Summary and Discussion
The circle of Willis, located at the base of the brain, provides the main route for collateral blood flow in patients with severe carotid artery occlusive disease. Several studies indicated that in such patients a well-functioning circle of Willis might protect the brain against ischemia. Transcranial color-coded duplex ultrasonography is a recently evolved technique which enables visualization of blood flow in the major cerebral arteries and the simultaneous determination of blood flow direction and measurement of its velocity. With carotid artery compression tests, occlusion can be simulated, and the resulting changes in intracranial flow dynamics can be assessed with TCCD. In this way, it is possible to determine the potential collateral ability of the circle of Willis.

In this thesis several aspects of TCCD examination of the circle of Willis are described. The results of the reported studies were achieved by examination of three different cohorts of subjects:

1. Patients with peripheral arterial occlusive disease who did not have symptoms of cerebrovascular disease. These patients were recruited from the vascular surgical ward or the vascular outpatient clinic of the Academic Medical Center in Amsterdam
2. Patients with clinical signs of ischemic stroke admitted to the Stroke Unit of the Department of Neurology of the Debrecen University Medical School in Hungary
3. Healthy persons, recruited via a local Dutch newspaper.

After a short note on the history of the ‘circle of Willis’, the following issues are described in the separate chapters of the thesis:

• the technical aspects of TCCD examination of the circle of Willis and its collateral function
• the success rate of TCCD in visualizing the major cerebral arteries of the circle of Willis
• reference values of intracranial hemodynamics
• the range of collateral variations in the circle of Willis
• the influence of the individual collateral pathways on hemispherical perfusion during carotid occlusion
• the arterial threshold diameter for supplying collateral flow through the circle of Willis
• the use of magnetic resonance angiography as a competitive noninvasive technique to assess the collateral integrity of the circle of Willis
• absent collateral flow in the circle of Willis as risk factor for ischemic stroke.
Chapter 2

In this chapter, an outline is presented of the possibilities and the clinical and experimental value of TCCD in the assessment of intracranial hemodynamics. The history of the development of TCCD and its advantages compared with conventional transcranial Doppler sonography are discussed. The TCCD examination technique and especially the examination of the collateral function of the circle of Willis are described in a detailed way and limitations are discussed.

Chapter 3

In this chapter, the results of a study are shown which was conducted to ascertain the as yet unknown success rate of TCCD in visualizing the basal cerebral arteries in atherosclerotic patients older than 60 years. In 112 patients, 70 men (mean age 70 years) and 42 women (mean age 71 years), who attended the vascular laboratory because of peripheral arterial occlusive disease but who did not have symptoms of cerebrovascular disease, TCCD was performed. All subjects were of Caucasian descent. The anterior, middle, and posterior cerebral arteries were insonated through the temporal window, and the vertebral and basilar arteries through the suboccipital window. In the female patient group, 23% of the temporal windows was impenetrable (no signal from any of the intracranial arteries could be picked up), whereas only 1% of the temporal windows in men could not be penetrated by ultrasound (p<0.001). Suboccipital window failure was more equally distributed among men and women (6% versus 5%, respectively). In women, the ability to penetrate the temporal window worsened with increasing age. Window failure in the 60- to 75-year age group was 13%, whereas window failure in the >75-year age group was 46% (p=0.001). All intracranial arteries were insonated at a deeper level in men. In both men and women a very broad range of blood flow velocities was found in all arteries and intracranial blood flow velocities were higher in women than in men.

From these results it can be concluded that in atherosclerotic Caucasian men older than 60 years of age, TCCD is a very successful technique to examine intracranial blood flow, with vessel identification rates exceeding 90%. In women of the same age there is a much higher failure rate due to impenetrable temporal windows. Investigators of cerebral hemodynamics have to take into account that in subjects without cerebrovascular disease a broad range of intracranial blood flow velocities exists and that intracranial blood flow velocities are higher in women than in men.
Chapter 4
Data on the collateral ability of the circle of Willis in atherosclerotic subjects without symptoms of cerebrovascular disease is largely lacking. Therefore, the aim of the study reported in this chapter was to establish the range of collateral variations in the circle of Willis as determined by TCCD and carotid compression tests. Criteria to define a functional anterior collateral pathway were flow reversal in the ipsilateral precommunicating part of the anterior cerebral artery (A1) during carotid compression, combined with a velocity increase in the contralateral A1-segment. The posterior collateral pathway was defined functional if the peak-systolic velocity in the ipsilateral precommunicating part of the posterior cerebral artery (P1) increased more than 20% during carotid compression. In this study 76 patients with a mean age of 61 years were examined. No neurological or cardiovascular complications occurred during carotid compression. The anterior collateral pathway was nonfunctional in 4 out of 76 (5%) subjects. This was due to the absence of a functional AcoA in 3 patients and a hypofunctional A1-segment in 1 patient. The posterior collateral pathway was nonfunctional in 68 of the 152 (45%) hemispheres. Unilateral hypofunctional PcoAs were found in 34 (22%) hemispheres, and bilateral hypofunctional PcoAs were found in 12 (16%) patients. In 10 (7%) hemispheres, persistence of the fetal origin of the posterior cerebral artery with a hypofunctional P1-segment was found. These results show that in atherosclerotic subjects without cerebrovascular symptoms the anterior collateral pathway of the circle of Willis is nearly always functional, as opposed to the posterior collateral pathway which is nonfunctional in almost half of the hemispheres.

Chapter 5
In this chapter, the influence of a functionally incomplete circle of Willis on middle cerebral artery blood flow during carotid occlusion was described. The velocity decrease in the middle cerebral artery, caused by manual compression of the common carotid artery as simulation of carotid occlusion, was compared between functionally complete and incomplete circles of Willis in 46 atherosclerotic vascular patients without hemodynamic significant extracranial arterial stenosis and without symptoms of cerebrovascular disease. In hemispheres with collateral supply through both the AcoA and the PcoA, the median peak-systolic velocity decrease in the middle cerebral artery during carotid compression was 43%. When the PcoA, the AcoA or both communicating arteries (1 hemisphere only) were missing, the peak-systolic velocity decrease was 58% (p=0.003), 70% (p=0.001) and 75%, respectively. From this study we can conclude that a complete collateral circulation consisting of a functional AcoA and PcoA, provides better perfusion of the middle cerebral artery.
during carotid occlusion than collateral supply through only the AcoA or the PcoA in the case of an incomplete circle of Willis. This data also indicates that the AcoA is a more important collateral than the PcoA.

Chapter 6
The varying definitions of hypoplasia of circle of Willis collaterals, some authors use 0.5 others 1.0 mm as threshold diameter, and the different populations studied, have resulted in a large variability of anomalous or 'incomplete' circles of Willis throughout the literature. The prevalence of the 'normal' textbook polygon ranges from 21% to 76%. However, these arbitrary diameters have never been discussed in terms of functional significance. It is unknown whether collateral flow through the Willisian collaterals is still possible at an arterial diameter of 0.5 or 1.0 mm. In the study described in this chapter, the collateral artery threshold diameter for supplying collateral flow through the circle of Willis was determined. This study was part of a larger study performed to determine the collateral function of the circle of Willis in acute stroke patients (chapter 8). Because of the severity of stroke, a number of patients included in this larger study died. The circle of Willis of these patients was removed at autopsy, and the size of the collateral arteries was measured. These data were compared with the premortem TCCD data. In total 12 acute stroke patients could be included in this study. The median diameter of the functional (n=19) and nonfunctional (n=16) collateral arteries of the circle of Willis was 1.1 (range, 0.4-2.0) and 0.5 (range, 0.3-0.7) mm, respectively (p=0.003). This proves that the ultrasound criteria used to discern functional from nonfunctional collateral arteries reflect significant differences in arterial size. An important finding in this study was that AcoAs and PcoAs with a diameter considerably less than 1.0 mm are still able to supply collateral flow, which can be detected by TCCD. The arterial threshold diameter allowing for collateral flow through the circle of Willis lies between 0.4 and 0.6 mm. These results confirm those from fluid-dynamic mathematical models of the circle of Willis which showed that the smallest luminal diameter allowing for cross-flow through the AcoA was 0.4 mm and for the PcoA it was smaller than 0.5 to 0.6 mm. Therefore, we suggest that the term hypoplasia is reserved for those vessels that cannot supply collateral flow. Our results indicate that in practice, collateral arteries with a diameter smaller than 0.5 mm should be labelled hypoplastic.

Chapter 7
Magnetic resonance angiography is an attractive, noninvasive technique to assess the anatomy of the circle of Willis. In the study described in this chapter, we investigated the ability of
MR angiography to identify functional collaterals of the circle of Willis. The results of MR angiography were compared with TCCD and carotid compression tests as the standard of reference for the identification of functional intracranial collaterals. In 50 healthy volunteers, 25 men and 25 women (mean age 51 years) without a history of cardiovascular and cerebrovascular disease, TCCD including carotid compression tests was performed. Three-dimensional time-of-flight (3D TOF) MR angiography of the circle of Willis was performed on a 1.5-T MR system within approximately 30 minutes following TCCD examination. Contrast-enhancers were not used. It was hypothesized that visible collateral arteries on MR angiography were patent and potentially capable of supplying collateral flow. Arteries that could not be visualized were defined as hypoplastic or absent. Collateral flow through the anterior collateral pathway of the circle of Willis was deemed possible if both A1-segments connected by an AcoA were visible. The posterior collateral pathway was defined as functional if the P1-segment and its accompanying PcoA were visible. Visibility on MR angiography of the vessels forming the anterior and two posterior collateral pathways was judged independently by two neuroradiologists who were unaware of the TCCD findings. MR results were then compared with the findings of TCCD. The sensitivity of MR angiography was 85%-87%, the specificity 67%-81%, the positive predictive value 92%-95%, and the negative predictive value 53%-55%. The low negative predictive value indicates that MR angiography fails to identify potentially functional collaterals in a relatively high number of cases. However, the results also indicate that if the collaterals are visible on MR angiography, it can be assumed with a high level of confidence that collateral flow is possible. The interobserver agreement was moderate (κ= 0.57, 95% CI: 0.42-0.72). Disagreement between the observers was mainly caused by the often very small size of the collaterals. From this study it can be concluded that 3D TOF MR angiography might fail to show functional collateral pathways in the circle of Willis when compared with TCCD and carotid compression tests. This indicates that in those cases where MR angiography fails to identify intracranial collaterals, supplementary TCCD examination is required.

Chapter 8
Finally, in this chapter the collateral function of the circle of Willis in patients who recently suffered from an ischemic stroke was described. A cohort of Hungarian stroke patients was studied with TCCD and carotid compression tests. These data were compared with a group of Dutch patients with atherosclerotic vascular disease, but without a history of cerebral ischemia. In this case-control study 109 patients (mean age of 66 years) with an acute ischemic stroke and 113 patients (mean age of 62 years) without a history of cerebral ischemia, were
included. TCCD could be successfully performed in 75 out of the 109 case patients and in 100 out of the 113 control patients. In 26 cases and 19 controls a ≥70% stenosis or occlusion of the ICA was found. A nonfunctional anterior collateral pathway in the circle of Willis was found in 33% of the total case group and in 6% of the control group (p<0.001). The posterior collateral pathway was nonfunctional in 57% of the case hemispheres, and in 43% of the control hemispheres (p=0.02). In cases and controls with severe ICA occlusive disease the anterior collateral pathway was nonfunctional in 48% and 11%, respectively (p=0.03). The ipsilateral posterior collateral pathway was nonfunctional in 60% and 33% (p=0.13). In cases and controls without severe ICA occlusive disease, the anterior collateral pathway was nonfunctional in 26% and 5%, respectively (p=0.002). The posterior collateral pathway was nonfunctional in 58% and 45% (p=0.19). In patients with severe ICA occlusive disease, the odds ratios of a nonfunctional anterior and a nonfunctional posterior collateral pathway were 7.33 (95% CI: 1.19-76.52) and 3.00 (95% CI: 0.77-12.04), respectively. The anterior collateral pathway was found to be nonfunctional in 75% of the patients with severe ICA occlusive disease, but no cardiac source of emboli, as compared with 22% in those with severe ICA occlusive disease and with a cardiac source of emboli (p=0.04). In patients without ICA occlusive disease, but with a cardiac source of emboli the anterior collateral pathway was nonfunctional in 18% (p=0.004). These results indicate that the absence of a functional anterior collateral pathway in patients with severe ICA occlusive disease is strongly associated with ischemic stroke. The association of a nonfunctional posterior collateral pathway and ischemic stroke is weak. The findings of this case-control study underline the vital importance of adequate collateralization in patients who have severe carotid artery occlusive disease as a predominant risk factor for stroke.
Transcranial color-coded duplex ultrasonography can be used to study all sorts of physiological and pathological changes in cerebral blood flow, in both clinical and experimental settings. The reason to start this research project was that an increasing number of studies reported that failure of the collateral function of the circle of Willis might be a risk factor for ischemic stroke. We explored the diagnostic potential of TCCD to assess basic cerebral hemodynamics and the collateral function of the circle of Willis. MR angiography as a competitive noninvasive technique to determine the collateral configuration of the circle of Willis was compared with TCCD. Additionally we investigated whether absent collateral flow in the circle of Willis is a risk factor for stroke. The studies described in this thesis show that:

- TCCD is a useful noninvasive technique to assess cerebral hemodynamics. TCCD provides insight in the hemodynamics of most subjects from target populations like atherosclerotic patients with vascular disease including patients with ischemic stroke. Failure to insonate the temporal window is a problem mainly found in elderly women. Furthermore, users of the TCCD technique should realize that there is a large variability in intracranial blood flow velocities.
- Based on blood flow velocity data, hemispherical perfusion via the middle cerebral artery in patients with carotid artery occlusion is better preserved when the anterior and the posterior collateral pathway are both functional. The anterior collateral pathway, however, seems to be more important than the posterior collateral pathway.
- The arterial threshold diameter allowing for collateral flow is approximately 0.5 mm.
- MR angiography, when compared with TCCD and carotid compression tests, might fail to identify functional collaterals in the circle of Willis in some cases. This limitation should be realized when MR angiography is used for this purpose.
- In healthy persons and atherosclerotic patients without symptomatic cerebrovascular disease, collateral flow through the anterior part of the circle of Willis is possible in more than 90% of the subjects. Collateral flow through the posterior part of the circle of Willis is hampered by hypoplastic posterior communicating arteries or hypoplastic P1-segments of the posterior cerebral artery in 25% to nearly 50% of the hemispheres. However, in patients suffering from ischemic stroke the number of nonfunctional anterior collateral pathways is significantly higher, with a highest percentage of 48% in ischemic stroke patients with severe (more than 70% diameter reduction) carotid artery occlusive disease. The number of nonfunctional posterior collateral pathways is also significantly higher in ischemic stroke patients, with a highest percentage of 60% in the hemispheres.
of patients with severe carotid artery occlusive disease. From this we can conclude that especially failure of the anterior collateral pathway seems to be an important risk factor for ischemic stroke. Whether the patients in our study suffered from a stroke caused by hemodynamic insufficiency or by embolization remains unknown. However, according to a recently proposed hypothesis, reduced perfusion to the brain limits the ability of the bloodstream to clear or 'washout' emboli and reduces available blood flow to regions rendered ischemic by emboli that block supply arteries.¹ This hypothesis suggests that impaired washout is an important concept that intertwines hypoperfusion, embolization, and brain infarction. This would also mean that the collateral function of the circle of Willis does not only play a role in ischemic stroke pathogenesis in case of pure hemodynamic insufficiency, but also in some cases with an embolic etiology.

Carotid compression
In patients with carotid lesions not severe enough to provoke spontaneous intracranial collateral flow, TCCD in combination with short carotid compressions is a fast and relatively simple technique to obtain a reliable insight in the collateral ability of the circle of Willis. The carotid compression tests are essential, as other studies showed that the AcoA and PcoA are not recruited until stenosis in the ICA has reduced the lumen by 80% or more.²-⁴ In case of lesser degrees of stenosis, intracranial collateralization is absent and flow through the communicating arteries is low or negligible. Many investigators are hesitant in performing carotid artery compressions, especially in patients with severe carotid lesions or patients who suffer from stroke. However, in the past and present carotid artery compressions have been widely performed in both asymptomatic and symptomatic patients with varying degrees of carotid stenoses and/or occlusions. The incidence of ischemic complications ranged from 0 to 5%.⁵-⁸ All reported complications were short-lasting, completely reversible focal cerebral ischemic deficits. However, minor stroke and even death after reverberating(I) compression of the common carotid artery or carotid sinus massage and compression have been reported as well.⁹-¹⁰ In the studies reporting ischemic complications, the status of the common carotid artery was often unknown. We think that it is essential to be informed about the atherosclerotic status of the common carotid artery, before compression is applied. In our studies all patients with plaques in the proximal common carotid artery were excluded. Furthermore, we did not perform reverberating compressions and we tried to avoid compression of the carotid sinus. Since we did not encounter any ischemic complications of common carotid artery compression, we think that it is an acceptable and safe procedure.
Limitations
Although the assessment of cerebral hemodynamics with TCCD is possible in most subjects, in specific groups visualization of the cerebral arteries is seriously hampered by temporal windows that cannot be penetrated with ultrasound. The visualization failure rate in our studies ranged from 7% in healthy subjects with a mean age of 51 years to 48% in female stroke patients with a mean age of 69 years. A hopeful new development in this light is the advent of echo-contrast enhancing agents. Recent studies showed that the main shortcoming of TCCD can largely be overcome with the use of such agents.\textsuperscript{11-15}

In this thesis, collateral vessels were defined as functional when they showed the ability to act as a collateral. However, this does not necessarily imply that these vascular pathways would or would not serve as an \textit{adequate} collateral to maintain normal tissue perfusion in case of occlusion of the carotid arteries. Although TCCD can be used to assess the presence of cross-flow through the AcoA and PcoA, it should be kept in mind that volumetric blood flow cannot be measured with this technique. A TCCD diagnosis that collateral flow to an endangered hemisphere is present or can be provoked, does not warrant that it will be sufficient to protect the hemisphere against ischemia. Moreover, the presence of leptomeningeal collaterals over the surface of the brain, which might be vital for hemispherical perfusion in some cases, cannot be assessed by TCCD. Conventional angiography, however, has the same shortcoming. Collateral flow can be identified, also in the leptomeningeal collaterals, but collateral flow cannot be qualified as adequate or inadequate to maintain normal tissue perfusion.

Suggestions for further research
So far several studies, including our own, indicated that in patients with ICA occlusive disease a deficient collateral function of the circle of Willis is a risk factor for ischemic stroke. However, none of these studies proved in a prospective way that the relation is causal. Further studies are needed to give definite answers. The design of such studies should be of the highest level. Since the NASCET trial\textsuperscript{16} showed a strong benefit of carotid endarterectomy in patients with a symptomatic high-grade ICA stenosis, the debate on best treatment in such patients is more or less closed. However, for the group with asymptomatic high-grade ICA stenosis the debate on best treatment still continues. The annual stroke event rate for asymptomatic patients with hemodynamically significant carotid artery stenosis ranges from 2% to 5%.\textsuperscript{17-21} In our hospital, surgery for asymptomatic carotid artery stenosis is not performed. However, in other hospitals yearly thousands of patients with asymptomatic severe carotid artery disease are 'treated' by surgery or percutaneous transluminal angioplasty.
Although carotid endarterectomy offers a slightly better outcome than best medical treatment\textsuperscript{21}, many neurologists and surgeons believe that the minimal benefit is not worth the risk of surgical complications. Nevertheless, among these asymptomatic patients there will be subjects with a high and subjects with a low risk of stroke. Patients with a high-risk profile, presumably those with functionally incomplete circles, might benefit more from a surgical or endovascular intervention. To definitely prove that the collateral configuration of the circle of Willis is a clinically relevant factor in ischemic stroke pathogenesis, two types of studies could be performed:

1. A long-term natural history study in which patients with high-grade asymptomatic ICA stenosis, stratified for functionally complete and incomplete circles of Willis, are followed in time, or
2. Randomization of patients with high-grade asymptomatic ICA stenosis and functionally incomplete circles of Willis to receive a surgical/endovascular intervention or best medical treatment.

Both studies would require the screening of thousands of patients with atherosclerotic vascular disease. TCCD is an attractive research tool for such a purpose, especially since echo-contrast enhancing agents have shown to improve the detection of intracranial collaterals in patients with poor temporal windows. Combination with MR angiography and perfusion MR imaging to assess the relative regional cerebral blood volume and relative regional cerebral blood flow could offer valuable additional information. Such studies might finally elucidate the relation between the anatomical configuration of the circle of Willis, the possibility and quality of intracranial collateral flow, regional cerebral perfusion conditions and the risk of ischemic stroke. This might help doctors in their struggle against the devastating consequences of stroke.

References
Summary and Discussion


