3

Methods
3.1 PARAMETERS

Investigation of the cardio- and cerebrovascular response to physiological stress requires (simultaneous) monitoring of systemic, cerebral and respiratory parameters. This chapter provides an overview of the parameters that were measured in the studies described in this thesis.

3.1.1 Systemic

Blood pressure
Continuous arterial blood pressure (BP) can be measured non-invasively by finger plethysmography (Nexfin, Edwards Lifesciences BMYE, Amsterdam, the Netherlands) using a volume-clamp technique. An optical plethysmograph in the finger cuff measures arterial volume continuously whereas the volume is clamped by applying variable pressures in an inflatable cuff around the finger countering the pulsatile arterial pressure. The cuff is placed around the midphalanx of the non-dominant hand and held at heart level. A height reference system is placed around a finger next to the cuff and at heart level to account for the hydrostatic pressure difference. To detect changes in BP correctly, an automatic built-in calibration system (Physiocal) tracks the unloaded diameter of the finger artery to establish and adjust the arterial unloaded volume. Finger arterial pressure is fundamentally different from brachial pressure in terms of wave shape and absolute levels such that waveform transformation and level corrections are applied in the Nexfin system to reconstruct brachial pressure.

In case finger plethysmography is not available (for instance in the MRI environment), BP measurements are taken every 2–4 min using an inflatable arm-cuff (Magnitude, Invivo, Orlando, FL) while HR can continuously be monitored by means of an MRI compatible finger pulse-oximetry unit.

Heart rate, stroke volume and cardiac output
A pulse contour method (Nexfin CO-trek, Edwards Lifesciences BMYE, Amsterdam, the Netherlands), adapted for age, sex, height and weight, provides left ventricular stroke volume (SV) and CO (equal to SV multiplied by instantaneous HR). This method has been thoroughly validated against invasive thermodilution measurements.

In Chapter 6, CO was also measured by means of inert gas rebreathing (Innocor, Innovision A/S, Odense, Denmark). During the rebreathing procedure, blood-soluble N₂O diffuses from the lung alveoli to the systemic circulation, and blood-insoluble NF₆ remains in the pulmonary fields. The disappearance rate in the bag volume is proportional to the pulmonary blood flow which is assumed to be equal to the CO of the left ventricle.
3.1.2 Brain

In this thesis, we assessed with two non-invasive modalities the CBF response to a variety of physiological challenges. First, transcranial Doppler ultrasound (TCD) provides high temporal resolution assessments of CBF velocity (CBFv) in large brain-feeding arteries. This method is a relatively simple and low-cost bedside technique and is assumed to reflect mean CBF over a large area of the brain; that is, the flow territory perfused by the insonated artery. In addition, MRI techniques such as arterial spin labeling (ASL) and blood-oxygen-level dependent (BOLD) imaging enable the measurement of whole brain CBF and oxygenation at the microvascular tissue level. MRI is a complex, costly and time-consuming procedure that offers a non-invasive measure of brain perfusion and oxygenation at a high spatial resolution. A combination of TCD and MRI based quantifications of CBF has the potential to complement each other in obtaining a more complete understanding of brain perfusion at both the macro- and microvascular level. In the following paragraphs we will discuss both modalities into more detail.

Middle cerebral artery blood flow velocity

Measuring CBFv in the basal cerebral arteries by TCD was introduced in the early eighties of the twentieth century by Aaslid and coworkers,\textsuperscript{104} and has found wide acceptance in both clinical and research settings. The ultrasound probe emits a high-pitched sound wave through the intact skull, which is then reflected back from erythrocytes moving in its path. The CBFv is recorded from the Doppler shift spectrum of the reflected sound waves.\textsuperscript{105} Mean CBFv reports the velocity associated with the maximal frequency of the Doppler shift (‘the envelope’) rather than the intensity-weighted mean flow velocity or the total signal power. These latter two variables are sensitive to small changes in insonation angle of the artery and therefore the maximum velocity is preferred as reported entity (Secher, Seifert et al. 2008). In the studies described in this thesis, a TCD system (DWL Multidop X4, Sipplingen, Germany) with a pulsed ultrasound frequency of 2 MHz was used in order to satisfactory penetrate the skull. As the bone of the of temporal region is thin and therefore the best promising area for penetration,\textsuperscript{104} CBFv was measured in the proximal segments of the left or right middle cerebral artery (MCA). The ultrasound probe was placed on the temporal region of the skull just above the zygomatic arch (Figure 3.1.1). Once the optimal signal-to-noise ratio was obtained at an insonation depth between 45 and 60 mm, the probe was secured in position by a head-band.
Methods

A. B.

Figure 3.1.1. The ultrasound probe is placed on the temporal region of the skull (dotted line indicates the ‘temporal window’) just above the zygomatic arch (A). A frontal view of the ultrasound probe directed toward the MCA (B). The cylinder around the MCA indicates the observation region (sampling volume) and the distance from the middle of the cylinder to the probe corresponds to the depth setting. Reprinted with permission from J Neurosurg.104

The relation between calculated versus actual CBFv depends on angle of insonation.106 When the angle increases from 0° to 30°, its cosine will decrease from 1 to 0.86 resulting in a maximum error up to 15%.105 By immobilizing the probe by a head-band, we minimized the influence of a potential change in angle as might occur during the experiments. A critical issue of TCD is to what extent blood flow velocity reflects actual blood flow. Changes in blood flow velocity reflect those in blood flow when the cross-sectional area of the insonated vessel remains constant (blood flow = blood flow velocity x cross-sectional area). Direct observations made during craniotomy reveal that the vessel diameter does not change significantly during moderate variations in mean BP or CO₂ partial pressure.107 Also orthostatic stress, as stimulated by lower body negative pressure (LBNP), does not alter the diameter of the MCA significantly as assessed with 3 Tesla MRI.108 These findings suggest that the MCA diameter remains constant and that changes in TCD determined CBFv will track those in CBF. In two studies presented in Chapters 5.1 and 5.2, we examined this assumption under different circumstances using high-resolution MRI at 7 Tesla.

Whole brain blood flow

Since the proposal of ASL two decades ago,109 the non-invasive quantification of regional CBF with MRI has progressively developed into a well-accepted and clinically suitable technique. ASL-MRI is based on the detection of a tracer that is delivered to and cleared from the tissue by blood flow,110 and usually expressed in ml·min⁻¹ per 100g tissue.111,112 With ASL, an endogenous tracer is created by inverting the proton spins of blood, mainly located in water molecules (H₂O). Magnetic labeling of arterial blood water spins is done by a long series of radiofrequency pulses (in pseudo-continuous ASL) that are applied in a plane perpendicular to the neck. Subsequently, the labeled protons in arterial blood water act as freely diffusible tracers. From the labeling location the labeled
protons migrate within 1-2 seconds via the arterial vessels and capillaries into the brain tissue where the label accumulates, thereby altering the local tissue magnetization. The change in tissue magnetization is measured by comparing multiple image slices covering the whole brain with identical control scans in which the inflowing blood was not labeled. A 3-dimensional perfusion map can be obtained by subtracting the labeled image volume from the control image volume (no label) (Figure 3.1.2).

Figure 3.1.2. Principle of arterial spin labeling. The magnetic labeling of arterial protons is carried out upstream from the volume of interest, at the neck vessels, by radiofrequency pulses. The labeled protons then migrate via the arterial vessels towards the brain tissue where they extravasate from the capillary compartment to the extravascular compartment. After the labeling, a delay time (the so-called post-labeling delay; PLD) of approximately 1.5-2.0 seconds allows the labeled protons to reach the tissue compartment, after which the images are acquired. The control acquisition is obtained in a highly similar manner, but without inverting the arterial magnetization.

The ASL-signal, i.e. the difference in signal intensity between label and control images, is small (~1%). To obtain a sufficient signal-to-noise ratio (SNR), many repetitions of the control and label pairs are acquired during 3-5 minutes. The ASL technique applied here is pseudo-continuous ASL, as the recommended standard for use in a clinical setting, and which has been recently compared with $^{15}$O H$_2$O positron emission tomography (PET) CBF measurements. Background suppression RF pulses were used to enhance the SNR of the CBF signal. In addition, the imaging module was extended with an extra echo block to obtain the BOLD fMRI signal with minimal additional scan time. The BOLD signal is mainly sensitive to the concentration of deoxy-hemoglobin, and also depends on blood flow, blood volume and tissue properties, such as diffusion. This makes this method less specific than ASL. Changes in ASL or BOLD determined regional are often used as proxy for neuronal activation. Chapter 5.3 describes a comparative study of the determination of CBF changes upon small muscle group exercise as measured by either ASL-MRI and TCD.
3.1.3 Respiration

Brain perfusion is highly sensitive to changes in PaCO₂. To enable a correct interpretation of the CBF and CBFv responses, it is highly recommended to also monitor (changes in) PaCO₂. The partial pressure of CO₂ in exhaled air (designated as end-tidal CO₂ partial pressure; PetCO₂) is generally used as a non-invasive proxy for PaCO₂ and therefore measured in the studies described in this thesis.

Partial end-tidal carbon dioxide pressure

PetCO₂ was continuously monitored, via a nasal cannula, by a sampling infrared capnograph (Tonocap, Datex-Ohmeda, Madison, USA or Datex Normocap 200, Helsinki, Finland). This technique is based upon the absorption of infrared radiation by CO₂, with the amount of absorbed radiation having a nearly exponential relation to the CO₂ concentration. Detecting a change in infrared radiation levels by means of photo-detectors, allows for the calculation of the CO₂ concentration in the gas sample.

3.2 PHYSIOLOGICAL CHALLENGES

The present thesis discusses various physiological methods (including orthostatic stress tests, small muscle group exercise and inhalation of a gas mixture containing CO₂) that were applied to address autonomic cardio- and cerebrovascular control. This section summarizes these methods with respect to their known physiological mechanism and the way in which they were performed.

3.2.1 Orthostatic stress tests

Passive head-up tilt (HUT), lower body negative pressure (LBNP) and orthostasis (standing up) all lead to a gradual translocation of blood from the intra-thoracic region into the lower parts of the body. As a result, CO decreases and a series of cardiovascular regulating mechanisms and reflexes come into action to maintain arterial blood pressure and cerebral perfusion.

Passive head-up tilt

Passive HUT is performed with the subject lying supine and safely strapped on a tilt table (custom built by AMC Medical Technological Development / Dr. Kaiser Medizintechnik, Bad Hersfeld, Germany) and then either mechanically or manually tilted to a semi-supine (30°), semi-upright (45°) and/or almost completely upright (70°) position. Tilting back from 70° HUT to the supine position lead to central blood volume repletion and mimics a fluid challenge.
Translocation of blood takes place because the hydrostatic indifference point for intravascular pressure (i.e. point in the vascular tree at which pressure remains constant independent of body position) is situated at the level of the diaphragm. Moreover, the indifference point for volume as monitored by electrical impedance is even lower positioned between the navel and iliac crest. As a consequence, from supine to upright approximately 70% of the blood volume becomes positioned below the level of the heart, largely being contained in the (compliant) venous compartment and, therefore, not contributing to the effective arterial blood volume. This shift in blood volume distribution is estimated to be 300-800 ml of which 50% takes place within the first few seconds. The central blood volume is challenged further by an estimated 10% or ~500 ml reduction after 5 min and 15% or ~750 ml reduction after another 5 minutes in the HUT position.

Lower body negative pressure
During LBNP, sub-atmospheric pressure is applied to the lower limbs in a supine subject such that blood redistributes from the upper parts of the body into the compliant compartment of the lower extremities. In preparation for LBNP, the lower body of the subject is positioned inside a LBNP box (Dr. Kaiser Medizintechnik, Bad Hersfeld, Germany / Dept. Instrumental Development, Leiden University Medical Center, Leiden, the Netherlands) and sealed at the level of the iliac crest. An advantage of this technique compared to passive HUT, is its usability within the static and horizontal setup of the MRI scanner. Chapter 4.2 provides a formal comparison of the cardio- and cerebrovascular response to LBNP and passive HUT. In Chapter 4.3, we introduce a custom designed compact LBNP box that meets MRI requirements more closely.

Standing up
The presumed mechanism behind the gravitational translocation of blood from the intra-thoracic region to the veins in the legs during passive HUT is similar to that when humans stand up from the supine position. However, the active change in posture during standing-up produces a hemodynamic response that is different from what happens with passive tilt during the first 30 s of upright posture.

3.2.2 Small muscle group exercise
Rhythmic handgrip exercise is a form of small muscle group exercise that increases HR and CO, with modest changes in blood pressure. An advantage of rhythmic handgripping is that it can be performed in the supine position while ensuring minimal (head) motion, which makes it a suitable exercise method during MRI monitoring. Moreover, a mild to moderate handgrip exercise level can be maintained for a longer period of time to achieve the steady state needed for acquisition of ASL measurements, which
typically take about 3-5 minutes. To standardize the workload between individuals, the subjects were first instructed to squeeze a handgrip dynamometer (Gripforce 500N, Current Designs Inc., Philadelphia PA, USA) to the maximum extent possible for 2-3 s without tensing the entire body. The so-measured maximum force was taken as 100%. The exercise experiments described in this thesis consisted of 0.5 Hz intermittent handgrip contractions performed for the first minute at 80% of the maximum force followed by 4 minutes at 60%. The decreasing force protocol was used to achieve a steady-state in minutes 3 to 5.

3.2.3 Inhalation of a gas mixture containing CO₂
In order to quantify the cerebral vasomotor reactivity, a wide range of PetCO₂ was established by inhalation of a gas mixture containing 5% CO₂ and 95% O₂ (Carbogen) for 2 minutes, followed by 2 minutes of breathing room air and, finally, hyperventilating for another 2 minutes.