. Because only indirect and weak end points have been used in past studies to evaluate collateral growth, we propose that the method described in the present study may possibly be used as a gold standard in future clinical studies.
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