BACHELOR THESIS TECHNICAL MEDICINE

THE INFLUENCE OF THE COLD PRESSOR TEST ON THE CEREBRAL BLOOD FLOW

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On this page we would like to express our gratitude for the welcoming embrace of our supervisors. Due to their enthusiasm in our research we were able to explore depths not expected when we first took on this assignment. It was a process where we were given all the opportunities that made this thesis possible.

To the persons dedicated to this thesis:

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ABSTRACT

Introduction: The response of cerebral hemodynamics to a transient elevation of blood pressure is not fully understood. The cold pressor test provokes such an elevation due to activation of sympathetic pathways. The test may be a good standard stimulus in order to study cerebral hemodynamics.

Objective: The physiological effects of the cold pressor test on the cerebral blood flow is not fully known. In order to provide more insights, the effect of the cold pressor test on the blood flow on three levels will be studied: the carotid artery as supplier, the middle cerebral artery (MCA) as the distributor and the cerebral microvasculature as the receiver.

Methods: Ultrasound will be used to quantify the flow in the carotid artery. Cerebral blood flow velocity (CBFV) of the MCA will be measured with transcranial Doppler and the diameter will be determined with MRI, using time of flight angiography. The cerebral microvasculature will be examined using near infrared spectroscopy (NIRS) for the frontal cortex. In addition to NIRS, arterial spin labeling will be used in order to monitor the global cerebral perfusion. The reactions of healthy subjects will be averaged in order to determine a general response to the effects of the cold pressor test.

Results: The flow in the left carotid artery (+5.44%) and CBFV in the MCA (+6.36%) increased during the cold pressor test. Besides, an increase in oxygenated hemoglobin (oxy-Hb) of 1.32 µmol/L and a decrease in deoxygenated hemoglobin (deoxy-Hb) of -0.311µmol/L was found in the microvasculature of the frontal cortex.

Conclusion: This research showed that the cold pressor test induced an increase in flow on all observed levels. Also, is was found that there was a time correlation between the CBFV in the MCA and the flow in the cerebral microvasculature of the frontal cortex.

CONTENTS

1 INTRODUCTION

Many research has been done to explore the mechanism of cerebral hemodynamics. A particular stimulus which causes a transient elevation in blood pressure is needed in order to examine this mechanism. In 1932 researchers preferred a cold stimulus over other stimuli such as electro-shock because of its reproducible response and relatively non violent character [1, 2]. Unlike other tests, the cold pressor test does not invoke a response through the baroreflex but directly on the sympathetic nervous system.

Rubenfire et al. applied the cold pressor test to detect a biomarker for cardiovascular diseases such as ischemic attacks. Subjects with coronary artery disease showed a constriction of the carotid artery as a result of the cold pressor test, while healthy subjects showed a dilatation [3]. Furthermore Schächinger et al. proved that coronary endothelial vasodilator dysfunction that is provoked by the cold pressor test predicts cardiovascular diseases [4]. Research also showed that the cold pressor test has an impact on for instance the blood flow velocity of the middle cerebral artery [5]. An increase in velocity was detected during the test. The particular response of the cold pressor test shows promise that it might be useful for research on biomarkers for cerebral vascular diseases.

In order to analyze this possibility, there is a need for more information on how the cerebral hemodynamics normally operate in response to the cold pressor test. There have been studies that measure the blood flow velocity in the middle cerebral artery, but these did not study the cerebral blood flow, which is an outcome that quantifies the cerebral hemodynamics [5-7]. In order to quantify the flow, the diameter is needed in addition to the velocity. Therefore it is necessary to evaluate the radial changes of the middle cerebral artery. MRI can be of value in measuring these changes in cerebral blood flow because of its ability to measure vessel diameters and general cerebral blood flow changes. The cold pressor test offers the possibility to measure cerebral hemodynamics because of its promising applicability within MRI and other technologies.

Cerebral hemodynamics can be measured in different vascular levels. In this research the focus will be on three distinct levels: the carotid artery as main supplier of the cerebral blood, middle cerebral artery as distributer of the blood and the microvasculature as receiver of the blood. The individual reaction and correlation of the vascular levels can answer questions about how the flow is being altered by the cold pressor test. This provides more information about how this test alters cerebral hemodynamics.

1.1 READING GUIDE

This reading guide will provide a short overview of this research. The global contents are described for each section. Chapter 2 will give an overview of the used abbreviations in this research. The objectives and approaches are described in chapter 3. In chapter 4 the anatomy of the cerebral vasculature, cerebral autoregulation, the cold pressor test and other tests are described. The hypothesis of this thesis is discussed in chapter 5 and the materials and methods in chapter 6. Furthermore, the results are shown in chapter 7. Chapter 8 includes the conclusion of the research and the research is discussed in chapter 9. Finally, the recommendations are given in chapter 10.

2 ABBREVIATIONS

A	Cross sectional area
ASL	Arterial spin labeling
BPM	Beats per minute
CPT	Cold pressor test
CBF	Cerebral blood flow
CBFV	Cerebral blood flow velocity
CVRi	Cerebrovascular resistance index
d	Diameter
dBP	Diastolic blood pressure
Deoxy-Hb	Deoxygenated hemoglobin
ECG	Electrocardiogram
$EtCO_2$	End-tidal CO_2
f	Frequency
MAP	Mean arterial pressure
MCA	Middle cerebral artery
min	Minutes
MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
NIRS	Near infrared spectroscopy
Oxy-Hb	Oxygenated hemoglobin
$PaCO_2$	Partial pressure CO_2
PaO_2	Partial pressure O_2
Q	Flow
s	Seconds
sBP	Systolic blood pressure
SNR	Signal-to-noise ratio
SOP	Standard operating procedure
t	Time
TCD	Transcranial Doppler
TI	Inversion time
TOF	Time of flight
v	Velocity

3 OBJECTIVES AND APPROACHES

Although the cold pressor test has been studied several times since 1932, the mechanism is not fully understood [1]. The primary goal of this study is to provide more insight in the effect of the cold pressor test on the physiology and specifically its effect on cerebral blood flow in healthy subjects. This thesis will focus on the flow measured on three levels: the carotid artery, the middle cerebral artery and the cerebral microvasculature. To achieve the goals of this study, the main question that will be answered is:

What is the effect of the cold pressor test on the blood flow in the carotid artery, middle cerebral artery and cerebral microcirculation in healthy subjects?

This thesis contains a literature and experimental research. The literature study is performed to provide relevant information about the cerebral blood flow regulation and the physiology of the cold pressor test in order to create links between the experimental data. To answer the main question, sub questions that will be answered are:

- *How are fluctuations in cerebral blood flow regulated?*
- *What is known about the mechanism of the baroreflex during the cold pressor test?*
- *What is known about the effect of the cold pressor test on the sympathetic nervous system?*
- *How does the flow in the carotid artery, middle cerebral artery and cerebral microvasculature correlate during the cold pressor test?*

4 BACKGROUND

4. 1 ANATOMY OF THE CEREBRAL VESSELS

The cerebrum receives about one sixth of the cardiac output and consumes 20% of the oxygen at rest. It is highly vascularized and is supplied by the internal carotid artery and the vertebral arteries. The common carotid arteries give rise to the internal carotid arteries. The vertebral arteries are branches of the subclavian artery and join to form the basilar artery.

In the cerebrum there are anastomoses between important arteries to protect against ischemia. The most important cerebral anastomoses are part of the Circle of Willis, a pentagon shaped circle of vessels in the cerebrum [8]. This ring at the base of the brain consists of three main arteries: the anterior, middle and posterior cerebral arteries and two communicating arteries, as seen in figure 4.1 [9, 10]. In this research the segments of the middle cerebral artery (MCA) will be at interest, which is divided into four segments, namely the M1, M2, M3 and M4 segment [11]. The major cerebral arteries supply the whole cerebrum through the capillaries which arise of the main arteries and their bifurcations. The capillaries belong to the microcirculation, along with venules and arterioles.

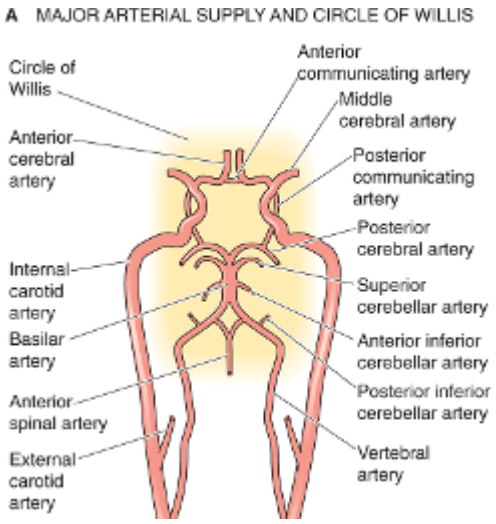

Figure 4.1. Circle of Willis and the three major cerebral arteries [10].

4.2 CEREBRAL AUTOREGULATION

Specifically for the cerebrum, it is important to maintain an adequate and stable blood flow which is provided by cerebral autoregulation. This mechanism keeps the cerebral blood flow constant, despite fluctuations in the mean arterial pressure between 70-150 mmHg [10]. Although there are local fluctuations throughout the brain, the total cerebral blood flow is maintained at a constant level.

Cerebral autoregulation is known to function as a low pass filter. Low fluctuations (<0,1 Hz) in blood pressure and flow are corrected by cerebral autoregulation better than fast fluctuations, which are transmitted almost linearly [12]. There can be made a distinction between static and dynamic cerebral autoregulation. In static cerebral autoregulation, the steady-state relationship between arterial blood pressure and cerebral blood flow is being controlled over a longer period

of time, from several minutes to hours. In dynamic autoregulation, the relation between a transient rise in blood pressure is being followed over a shorter period of several seconds.

Cerebral autoregulation uses neural, myogenic and metabolic mechanisms to protect the cerebrum against possible blood pressure fluctuations [13]. These mechanisms are studied for several years, but it is not fully understood at the moment. The myogenic control mechanism will cause vasoconstriction as a result of an increase in blood flow, detected by smooth muscle cells. This results in a decreased blood flow [12, 14]. This mechanism keeps tissue perfusion relatively constant, despite variations in systemic pressure. Besides, it prevents damage to the small vessels [15]. The neural control of autoregulation is divided into two groups: sympathetic and parasympathetic nerve fibers. Activated sympathetic fibers release norepinephrine, which result in contraction of the vascular smooth-muscle cells and thus vasoconstriction. The parasympathetic nerve fibers induce vasodilatation, as a consequence of acetylcholine release. The neural control is relatively weak compared with the metabolic control [10]. The metabolic control counteracts an imbalance between cerebral metabolism and oxygen delivery. An increased metabolism causes a decrease in PaO_2 and pH and an increase in $PaCO_2$. The cerebrum is extremely sensitive to the changes in pH and $PaCO_2$. An increase in $PaCO_2$ will result in vasodilatation because of the cerebrovascular vasoreactivity [10, 12]. A difference in PaO_2 is not a considerable stimulus for autoregulation [15].

Besides, respiratory changes can also contribute to a change in cerebral blood flow. Hyperventilation causes a decrease in $PaCO_2$, which will cause cerebral vasoconstriction and thereby, a decreased blood flow [10]. A decrease in $PaCO_2$ corresponds linearly with a decrease in $EtCO_2$ [16]. A lower $PaCO_2$ is known to decrease the velocity in the MCA and decreases the diameter, velocity and thereby flow of the internal carotid artery [16-18]. There is a logistic and not a linear regression between the $EtCO_2$ and cerebral blood flow velocity (CBFV) where the CBFV alters more slowly when reduction in $PaCO_2$ occurs. Willie et al. stated a decrease in CBFV of ±34% when the $PaCO_2$ decreased with 10 mmHg [16].

4.3 COLD PRESSOR TEST

4.3.1 PRACTICAL AND CLINICAL USE

The cold pressor test is a test in which an extremity is submerged in ice water for the purpose to increase the blood pressure by stimulating the sympathetic efferent pathways [19]. The test has been used for many years to study sympathetic responses that are not mediated by the baroreflex [20, 21]. In the past, the test was frequently applied in cardiovascular research. For example, hyperresponsiveness of the test in healthy subjects has been linked to future development of hypertension [2, 22]. Also, coronary vascular response differs between young and older men and an altered response in the carotid artery is present in subjects with coronary risk factors and coronary artery diseases [3, 4, 23]. The cold pressor test is found to give a reproducible response within the same person. Hines et al concluded in early research that over a year, the average response varied approximately 10% [1]. Saab et al. concluded that the systolic and diastolic blood pressure, total peripheral resistance and heart rate gave similar responses over the course of two weeks of repeated examinations [2].

Different approaches of the test were used in the past, which vary mainly in submersion time and part of the body that is affected by the cold. In early research with the cold pressor test, a hand was emerged for 60 seconds [21, 22]. More recent studies have chosen to perform the cold pressor test mainly for 1-5 minutes [2, 5, 6, 24]. One reason for the usage of a longer submersion time is that the temperature of the hand after withdrawal was too variable to compare between

responses [25]. Additionally, different outcomes that were of interest, such as cold adaptation, influenced the chosen immersion time. Between studies, it also differed which body part was exposed to the cold. Mainly, the hand was chosen, but also foot submersion and applying a cold pack to the forehead region was performed frequently. The cold pressor test shows similar response between foot and hand immersion and it is found that the response did not change much if the subject places both hands or feet in cold water [1, 2].

4.3.2 SYMPATHETIC RESPONSE OF THE COLD PRESSOR TEST

Many studies are performed to gain insight in the physiology of the cold pressor test. A lot of outcomes are described, but the actual mechanism is not fully understood. The cold pressor test has shown to cause a great sympathetic discharge at the spinal cord and endings of the sympathetic nervous system, so it is assumed that the cold pressor test activates the sympathetic nervous system [26, 27]. The activation of this division of the central nervous system causes an increase in heart rate and contractility of the heart, as a result of released norepinephrine. It causes peripheral vasoconstriction as well.

The primary response of the cold pressor test, that may be responsible for the sympathetic activation, is suggested to be caused by the activation of nociceptive C-fibers, which causes perception of pain and cold. A study of Kregel et al. looked at the impact of different amounts of skin cooling on muscle sympathetic nerve activity (MSNA), a way to measure the activity of sympathetic nerves, in the peroneal nerve. Activation of MSNA only occurred during severe skin cooling, associated with high temperature drops in the skin and intense sense of pain [28]. Additionally, a weak but statistically significant correlation is found between the perception of pain and the activation of MSNA . The response of the body however is not only because of pain sensation [29].

The activation of nociceptive fibers may evoke selective autonomic responses because of different sensations in humans [28]. Many studies have been performed to quantify these autonomic responses to the cold pressor test. An important effect of the cold pressor test is found within the blood pressure. Many studies describe an increase in blood pressure of approximately 20 mmHg for the MAP as a result of the test [5, 6, 20, 21, 25]. It was found that the heart rate is initially increased because of the primary response to the skin cooling [27, 28]. Kregel et al. stated that the origin of this response was not clear because a rise in heart rate was associated with an inhibition in MSNA at different water temperatures, so the two responses might not have the same origin [28]. These findings are displayed in figure 4.2.

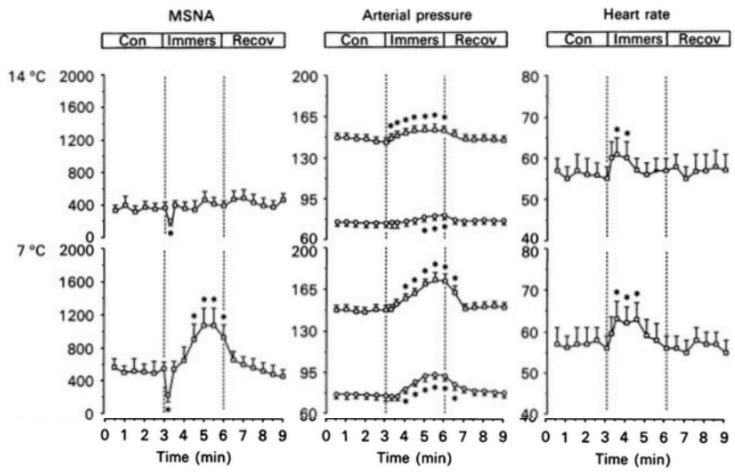

Figure 4.2. The reaction of the MSNA related to arterial pressure and heart rate before, during and after the cold pressor test. The image is obtained and modified from a study of Kregel et al [28].

Another signal of importance for sympathetic activation is norepinephrine. Victor et al. have reported an increase in norepinephrine as a result of the cold pressor test [27]. Peak changes in MSNA are correlated with a small increase in norepinephrine. The cold pressor is expected to have an influence on norepinephrine release because of its sympathetic activation. The only low increase that is subscribed can be explained by the fact that many factors affect the norepinephrine concentration. Victor et al. described a change of norepinephrine that only occurred after the first 2 minutes of the cold pressor test [27].

4.3.3 Baroreflex response of the cold pressor test

The baroreflex is sensitive to changes in blood pressure. Normally, the baroreflex causes vasodilation and a decreased heart rate when there is an increase in blood pressure. Another way by which the baroreflex compensates, is by inhibiting MSNA, which is normally controlled by a negative feedback loop. When there is a decrease in blood pressure, this mechanism results in activation of the sympathetic nervous system and causes vasoconstriction and an increased heart rate [10]. The sympathetic activation mediated through the baroreflex is important in short term blood pressure regulation. It is interesting to consider the baroreflex during the cold pressor test. Expected may be that the baroreflex is inhibited, otherwise a rise in blood pressure would be compensated. It is described that in the response of the cold pressor test, MSNA can be increased, partly independent of the baroreflex. In a study of Cui et al. the sensitivity of the baroreflex on pressure alterations during the cold pressor test is quantified. It is suggested that the new values for blood pressure and MSNA that occur directly after the cold pressor test function as new thresholds. Supplementary, the sensitivity of the baroreflex control of MSNA is increased, but can only partly undo the changes the cold pressor test induced, for example the delay in heart rate which occurs 1-2 minutes after the start of the cold pressor test [5, 20]. In conclusion, the cold pressor test can override the outcome of the baroreflex on the blood pressure, when looking at MSNA.

4.3.4 Cerebral hemodynamics during the cold pressor test

There have been studies that measured the diameter of the carotid artery, velocity in the MCA, cerebral blood volume and cerebral blood flow during the cold pressor test. Rubenfire et al. measured the reaction of the carotid artery as a result of the cold pressor test, primarily to describe coronary risk assessment. Subjects with average risk showed a dilatation of the carotis with a maximum of 7.9%±3.3% at 90 s after the start of the cold pressor test. However, subjects with high risk showed barely dilatation and subjects with coronary artery disease showed a large constriction [3]. There is no mention of possible risk assessment regarding cerebral hemodynamics. Roatta et al. reported an increase in velocity in the middle cerebral artery of 4.4% contralateral and 2.4%, ipsilateral, relative to the affected hand, which may be explained by the activation of the motor-sensory cortex [5]. Zvan et al. measured an average increase of 9.8%. The study also showed that higher increases were found in the contralateral hemisphere [7].

In many studies, an increase in cerebrovascular resistance was found due to the cold pressor test [2, 5, 7, 25, 30]. It is pointed out that the sympathetic response is most likely to contribute to vasoconstriction via a neurogenic factor above metabolic and myogenic factors because of its time response [5, 7]. In a study of Wilson et al., a constant CBF is found together with a decrease in volume in the measured compartments. Vasoconstriction during the cold pressor test may have contributed to the changes [6]. The mechanism of protection against an increased blood pressure is probably at the level of the large arteries [5]. In none of all the studies described, the diameter of the MCA was measured so no clear statements could be made about the flow in the MCA. However, both small cerebral arterioles and large cerebral arteries contribute significantly

to the vascular resistance. Giller et al. reported that the diameter of the MCA did not change significantly, less than 4%, during blood pressure alterations of 30±16 mmHg. However, the anterior cerebral artery and M2 segment of the MCA showed larger changes up to 21% [31]. The study is limited for statements about the cold pressor test response because an adrenergic receptor agonist was used to induce an elevation in blood pressure. Also, the changes that are detected referred to both an increase and decrease of the diameter [31]. Besides the blood pressure, the $PaCO_2$ has an impact on cerebral hemodynamics. During research of Wilson et al. and Zvan et al. it was found that the cold pressor test has no direct impact on the $PaCO_2$, stating that when it does occur this is a confounding element in the results [6, 7].

4.4 Other tests for research on cerebral hemodynamics

In pursuit of finding ways to research the cerebral hemodynamics there are multiple tests. Each test interacts differently with this mechanism. The first test to discuss, which is similar to the cold pressor test, is the isometric sustained handgrip. With this test the subject is asked to clench their hand into a fist forcefully and maintain it until fatigued. With this test the total peripheral resistance is increased. As the peripheral resistance increases the blood pressure will increase in response to counteract this phenomenon [32]. With the general increase in blood pressure there will be a flow increase to the rest of the body because the peripheral resistance increase is a local occurrence. In essence this is a sympathetic activation similar to the cold pressor test, where the body reacts to an altered state [33].

Another test to alter the blood pressure is the Valsalva maneuver. The subject is asked to exhale while keeping the exits of the airways closed. It increases the internal pressure on the chest, especially the head. The test initially increases the stroke volume and cardiac output, because blood is forced out of the pulmonary circulation. After this occurrence, the cardiac output will decrease because the venous return is diminished [34]. However studying this maneuver in relations to hemodynamics, there are other factors which come into play. By holding ones breath the $EtCO_2$ decreases. This makes the response more complex for studying particular effects of an increased blood pressure on cerebral hemodynamics because of the effects of the $PaCO_2$.

Passive head tilt is a test where the head of a subject is elevated while lying in supine position. The elevation causes a decrease in blood pressure in the cerebrum. During this exercise it is observed that the cerebral blood flow and $EtCO_2$ decrease while maintaining an unchanged breathing rate [35]. A similar way to induce hemodynamic changes is with the sit-to-stand procedure. A benefit with this test is that it contains a normal daily movement. The blood pressure decreases immediately after the patient starts standing up, which is called initial orthostatic hypotension. The baroreflex will increase the blood pressure to counteract this new state [36]. This reaction is absent in the passive head tilt [37].

The final two tests that will be discussed are the thigh cuff method and lower body negative pressure test. The goal of these tests is to create a pressure drop. Thigh cuff method is performed by placing a cuff around a leg for at least 2 minutes. Then rapidly the pressure is released causing increased flow to the legs and a blood pressure drop in the rest of the body including the brain. The decrease in blood pressure is detected by the baroreceptors which initiate the baroreflex. The thigh cuff procedure is a painful procedure for the patient to endure [38]. With the negative lower body pressure the lower extremities are placed in a box. This box will create a lower atmospheric pressure inside it, which reduces the external pressure on the legs. As the upper body is outside the box and the lower body inside, the blood will flow from the upper to the lower body [39]. The systemic blood pressure will decrease, which will cause activation of the baroreceptors. Therefore, heart rate will increase, while cardiac output and

stroke volume will decrease [40]. This will result in a decrease in velocity in the MCA by 16% [41]. The negative side of this examination is that the patient must be able to fit inside the box [39]. In some cases the lower negative body pressure test had to be cancelled during the procedure because the body could not compensate for the new state, which is caused by symptomatic blood pressure drops and heart rate fluctuations [41].

5 HYPOTHESIS

The main goal of this thesis is to study the effect of the cold pressor test on the blood flow in the carotid artery, the middle cerebral artery and the microcirculation in healthy subjects. It is expected that the flow in the carotid artery, the middle cerebral artery and the cerebral microvasculature increases in response to the cold pressor test.

Rubenfire et al. showed a maximal dilatation of 7.9%±3.3% in the carotid artery as a consequence of the cold pressor test and that did not change for three minutes after release. It is expected that the flow in the carotid artery increases, because of an increase in blood pressure and diameter of the carotid artery.

Studies of the effect of the cold pressor test on the velocity in the MCA assumed that the diameter of this artery is constant [5, 6]. For blood pressure changes of less than 30 mmHg, it is expected that the diameter of the MCA is not affected in response to the cold pressor test. This is based on research of Giller et al., that showed no significant changes in the diameter of the MCA during blood pressure alterations of 30±16 mmHg [31]. Additionally, research shows a higher blood flow velocity in the middle cerebral artery in response to the test [5, 7]. Therefore it is expected that the flow in the MCA will increase as a result of the cold pressor test. The flow in the right middle cerebral artery will increase more than the left side, because the contralateral cortex might be activated as a result of left hand submersion [5].

It is reported that the central venous pressure does not change during the cold pressor test [6, 27]. Besides, dynamic cerebral autoregulation only partially compensates for a transient increase in MAP by increasing the resistance. Therefore, the increased flow in the MCA will result in an increased flow in the microvasculature. In addition, the flow in the right hemisphere increases more than the concentration in the left hemisphere, because of the contralateral cortex activation. The flow in the microvasculature is maximal direct after the maximum flow in the carotid artery and the MCA, because it does not take a long time for the blood to reach the capillaries.

6 MATERIALS AND METHODS

In this research there are three different experiments that take place. All the experiments apply the cold pressor test. First the criteria for the subjects are discussed. Then the usage of the cold pressor test will be explained and the techniques used in the experiments. After that, the three experiments will be explained and finally the analysis will be discussed.

6.1 SUBJECTS

Four healthy subjects take part in this research that comply with the following criteria. The subjects are aged between 18-30 years, in order to minimize the influence of age within the group. The subjects should have a low risk of heart disease. Therefore their body mass index should be under 30 kg/m2, their blood pressure in rest should be less than 140/90 mmHg and they must be non-smokers. Subjects must abstain from caffeine, alcohol and any drugs for at least 24 hours, because it may influence the outcomes of the research [6].

As seen in figure 6.1, three subjects were used for the standard analysis, because of a confounding element present within the results of one subject. The $EtCO_2$ of one of the subjects decreased during the cold pressor test, so this subject was taken separately as a case to evaluate [42]. Additionally, another subject was excluded from the analysis of the carotid artery measurements, because the ultrasound probe moved during the experiment which caused an incorrect measurement of the velocity. Therefore, the flow could not be calculated properly.

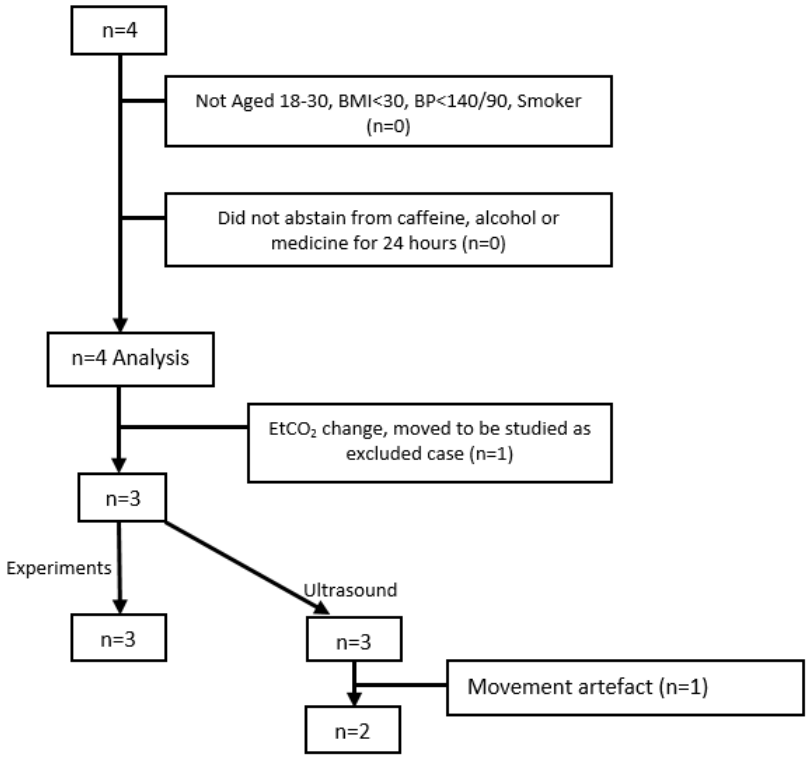

Figure 6.1. Flowchart of the excluding procedure, no subjects were excluded before the experiments were performed. 1 subject was excluded from the analysis because of the change in $EtCO_2$ and 1 subject was excluded from the analysis of the ultrasound data because of a movement artefact.

6.2 COLD PRESSOR TEST

During the experiments, the cold pressor test was performed by submerging the left hand to the wrist in a container filled with ice water for three minutes. The left hand was chosen because it was practical to use in the design of the first experiment and because it is proven that the left hand evokes a greater response [43]. The temperature of the ice water must be between 0-7 ℃, because data of the research of Kregel et. al suggest that sympathoexcitation only occurs when the skin temperature falls to less than 15 ℃ [28]. The maximal level of the mean arterial blood pressure is reported to be achieved at 90-120 s [30]. The same applies to the carotid artery, where the maximal level of dilatation occurred at 90 s [3]. It is important that the maximum is within the measurements, therefore a period of three minutes is chosen.

6.3 APPLIED TECHNIQUES

During the experiments there are different techniques used to detect a change in flow in the three examined levels.

6.3.1 ULTRASOUND

Ultrasound is used to image the carotid artery, so that the diameter can be measured in order to calculate the flow. Ultrasound is measured on the left carotid artery for practical reasons. Ultrasound waves are produced by a piezoelectric transducer, which emits and receives the waves. These waves are scattered or partially reflected by different layers of tissue and detected by the transducer. Ultrasound uses high frequencies of 1 to 15 MHz. A higher frequency will result in a higher resolution image, which is desirable to detect a change in diameter of the carotid artery. However, the higher the frequency, the less the waves penetrate the tissue. To determine the flow, the velocity in the carotid artery is measured by using the Doppler effect of ultrasound. The Doppler effect is caused by waves reflected by moving objects, which cause a frequency shift. The blood velocity can be calculated out of this frequency shift.

6.3.2 TRANSCRANIAL DOPPLER

Transcranial Doppler (TCD) is used to measure the velocity in both the MCAs in order to determine the flow. The skull is an obstacle for ultrasound to penetrate, because it strongly attenuates ultrasonic waves due to the high density. Therefore the frequency that is used has to be in the low region of ultrasound, 1 or 2 MHz. However, the resolution of the image decreases as the frequency is low. Hence the MCA can not be imaged by TCD, but the velocity can be measured using Doppler. The middle cerebral artery is often chosen because it is easy to visualize with TCD [44]. TCD can be used to measure fluctuations in the velocity with a high temporal resolution [18].

6.3.3 TIME OF FLIGHT ANGIOGRAPHY

In order to calculate the flow in the MCA, not only the velocity but also the diameter is needed. Time of flight angiography (TOF) is used to determine the diameter of the MCA. TOF is an MRI sequence that can image blood vessels without the use of a contrast agent. This technique manipulates the magnitude of the magnetization, such that the moving spins will give a larger signal than the stationary spins. When there are more excitation pulses given to spins, the signal will be less until eventually a saturation value is reached. In TOF, the intention is that the stationary spins receive a large number of excitation pulses and that the moving spins receive only a few excitation pulses. The repetition time of the pulses is adjusted to be long enough so that the blood can flow in the imaging plane. This will give a contrast between inflowing blood,

which has a high signal, and the stationary spins, which will be saturated and therefore have a low signal [45].

6.3.4 Arterial spin labeling

Not only can the diameter of the MCA be imaged in an MRI, but also the change in cerebral blood flow in the microvasculature. Arterial spin labeling (ASL) is used to look at this change. ASL is an MRI sequence that is capable of measuring perfusion without the use of an exogenous contrast agent. It is a non-invasive technique, because it uses the blood water protons itself as a contrast agent. Arterial blood water protons are magnetically labeled so they can be imaged. ASL uses a 'tag image' and a 'control image', the signals of the static tissue in these images are the same, but the magnetization of the inflowing blood is different [46]. In order to acquire this magnetic labeling, a region downstream of the region of interest will be given a radiofrequency pulse that inverts water protons. After some time, the magnetically labeled blood water will flow in the region of interest where the image can be acquired. There has to be a delay between labeling and the image acquisition, this is called the inversion time (TI), because the labeled blood has to reach the capillaries [47]. While traveling upstream the labeled protons will not be fully relaxed and when excited again to acquire the tag image, they will give a different signal than the surrounding fully relaxed stationary particles. In order to eliminate these stationary particles, a control image is acquired when there is no labeled blood in the region of interest. Subtraction of the tag image from the control image gives the signal of the labeled blood which is a relative measure of perfusion proportional to cerebral blood flow [48]. There are several ASL techniques that differ mainly on how they label blood. In this experiment, pulsed ASL is used. Instead of labeling blood as it flows through a plain, pulsed ASL uses short radiofrequency pulses to saturate or invert a thick slab of tissue proximal to the imaging region [49]. A single TI image is a single subtraction image. In order to improve the signal-to-noise ratio (SNR) a single-TI sequence was used that gave multiple ASL images. These images are complementary to near infrared spectroscopy, which is described below.

6.3.5 Near infrared spectroscopy

To investigate the flow in the microvasculature with a high temporal resolution, near infrared spectroscopy (NIRS) is used on the frontal lobe. NIRS is a non-invasive technique whereby near infrared light with a wavelength of 650 to 1100 nm is emitted into the human body. Near infrared light can penetrate the skull and the adjacent structures for several centimeters [50]. It is possible to detect the backscattered photons and to measure the absorption spectrum of the near infrared light. The absorption rate depends on the substances in the analyzed region [51]. Deoxyhemoglobin (deoxy-Hb) and oxyhemoglobin (oxy-Hb) have different absorption profiles, so it is possible to calculate the ratio between those variables [52]. NIRS provides valuable information about the cerebral microcirculation and oxygenation in the cortical regions [53]. There are different kinds of NIRS and in this experiment the continuous wave NIRS is used. The light is emitted into tissue with a constant amplitude and frequency. There is an amplitude decay of the light intensity as a consequence of absorption [51]. This NIRS method does not have any information about the path-length of the photon, so the changes are relative to an unknown path-length. Therefore, a modified version of the Beer-Lambert law should be used to determine the absorption. The outcome of the Beer-Lambert law does not give a correlation between the absolute values of oxy-Hb with CBF. Though it is a good way to detect changes of CBF [52]. The cerebral venous compartment contains 70%-80% of the blood. Therefore, NIRS mainly measures the amount of oxy-Hb in the venous compartment. The oxygen content of this compartment represents the relationship of CBF to the metabolic rate of oxygen in the

cerebrum. If the metabolism in the cerebrum is constant, the change of oxy-Hb is directly proportional to the change of CBF [52].

6.4 Experiment 1

Experiment 1 contains the following techniques: ultrasound, transcranial Doppler, near infrared spectroscopy, CNAP, ECG and CO_2 meter. This experiment is executed according to 'SOP Cerebral blood flow measurements' which is included in appendix A. It will be briefly discussed how this protocol has been established.

As many circumstances as possible must remain constant during the experiment, therefore the room temperature has to be constant and the curtains have to be closed. The subject was instructed to abide by certain rules: do not speak, stay in position and move minimally, prevent the Valsalva maneuver, breath normally and try not to hyperventilate, avoid swallowing or coughing, do not close the eyes for a longer period of time and breathe through the nose. These actions could have a confounding impact on the experiment. During the preparation, the subject will be connected to all the devices that are used. The subject has to lie still in supine position for two minutes before the measurements are started to ensure that the blood pressure levels are stabilized and the patient is at rest.

The measurement will take thirteen minutes, as illustrated in figure 6.2. First a baseline of five minutes is measured and then the cold pressor test is performed. After three minutes cold pressor test, a five minute recovery is measured.

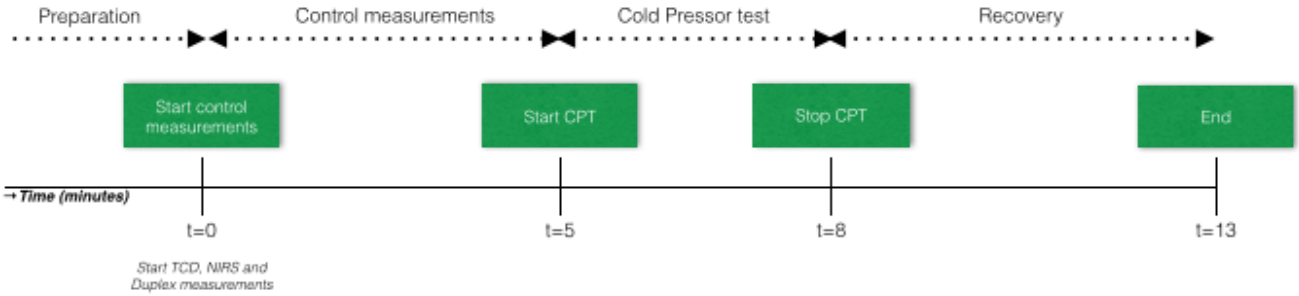

Figure 6.2. Timeline of experiment 1, the TCD, NIRS and ultrasound measurements are started at t=0min and these measure during the whole experiment, at t=5min the cold pressor test is started and at t=8min the test is ended, the measurements end at t=13min.

Not only are the parameters to determine the flow measured, but also the parameters that might influence the flow: blood pressure, heart rate and $EtCO_2$. The CNAP is used to measure the blood pressure real time by means of a finger cuff. The ECG leads measure the heart rate in order to evaluate the cardiac reactivity to the cold pressor test. A CO_2-meter measures the percentage of CO_2 that is exhaled. The $EtCO_2$ can be derived out of this data.

6.5 Experiment 2

In this second experiment, the cold pressor test is performed in an MRI scanner, using arterial spin labeling and time of flight angiography. This experiment is used according to 'SOP MRI' which is included in appendix B. It will be briefly discussed how this protocol is established.

This experiment has to be executed under similar circumstances as experiment 1. As seen in figure 6.3, the ASL and TOF sequences are performed alternately. Before the cold pressor test is performed, one ASL sequence and one TOF sequence are used to acquire reference images. One

minute after the start of the second ASL, the cold pressor test is started. The ASL sequence will be completed around 1min 30s cold pressor test, so that the second TOF sequence can be taken around the two minutes. The results of experiment 1 showed that the maximum level of the outcomes was around two minutes and therefore it is assumed that the maximum diameter of the MCA is at about two minutes. After that, another ASL sequence and TOF sequence are taken.

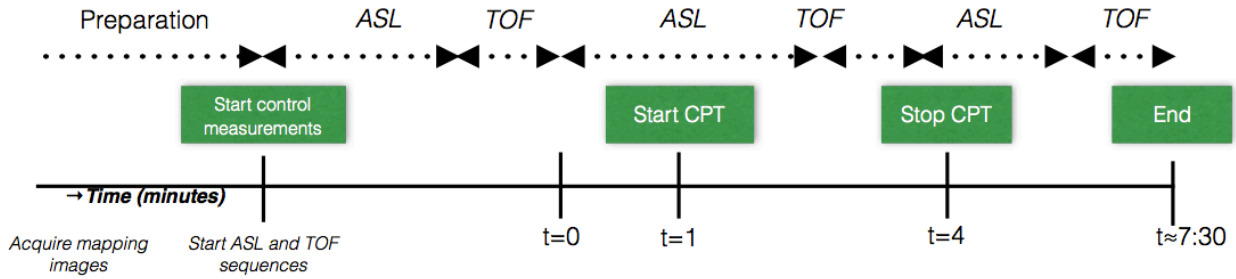

Figure 6.3. Timeline of experiment 2, first reference ASL and TOF images are made, the second ASL sequence starts at t=0min and at t=1min the cold pressor test is performed, the second TOF sequence starts at t=2min30s, the third ASL sequence starts just before the cold pressor test ends at t=4min, at last another TOF image is made.

6.6 Experiment 3

In this third experiment, the cold pressor test is performed and the blood pressure will be measured by an automatic blood pressure monitor. In experiment 1, the change in blood pressure measured by the CNAP contradicted the expected increase that was found in the literature. The results of this experiment might provide insight into the validity of CNAP during the cold pressor test.

The subject had to abide by the same rules established in 'SOP Cerebral blood flow measurements'. The subject had to lie in supine position on the examination table for two minutes to assure stable body conditions. The blood pressure was measured to acquire a baseline value. Two minutes after this measurement, the cold pressor test was started and the second measurement was performed. The data of the first experiment showed there was a significant decrease of blood pressure in the first thirty seconds. The measurement of the blood pressure monitor takes about twenty seconds and therefore the cold pressor test and measurement are started at the same time. It is expected that the maximum level of blood pressure will be reached in about two minutes. Hence, the third measurement of blood pressure is started 1min 40s after the start of the cold pressor test.

6.7 Analysis of data

The data acquired out of these experiments is analyzed in Matlab and Microsoft Excel. The data of TCD, NIRS, CNAP, ECG and $EtCO_2$-meter were acquired by Biopac with a sample frequency of 200Hz. The ultrasound data was stored in a .csv-file and has a sample frequency of around 100 Hz. The ASL and TOF images were stored as DICOM files and converted to NIfTI.

Every measured parameter is averaged at different stages in the analysis. This averaging was established by using the following calculation which is referred to as the 'average calculation'. First, the parameter was averaged over the five minutes of the baseline per subject. After that, the average value of the cold pressor test and the averages per ten seconds during the cold pressor test were determined per subject. Then, the change in percentage between baseline and

cold pressor test was determined per subject for the average value of the cold pressor test and the averages per ten seconds. Finally, these relative changes were averaged over all the subjects.

The flow of the carotid artery is calculated with the diameter and velocity that are acquired by ultrasound. The equation for flow and the equation for the cross sectional area are:

$$Q = v \times A \qquad (6.1)$$

$$A = \frac{1}{4} d^2 \times \pi \qquad (6.2)$$

The diameter, velocity and flow were analyzed by using the average calculation.

The flow of the MCA is calculated with the velocity acquired by TCD and the diameter derived from the TOF images, using the equations 6.1 and 6.2. The velocity of TCD was analyzed by using the average calculation. Each subject had three TOF images and at every image the diameter of the left and right MCA were determined. First it was decided at which slide the MCA was most evident. Then the diameter was determined manually in each image at approximately the same point using the Pythagorean Theorem. It is not sure whether the diameter will change during the cold pressor test. If it remains the same, the calculation of the flow will not be necessary because changes in velocity will be proportional to changes in flow.

The change in flow can be derived out of the NIRS values and ASL images. The NIRS values were analyzed by using the average calculation, except for the change in percentage. Instead of the change in percentage, the absolute change was calculated and averaged over all the subjects. Only the absolute change could be calculated, because the baseline was set to zero at the beginning of the experiment. The mean of the ASL images before and the mean of the ASL images during the CPT will be subtracted from one another to see if there is a change in flow.

To calculate the mean arterial pressure (MAP), the systolic (sBP) and diastolic (dBP) blood pressures have to be determined in the CNAP data. Peak detection in Matlab is used to determine all systolic and diastolic values. The diastolic and systolic values were analyzed by using the average calculation, except for the change in percentage. Because before the changes in percentage were calculated, the MAP had to be determined. To evaluate an increase or decrease in overall blood pressure, the MAP is calculated according to the following equation:

$$MAP = \frac{2dBP + sBP}{3} \qquad (6.3)$$

Using peak detection in Matlab on the ECG-signal, the locations of the R-peaks could be determined. The heart rate between two R-peaks was calculated according to the following equation: $f = 1/t$ (6.4). After this, the heart rate was analyzed by using the average calculation.

CO_2-meter measured the percentage of exhaled CO_2. Peak detection is used to determine the $EtCO_2$, because at the location of the peak the concentration of exhaled CO_2 is represented. The $EtCO_2$ was analyzed by using the average calculation, except that the averages per ten seconds were not calculated. The respiratory rate between two breaths was determined using equation 6.4. The respiratory rate was also analyzed by using the average calculation with the same exception as $EtCO_2$.

The total cerebral vascular resistance index is calculated using the equation:

$$CVRi = \frac{MAP}{CBFV} \qquad (6.5)\ [36]$$

This is calculated using the means from the MAP derived out of the CNAP and the means of the velocity in the MCA, before and during the cold pressor test. Also, the resistance is calculated by using the MAP derived out of experiment 3 and the velocity corresponding to the same time interval. The change in percentage of CVRi is calculated compared to the baseline.

7 RESULTS

The recovery period of the cold pressor test was not included in the analysis due to the extent of this study of ten weeks. Also, the results were not necessary for the aim of this study.

In appendix C, the unfiltered data is included of the measured parameters.

The absolute and relative changes during the cold pressor test are displayed in table 7.1. All the measured parameters are increased during the cold pressor test, except for the NIRS deoxygenated signal.

Table 7.1. List of the average change, baseline measurement and cold pressure test measurement of different parameters

Parameter	Average change	Baseline	Cold pressor test
HR	4.61%	82.5	86.3
MAP (CNAP)	1.66%	102.8	104.6
MAP (automatic blood pressure device)	11.9%	91.8	102.1
Diameter carotid artery	0.189%	0.562	0.564
Velocity carotid artery	5.35%	22.0	22.9
Flow carotid artery	5.34%	6.12	6.20
CBFV left MCA	7.04%	63.7	68.3
CBFV right MCA	5.68%	72.9	77.1
Oxy-Hb left	1.67	0.96	2.63
Oxy-Hb right	0.961	1.47	2.43
Deoxy-Hb left	-0.331	-0.208	-0.539
Deoxy-Hb right	-0.291	-0.310	-0.601
CVRi (CNAP)	1.66%	1.33	1.35
CVRi (automatic blood pressure monitor)	8.00%	1.35	1.46

Average change represents the averaged changes of individual subjects during the cold pressor test compared to their baseline. Baseline and cold pressor test represent the absolute averages of the subjects during respectively the baseline and the cold pressor test. HR, heart rate (beats/min); MAP, mean arterial pressure (mmHg); diameter (cm); velocity (cm/s); flow (cm^3/s); CBFV, cerebral blood flow velocity (cm/s); oxy-Hb, oxygenated hemoglobin (µmol/L), deoxy-Hb, deoxygenated hemoglobin (µmol/L); CVRi, cerebrovascular resistance index (mmHg/(cm/s)). Changes are shown in percentage, except for oxy-Hb and deoxy-Hb which are shown as µmol/L.

7.1 PARAMETERS OF THE CAROTID ARTERY

In order to calculate the flow, the diameter and velocity were measured using ultrasound. In figure 7.1 the change of the diameter during the cold pressor test is shown. During the cold pressor test, the diameter increases on average 0.189%. The flow increases with a maximum of 1.14% at 40-50 seconds after the start of the test.

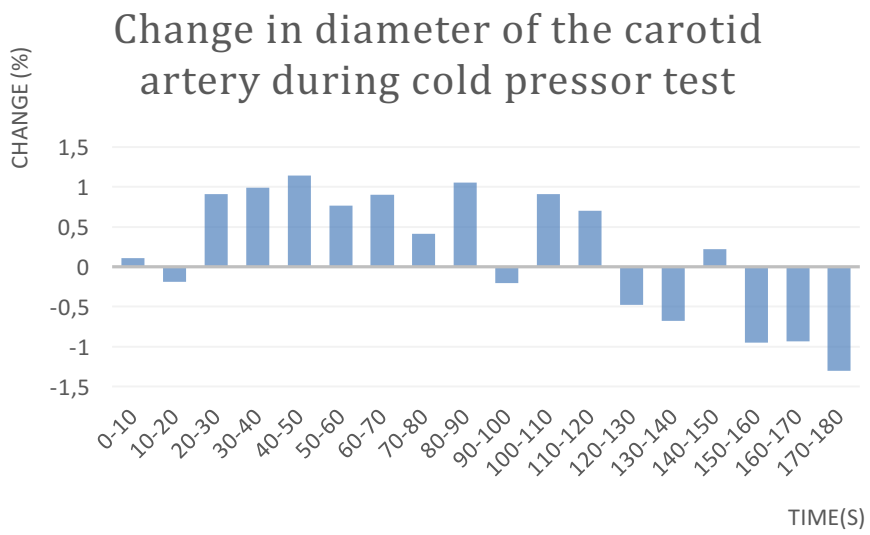

Figure 7.1. Change in the diameter of the carotid artery during cold pressor test compared to baseline. A maximum increase of 1.35% occurs at 80-90 s.

The change of the velocity is shown in figure 7.2. During the cold pressor test, the velocity in the carotid artery is increased by 5.35% on average. The maximum increase of the velocity is 20.5% at 150-160 s.

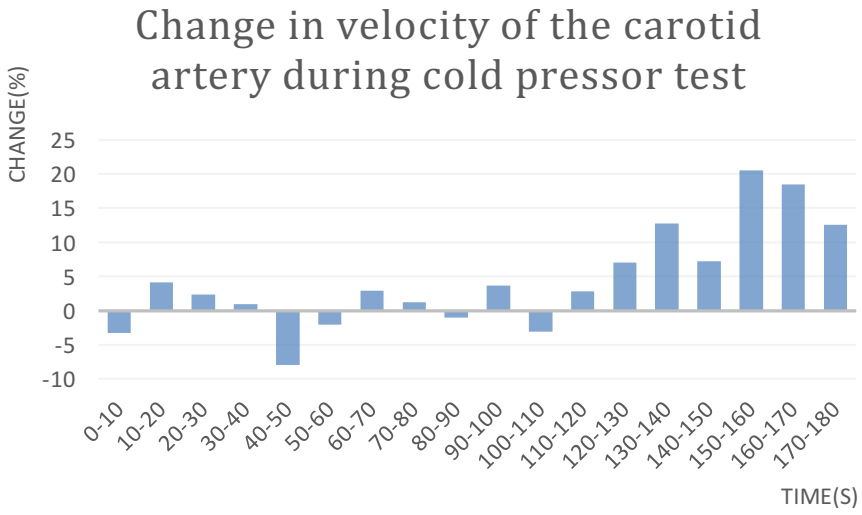

Figure 7.2. Relative change of the velocity of the carotid artery during cold pressor test compared to baseline. A maximum increase of 20.5% occurs at 150-160 s.

The flow was calculated using the velocity and diameter of the carotid artery. In figure 7.3, the relative change of the flow compared to the baseline is shown. The flow in the carotid artery increases with an average of 5.34% during the cold pressor test. The maximum flow increase is at 160 s with an increase of 18.5%. The flow particularly increases from 100-110 s until the end of the test.

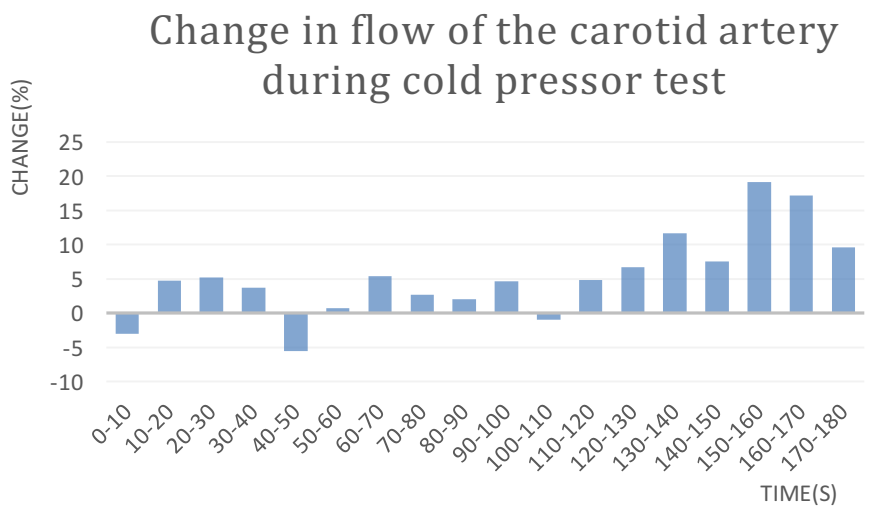

Figure 7.3. Change of the flow in the carotid artery during cold pressor test compared to baseline. A maximum increase of 18.5% occurs at 150-160 s.

7.2 PARAMETERS OF THE MIDDLE CEREBRAL ARTERY

To detect a change in the flow in the MCA, the diameter and CBFV of this artery are measured. In figure 7.4, the relative change of the CBFV in the MCA is shown compared to the baseline. The CBFV in the left and right MCA increases during the cold pressor test, with respectively 7.04% and 5.68%. This gives an average increase in CBFV in the MCA of 6.36%. The maximum increase appeared at 120-130 s after the start of the test. On average during the interval, the CBFV of the left MCA increases with 11.0% and the CBFV of the right MCA increases with 11.3%.

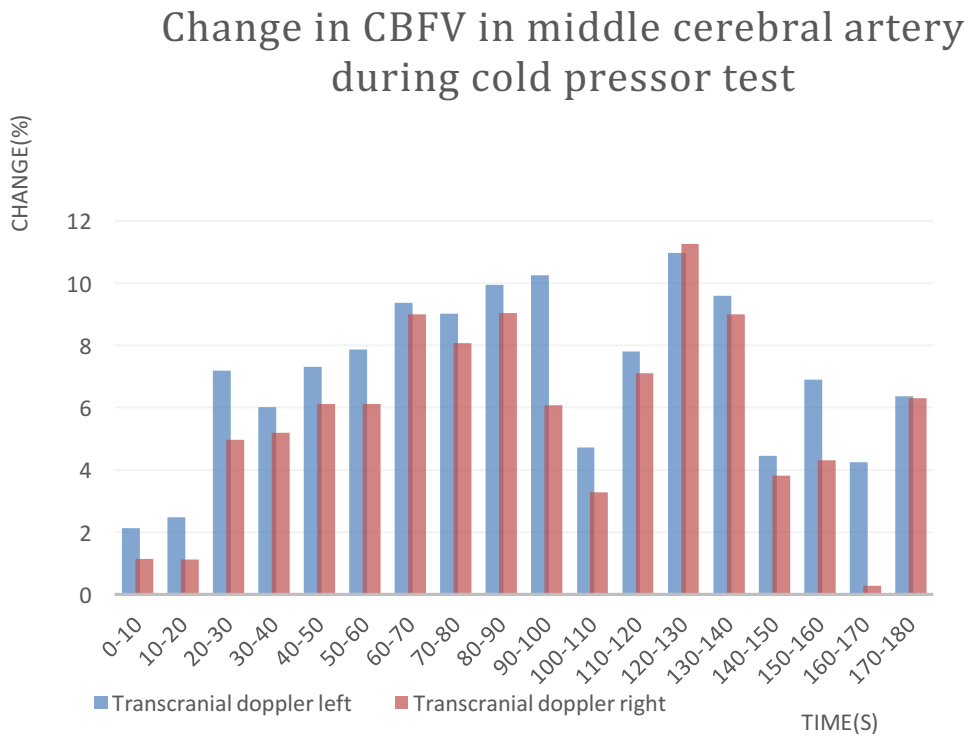

Figure 7.4. Change of the velocity in the middle cerebral artery during cold pressor test compared to baseline. The maximum increase occurs at 120-130 s, with 11.3% on the right side and 11.0% on the left side.

The diameter of the MCA is derived from TOF angiography images. In figure 7.5 there is seen an example of a representative TOF image during the cold pressor test. One voxel length and width are both equal to 0.651 mm. In table 7.2, the diameters of each subject before and during the cold pressor test are shown. The change in diameter has a maximum of 0.920 mm which is found in subject 3.

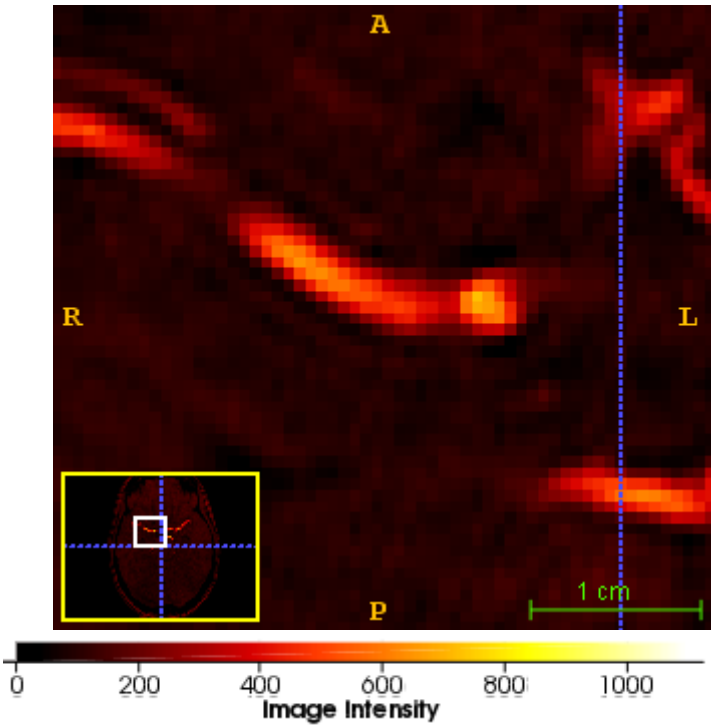

Figure 7.5. Example of a representative TOF image during the cold pressor test.

Table 7.2. Diameter of the middle cerebral artery at baseline and during cold pressor test

Subject	Diameter left MCA before CPT	Diameter left MCA during	Diameter right MCA before CPT	Diameter right MCA during
1	3.26	3.26	3.26	3.26
2	2.76	2.76	2.76	2.35
3	2.76	1.84	2.76	1.84

MCA, middle cerebral artery; CPT, cold pressor test. Values are shown in mm.

7.3 PARAMETERS OF THE CEREBRAL MICROVASCULATURE

To detect a change in flow in the microvasculature, NIRS and ASL were used. In figure 7.6, the change in concentration of oxy-Hb and in figure 7.7, the change in concentration of deoxy-Hb of the frontal cortex is shown. Both the left and the right frontal cortex were measured. As seen in figure X6, the concentration of oxy-Hb increases during the cold pressor test, with an average of 1.67 µmol/L on the left side and 0.961 µmol/L on the right side. This gives an average increase of 1.32 µmol/L in oxy-Hb. There was a maximum of 2.54 µmol/L at the right side and 1.65 µmol/L at the left side. This occurred at 120-130 s. It is notable that this peak is at the same moment as the peak velocity in the MCA. As seen in figure X9, deoxy-Hb decreases during the cold pressor test, with an average of -0.331 µmol/L on the left side and -0.291 on the right side. This gives an average decrease of 0.311 µmol/L in deoxy-Hb. The concentration of deoxy-Hb on the left side is at a minimum of -0.650 µmol/L at 170 seconds and the right side has a minimum

of -0.554 µmol/L at 120 seconds. The minimum of the right side occurs at the same moment as the peak in oxy-Hb and the peak velocity in the MCA.

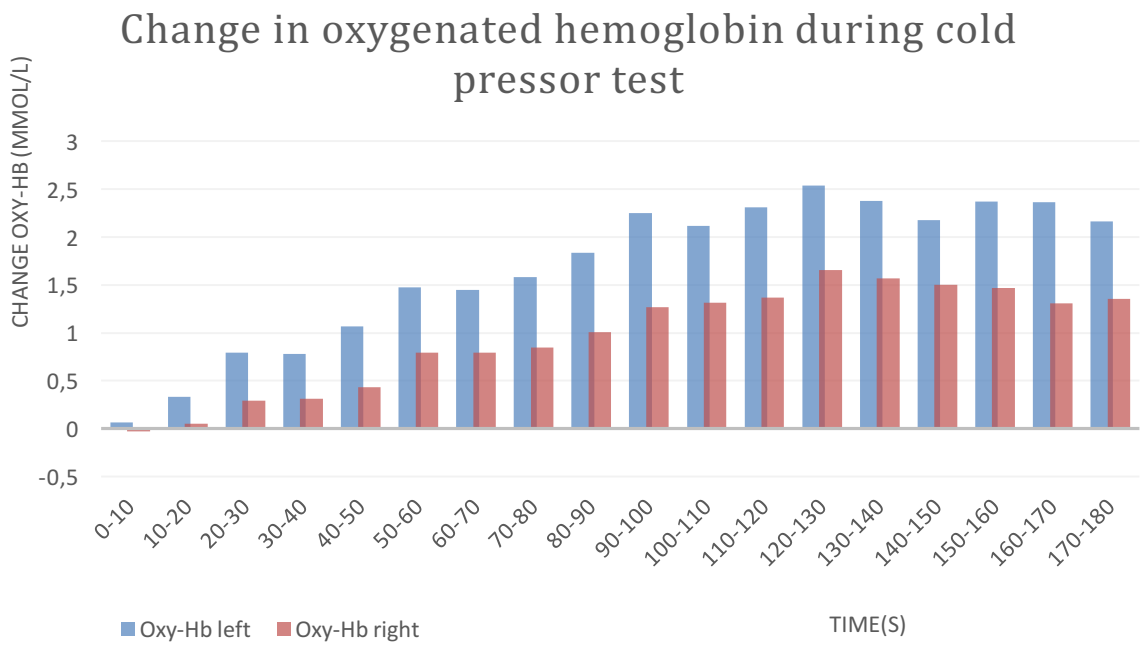

Figure 7.6. Changes in oxygenated hemoglobin of the left and right frontal cortex compared to baseline. The maximum increase occurs at 120-130 s, with 1.67 µmol/L on the left side and 0.961 µmol/L on the right side.

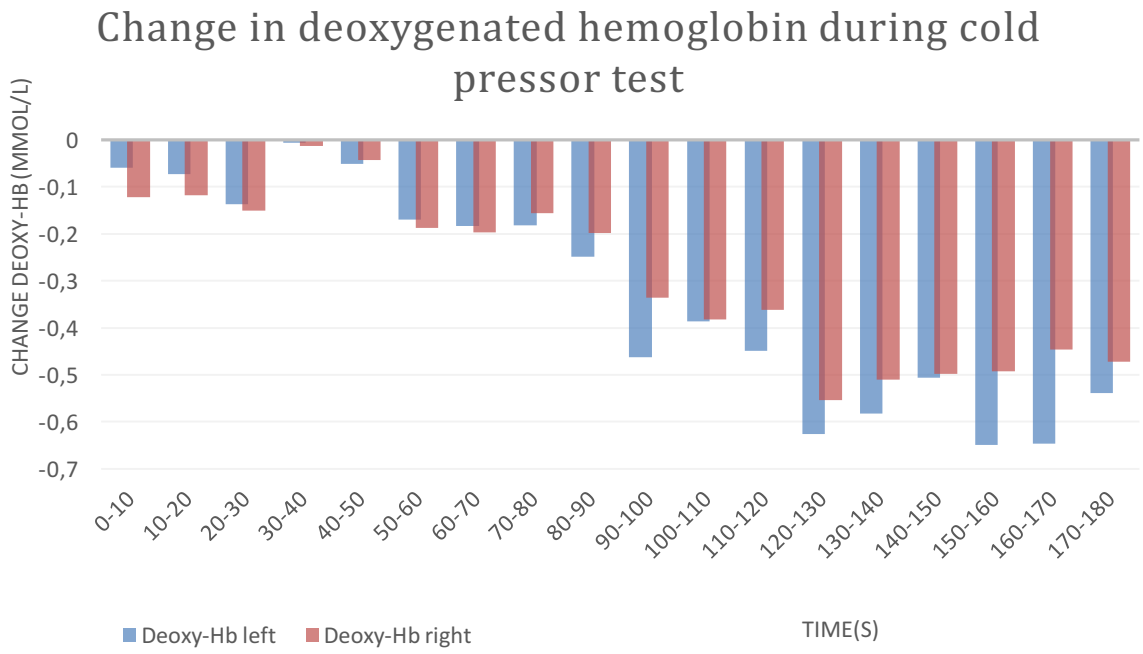

Figure 7.7. Changes in deoxygenated hemoglobin of the left and right frontal cortex compared to baseline. The maximum decrease of the left cortex occurs at 160-170 s with -0.650 µmol/L. Of the right cortex, the maximum decrease occurs at 110-120 s with -0.554 µmol/L.

The difference images of ASL are represented in figure 7.8. A blue color represents a flow decrease and the red color represents a flow increase during the cold pressor test compared to the baseline. In the images of subject 2 and 3, there is a movement artefact present, despite movement correction. The movement artefact is the strong negative edge of the brain. All images have a low signal-to-noise ratio (SNR) and are therefore difficult to interpret.

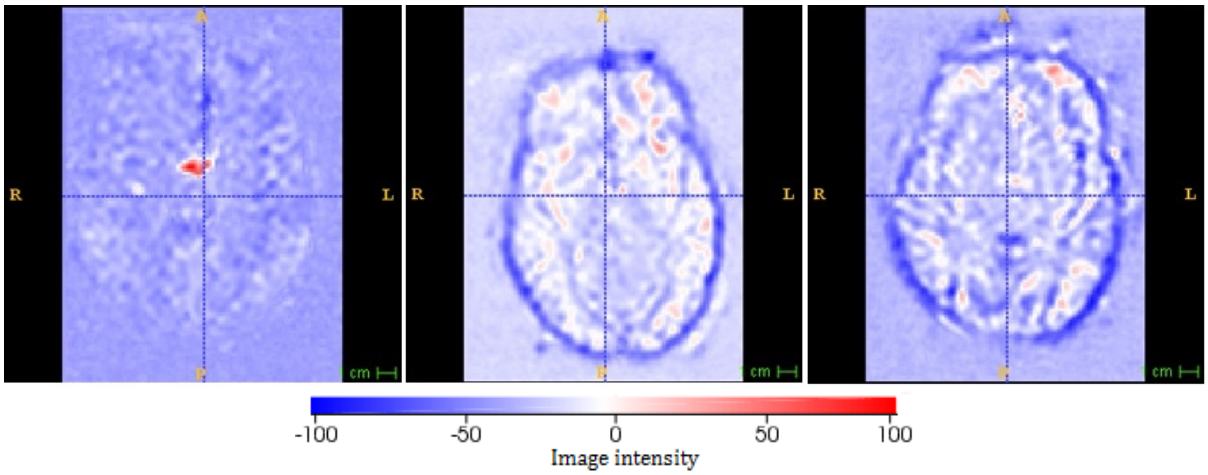

Figure 7.8. Representable difference arterial spin labeling images of all three subjects. From left to right from top to bottom: subject 1, subject 2, subject 3. Subject 2 and 3 showed a movement artefact.

7.4 Mean Arterial Pressure

The blood pressure in experiment 1 is measured by the CNAP. In experiment 3, it was measured by an automatic blood pressure monitor. In figure 7.9, the relative change of the mean arterial pressure measured by CNAP is shown in comparison to the baseline. Overall, the mean arterial pressure is slightly increased by 1.66% during the cold pressor test. As seen in figure X1, at the start of the cold pressor test the MAP decreases and after about 50 seconds, the MAP increases.

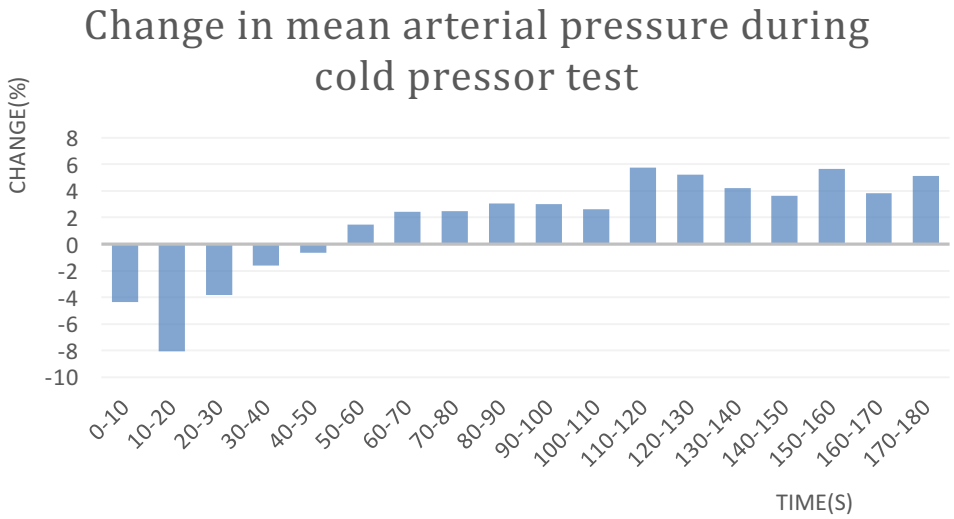

Figure 7.9. Change of the mean arterial pressure, measured by CNAP.

In table 7.3 the MAP calculated from the automatic blood pressure measurements and the CNAP are shown at 20-30 and 120-130 s. An initial decrease is seen in the mean arterial pressure measured by CNAP at 20 seconds after the start of the test. In contrast, the mean arterial pressure measured by the automatic blood pressure monitor rises in all three subjects at 20-30 s. The average increase of the mean arterial pressure over the whole cold pressor test, measured by automatic blood pressure device, is 11.9% compared to the baseline, which is equal to an increase 10.3 mmHg. The average increase of the MAP over the whole cold pressor test measured by CNAP amounts 1.66%, which is equal to an increase of 1.8 mmHg.

Table 7.3. Mean arterial pressure measured by CNAP and digital blood pressure device at baseline and during cold pressor test

Parameter	**Baseline**	**20-30 s**	**ΔBaseline 20-30 s**	**120-130 s**	**ΔBaseline 120-130 s**
MAP (CNAP)	102.8	99	-3.8	108.03	5.23
MAP (automatic blood pressure measurement)	91.8	101	9.2	103.2	11.4

MAP, mean arterial pressure. Values are shown in mmHg.

7.5 HEART RATE

In figure 7.10, the change of the heart rate is shown. The average rise in heart rate amounts 3.7 BPM, from 82.5 BPM to 86.3 BPM during the cold pressor test. The maximum increase of the heart rate appeared at the start of the cold pressor test with an increase of 5.44%. After the maximum occurred, the heart rate decreased and is relatively constant at a new setpoint of about 86 BPM.

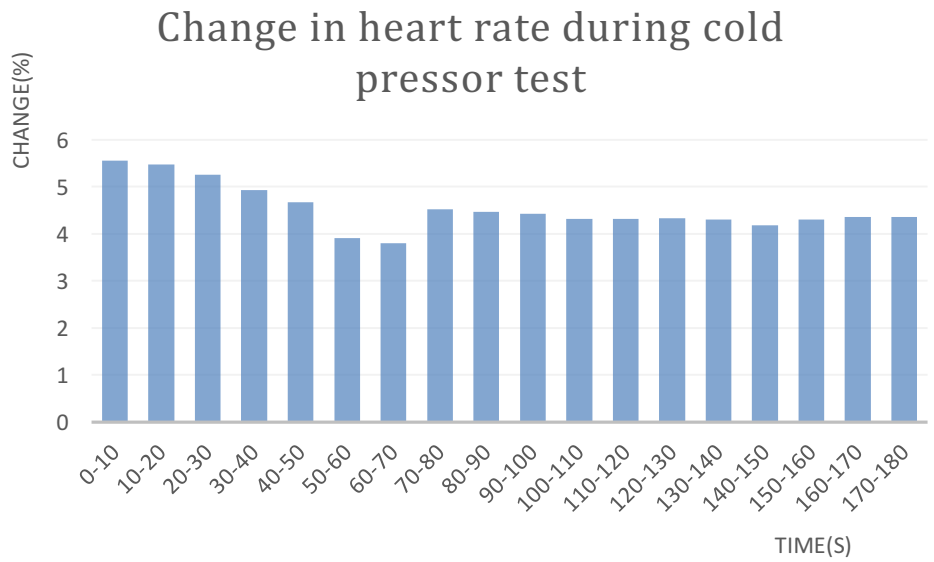

Figure 7.10. Change in heart rate during cold pressor test compared to baseline. The maximum increase occurs initially with an increase of 5.44%.

7.6 $ETCO_2$ AND RESPIRATORY RATE

The increases in breathing frequency and $EtCO_2$ are displayed in table X6. It is noticeable that the respiratory rate of subject 2 increases with 13.6% which was about 21 breaths per minute during the cold pressor test. The $EtCO_2$ of this subject decreased with 3.49%.

Table 7.4. Change in respiratory rate and $EtCO_2$

Subject	**ΔRR**	**Δ$EtCO_2$**
1	1.90 %	-2.27 %
2	13.6 %	-3.49 %
3	-4.18 %	-1.51 %

RR, respiratory rate; $EtCO_2$, end tidal CO_2. Changes are compared to baseline.

7.7 CVRI

The CVRi, measured with values of the CNAP and automatic blood pressure monitor, are shown in table 7.1. The CVRi by using CNAP blood pressure values increase slightly during the cold pressor test, with 1.66%. In contrast, the CVRi the using automatic blood pressure values increased during the cold pressor test with 8.00%.

7.8 CASE

The results of the case are separately discussed. The figures and tables in which these results are displayed, can be seen in appendix D. In contrast to the subjects, the mean arterial pressure, the CBFV in the left and right MCA and the CVRi (CNAP) were not increased during the cold pressor test. This is shown in table E.1.

7.8.1 PARAMETERS OF THE CAROTID ARTERY

The average diameter of the carotid artery does not change during the cold pressor test. The maximum decrease was 1.74% at 80 seconds and the maximum increase was 1.70% at 180 seconds. The average velocity in the carotid artery of the case increases with 9.00%. At 90 seconds after the test, there is a maximum decrease of 17.9% and at 130 seconds there is a maximum increase of 22.80%. The flow in the carotid artery increases with an average of 9.19% during the test. There is a maximal decrease of 19.7% at 90 seconds after the test as well, which occurred at the same time as the velocity minimum. The maximal increase occurs at 120-130 s with an increase of 24.3%

7.8.2 PARAMETERS OF THE MIDDLE CEREBRAL ARTERY

In contradiction to the increase of the CBFV in the MCA of the subjects, the CBFV of the case decreases with an average of 3.15% at the left MCA and 7.78% at the right MCA. This equals an average decrease in CBFV of 5.47%. There is a minimum of -13.8% at 160 s after the test.

7.8.3 PARAMETERS OF THE MICROVASCULATURE

The concentration oxy-Hb in the frontal cortex of the case increase during the cold pressor test. The average increases of oxy-Hb during the cold pressor test were 2.42 µmol/L and 1.15 µmol/L, for the left and right frontal cortex, respectively. This equals an average increase in oxy-Hb in the frontal cortex was 1.79 µmol/L. The average change in deoxy-Hb was -0.156 µmol/L for the left frontal cortex and 0.0620 µmol/L for the right frontal cortex during the cold pressor test. The

maximum increase was, just like the measurement of the subjects, at 130 seconds after the start of the cold pressor test with a maximum of 4.06 µmol/L on the left side and 2.43 µmol/L on the right side. After the maximum, the concentration slightly decreases. In contrast to the subjects, the case shows an increase of deoxy-Hb during the cold pressor test at the right side, though a decrease at the left side occurred.

7.8.4 MEAN ARTERIAL PRESSURE

The mean arterial pressure of the case, measured with CNAP, decreases with an average of -8.41% during the cold pressor test. The maximal decrease during the test is -25.8%, and occurs at 20-30 s after the start of the cold pressor test. After about 100 seconds, the MAP is recovered. The automatic blood pressure measurements showed an average increase of 13.2% in MAP.

7.8.5 HEART RATE

The heart rate of the case increased from 81.9 to 83.2 BPM during the cold pressor test, which is an increase of 1.60%. Just after the start of the cold pressor test, there is a maximum of 2.12%. After the maximum, the heart rate decreased to the baseline value in approximately 60 seconds.

7.8.6 $ETCO_2$ AND RESPIRATORY RATE

The $EtCO_2$ decreases with -14.8% where the subjects showed smaller decreases. This decrease in $EtCO_2$ is accompanied by a high increase in respiratory rate, compared to the subjects. The respiratory rate increased with 29.7%, which corresponded with a respiratory rate of about 26 breaths per minute during the cold pressor test.

7.8.7 CVRI

The CVRi of the case decreases with 1.36% when using the CNAP measurement. The CVRi in combination with the automatic blood pressure values increases during the cold pressor test with 19.95%.

8 CONCLUSION

8.1 SUBJECTS

The flow in the carotid artery, middle cerebral artery and the cerebral microvasculature as a consequence of the cold pressor test were observed. As expected, it was found that the flow in the left carotid artery (+5.34%) and the CBFV in the MCA (+6.36%) increased during the cold pressor test. Besides, the flow in the cerebral microvasculature of the frontal cortex increased with an increase in oxygenated hemoglobin of 1.32 μmol/L.

Contrary to the hypothesis, the diameter in the left carotid artery did not change much on average during the cold pressor test (+0.188%). The increased flow was mainly caused by an increase of the blood velocity in the carotid artery (+5.35%). The spatial resolution of the TOF sequence was too low to accurately estimate changes in the MCA during the cold pressor test. In the left and right MCA an increase in CBFV of respectively 7.04% and 5.68% was found. Oxy-Hb increased by 1.67 μmol/L in the left cortex and 0.961 μmol/L in the right cortex. Deoxy-Hb decreased by respectively 0.331 μmol/L and 0.291 μmol/L. The increase in oxy-Hb and decrease in deoxy-Hb demonstrated an increase in flow. The findings of a bigger increase in CBFV and flow in respectively the left MCA and left cortical microvasculature did not correspond with the hypothesis. ASL was used to complement flow in the cerebral microvasculature estimated by NIRS, but the SNR was too low to make statements.

There seemed to be a clear correlation in the time response of the flow in the MCA and the cerebral vasculature with a peak flow at 120-130 seconds after the initiation of the cold pressor test. It was not possible to distinguish a relationship between the flow in the carotid artery and higher observed levels.

The MAP measured by CNAP increased with 1.8 mmHg. It was found that the CNAP measurements provided possibly incorrect values for the arterial blood pressure. This conclusion was made because of contradictory information found during the literature study and additional measurements. The additional measurements showed that the MAP increased with 10.3 mmHg which came closer to the expected value. In addition to this blood pressure elevation, an unknown mechanism probably apart from MSNA activation or baroreflex inhibition initiated an initial rise in heart rate (+3.7 BPM). This increase seemed to be partly compensated by the baroreflex control during the cold pressor test. The eventual cerebral flow alterations occurred, because dynamic cerebral autoregulation does not compensate entirely for transient elevations in blood pressure and cardiac output. The CVRi obtained by the automatic blood pressure measurements increased with 8.00%.

8.2 CASE

In the case subject, the flow in the carotid artery increased (+9.19%) and the CBFV in the MCA decreased (-5.47%). The flow in the cerebral microvasculature of the frontal cortex increased with an increase in oxygenated hemoglobin of 1.79 μmol/L. The observed values were expected to be confounded by a decrease in $PaCO_2$ due to hyperventilation that occurred during the cold pressor test. The detected decrease in the CBFV corresponds to the expectation. However, the increase in flow in the carotid artery and cerebral microvasculature do not. This could not be declared.

9 DISCUSSION

The described study was performed to gain more insights in the cerebral blood flow at different levels as a result of the cold pressor test. As expected, the flow at different levels in the cerebral perfusion increased and the cold pressor test showed to be a test that has a great impact on the hemodynamics of healthy subjects.

9.1 CAROTID ARTERY

One subject was excluded from the velocity data due to incorrect measurements. Although the diameter measurement of the subject was correct, the measurement was also excluded in order to make a correct comparison. The diameter of the carotid artery including this subject is included in appendix E. The dilatation of the carotid artery did not correspond with the hypothesis, which was based on the results described in literature. Rubenfire et al. reported an increased diameter of the carotid artery with a maximum of 7.9+3.3% after 90 s which retained for three minutes [3]. The maximum dilatation in this study appeared at 40-50 s and was 1.14%, which decreased afterwards. When the diameter measurement of the excluded subject is included, a maximal increase of 2.97% at 110-120 s was found.

The cold pressor test has a significant influence on the velocity in the carotid artery, but notable is that this mainly occurs at 150-160 s, with a big maximal increase (+20.5%). Due to the little changes in diameter, the velocity mostly contributed to the alterations that were observed in flow. The flow measurements show that the small decrease in diameter that occurs after 120 s is not responsible for the increase in velocity. It is remarkable that the flow in the carotid artery shows relatively little changes in the interval 0-120 s compared to the maximum increase. Regarding the velocity measurements, there should be noted that the measurements may not be completely reliable because of the exertion for the examiner to manually perform the measurements.

In the case, the flow in the carotid artery increased in a greater extent than in the subjects. The $PaCO_2$ is known to decrease the flow in the carotid artery, so the finding is contradicts the expectation. A different individual response might declare this response. Besides, a possibly incorrect velocity measurement may play a role.

9.2 MIDDLE CEREBRAL ARTERY

The diameter of the MCA, which is provided by the TOF sequence, is limited by the resolution of the image. One voxel equals 0.651mm and the diameter of the MCA equals 3-5 voxels in all subjects. A difference in terms of one voxel suggests a change in the diameter of the MCA of 20%, which is not in the range of the expected changes of the MCA. Also, the TOF measurement is unreliable because a small movement of the subject or the manual estimation that is performed can cause such a difference. Because of the inadequate diameter measurements, there should be argued about the diameter response of the MCA. As stated earlier, the diameter of the MCA does not change significantly for blood pressure alterations of less than about 30 mmHg, but this may only be an indication due to the different character of the study [31]. Therefore, there can not be concluded, and only speculated what the change of the diameter might be in response to the cold pressor test. Under a constant diameter of the MCA, the CBFV is proportional to the flow. Assuming that the larger increase in the left MCA corresponds with a larger increase in flow, this

did not correspond with the hypothesis. This was also found in the cerebral microvasculature and is discussed in that section.

The case was excluded from normal statements due to a decrease in $PaCO_2$, which is known to decrease CBFV in the MCA. The CBFV in the MCA did decrease in a large extent compared to the subjects. The difference between $EtCO_2$ before and during the cold pressor test was 0.85%. When scaled to mmHg, a decrease of 760*0.0085=6.46 mmHg $PaCO_2$ was found (assuming a 1:1 linear relationship between $EtCO_2$ and $PaCO_2$). According to findings of Willie et al., this should match with a 20% decrease in CBFV when a linear correlation of the CBFV between 0 and 10 mmHg is assumed [16]. The case showed a difference of about 27.5% in CBFV compared to the subjects, which is in the range of 20% based on the calculation. Therefore, it is realistic that both the increasing effect of the cold pressor test and the decreasing effect of the hyperventilation together resulted in the values of the CBFV as observed.

9.3 MICROVASCULATURE OF THE FRONTAL CORTEX

There are three components which attribute to the NIRS signal: changes in cerebral flow in the frontal lobe, in skin perfusion and in cognitive activation. It is not known whether the skin blood flow is changed during the cold pressor test or not. Therefore, it is not certain whether the alterations in NIRS signal are a consequence of a change in CBF. Also, the NIRS optodes could have been moved during the measurements as a consequence of wrinkling what might affect the results by measuring other regions. The risk was minimized by tightly securing the helmet and optodes. Also, signal drifting can occur which makes the signal of NIRS over the time more unreliable compared to the initial setpoint. It is assumed that the metabolic rate (e.g. by cognitive activation) during the measurements is constant. If this is not constant, the results of NIRS are affected, because the increase of oxy-Hb does not happen due to the increase in cerebral blood flow. Because NIRS detects alterations compared to a selected setpoint, it was not possible to calculate changes in percentage the cold pressor test causes. However, the results showed a clear increase in oxy-Hb compared to that setpoint. The peak in the NIRS signal corresponded with the maximum peak in the TCD signal, at 120-130 seconds, which suggests that the increase found in the NIRS signal is highly contributed by an increase in cerebral blood flow in the MCA.

Another outcome of the NIRS measurements was that oxy-Hb and deoxy-Hb showed a bigger response ipsilateral than contralateral compared to the submerged left hand. Therefore, it is assumed that the flow in the microvasculature of the ipsilateral cortex increased more. In addition to the bigger increase in the TCD signal, this contradicts the hypothesis that the flow increases more in the contralateral cortex relative to the submerged hand. It is questionable whether activation of the motor cortex is detectable in the TCD and NIRS signal as an increase in cerebral blood flow and no cognitive activation. A reason for the greater increase in the ipsilateral signal is unknown.

Although it is possible to perform the cold pressor test in an MRI scanner, the results of the ASL sequence are inconclusive. The difference images between the baseline and the cold pressor test showed moving artefacts in two subjects, despite correction. Besides, in general the SNR was too low to correctly interpret the results as well because of the short single TI sequence. Also, no correction was made for the extra water in the magnetic field which may have contributed to this.

In the case, a bigger increase in oxy-Hb and lesser decrease in deoxy-Hb was observed, which is contradictory with the expected vasoconstriction because of the lower $PaCO_2$. A possible reason for this finding is unknown. Possibly, there was a complex interaction of both the constrictor effects of the decreased $PaCO_2$ and the physiological mechanisms of the cold pressor test.

9.4 Correlation between the different levels

The flow in the carotid artery did not show a clear time correlation in comparison to both the TCD and NIRS signals. A clear increase before 120-130 s is detected for the CBFV in the MCA and the flow in the microvasculature. In contrast, great a peak value in the flow of the carotid artery was found at 150-160 s, which is not seen at the level of the MCA or microcirculation. The outcome did not correspond with the hypothesis and is not well-understood. As stated, the velocity measurements may have been unreliable. When taking the excluded subject in consideration, it is remarkably that the diameter apart did show a corresponding peak with the TCD and NIRS signals. Also, the exclusion of the subject in the flow measurements of the carotid artery may have been of influence since the data of two subjects from the carotid artery was compared to the data of three subjects for higher levels.

A different peak in the flow in the carotid artery and the flow in both the MCA (assuming a constant diameter) and the flow in microvasculature can also be explained by different vasoreactivity of the cerebral arteries. The vertebral artery might have contributed differently to the cerebral flow than the carotid artery, but this artery does not contribute much to the CBFV in the MCA. Also, other cerebral arteries than the MCA may have shown different reactions in terms of constriction or dilatation. There can also be the hypothesis that the diameter of the MCA itself contributed to the change in CBFV. Complex vasoreactivity may play a role in regulating the flow in the cerebral vessels, since small flow alterations in the carotid artery correspond with a high increase in CBFV, while bigger flow alterations do not. However, the flow in the microvasculature increased, so it is suspected that not only constriction of the MCA contributed to the increase in CBFV.

The case did not confirm the hypothesis of the time correlation between the three different levels. The flow in the carotid artery increased while the CBFV in the MCA decreased and the flow in the microvasculature increased. Apart from the CBFV in the MCA, this does not correspond in contrast to what is expected based on a decreased $PaCO_2$. Also, no clear time response can be distinguished between the signals. A reason for this observations, associated with a decreased $PaCO_2$ and sympathetic activation by the cold pressor test, is unknown.

9.5 $EtCO_2$

As stated, the $EtCO_2$ of the case decreased during the whole cold pressor test due to hyperventilation. An increase of 29.7% in respiratory rate was detected, with an average respiratory rate of 26 per minute during the cold pressor test, which is quantified as hyperventilation. Hypothesized is that within this subject, the cold pressor test was the trigger of the hyperventilation which caused the changes in $PaCO_2$. Due to hyperventilation and the influence on the CBF, the results are taken apart from the measurements because of this other physiological response that is provoked. It could be stated that this is a different kind of reaction to the cold pressor test that has a bigger prevalence in the population than expected based on other researches [6, 7]. Noted should be, that besides the case, one other subject showed a slight increase in respiratory rate. The $EtCO_2$ however, differed not much, so it was not necessary to exclude that subject.

9.6 BLOOD PRESSURE, HEART RATE AND CEREBRAL VASCULAR RESISTANCE INDEX

A remarkable outcome of the study is that the CNAP measurements showed no great increase in mean arterial pressure during the cold pressor test. These measurements were contradictory with results obtained in literature. In the initial response of the cold pressor test a decrease in blood pressure was found in all three subjects which after 50 seconds was followed by an elevation compared to baseline. The drop in blood pressure went along with a strong initial rise in heart rate. When the heart rate partly recovered, the blood pressure rose. A possible reason for this remarkable observation is that the CNAP measures the blood pressure through a finger cuff. A pressure in the cuff is applied, which corresponds with the intra-arterial pressure. The intra-arterial pressure in the finger represents a peripheral pressure, which is scaled through a calibration to the systemic pressure. When the cold pressor test has a specific effect on peripheral arteries, the measurement might not represent the systemic pressure correctly. However, Parati et al. concluded that a FINAPRES device, a device that works by a similar mechanism, gave accurate values that corresponded with intra-arterial measurements during the cold pressor test [54]. The changes detected by the automatic blood pressure monitor during the cold pressor test came closer to the expected values: an increase of 11.9% or 10.3 mmHg. There should be noted that the measurements by automatic blood pressure were primarily made for exploration. It is assumed that the reactions of both the experiments can be equivalent, but it is not certain whether the circumstances of experiment 1 and 3 were exactly the same.

There was seen a strong initial rise in heart rate, that has to do with sympathetic activation of which the exact mechanism is found to be unknown in literature [28]. The decrease in heart rate compared to the initial increase that occurred during the cold pressor test is believed to be because of the partial recovery that the baroreflex causes. Remarkably, one subject showed a substantially constant heart rate of 62 BPM during the cold pressor test, where other subjects showed variation. This constant heart rate is believed to be because of a junctional escape rhythm that was provoked by the cold pressor test. This is shown in appendix F.

In the case, a lesser transient increase in heart rate was found, that was probably completely compensated by the baroreflex. Expected would be that hyperventilation causes an increased heart rate, so this is a curious finding which may be due to a different individual response.

The CVRi, which is an index for the resistance because a correct value for the flow could not be determined, increased during the cold pressor test. Both MAP values measured by the CNAP as values measured by the automatic measurements are used to calculate the resistance indices, while the CNAP measurements are being questioned. The CVRi derived out of the CNAP values gave a slight increase. The increase in MAP combined with the small increase CVRi, assuming a corresponding increase in resistance, should be responsible for the increase in CBFV. In addition of the many studies that reported an increase in resistance, this would be an abnormal finding because the cold pressor test is known to cause sympathetic activation and increase norepinephrine. These mediators are known for their vasoconstrictor effects and therefore it is very unlikely that the cold pressor test increases the flow by means of vasodilatation. It is suggested that an increase in blood pressure was the reason of the flow alterations.

There is an increased CVRi found with the automatic blood pressure measurements. This resistance does not fully compensate for the blood pressure alteration because there is still found an increase in flow in all the measured levels. This is typical for transient changes in blood pressure, regulated by dynamic cerebral autoregulation so this response is as would be expected. The case showed a higher increase in CVRi than the subjects. This is due to the big decrease in CBFV and a similar increase in blood pressure. There should be noted that the

resistance indices were calculated using the velocity measurements that corresponded with the moment the automatic blood pressure values were obtained. The velocity measurements of experiment 1 were used, assuming a similar reaction in both the experiments. This CVRi is specific for the intervals that the measurements were taken and it does not take fluctuations between the intervals into account.

9.7 STUDY LIMITATIONS

Three experiments were performed, all using 4 subjects. The goal of the study was to gain global insights in the physiology of the cold pressor test. Therefore the outcomes may not be generalized over a population due to the low number of subjects. The cold pressor test evokes different responses in different subjects, as was seen within the results.

From the TOF measurements, no correct values for the diameter of the MCA could be derived. Therefore, there could only be suggested about what happens with the flow in the MCA.

Due to an incorrect velocity measurement of the carotid artery statements regarding flow in the carotid artery regarded two subjects where in the other levels data of three subjects was used.

The reproducibility of the experiments may be of influence. Although it is stated in literature that the response of the cold pressor test within persons does not vary much, this may have been of influence [1, 2]. This may have been of influence, because the outcomes of different experiments with different environments were compared. Also, there were different conditions for the subjects. Relevant for the reproducibility, but also for the measurements between subjects was that the hand was not always totally submerged until the wrist, which might was of influence.

The response of the vertebral artery is not measured in this study. The majority of the cerebral blood supply takes place via the carotid artery, but the response of the vertebral artery is reported to be different in conditions of a decreased $PaCO_2$ compared to other arteries [16]. The vertebral artery may have contributed to the CBFV in the MCA and flow in the microvasculature.

10 RECOMMENDATIONS

As a result of this thesis, these recommendations are established that should be considered in further research regarding the cold pressor test and cerebral hemodynamics.

Because there is a variety in responses between different subjects to the cold pressor test, there should be measured with an increased amount of subjects to determine the average effect of the cold pressor test on the flow more accurately. Then statistical analysis could be performed to determine whether the alterations that are observed in this thesis are statistically significant.

In order to determine the diameter of the MCA more accurately, it is desirable to image the vessel with a higher spatial resolution. Besides, determining the cross sectional area could give a smaller error instead of determining the diameter, based on a 2-dimensional image.

The NIRS used in this thesis detects alterations of the concentration oxy-Hb and deoxy-Hb compared to a selected setpoint, which makes it impossible to detect relative changes in percentage. It is recommended to measure absolute values by NIRS.

There was seen a low SNR in the ASL images, which should be improved to make statements about the cerebral blood flow. This could be improved when there is a lower acquisition time per image, so there can be made more images within the same time period. Another issue that arises is movement artefacts. These will need an adequate correction during analysis. Additional bias field corrections are needed as the container of ice water interrupts with the magnetic field of the MRI.

The CNAP values during the cold pressor test need to be validated with additional research.

More research is necessary to find out whether the cold pressor test might induces hyperventilation and therefore causes a decrease in $EtCO_2$.

Although the majority of the cerebral blood supply takes place via the carotid artery, there could be a specific role for the vertebral artery in the cerebral blood supply during the cold pressor test. Therefore measuring this artery could provide extra information in how the cold pressor test effects the cerebral hemodynamics.

Therefore, there should be done more experimental research to complete the knowledge about the exact mechanism. Finally, the recovery after the cold pressor test might be interestingly to investigate. It might give new insights in the responses of the cold pressor test.

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APPENDIX A: SOP CEREBRAL BLOOD FLOW MEASUREMENTS

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Introduction

This standard operating procedure (SOP) will be used as a guideline to acquire data. This data will be used to answer the research question: *"What is the effect of the cold pressor test on the blood flow in the carotid artery, middle cerebral artery and cerebral microcirculation in healthy subjects?"*

Inclusion criteria

- 24 hours before exam no medicines, no alcohol, no caffeine [19].
- BMI below 30
- Age between 18 and 30
- Blood pressure at rest <140/90 mmHg
- Non smoker

Requisites

- Examination table
- OxyMon MkIII (NIRS)
- DWL Multidop 4x device with accessories
- Ultrasound system with Duplex Doppler
- Isolated container with ice-water
- Thermometer
- ECG
    - 3-leads ECG
    - 3 ECG electrodes
- CNAP (Finapres)
- $EtCO_2$-meter
- Towel (2x)
- Razor

Method

Preparation

- Ensure a constant temperature at the control room.
- Close curtains, prepare the examination table, lay materials ready, boot computers and programs that are used.
- Put the container with ice-water on a small platform at half the height of the examination table on the left side of the subject.
- Inform the subject about the protocol and answer questions. Obtain informed consent.
- Instruct the subject to take off his or her shoes and to keep wearing the socks. The subject may have to take off his or her shirt in order to place the ECG-electrodes. During the measurements a shirt may be worn.
- Instruct the subject to lie in a supine position on the examination table.
- Shave at the positions of the electrodes, if necessary. Use soap and water on a mesh to clean the skin. Connect the ECG-leads to the electrodes. Place the electrodes as in the figure below, by pressing the surrounding part of the electrodes. Do not press the centre

of the electrode, because you can create a bubble. Start the acknowledge measurement and check the ECG signal.

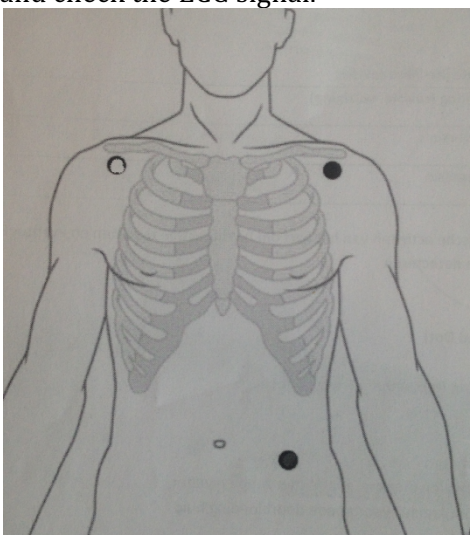

Figure 1. location of ECG-electrodes

- Place the Et-CO_2 meter around the neck.
- Fixate the helmet on the subject's head, including TCD probes and NIRS optodes at left and right side. Leave a gap of 2 centimeters between the headband and the eyebrow. The helmet must be tight, but it may not pinch the forehead. Determine the correct probe and optode location.
- Place the NIRS optodes in the openings nearest to the ears and fixate them. Prepare the software for the measurements according to the document 'SOP NIRS' and check the quality of the signal.
- Place the CNAP controller over the subject's right wrist. Place the CNAP cuff on the index- and middle-finger proximal the second phalanx. Fixate the right wrist at heart level. Perform the CNAP measurement and check for correctness.
- Place and fixate the tube of de $EtCO_2$-meter inside the nose. Perform $EtCO_2$ measurement and check for correctness.
- Locate the middle cerebral artery on both sides by using Doppler function. Place the Doppler probes anterior to the ear, about 1 cm above the eye-ear axis. Identificate the middle cerebral artery by taking its characteristics into account. More information about the usage and settings of the DWL Multidop can be found in the document 'SOP TCD'.
- Place the thermometer in the container of ice-water to ensure a constant temperature throughout the measurement.
- Instruct the subject to hold his or her head slightly in hyperextension. Use a pillow for comfort to support the subject's neck. Check whether the TCD and NIRS signals are correct or not.
- Instruct the subject to (during the measurements):
    - do not talk.
    - stay in position and move minimally.
    - prevent the Valsalva maneuver.
    - breath normally and do not hyperventilate.
    - avoid swallowing or coughing.
    - do not close the eyes for a longer period of time.
    - breath through the nose.
- Due to the non-talking, agree signs to communicate with the subject. Firstly, let the subject blink once for 'yes' and twice for 'no' in response to a question of the executor. Secondly, let the subject know, he or she must curl the toes of both feet repetitious in case something is wrong.

- Locate the carotid artery using duplex mode of the ultrasound device and hold the probe at an angle of 45 degrees relative to the artery. Check during the measurement whether the artery is being displayed or not.
- During the measurements, make sure to ask the subject every minute how he or she is doing. Be aware of signs that might indicate that something is wrong.
- Be sure the subject is lying quietly for at least 2 minutes before starting the measurements.

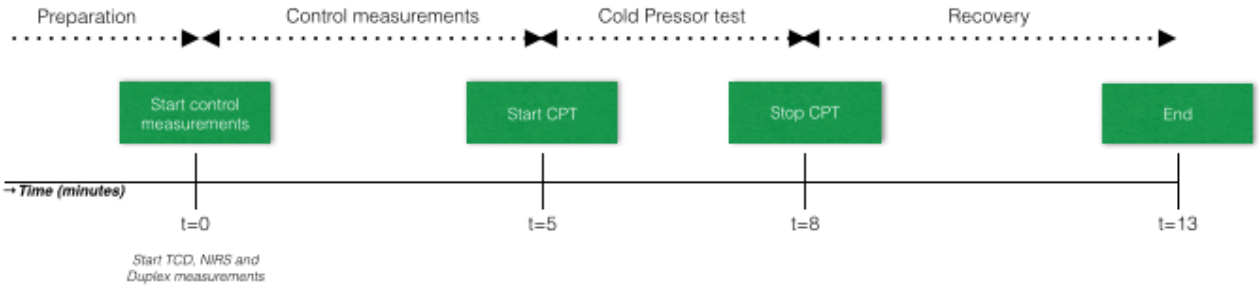

Figure 2. Timeline measurements

Measurements

The measurements will take 13 minutes. This is displayed by the timeline above. Time of interest starts at t = 0 and ends at t = 13. Highlight the events as below in the dataset.
Perform the measurements as followed:

- Mark the start of the TCD, NIRS and duplex measurements to create t=0.
  - TCD, NIRS and duplex should be set to acquire the data continuously
- Start the cold pressor test at t = 5.
- The executor should place the left hand and arm of the subject in the container which contains ice-water. This should be executed smoothly. The hand should be placed under water for about 5 centimeter proximal of the wrist.
  - The subject must stay in this position for 3 minutes.

  To ensure a minimum of artefacts, practice this beforehand.
- Stop the cold pressor at t = 8.
  - The subject should remove his or her arm from the container and place the arm on a towel next to the body.
- Stop the TCD, NIRS and duplex measurements at t = 13. This marks the end of the measurements.

APPENDIX B: SOP MRI

Document information:

Authors: Jasper ten Dam, Lieke Numan, Mark Scheeren, Babette van de Werff
Date: 28-05-2015

Introduction

This standard operating procedure (SOP) will be used as a guideline to acquire data. This data will be used to answer the research question: *"What is the effect of the cold pressor test on the blood flow in the carotid artery, middle cerebral artery and cerebral microcirculation in healthy subjects?"*

Inclusion criteria

- 24 hours before exam no medicines, no alcohol, no caffeine [19].
- BMI below 30
- Age between 18 and 30
- Blood pressure at rest <140/90 mmHg
- Non smoker

Requisites

- MRI device
- 'MRI controlelijst'
- Isolated container with ice-water
- Thermometer

Method

Preparation

- Ensure a constant temperature in the examination room.
- Inform the subject about the protocol and answer questions. Obtain informed consent.
- Let the subject fill in the 'MRI controlelijst'.
- Make sure the subject does not carry any metal.
- Let the subject lay down in supine position in the MRI device.
- Secure the subject.
- Check and note the temperature of the ice-water
- Make sure that the subject can put his/her left hand in the isolated container with ice-water.
- Be sure the subject is lying quietly for at least 2 minutes before starting the measurements.

Measurement

The measurements will take about 8 minutes. This is displayed in figure 1 below. Time of interest starts at t = 0 and ends at t ≈ 7:30. Highlight the events as below in the dataset.
Perform the measurements as followed:

- Talk to the subject during the measurements. Inform the subject about when to start and stop the cold pressor test.
- Start the first ASL and TOF sequences.
- Start the second ASL measurement at t=0.
- Start the cold pressor test at t=1.
- Start the second TOF measurement direct after the ASL sequence.
- Start the third ASL sequence direct after the second TOF sequence.

- Stop the cold pressor test at t=4.
- Start the third TOF sequence direct after the third ASL sequence.

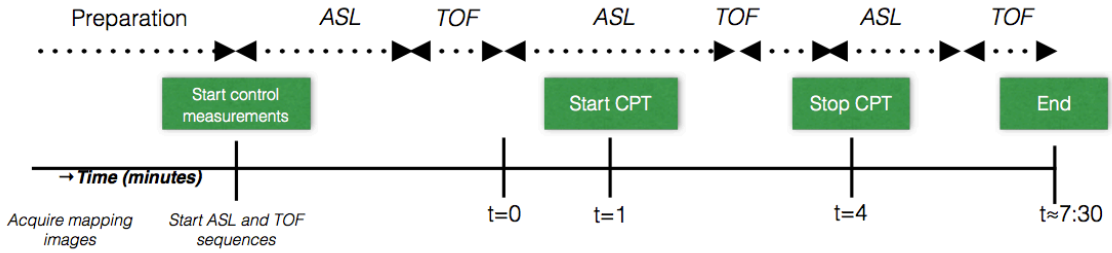

Figure 1. Timeline for the MRI measurements

APPENDIX C: EXAMPLES OF UNFILTERED DATA

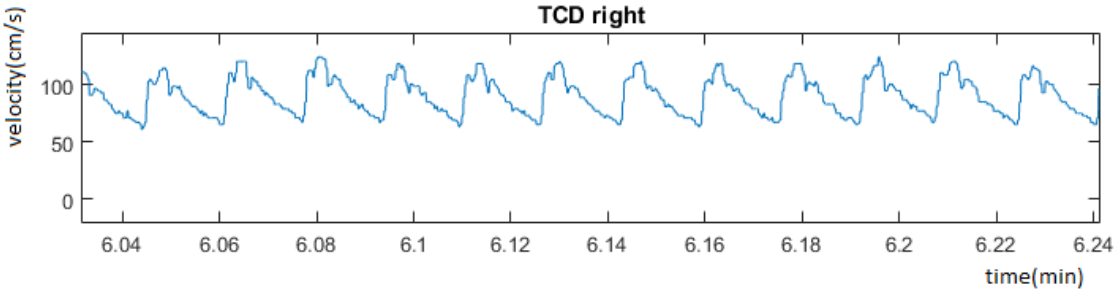

Figure C.1. Example of a representative cerebral blood flow velocity measurement in the middle cerebral artery during the cold pressor test

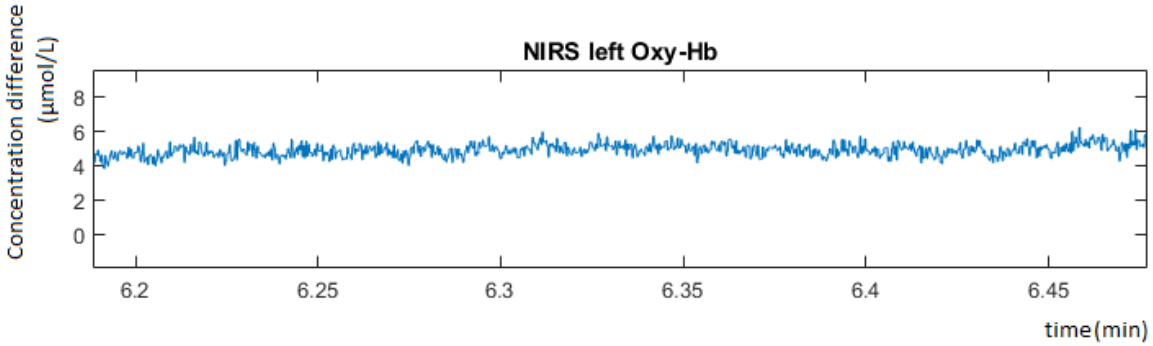

Figure C.2. Example of a representative oxy-Hb measurement of the left frontal lobe during the cold pressor test

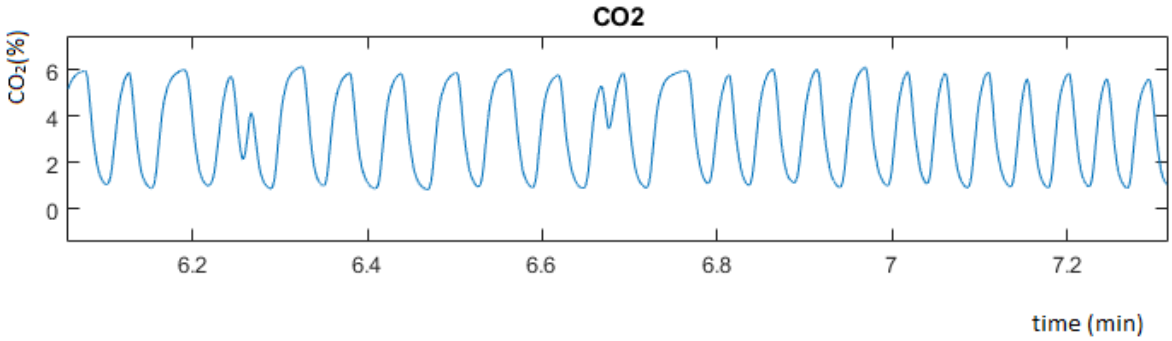

Figure C.3. Example of a representative CO_2 measurement during the cold pressor test

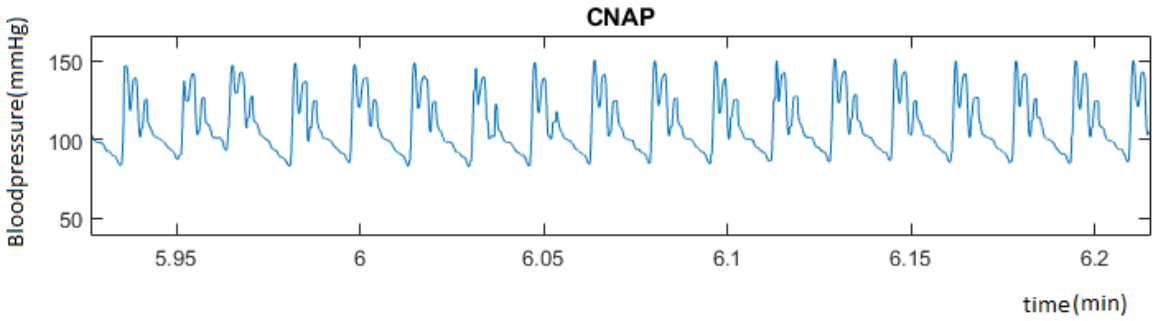

Figure C.4. Example of a representative mean arterial pressure measurement, measured by CNAP, during the cold pressor test.

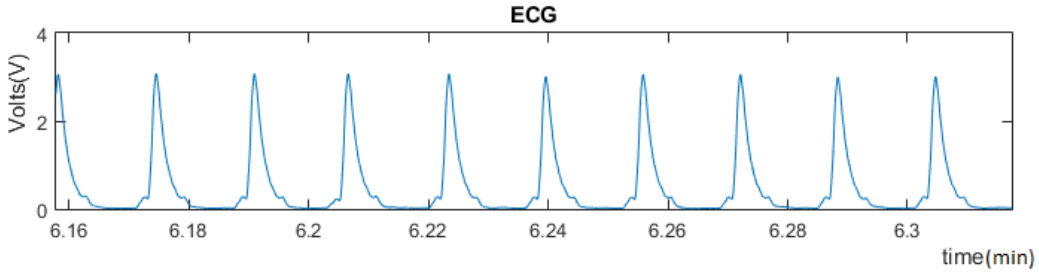

Figure C.5. Example of a representative ECG measurement during the cold pressor test.

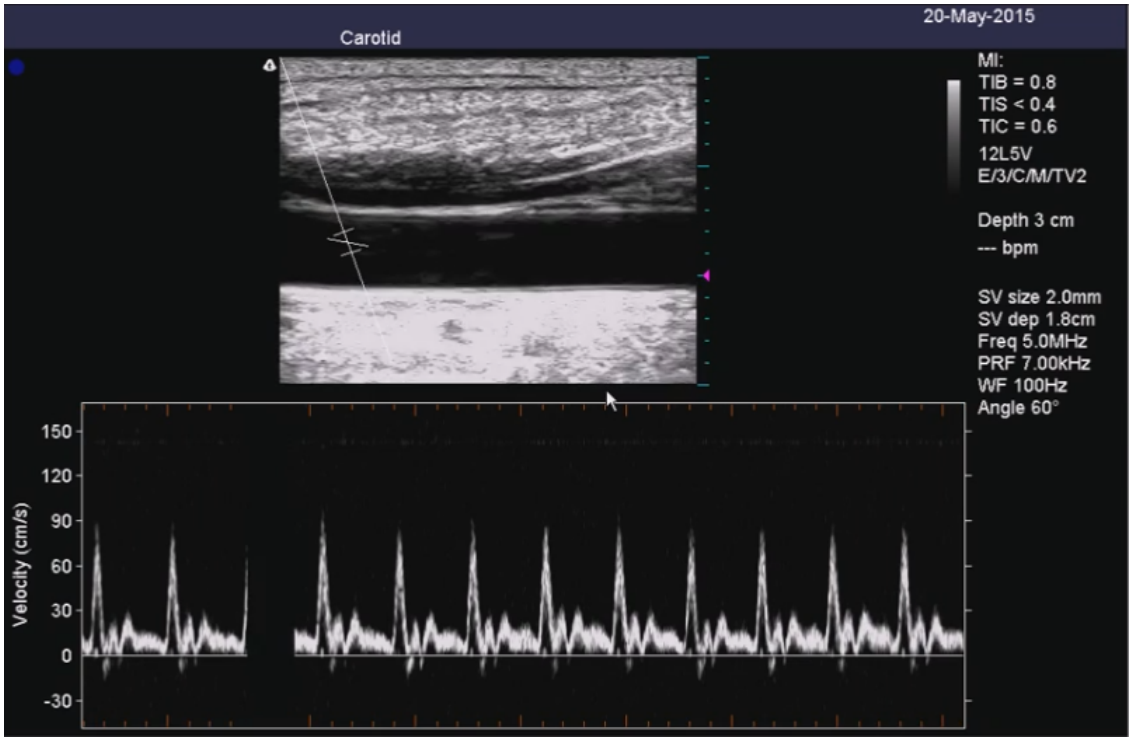

Figure C.6. Example of a representative example of the ultrasound velocity and diameter measurement of the carotid artery during the cold pressor test.

APPENDIX D: TABLES AND FIGURES OF THE CASE

Table D.1. List of the average change, baseline measurement and cold pressure test measurement of different parameters.

Parameter	Average change	Baseline	Cold pressor test
HR	1.60%	81.9	83.2
MAP (CNAP)	-8.41%	98.3	90.1
MAP (automatic blood pressure device)	13.2%	102	115.5
Diameter carotid artery	~0%	0.586	0.586
Velocity carotid artery	9.00%	26.3	28.6
Flow carotid artery	9.19%	7.17	7.83
CBFV left MCA	-3.15%	49.7	46.6
CBFV right MCA	-7.78%	63.8	58.9
Oxy-Hb left	2.42	-0.0268	2.39
Oxy-Hb right	1.15	0.939	2.09
Deoxy-Hb left	-0.156	0.243	-0.399
Deoxy-Hb right	0.0620	-0.0710	-0.00900
CVRi (CNAP)	-1.36%	1.72	1.71
CVRi (automatic blood pressure monitor)	19.95 %	1.80	2.16

Average change represents the averaged changes of the case during the cold pressor test compared to the baseline. Baseline and cold pressor test represent the absolute averages of the case during respectively the baseline and the cold pressor test. HR, heart rate (beats/min); MAP, mean arterial pressure (mmHg); diameter (cm); velocity (cm/s); flow (cm^3/s); CBFV, cerebral blood flow velocity (cm/s); oxy-Hb, oxygenated hemoglobin (µmol/L), deoxy-Hb, deoxygenated hemoglobin (µmol/L); CVRi, cerebrovascular resistance index (mmHg/(cm/s)). Changes are shown in percentage, except for oxy-Hb and deoxy-Hb which are shown as µmol/L.

Table D.2. Diameters of the middle cerebral artery of the case, both left and right, before and during the cold pressor test, determined by TOF angiography.

Subject	Diameter before CPT left (mm)	Diameter during CPT left (mm)	Diameter after CPT left(mm)	Diameter before CPT right (mm)	Diameter during CPT right (mm)	Diameter after CPT right (mm)
Case	2.76	3.26	2.76	3.26	2.35	3.26

MCA, middle cerebral artery; CPT, cold pressor test. Values are shown in mm.

Table D.3. Increase of the case in respiratory rate and $EtCO_2$ during the cold pressor test compared to the baseline.

Subject	Change respiratory rate	Change $EtCO_2$
Case	29.7 %	-14.8 %

RR, respiratory rate; $EtCO_2$, end tidal CO_2. Changes are compared to baseline.

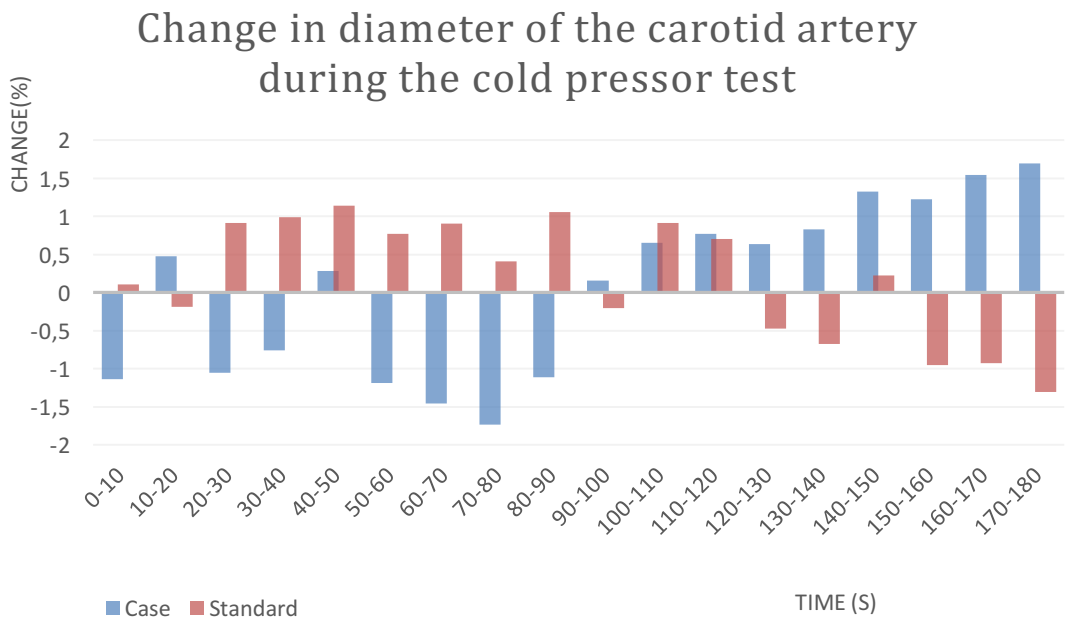

Figure D.1 Change in the diameter of the carotid artery during cold pressor test of the case compared to baseline. A maximum increase of 1.70% occurs at 170-180 s.

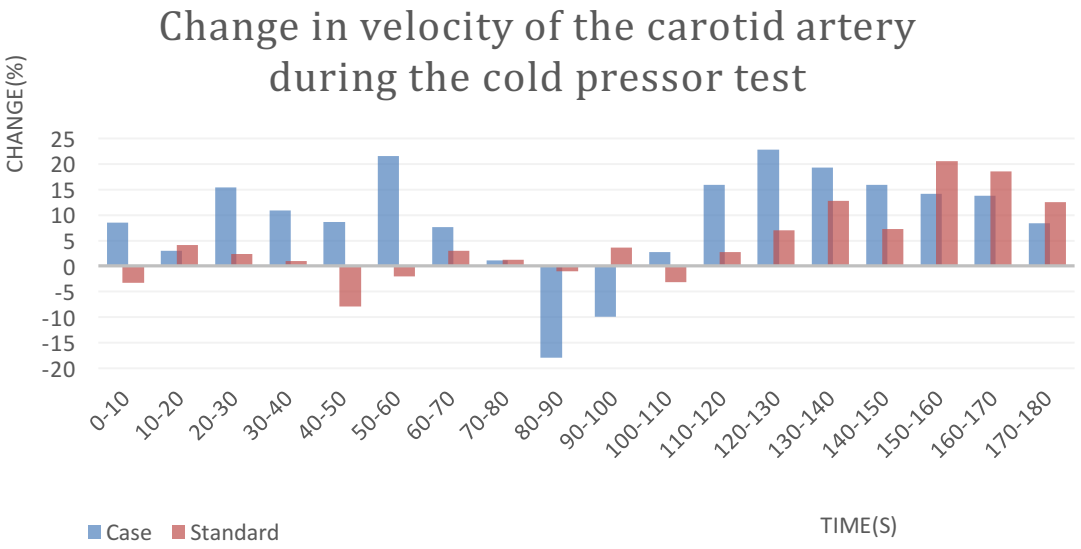

Figure D.2: Relative change of the velocity of the carotid artery of the case during cold pressor test compared to baseline. A maximum increase of 22.8% occurs at 120-130s.

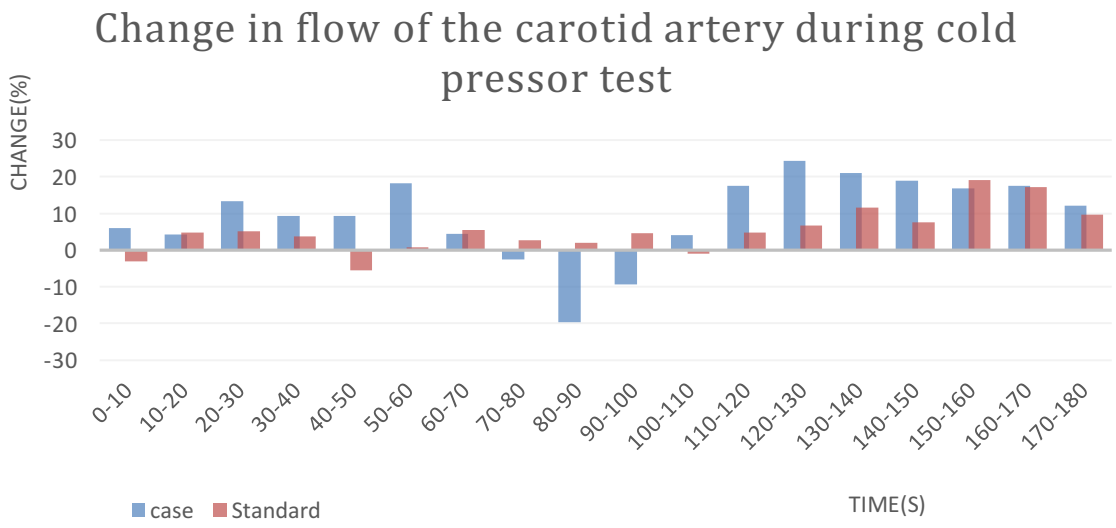

Figure D.3: Change of the flow in the carotid artery during cold pressor test compared to baseline. A maximum increase of 24.3% occurs at 120-130s and a maximum decrease of -19.7% at 80-90s.

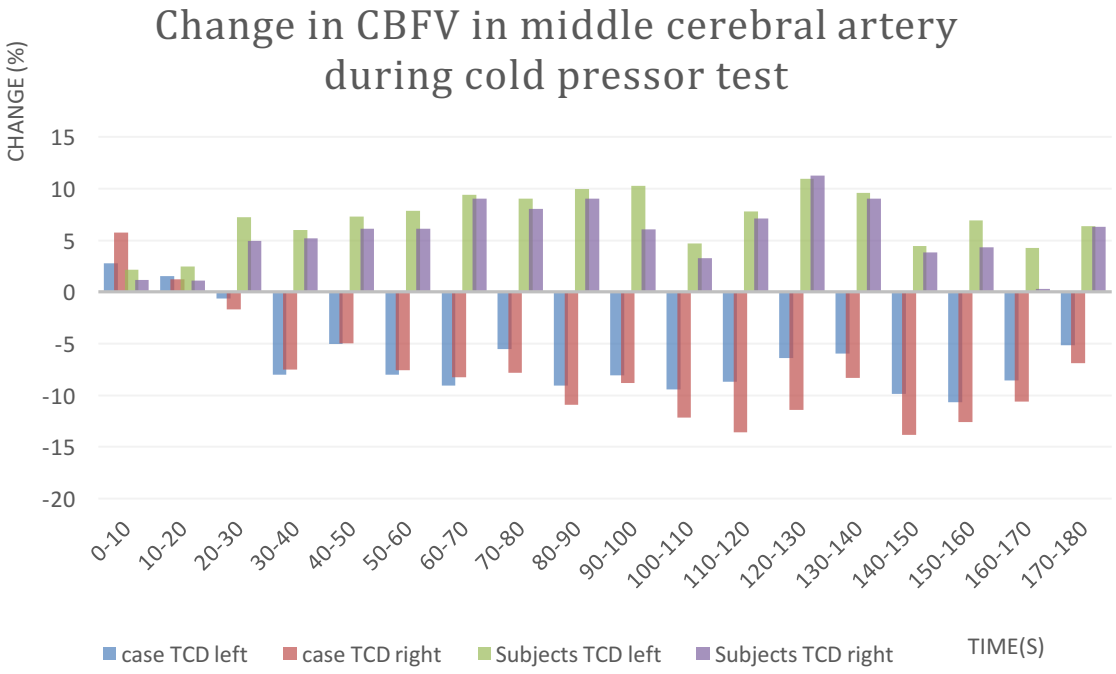

Figure D.4: Change of the CBFV in the middle cerebral artery during cold pressor test of the case. The maximum decrease on the right side occurs at 140-150 s with -13.8% and the maximum decrease on the left side occurs at 150-160 with -10.7.

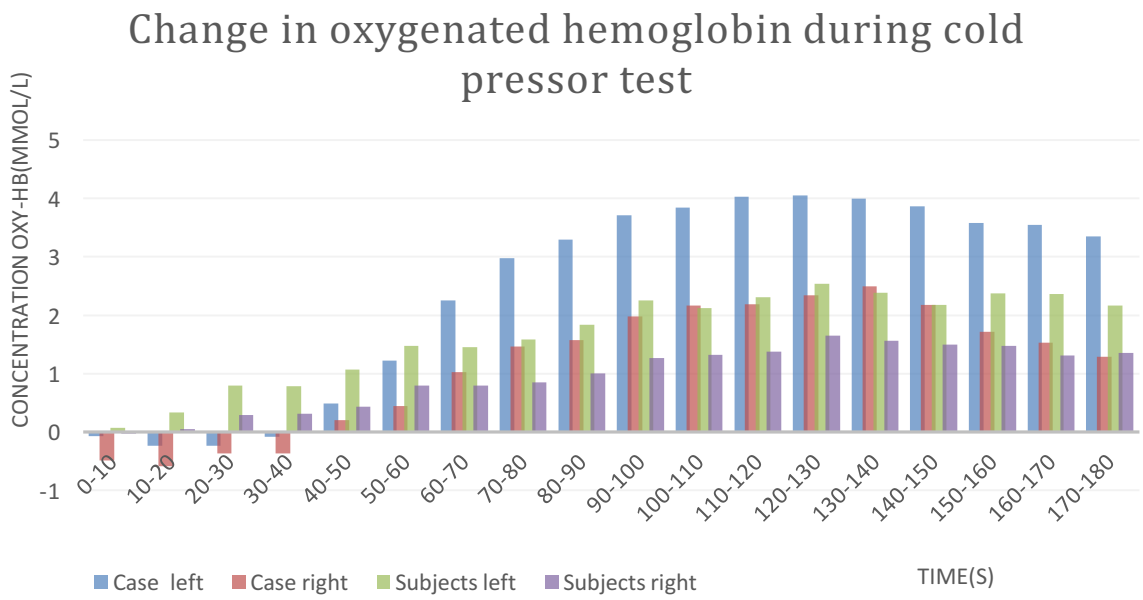

Figure D.5: Changes in oxygenated hemoglobin of the left and right frontal cortex of the case, compared to baseline. The maximum increase of 4.06 µmol/L occurs at 120-130 s at the left side and the maximum increase of 2.49 µmol/L occurs at 130-140s at the right side.

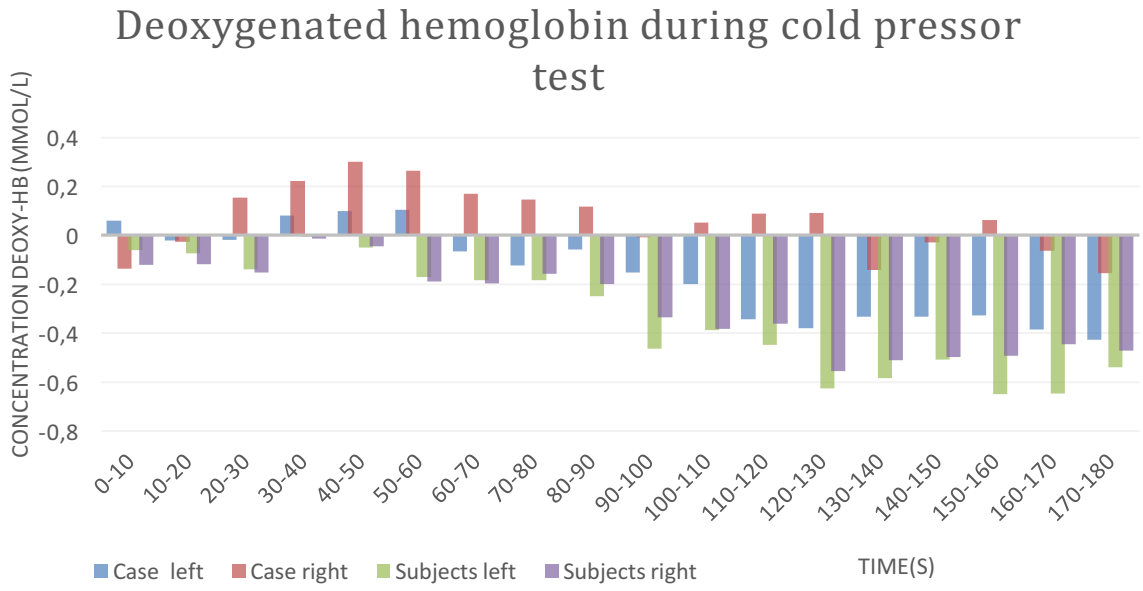

Figure D.6. Changes in deoxygenated hemoglobin of the left and right frontal cortex of the case compared to baseline. The maximum decrease of the left cortex occurs at 170-180s with -0.423 µmol/L and with -0.154 µmol/L at the right cortex. The maximum increase of 0.106 µmol/L occurs at the left cortex occurs at 50-60 s and the maximum increase of 0.301 µmol/L occurred at 40-50 s.

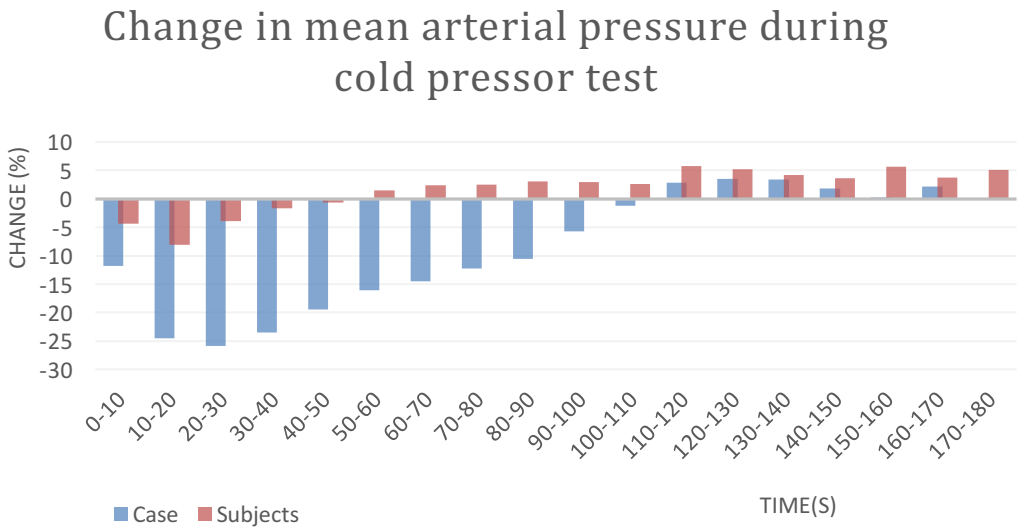

Figure D.7. Change of the mean arterial pressure of the case, measured by CNAP. The case shows a maximal decrease of -25.8 % at 20-30s.

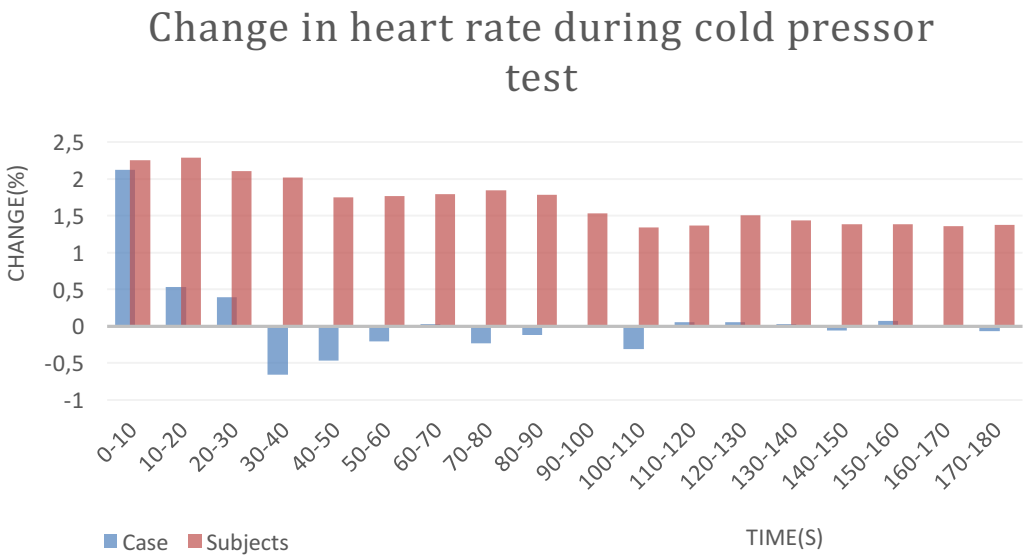

Figure D.8. Change in heart rate during cold pressor test of the case compared to baseline. The maximum increase occurs initially with an increase of 2.12%.

APPENDIX E: DIAMETER OF THREE SUBJECTS

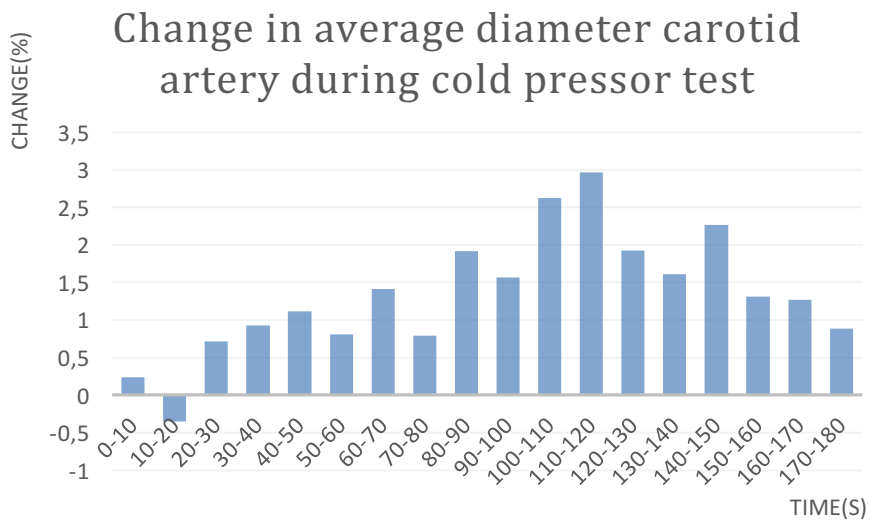

Figure E.1. Change in average diameter of the carotid artery during the cold pressor test. In this figure, all three diameters were used instead of the two included in the velocity measurements.

APPENDIX F: JUNCTIONAL ESCAPE RHYTHM

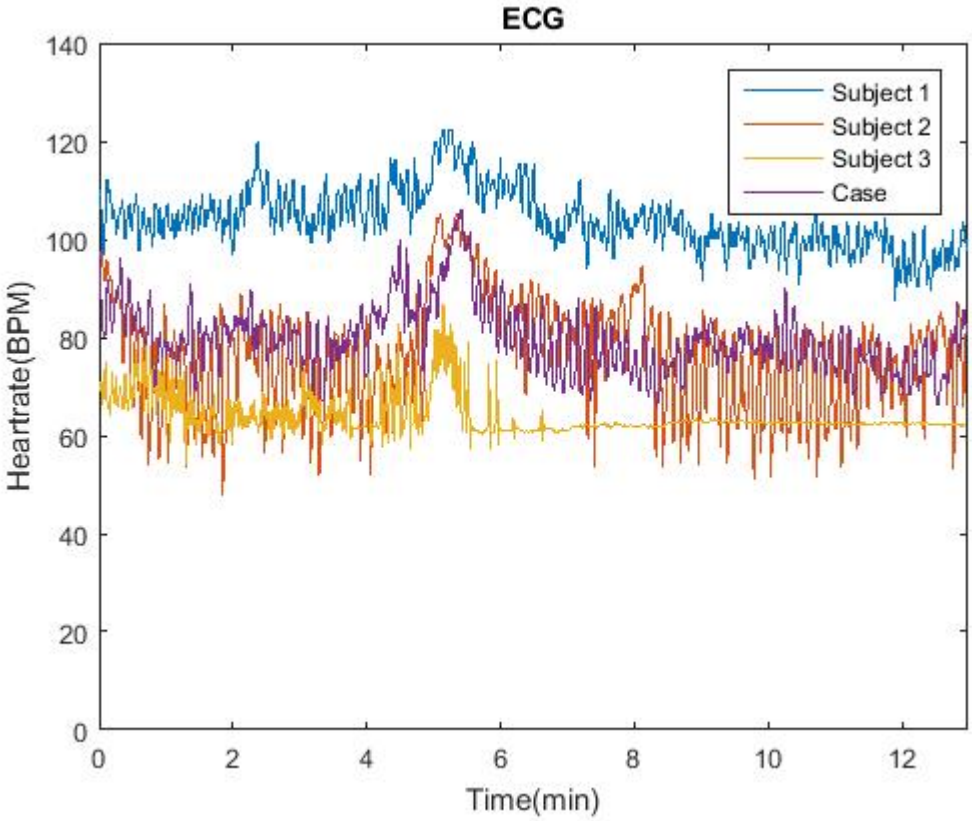

Figure F.1. In this figure the heart rate during the entire experiment is shown. In subject three a possible junctional escape rhythm is detected.