# Rational use of dopamine in hypotensive newborns: Improving our understanding of the effect on cerebral autoregulation

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## 1. INCLUDED MANUSCRIPTS

(I) Cerebral autoregulation in the preterm newborn using near-infrared spectroscopy: a comparison of time-domain and frequency-domain analyses. Eriksen VR, Hahn GH, Greisen G. J Biomed Opt 2015; 20(3):037009 (doi: 10.1117/1.JBO.20.3.037009).

(II) Dopamine therapy is associated with impaired cerebral autoregulation in preterm infants. Eriksen VR, Hahn GH, Greisen G. Acta Paediatr 2014; 103(12):1221-6 (doi: 10.1111/apa.12817).

(III) Dopamine therapy does not affect cerebral autoregulation during hypotension in newborn piglets. Eriksen VR, Rasmussen MB, Hahn GH, Greisen G. PLoS One 2017;12(1):e0170738 (doi: 10.3171/journal.pone.0170738).

(IV) Mechanical and vasomotor properties of isolated piglets' middle cerebral artery. Eriksen VR, Abdolalizadeh B, Trautner S, Greisen G, Sheykhzade M. Pharmacology Research & Perspectives 2017;5(1):e00279 (doi: 10.1002/prp2.279).

## 2. ABBREVIATIONS

| | |
|---|---|
| AWT | Active wall tension |
| CA | Cerebral autoregulation |
| CBF | Cerebral blood flow |
| CBFV | Cerebral blood flow velocity |
| COx | Cerebral oximetry index |
| $EC_{50}$ | Molar concentration of agonist required to produce half-maximal response |
| HIP | Hypotension in preterm infants (trial) |
| IC | Internal circumference |
| LDF | Laser doppler flowmetry |
| MAP | Mean arterial blood pressure |
| MCA | Middle cerebral arteries |
| NIRS | Near-infrared spectroscopy |
| OI | Oxygenation index |
| PSS | Physiological salt solution |
| PU | Perfusion units |

## 3. BACKGROUND

Being critically ill in the neonatal period can be associated with severe sequelae. Reducing the risk of cerebral injury is one of the main focus areas when treating this group of vulnerable infants.

One factor that is known to be associated with an impaired neurodevelopmental outcome is if the infant has been hypotensive [1–5]. Though it seems like an 'easy' intervention to improve the blood pressure and thereby avoid this risk factor, unfortunately treating hypotension does not improve the outcome. Paradoxically, hypotensive infants who were treated with vasopressors did worse than comparable infants who were not [6,7]. Therefore, we hypothesized that the most commonly used vasopressor, dopamine, might have a negative effect on the brain's protective mechanism – cerebral autoregulation. In this thesis, I will seek to clarify this notion.

### CEREBRAL AUTOREGULATION

Cerebral autoregulation (CA) is a protective mechanism that ensures a fairly constant cerebral blood flow (CBF) despite fluctuations in mean arterial blood pressure (MAP) (figure 1). CA describes the cerebrovascular alterations in response to changes in cerebral perfusion pressure [20] – not MAP. But in a clinical setting, continuously measuring cerebral perfusion pressure in newborn infants is not possible, and when intracranial pressure is constant, cerebral perfusion pressure is linear related to MAP, which has also been described in preterm infants [144]. Therefore, in this thesis, I will describe CA as cerebrovascular adjustments to changes in MAP.

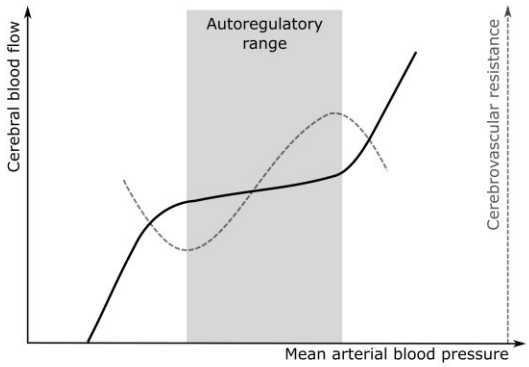


**Figure 1.** Cerebral autoregulation curve (black line) with indication of the autoregulatory range. Cerebrovascular resistance (dashed line) is altered in response to changes in MAP to maintain a fairly constant cerebral blood flow. Based on [8].

When MAP is within the autoregulatory range, CBF is fairly constant. To maintain this autoregulatory plateau, the cerebral arteries must be able to regulate their resistance according to changes in MAP (figure 1). When MAP is outside the autoregulatory range, the cerebral arteries fail to compensate for the changes in MAP, and therefore CBF will passively follow changes in MAP.
The classical CA curve, as described by Lassen, had a horizontal plateau [9]; but it is more appropriate to consider the plateau to have a slope [10]. According to this refinement, CA is better described as a quantification of how much CBF is changing in response to changes in MAP, and only determining the presence or absence of CA may be insufficient [11]. Hypotension is more common than hypertension in newborns. When MAP is below the autoregulatory range, we are concerned that CBF will decrease to a level where metabolic demands are not met, resulting in cerebral ischemic lesions. The lower limit of CA is not defined in preterm infants. Some studies have found that CA is impaired when MAP is below 30mmHg in preterm infants [12–14], whereas an alternate study found an intact CA well below this threshold [15]. This thesis will focus on the area around the lower limit of the autoregulatory range.

***Mechanisms maintaining cerebral autoregulation***
To maintain a fairly stable CBF within the autoregulatory range, the cerebral arteries must be able to compensate for the changes in MAP by altering the cerebrovascular resistance (figure 1). In short, when MAP decreases the cerebral arteries dilate, and conversely, the arteries constrict when MAP increases [16]. The myogenic response of the smooth muscle cells is considered the most important factor in maintaining CA, especially in the lower spectrum of the autoregulatory range [17], and alterations in the transmural pressure induce changes in the myogenic tone [10]. CBF and regulation of CBF are also influenced by other factors. These factors may affect the shape and/or slope of the CA curve. It is well documented that CBF is highly influenced by changes in $CO_2$-tension, $O_2$-tension, pH, neurogenic factors, and changes in metabolic demand [10,18–22].

CEREBRAL ARTERIES
Blood to the brain is supplied by four arteries: the two internal carotid arteries and the two vertebral arteries. The vertebral arteries merge to form the basilar artery. The internal carotid arteries and the basilar artery form the Circle of Willis at the base of the brain. From the Circle of Willis three pairs of arteries arise: the anterior, middle, and posterior cerebral arteries that supply the pial network of arteries covering the entire surface of the brain. At a certain point, the pial arteries penetrate into the brain parenchyma to supply the brain tissue [23]. At this point, the cerebral circulation becomes unique in the sense that it has a blood-brain barrier [24] that selectively regulates the transport of substances from blood into the cerebral tissue [25].
As a general rule, up to 50% of the peripheral resistance lies in arteries with a diameter larger than 100µm. However, the decrease in pressure depends on the type of vascular bed [26]; and in cerebral circulation the larger arteries (i.e. the internal carotid arteries) and the arteries forming the circle of Willis, as well as smaller arteries and arterioles, contribute substantially to regulation of CBF [10,19,25,27–29]. In the in vitro experiments conducted for study IV, 2nd order middle cerebral arteries (MCA) were examined (figure 2).

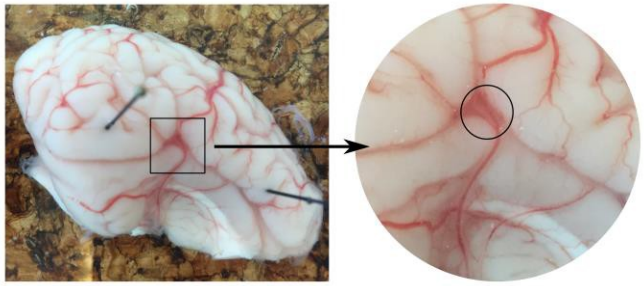

**Figure 2.** Lateral view of a brain from a mature newborn piglet. 2nd order branches of middle cerebral artery are encircled.

***Structure of cerebral arteries***
The arteries' structures vary: ranging from the large elastic conductance arteries originating from the aorta, to the smaller muscular resistance arteries and arterioles, to the capillary bed.

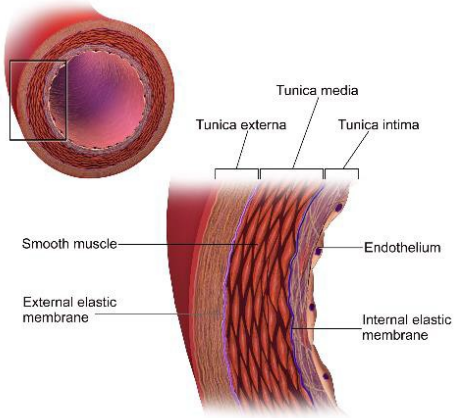


**Figure 3.** The histological structure of a resistance artery. From http://en.wikipedia.org/tunica_intima#/media/.

A resistance artery consists of three layers (figure 3). Outermost is the tunica adventitia consisting mainly of connective tissue with elastin and collagen. The middle layer, tunica media, contains smooth muscle cells that enable the arteries to constrict. The innermost layer, tunica intima, consists of endothelial cells on a thin layer of connective tissue. Tunica media is separated from the other layers by elastic membranes. The internal elastic membrane, between intima and media, has fenestrations that allow contact between the endothelium and the smooth muscle cells [26].

***Flow in arteries***

If we consider an artery as a cylinder, flow (F) depends on the driving pressure (ΔP) and resistance (R) as described by the law of Darcy (equation 1):

$$F = \frac{\Delta P}{R} \qquad \text{(equation 1)}$$

According to Poiseuille's law (equation 2), resistance is highly influenced by the radius (r) of the artery:

$$R = \frac{8 * \eta * L}{\pi * r^4} \qquad \text{(equation 2)}$$

Where η is the viscosity of the blood and L is the length of the artery segment.

As per the law of Poiseuille, even a small reduction in radius leads to a large increase in the resistance. This makes the arteries able to regulate the blood flow despite extensive changes in the driving pressure [30].

If the artery was a rigid tube, pressure-flow relation would be linear; however, this is not the case. Resistance arteries have both passive and active components [26]. Therefore, the artery distends (increases radius) when the driving pressure is increased. Consequently, resistance decreases, in accordance with the law of Poiseuille, and flow increases in line with Darcy's law.

The relation between distension of the artery (radius) in response to increased intraluminal pressure ($\Delta P_{transmural}$) and the passive wall tension (T) is mainly caused by passive components, and this relation is described by Laplace's law (equation 3) [30]:

$$T = \Delta P_{transmural} * r \qquad \text{(equation 3)}$$

***Control of flow in cerebral arteries***

As noted, the main factor in regulation of CBF is the myogenic response maintained by smooth muscle cells in the cerebral arteries [17], and several factors are involved in maintaining and modulating this myogenic response.

*Intrinsic factors*

Mechanisms that are contained within the artery itself are called intrinsic factors. Alterations in the transmural pressure, caused by changes in MAP, will lead to changes in tension and to stretching of the arterial wall [10]. Both increased tension and stretching of the arterial wall cause vasoconstriction via mechanisms that are not fully understood [31]; but the process involves depolarization of the smooth muscle cells' membranes [32] and altered membrane permeability to ions [18]. Changes in flow have also been demonstrated to influence the myogenic tone; but the extent to which altered flow is influential depends on the type and localization of the vessel, the species, and the age of the examined animal [32,33].

*Extrinsic factors*

Extrinsic factors are factors arising from outside the artery. Pial arteries have a 'classical' peripheral nerve supply that consists of three parts: (i) sympathetic innervation originating from the superior cervical ganglion, (ii) parasympathetic nerves from the sphenopalatine and otic ganglia, (iii) sensory nerves from the trigeminal ganglia [34,35].

Pial arteries are richly innervated by sympathetic nerve fibers [36–38], and cerebral arteries from newborn infants are more densely innervated than comparable adult arteries [37]. The sympathetic nerves release noradrenalin in response to stimulation, and noradrenalin causes vasoconstriction through α-adrenoceptors (mainly $\alpha_2$-adrenoceptors) on the smooth muscle cells[39]. The parasympathetic system does not seem to play a significant role in physiological cerebrovascular responses. The sensory nerves, in contrast, seem to be able to restore the artery's tone after a vasoconstrictive stimuli [34]. Also the before mentioned factors known to affect CA (such as $CO_2$-tension) are considered extrinsic factors.

HYPOTENSION IN NEWBORNS

Defining hypotension in the clinical setting and deciding when to treat hypotension in newborns are complex matters. A general consensus has not been reached [40]. Normative data is currently insufficient [41], and a threshold for hypotension has not been established for newborn infants [42,43]. Most neonatologists define hypotension as an MAP (in mmHg) below the infant's gestational age (in weeks) [44], despite the fact that no evidence exists to support this definition [42].

***Cerebral injuries in relation to hypotension***

Cerebral injuries associated with hypotension include both peri- and intraventricular hemorrhage as well as ischemic cerebral lesions [2,5,45,46]. It is especially the cerebral white matter which is vulnerable to hypoxia during hypotensive periods [13] because the arteries form vascular end zones here. These injuries lead to a heterogeneous group of clinical conditions, ranging from slight neurological impairment to hydrocephalus, cerebral palsy, loss of sensory functions and mental retardation.

***Treating hypotension and compromised blood flow***

The major concern relating to hypotension is that perfusion of the vital organs, especially the brain, is compromised. Hypotension is only one parameter in the clinical presentation of circulatory insufficiency, and because MAP is determined by both vascular resistance and cardiac output, it is a poor surrogate of the perfusion of the end organs [47]. Still, hypotension, as a single factor, has been associated with increased mortality and increased risk for neurodevelopmental impairment in newborn infants [1–5,48].

Dopamine is the most commonly used vasopressor in neonatology [44,49]. Unfortunately, improving MAP by administering dopamine has never been shown to improve outcomes. In fact, hypotensive preterm newborns, without other signs of circulatory insufficiency, who were treated with dopamine fared worse than comparable infants who were not treated with dopamine [6,7]. In fairness, compared to other vasopres-

sors, dopamine is not associated with a higher incidence of adverse neurological outcomes [48,50–52].
This uncertainty creates a sharp need for evidence-based knowledge in this area. Presently, we lack a true evidential basis for deciding who to treat, as well as when and how [53,54]. Two previous attempts to conduct randomized trials on antihypotensive treatment were not completed due to poor recruitment and physicians' unwillingness to enroll [55,56]. Currently, 'the hypotension in preterm infants (HIP) trial' (http://www.hip-trial.com) is running and it aims to compare standard treatment of hypotension with a more permissive approach in 830 extremely preterm infants [57].
Treatment of hypotensive and circulatory compromised preterm infants differs between neonatal intensive care units. Most neonatal intensive care units use fluid boluses as first line treatment followed by infusion of dopamine, and, if needed, addition of dobutamine, adrenaline and/or noradrenaline [44].

DOPAMINE AS A VASOPRESSOR
Dopamine is the most commonly used vasopressor in newborn infants; but it is also a naturally occurring catecholamine that acts as a neurotransmitter. When I refer to dopamine throughout this thesis, I refer to the exogenously administered, vasoactive drug dopamine.
When dopamine is used to raise blood pressure in newborn infants, it is administered as a continuously intravenous infusion. The dosage is determined at bedside, and adjusted according to the intended response on MAP. Normally, infusion rates are within the dosage range 2-20µg/kg/min [44,58,59].
Dopamine acts by stimulating both dopaminergic receptors as well as α- and $\beta_1$-adrenoceptors [59,60]. Dopamine receptors can be divided into subtypes and are grouped into D1-like (D1- and D5-receptors), and D2-like (D2-, D3- and D4-receptors) receptors. Not all subtypes are present in the cerebral vasculature. Briefly, D1-like receptors are located post-synaptic on the smooth muscle cells in the tunica media, and induce vasodilation of the arteries. D2-like receptors are located pre-synaptic on the sympathetic nerve endings on the border between the tunica media and the tunica adventitia. These receptors act by inhibiting the sympathetic induced vasoconstriction [61]. The effect produced by stimulation of the dopaminergic receptors is a vasodilation [62]. At higher infusion rates, dopamine also activates α-adrenergic receptors [63], leading to increased vascular resistance and raised MAP [64]. Hence, dopamine has a biphasic effect on the arteries, causing vasodilation at lower concentrations and vasoconstriction at higher concentrations [59,64]. In preterm infants, even low infusion rates of dopamine will raise MAP, which indicates a high α-adrenergic sensitivity [58].
Cardiac function may also be increased by dopamine due to increased heart rate and increased contractility of the cardiac ventricles, which may also account for at least a part of the increased blood pressure [42,65,66].

## 4. HYPOTHESIS
The overall hypothesis in this thesis is that dopamine induces a rightward shift of the CA curve (figure 4).

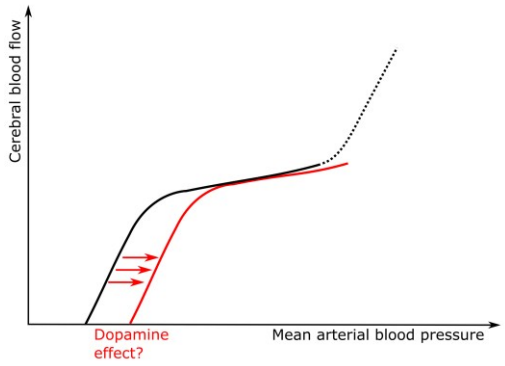


**Figure 4.** Hypothesis: dopamine induces a rightward shift of the cerebral autoregulation curve.

In theory, stimulation of α-adrenoceptors on the cerebral arteries' smooth muscle cells will result in vasoconstriction, and consequently a rightward shift of the CA curve. A rightward shift of the CA curve would, theoretically, leave infants who are treated with dopamine below the lower limit of CA at blood pressure levels that are otherwise considered 'safe'.

AIMS
The following questions were explored in the individual papers:

Study I: Estimation of CA – which mathematical method to use?

Study II: Is dopamine therapy associated with impaired CA?

Study III: Does dopamine induce a rightward shift of the CA curve at low blood pressure in a newborn piglet model?

Study IV: Do active and passive mechanical characteristics of isolated MCA from preterm and term newborn piglets differ? How does MCA respond to cumulative concentrations of dopamine assessed by wire and pressure myography? Does dopamine affect the pressure-induced myogenic response?

## 5. STUDY DESIGN AND METHOD
All methods and statistics are described in more detail in manuscripts I-IV.

CLINICAL STUDIES (STUDY I + II)
***Patients***
Study I and II were based on measurements from a previous study [67], where 60 very preterm infants with an indwelling arterial catheter were included. Infants were recruited within the first day of life. CA was estimated by using simultaneous measurements of MAP and cerebral oxygenation index (OI) measured by near-infrared spectroscopy (NIRS).

***Study designs***
*Study I*
Study I was a methodological study in which we compared the two most commonly used methods of describing CA: (i) time-domain analysis and (ii) frequency-domain analysis. In the time-domain analysis the correlation coefficient (cerebral oximetry

index (COx)) and regression coefficient were the outcome variables, whereas the outcome variables in frequency-domain analysis were coherence and gain. Results exhibiting the presence or absence of CA, estimated by COx and coherence, were compared. Further comparison was undertaken based on the regression coefficient and gain, as a quantification of the degree of impaired CA.

*Study II*

Study II was a retrospective observational study that was carried out prior to planning further studies for exploring our hypothesis. The aim was to test whether there was any association between impaired CA and dopamine. Thirteen out of 60 very preterm infants were treated with dopamine during the measurement periods. COx for the infants treated with dopamine was compared to COx for infants not treated with dopamine.

***NIRS***

NIRS is a non-invasive method that allows estimation of changes in cerebral oxygenation at a depth of 2-3cm. NIRS utilizes the fact that biological tissue has a relative transparency to near-infrared light (700-1000nm) [68] and that oxyhaemoglobin and deoxyhaemoglobin absorb near-infrared light with different wavelengths. OI is the difference between oxy- and deoxyhemoglobin divided by a factor of two, and continuous monitoring of changes in OI have previously been used to reflect change in CBF [69–71], provided that oxygen consumption is constant during the measurement.

***Methods for estimating cerebral autoregulation***

*Frequency-domain analysis*

Coherence analysis is a correlation in the frequency-domain that describes the strength of relation between OI and MAP at a particular frequency range. Coherence values are ranges between 0 and 1, where 0 indicates intact CA (no correlation between MAP and OI) and 1 indicates complete absence of CA (perfect correlation between MAP and OI) [72].

Gain is determined by the transfer function analysis and quantifies the extent to which a waveform is transferred from an input signal (i.e. MAP) to an output signal (i.e. OI) [73]. A reduced gain indicates that the output signal is damped and suggests intact CA; a high gain reflects a high CBF variability in proportion to MAP variability (figure 5).

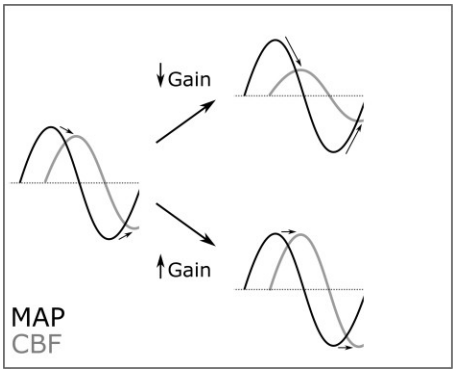


**Figure 5.** Illustration of increased and decreased gain. Black lines represent the input signal (MAP, mean arterial blood pressure) and grey lines represent the output signal (CBF, cerebral blood flow). Based on [8].

*Time-domain analysis*

Time-domain analysis is a statistical measurement of the linear relation between OI and MAP. COx corresponds to Pearson's r in the correlation analysis [74] and can have both positive and negative values. The more positive the COx, the more impaired CA. Negative values can be seen when increased MAP is followed by a decreased OI as a consequence of vasoconstriction (i.e. intact CA). The degree of impaired CA was quantified by the regression coefficient.

ANIMAL EXPERIMENTAL STUDY (STUDY III)

***Animals***

Eighteen newborn anaesthetized piglets were examined in the animal experiment. These piglets were born at term and were less than 72 hours old at the time of examination.

***Preparation of the piglet***

The femoral arteries were cannulated with: (i) an arterial line for MAP monitoring and blood gas sampling, and (ii) a 4 FR embolectomy-catheter with a balloon near its tip which was utilized to increase MAP in the upper part of the body by inflating the balloon. The femoral veins were cannulated with: (i) a 4 FR double lumen central venous line for infusion of glucose and dopamine, and (ii) another 4 FR embolectomy-catheter with the balloon in the inferior vena cava just before the inlet into the right atrium for the purpose of decreasing cardiac preload when inflated and hereby inducing arterial hypotension.

Micro-vascular perfusion of the brain was monitored by laser-doppler flowmetry (LDF). The LDF probe was placed, close to an intact dura, in the right parietal region. The superior sagittal sinus was cannulated and cerebral venous blood was sampled in order to determine cerebral venous saturation.

***Study design***

Each piglet was studied in two phases: a phase in which MAP was decreased and a phase in which MAP was increased (figure 6). Hypotension was induced by the balloon catheter in the vena cava. The piglet was randomized to receive dopamine in either the first or the second phase, and each piglet was randomized to receive an infusion rate of either 10µg/kg/min (n=6), 25µg/kg/min (n=6), or 40µg/kg/min (n=6). These dosages were chosen to represent: (i) a low-dosage that in theory would only affect the dopaminergic receptors, (ii) a blood pressure active dosage, and (iii) a supra-standard dosage [75–77].

At each level of hypotension, fluctuations in mean arterial blood pressure were induced by inflations/deflations of a balloon catheter in the descending aorta. Perfusion of the brain, monitored by LDF, and MAP was measured continuously.

At each level of hypotension, the following outcome variables were determined/calculated: cerebral venous saturation from the superior sagittal sinus, CBF, and CA capacity. All outcome variables were plotted against MAP and the relation between the variables and MAP was best described by a regression line with a breakpoint. Non-linear regression was used to determine this breakpoint.

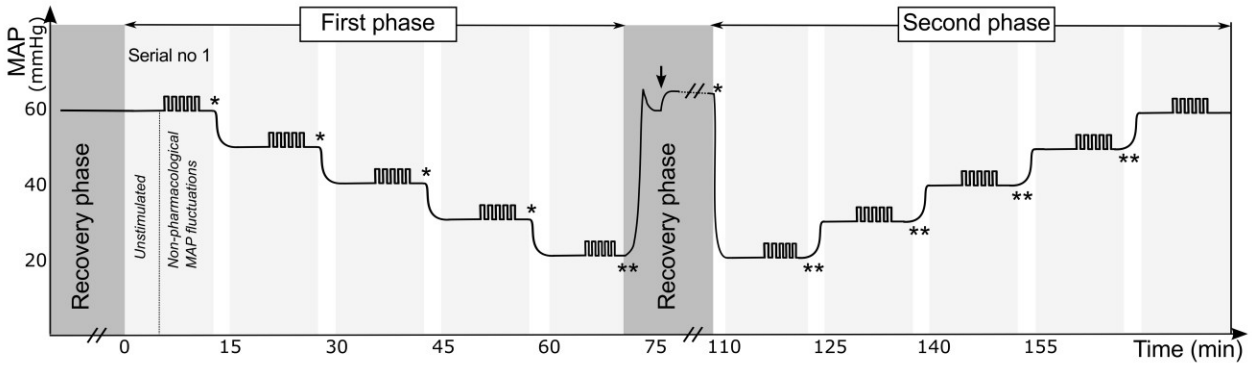


**Figure 6.** Study design in study III. The study had two phases: one phase of decreasing blood pressure and one phase of increasing blood pressure. The order of the phases was randomized. The process of infusing dopamine in either the first or second phase was also randomized. * Indicates inflation of the venous balloon catheter resulting in decreased blood pressure. ** Indicates deflation of the venous balloon catheter. The arrow points at the initiation of dopamine.

***Laser doppler flowmetry***

LDF was used to measure the perfusion units (PU) in a small area of parietal cortex in the piglets. The area that LDF measures is approximately 1mm$^3$, so only a very small part of cortex is represented. PU is a non-absolute value that depends on the number and the velocity of moving red blood cells [78]. Changes in PU have been validated as a method for continuously measuring changes in CBF [79–81].

IN VITRO MYOGRAPHY STUDIES (STUDY IV)

***Animals***

Eleven MCA segments from six preterm piglets and 22 MCA segments from nine term piglets were examined in the myograph study. Term piglets were less than 48 hours old. Preterm piglets were delivered at approximately 90% of full gestational age (full gestation is 116 days). They were examined when they were 4-5 days old (after participating in another study focusing on nutrition which took place at the Section of Comparative Pediatrics and Nutrition, University of Copenhagen).

After the piglets were euthanized, the brain was gently removed from the scull, and 2$^{nd}$ order MCA was identified and isolated. The segments were then mounted on either wire myographs or a pressure myograph (figure 7).

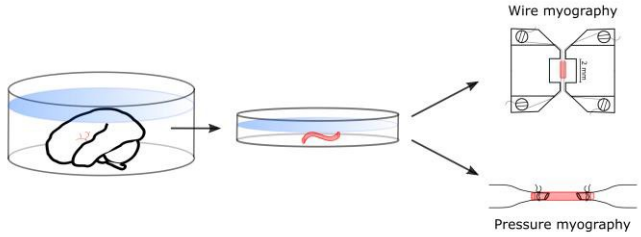


**Figure 7.** Preparation of the arteries for the studies in wire and pressure myographs.

***Study design***

*Wire myography*

In the first part of the study, the arteries were mounted as described by Mulvany and Halpern [82] on two wires (25µm) in the wire myograph. The wires were connected to a force transducer and a micrometer device (figure 8). This allowed us to measure isometric tension continuously. When the micrometer screw is increased, leading to increased distance between the myograph jaws, the stretch of the artery results in a cylindrical shape of the artery's circumference.

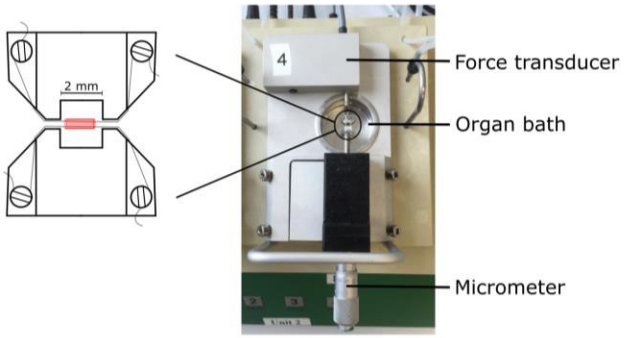


**Figure 8.** Schematic drawing of a mounted arterial segment and the wire myograph.

Firstly, the active and passive mechanical characteristics were described in an internal circumference (IC) tension relationship study [82] (figure 9). Passive wall tension (PWT) was determined in $Ca^{2+}$-free physiological salt solution (PSS) during a stepwise increase in IC. At each IC step, the segments were maximally contracted with a depolarizing bicarbonate buffer solution (K-PSS containing $10^{-5}$M noradrenaline) and active wall tension (ΔAWT) was estimated (figure 9).

Results from the passive IC-tension study were fitted to an exponential growth equation and results from the active IC-tension study were fitted to a Gaussian distribution equation. Maximal active wall tension ($AWT_0$) development is the amplitude of the curve and the optimal IC ($IC_0$) is the IC value at $AWT_0$. Subsequently, the pharmacodynamic characteristics arising from the cumulative concentration of dopamine (1nM-0.3mM) were examined (figure 10).

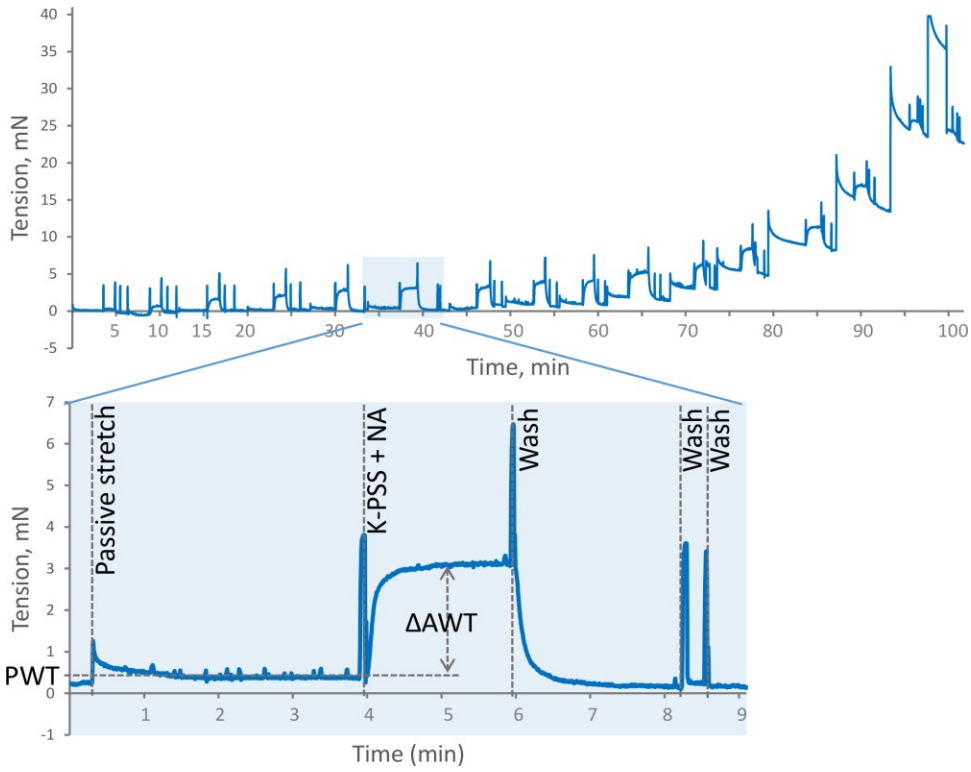


**Figure 9.** Example of an internal circumference (IC) tension study. The relationship between IC and active and passive properties is examined during stepwise increases in IC. Each sequence starts with a passive stretch, with the aim of determining the passive wall tension (PWT) at that specific IC. The artery is then stimulated by K-PSS with noradrenaline for determination of the active wall tension (ΔAWT). At the end, the artery is washed with $Ca^{2+}$-free PSS. Then the sequence is repeated.

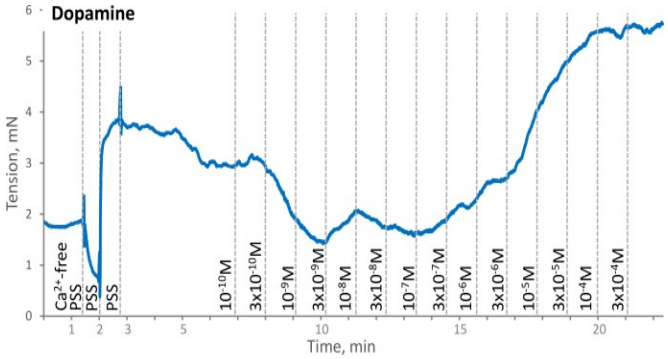


**Figure 10.** Dopamine concentration-response curve by wire myography.

The concentration-response curve was biphasic with vasodilation at low concentration and vasoconstriction at higher concentrations. The vasoconstrictive response to cumulative concentrations of dopamine was fitted to the four-parameter sigmoid equation. Sensitivity to dopamine was expressed as $pEC_{50}$ value, where $pEC_{50}$ = -log ($EC_{50}$ [M]), and $EC_{50}$ [M] is the molar concentration of agonist required to produce half-maximal response [83].

*Pressure myography*

In the second part of the study, the arteries were denuded of their endothelium and examined on the pressure myograph. The artery was cannulated by 140-160µm glass pipettes and each end was tied with 10-0 suture. The segment was visualized using an inverted microscope connected to a video camera, and the inner and outer diameters were determined using the software MyoVIEW (figure 11). The pressure myograph is often considered more physiological compared to the wire myograph because the artery preserves its normal shape, and the setup allows pressure and flow within the arterial segment [84]. However, in a similar manner to wire myography, the contribution from perivascular nerve endings is low in the pressure myograph.

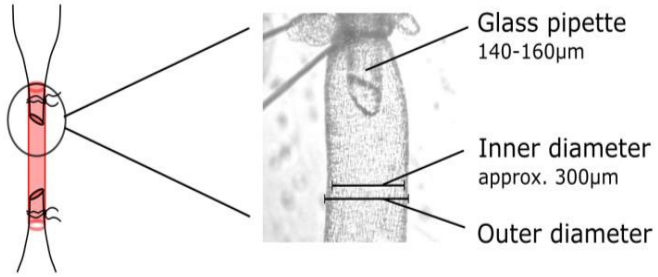


**Figure 11.** Schematic drawing and a picture of an artery mounted on the pressure myograph. Determination of inner and outer diameters by the pressure myograph.

The pharmacodynamic characteristics arising from cumulative additions of dopamine (1nM-1mM) were also examined by pressure myography. After an equilibration period, the intraluminal pressure was increased from 10 to 90mmHg in 20mmHg increments. The pressure-curves were repeated three times with the

artery exposed to PSS, PSS containing $10^{-4}$M dopamine, and $Ca^{2+}$-free PSS (figure 12).

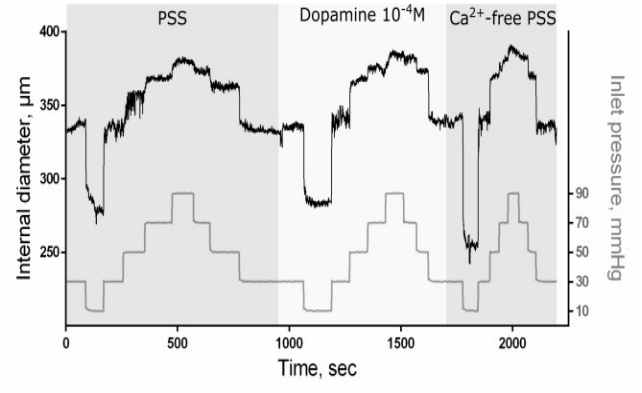


**Figure 12.** Example of pressure curves by pressure myography. The gray curve illustrates the changes in pressure within the artery (scale on the right y-axis). The black curve is the measured internal diameter in relation to the changes in the pressure. The first pressure curve was run while the artery was exposed to PSS, the artery was exposed to dopamine in the second pressure curve, and the last pressure curve was run in $Ca^{2+}$-free PSS.

*Wire myography versus pressure myography*

The two myograph techniques differ in several ways. One major difference is that contraction of the artery will lead to opposite effects on the wall tension. According to the law of LaPlace (equation 3), pressurized arteries decrease their wall tension in response to a contraction (i.e. reduction of internal diameter) under stationary pressure settings; whereas in the wire mounted arteries, wall tension increases in response to a contraction because IC is kept fixed by the wires.

## 6. SUMMARY OF RESULTS

STUDY I

In study I, frequency-domain analysis and time-domain analysis were compared. Both methods had a high relative repeatability, as tested by ANOVA, indicating that the methods were able to discriminate between the subjects. Correlation between coherence and COx was weak (r=0.215, p=0.097) (figure 13a). Accordingly, the two methods were not able to classify the same infants as having impaired CA ($Chi^2$=3.78, p=0.052). Also, the correlation between gain and the regression coefficient was weak (r=-0.206, p=0.115) (figure 13b).

The frequency-domain analysis was unable to detect whether signals were in phase or in counter-phase. Hence, if OI decreases in a similar pattern as MAP increases, the frequency-domain analysis will describe the signals as being similar and therefore indicate impaired CA. However, from a physiological viewpoint, if OI decreases in response to a rise in MAP, CA is working.

In conclusion, a high gain and high coherence may arise spuriously when OI decreases as MAP increases at low frequency. Hence, time-domain analysis appeared a more robust – and simpler – method for describing CA.

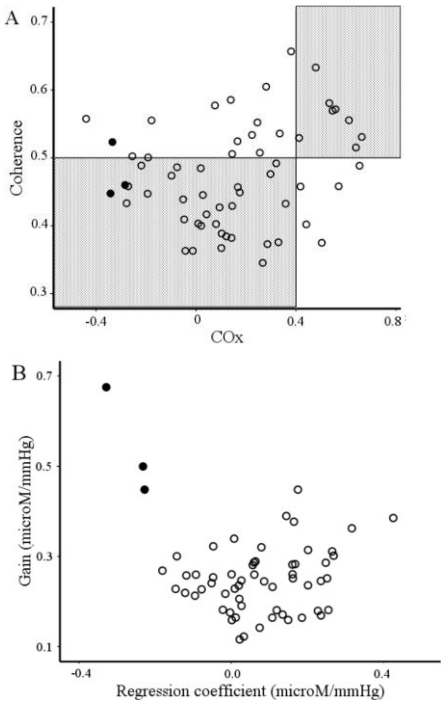


**Figure 13.** The consistency between frequency domain analysis and time domain analysis. (a) The coherence versus cerebral oximetry index (Cox). Thresholds for impaired CA are marked by gray lines (Coherence ≥ 0.5 and COx ≥ 0.4). The shaded areas indicate the concordance between the two methods. (b) The gain versus regression coefficient. The three most extreme cases with high gain and negative regression coefficient are marked by solid circles.

STUDY II

In study II, we compared COx from infants receiving dopamine (n=13) with infants who were not treated with dopamine (n=47). COx was higher in the dopamine group than in the control group (0.41±0.25 vs. 0.08±0.25, p<0.001) (figure 14), indicating impaired CA in the dopamine group.

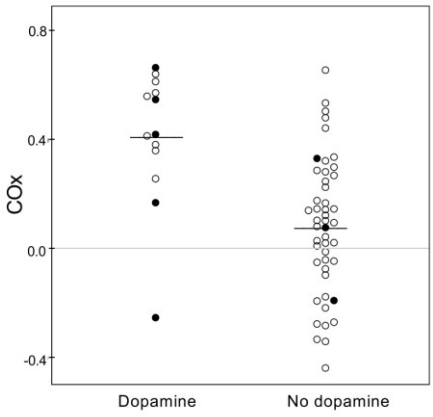


**Figure 14.** Cerebral oximetry index (COx) in infants receiving dopamine and untreated infants. Each circle represents an infant, and the horizontal lines indicate the mean. The infants who died in the neonatal period are marked by solid circles.

Compared to the control group, more infants in the dopamine group were treated with surfactant and needed mechanical ventilation. Also, MAP tended to be slightly lower in the

dopamine group. However, in the multiple regression analysis where the variables: dopamine, CRIB score, saline bolus, surfactant, mechanical ventilation and neonatal death, were tested for their influence on COx, only dopamine remained significant ($p<0.001$).

MAP was slightly lower in the dopamine group than in the control group. However, it seems unlikely that this should leave these infants below the lower knee of the CA curve and thereby explain the impaired CA.

If we assume that the observed difference in COx is caused by dopamine infusion, the results can be explained in two ways: firstly, due to a stimulation of the sympathetic nerves and a shift of the CA curve to the right [85] (figure 15a); or secondly, due to dopamine's potential interference with the efficiency of vascular smooth muscle cells' response to transmural pressure [86], thus inducing a steeper slope of the plateau of the CA curve (figure 15b).

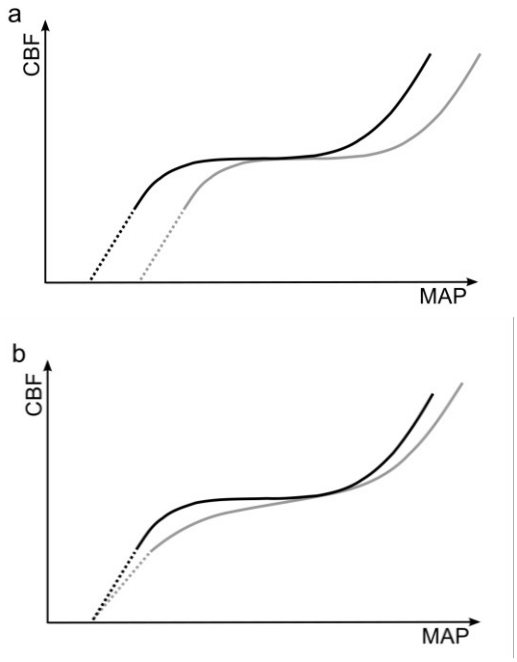


**Figure 15.** Hypothetical effects of dopamine on cerebral autoregulation. The 'original autoregulation curve' is marked with black, and the grey curves represent the hypothetical dopamine-induced changes. (a) Dopamine induces a rightward-shift of the autoregulation curve. (b) Dopamine induces a steeper slope of the autoregulation curve. CBF: cerebral blood flow; MAP: mean arterial blood pressure.

In conclusion, dopamine therapy was associated with impaired CA in preterm infants. We were unable to determine whether dopamine directly affected CA or whether dopamine was merely an indicator of illness.

STUDY III

In 18 newborn piglets we measured cerebral venous saturation, CBF, and CA capacity at different levels of hypotension with and without one of three dosages of dopamine. The relation between the outcome variables and MAP was best described by a regression line with a breakpoint. In order to visualise whether dopamine affected CA, the breakpoints, as an estimate of the lower limit of CA, were plotted against dopamine dosage. Neither cerebral venous saturation nor CBF were affected by dopamine infusion (figure 16a and 16b), whereas dopamine at higher infusion rates tended to decrease the breakpoints of CA capacity, indicating an improved CA at lower MAP (regression coefficient of -0.36mmHg/μg*kg$^{-1}$*min$^{-1}$, $p=0.057$) (figure 16c).

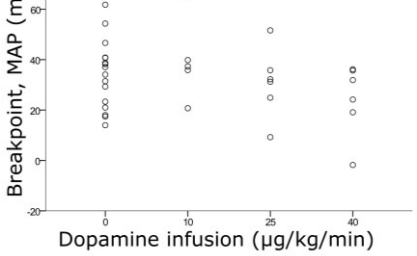


**Figure 16.** Relation between individual breakpoints of 'outcome variables' and dopamine infusion in 18 piglets. Each piglet was studied twice – with and without one of the three infusion rates of dopamine. (a) Cerebral venous saturation. (b) Steady-state cerebral blood flow. (c) Cerebral autoregulation capacity. MAP, mean arterial blood pressure.

In short, dopamine tended to improve CA capacity at low MAP; however, a beneficial effect of dopamine was not confirmed by improved CBF or cerebrovenous oxygen saturation. We concluded that dopamine therapy did not appear to affect CA in hypoten- sive newborn piglets.

STUDY IV

In the first part of the study, where MCA from preterm and term newborn piglets were mounted on wire myographs, active and

passive mechanical characteristics of MCA from preterm and term newborn piglets were comparable (figure 17a+b).

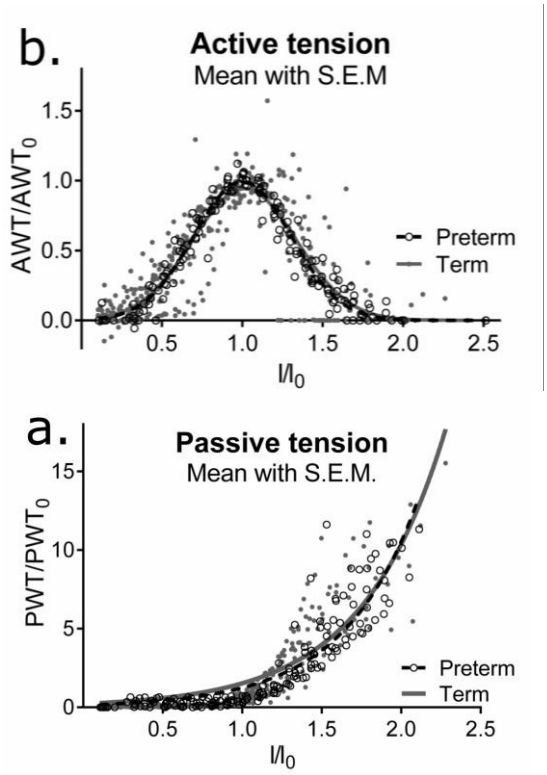


**Figure 17.** Relative passive and active curve internal circumference (IC) tension relationship. (a) Relative passive wall tension (PWT/PWT0) against relative IC (I/I0). (b) Relation between relative active wall tension (AWT/AWT0) and I/I0. Solid dots and lines represent the measurements from the preterm piglets, and circles and dotted lines represent the term piglets.

In wire myography, the response to cumulative concentrations of dopamine was biphasic, starting with vasodilation, followed by vasoconstriction at higher concentrations. The response was comparable in the two age groups (figure 18).

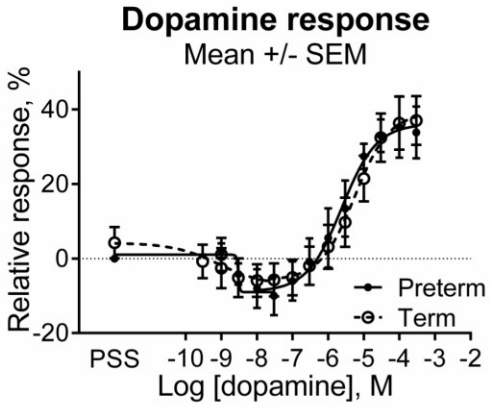


**Figure 18.** Dopamine concentration response curve by wire myography.

In contrast, response to dopamine was mainly dilatory when examined by pressure myography. However, at concentrations above 10-5M, dopamine slightly, albeit non-significantly, increased the myogenic tone (i.e. reduced IC). IC was still larger than the resting IC in PSS prior to the dopamine concentration-response study (figure 19).

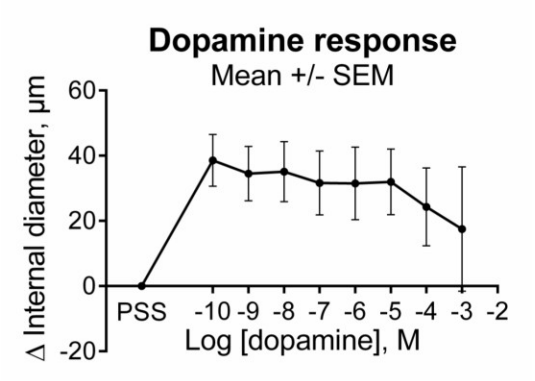


**Figure 19.** Dopamine concentration response curve by pressure myography.

The myogenic tone, prior to the pressure curves, was too low to allow proper examination of the interaction between dopamine and pressure-induced myogenic response, possibly due to dopamine exposure prior to the pressure curves.

In conclusion, active and passive mechanical characteristics of MCA from preterm and term newborn piglets were comparable. In the wire myograph, increasing dopamine concentration caused a biphasic response, starting with vasodilation at low concentrations, followed by vasoconstrictions at higher concentration. In contrast, in the pressure myograph, dopamine mainly induced vasodilation and the internal arterial diameter only tended to decrease slightly at the highest concentrations. As the myogenic tone was markedly reduced after the concentration-response experiment, we were not able to describe the active pressure-induced myogenic response.

## 7. DISCUSSION AND PERSPECTIVES

Despite hypotension being a relatively common condition in preterm and critically ill newborn infants, there is a significant level of debate concerning thresholds for treating hypotension in newborns and about which strategy to follow [42,43,49,56,87–90]. Dopamine therapy has been associated with higher mortality and poorer neurodevelopmental outcomes [6,7], and therefore questions have arisen as to whether dopamine itself could influence such outcomes.

In my view, three physiologically plausible explanations could explain this association between dopamine and poor outcomes: (i) dopamine may affect the intrinsic protective mechanism, CA, in a negative way; (ii) dopamine might penetrate the blood-brain barrier and have a directly deleterious effect on the developing brain; and (iii) dopamine therapy is solely an indicator of those infants who are the most ill and have the highest *a priori* risk of a poorer outcome.

When we, as physicians, confront decisions about whether an infant will benefit from treatment with dopamine, i.e. evaluate the risks versus the benefits from the treatment, it is important to

know whether there is a causal relationship between dopamine therapy and poorer outcomes.

The main hypothesis in this thesis was that dopamine affected CA negatively by inducing a rightward shift of the CA curve – an effect that could possibly be caused by stimulation of the α-adrenoceptors on the cerebral vasculature with a resultant vasoconstriction.

Before addressing this, it is necessary to consider how CA is best estimated. The ensuing section will address this issue.

## WHICH METHOD SHOULD BE USED TO DESCRIBE CA?

In the absence of a 'gold standard' method for determining CA, we must consider which method to use for estimating CA in newborns. CA is estimated based on relations between changes in MAP and changes in CBF. We used NIRS as a non-invasive method for estimating changes in CBF. However, cerebral blood flow velocity (CBFV) in MCA by transcranial doppler ultrasound can also be used. It is beyond the scope of this thesis to discuss differences and relationships between these two different measures of CBF, but it is worth noting that a good correlation has been shown between changes in NIRS signal and changes in CBFV [91] as well as between NIRS-deviated and CBFV-deviated estimates of CA [14].

Estimation of CA in newborns based on spontaneous fluctuations in MAP is most often performed by either frequency-domain analysis [67,92–95] or time-domain analysis [96–99] [study II]. The outcome variables from the frequency-domain analysis are coherence, gain, and phase; whereas COx and regression coefficient are the outcome variables from the time-domain analysis. COx, coherence, and gain were validated in piglet models. Gain, for those piglets having a high coherence, and COx showed a good correlation with the invasive methods of estimating CA [100,101]. Coherence had a poor correlation with CA capacity [101].

In Study I, we compared frequency-domain analysis and time-domain analysis applied to simultaneous measurements of OI and MAP from very preterm newborns. The concordance between the two methods was poor. We found that high gain and coherence may arise spuriously when OI decreases as MAP increases. Therefore, we concluded that time-domain analysis was more robust compared to frequency-domain analysis [study I]. In another study including very preterm infants, the correlation coefficient between CBFV and MAP was a poor estimate for determining CA [102]; however, these estimates were based on five-minute observation periods, and a linear relationship between CBF and spontaneous MAP changes may not be assessed properly within such an observation period [103]. In a population of adult patients with traumatic brain injuries, the time-domain correlation between CBFV and either cerebral perfusion pressure or MAP correlated well with Panerai's autoregulatory index and with the patients' outcomes; whereas the outcome variables from the frequency-domain analysis did not correlate with either Panerai's autoregulatory index or patient outcomes [104].

Frequency-domain analysis was initially introduced by Giller in an attempt to describe CA as a dynamic process [105]. The analysis is based on the assumption that the input signal (MAP) and the output signal (OI) are stationary. In this way, influence by noise and variance between the measurements is reduced [103]. This reduction of the influence of noise seems like an improvement as the signal-to-noise ratio is low and represents a major challenge when estimating CA, especially when observing spontaneous changes of MAP in newborns. However, the assumption of stationarity is not met [106]. In contrast, time-domain analysis does not assume stationarity. Yet this method has a different major drawback: it does not handle the lag-time between input and output signals. Consequently, the correlation between the signals may be underestimated [103]. Frequency-domain analysis is insensitive to lag-time between the signals; and similar patterns of change in the signals may be correlated in a frequency-domain analysis even though the response of the output signal is too far away from the input signal to be associated. In this way, the frequency-domain analysis may promote a false interpretation of impaired CA [103,107]. In an effort to avoid this misinterpretation, a new method for estimating CA has recently been described. The BiAR-COH is a method where both temporal and frequency dependences are considered, and in this way the method should be able to discriminate changes that are closely related in time from those that are not time-related and therefore have no direct dependence [108]. Unfortunately, two-thirds of all measurements were excluded in the BiAR-COH method as they did not meet the prerequisites, while in one-third of all infants an estimate of CA was not determined despite several hours of observation [108].

A further difference between time-domain and frequency-domain analyses is that time-domain analysis is sensitive to phase shift whereas coherence and gain are not able to discriminate between signals being in phase or counter-phase. For example, if MAP rises and OI decreases, then COx and the regression coefficient will be negative, indicating intact CA, whereas gain will be positive, indicating impaired CA [study I]. From a clinical viewpoint, it is more appropriate to consider CA to be intact if OI decreases in response to increased MAP. Caicedo et al. reported that correlation of time-domain analysis and gain constituted the two most robust estimates of CA, and concluded that gain was the best measure based on the fact that gain handles the time lag between the subjects [103]. However, the correlation coefficient in the study by Caicedo et al. was absolute. From a mathematical standpoint, this may make sense when comparing the correlation coefficient and coherence, but according to the interpretation of a negative value of COx as described above, an absolute value of the correlation coefficient does not match a physiologic concept [Study I].

Phase is also an outcome variable in frequency-domain analysis and phase-shift is a way to describe the time delay between the two signals. Phase-shift makes sense when examining a controlled fluctuation in the input signal and a corresponding fluctuation in the output signal [109]. But what does the average phase-shift in a frequency band actually mean? It probably makes little sense when applied to observations over several hours with spontaneous fluctuations in MAP, which may also be the reason that phase-shift has not been reported for studies of CA in neonates [67,72,73,94,95,105].

It has been shown in studies of patients with traumatic brain injuries that an individual optimal cerebral perfusion pressure can be determined in retrospective analyses, and that the time being outside the optimal cerebral perfusion pressure range is correlated with poorer outcome [110–112]. But the causality between being outside the optimal perfusion pressure range and poor outcome needs further evaluation. Similar to this approach, time-domain based estimates of CA obtained over several hours-to-days measurements have been introduced in newborns with the aim of determining the optimal MAP range with the strongest cerebral vaso-reactivity [14,96,113,114]. However, determination

of an individual optimal MAP/cerebral perfusion pressure range fails in almost one-fifth to one-fourth of all cases despite several hours of observations.
As noted in the Background section of this thesis, CBF, and consequently CA, is influenced by multiple other factors: $PaCO_2$, $PaO_2$, pH, changes in metabolic demands, temperature, intrathoracic pressure, and neurogenic factors [18,22,106]. These factors may contribute to the non-stationarity of CA. It has been suggested that more complicated models including more co-variates and time-varying techniques may be a better way to describe CA [106]. We can expect that new and improved methods will be introduced; but as methods grow more complicated, the measurements may often need to meet stronger prerequisites. At this point, the prerequisites of the suggested new methods result in rejection of up to half of the collected data [115]. Consequently, these methods may only be applicable on selected patients, and that will be a significant problem in the heterogeneous group of critically ill newborns.

In short, no 'gold standard' for estimating CA exists, and we are still quite some distance from a real-time, bedside estimate of CA. In view of this, the most robust and the most currently viable method for describing CA is based on time domain analysis.

ASSOCIATION BETWEEN DOPAMINE THERAPY AND CA
Several studies have demonstrated an association between hypotension and impaired CA in preterm newborns [12,93,96,98] which could explain the higher mortality and poorer outcomes observed in hypotensive infants [1–5,45]. The degree and duration of hypotension evidently matter since isolated hypotension and mild-to-moderate hypotension have not led to impaired CA [99,116].
Two retrospective, observational studies have raised the concern that treating hypotension with vasopressors might have a negative effect on the outcome since they observed that preterm infants treated with dopamine fared worse than a 'comparable' group of infants who were not treated [6,7]. However, the infants treated with dopamine may not be comparable with the control group because baseline information indicated that they were more ill [6,7].
It is still unknown whether dopamine therapy has an effect on CA. In study II, we observed impaired CA in very preterm infants during dopamine therapy, which is in line with previous studies in preterm infants [12,98,99,117]. As MAP was only slightly lower in the dopamine group compared to the control group (31.2±4.6 vs. 33.8±4.2, p=0.054), it seems unlikely that this could cause the observed differences in COx. Also, dopamine was the only factor that remained significant in the multiple regression analysis when variables were included that could indicate more severe illness, e.g. the CRIB score [study II]. Therefore, we speculated that dopamine may have a direct effect on CA, either by an α-adrenergic stimulation of the cerebral arteries, resulting in a rightward shift of the CA curve [85], or by an influence on the smooth muscle cells' response to altered intramural pressure [86], causing a steeper slope of the autoregulatory plateau [study II]. Causality between dopamine and impaired CA cannot be determined based on this study design.
A number of studies have estimated CA just prior to and during dopamine therapy. They found that the ability to autoregulate was unaffected by dopamine [98]. Infants who did not autoregulate continued to have impaired CA during dopamine therapy despite being normotensive [12]; and infants with a sufficient ability to autoregulate were not negatively affected by dopamine [118]. Infants who were included in these studies were hypotensive and treated according to routine clinical care in the relevant centers. So why do the infants in one group have an intact CA prior to dopamine therapy [118], whereas autoregulation was impaired in the other group [11]? Could this indicate that the infants with impaired CA were more ill in the study with impaired CA? Or does it indicate that the threshold for treating infants differed between the centers? Also, why did the infants with impaired CA not restore CA after achieving a normal MAP? Could it be that CA remained impaired based on previous hypoxic and/or hypotensive insults [50]? Such questions are best answered by a blinded randomized trial where infants are randomized to either no treatment or dopamine therapy in therapeutic dosages; however, this approach would not be accepted in a clinical study including either clinically well preterm infants or in severely circulatory compromised preterm infants and, therefore, the answer may have to be solved in a population with permissive hypotension like the ongoing HIP trial [57].

In summary, hypotension is associated with impaired CA, and impaired CA is associated with dopamine therapy in preterm newborn infants. Initiation of dopamine infusion does not affect CA in observational studies which may indicate that dopamine does not lead to impaired CA.

CAUSATION BETWEEN DOPAMINE THERAPY AND IMPAIRED CA
The causal relationship between dopamine therapy and impaired CA is best described in animal studies, where the factors that are known to affect CA can be reduced.
It has been demonstrated that dopamine increases CBF in adult and newborn animals, and the studies concerned have explained this observation as a cerebrovascular vasodilation [75,76,119,120]. But the observations are not consistent. In fetal lambs and newborn mature piglets CBF remains constant during dopamine infusion [75,121,122]. Like the observational studies in newborns, the designs of these studies have observed changes in CBF in relation to initiation of dopamine infusion. However, a general problem in this design is that the observed change in CBF may be affected by both a direct dopaminergic effect on the cerebral arteries as well as by an effect caused by a dopamine-induced rise in MAP. We intended to overcome this problem by estimating CA at comparable levels of hypotension with and without dopamine in study III. We observed that dopamine tended to improve CA capacity at low MAP, however CBF and cerebral venous saturation was unaffected by dopamine.
A previous study found that dopamine administered either prior to or after induced traumatic brain injury in newborn piglets prevented loss of CA and restored the cerebral arteries' ability to dilate during hypotension [123]. It is unknown whether these results can be compared to ours, but at least this study indicates that dopamine does not induce cerebral vasoconstriction. In a study of 10-day-old piglets, Nachar et al. found a dose-dependent relationship between MAP and cerebral oxygenation. This was not surprising as the piglets were anaesthetised by isoflurane that is known to impair CA [124]. But it was surprising that CA tended to improve when the piglets received dopamine infusions of 20 to 30µg/kg/min. The authors concluded that this restoration of a 'not-completely absent CA' was caused by stimulation of the α-adrenoceptors [120].
In study III, cerebral venous saturation was used as a simple indicator of CA failure. When MAP is lowered, oxygen extraction

increases to maintain a sufficient oxygenation of the brain. As a consequence, cerebral venous saturation decreases. Dopamine did not affect the MAP breakpoint where cerebral venous saturation began to drop in response to decreased MAP [study III]. It has been shown that oxygen extraction is much less affected by hypotension than CBF in newborn piglets [71], possibly due to reduced oxygen consumption [125]. Therefore, we might underestimate the effect on CBF when using cerebral venous saturation as a simple indicator for global CBF – however, the effect of dopamine may not be concealed by such insensitivity [study III].
Another factor that could exert influence on how well cerebral venous saturation reflects CBF is that it has been shown that dopamine promotes flow-metabolism coupling in preterm infants, meaning that CBF increases parallel with increased metabolic demands and as a consequence, cerebral oxygen extraction fraction and cerebral venous saturation is kept constant during dopamine therapy [126]. If the same was true in our piglets, we would have expected that the relation between cerebral venous saturation and CBF differed depending on whether the piglets received dopamine or not. We did not observe this outcome.

Overall, these studies indicate that administering dopamine has not led to impaired CA.

DOPAMINE EFFECT ON CEREBRAL ARTERIES

Thus far, we have described CA on a whole-scale basis – trying to clarify the effect of dopamine on regulation of CBF. As described previously, CA is influenced by several mechanisms, but the main factor in maintaining CA remains the myogenic response of the cerebral arteries. Therefore, studies focusing on these arteries will be prioritized in this section. The studies in this section, with the exception of one, differ from the clinical and experimental studies in that dopamine is applied on the abluminal surface of the arteries.
Before these studies can be set into a context of how dopamine affects CA, we must consider what the perivascular concentration of dopamine at the cerebral arteries will be when dopamine is infused in therapeutic dosages. The therapeutic dosage of dopamine in newborns is usually 2-20μg/kg/min [44,58,59]. Plasma-dopamine and cerebrospinal-fluid-dopamine concentration during dopamine therapy is highly variable, but it is most often reported to be in the order of $10^{-6}$M [127–130]. Although the concentration of dopamine in the synaptic cleft in the cerebral arteries has not been described during dopamine therapy, we would not expect that the concentration of exogenous dopamine will be significantly higher than the concentration in plasma or cerebrospinal fluid.
A cranial window can be used to visualize pial arteries in vivo. In newborn piglets, topical application of dopamine in the concentration range $10^{-7}$ to $10^{-5}$M did not affect the diameter of the pial arteries, and only concentrations above $10^{-4}$M induced vasoconstriction [131]. In line with this, infusion of 15μg/kg/min dopamine did not affect the ability of the pial arteries to dilate in response to hypotension. Actually, dopamine restored the arteries' ability to compensate for hypotension during experimentally induced traumatic brain injuries in these newborn piglets [123]. Also, in an adult cat model, dopamine ($10^{-6}$ to $10^{-3}$M) induced vasodilation and no vasoconstriction was observed [132].
The literature on dopamine's effect on isolated cerebral arteries is sparse. In study IV, MCA had a biphasic response to dopamine with vasodilation at the lower concentration range ($10^{-9}$M to $3x10^{-7}$M) followed by vasoconstriction at higher concentration when the arteries were examined by wire myography [study IV]. A similar biphasic response has been observed in human cerebral arteries and in cats [133,134]. We observed that the sensitivity to dopamine was higher in our newborn model compared to the adult models [133,134] [study IV]; and in line with this observation, newborn baboons have a higher sensitivity to contractile substances than adult baboons do [135].
To my knowledge, dopamine's effect on pressurized cerebral arteries has not been described previously, and to our surprise the pressurized MCA decreased their myogenic tone (i.e. dilated) in response to cumulative concentrations of dopamine. Only at the highest concentrations ($10^{-4}$ to $10^{-3}$M) did the internal diameter tend to decrease. However, the diameter was still larger than the resting diameter in PSS prior to the experiment [study IV]. We were surprised that dopamine did not induce a contractile response by the pressure myography comparable to the results we observed in the wire myography, but it has previously been described that sensitivity to vasoconstrictive agents differs in pressurized arteries compared to wire mounted arteries [84,136,137].

The overall conclusion of this section is that dopamine concentrations in the order $10^{-6}$M, which is the assumed concentration of dopamine in the perivascular tissue during dopamine therapy, do not induce a vasoconstriction either in the in vivo setup, with a cranial window, or in the in vitro setups by wire and pressure myography. Yet while dopamine does not induce a vasoconstriction, we cannot rule out dopamine's capacity to affect the pressure-induced myogenic response. Therefore, we planned to examine whether exposure to dopamine affected the pressure-induced myogenic response. However, the spontaneous myogenic tone was significantly reduced after the dopamine CRC experiment, and therefore we were not able to observe any myogenic response to increasing intraluminal pressure [study IV].

These studies of isolated cerebral arteries differ substantially from the clinical and experimental animal studies. Firstly, the application of dopamine on the abluminal surface is consequential. In a clinical situation dopamine is exposed to the luminal surface and diffusion over the endothelial barrier may affect the concentration in the perivascular space. Secondly, in these models, pial arteries were examined. CA is not solely based on pial arteries even though they contribute substantially [19,27]. Intraparenchymal arteries also play a significant role [10,19,25,27–29]. Thirdly, in the in vitro models, the contribution of the perivascular nerve endings as well as other extrinsic factors is sparse. Fourthly, flow within the artery as well as the endothelial influence on the myogenic response is different in the myograph setups compared to the in vivo situation.
Thus, the response observed in a myograph setup may reflect part of the in vivo response, but never the complex physiological response with modulating factors.

## 8. LIMITATIONS

MEASURING CBF

Measurement of CBF is the key when estimating CA. Unfortunately, we have no unimpeachable standard in measuring CBF continuously in newborns [107].
In study I and II, changes in OI by NIRS were used as a surrogate measure of CBF. Even though changes in OI have been demonstrated to reflect changes in CBF [69–71], we may recognize that cerebral oxygenation is only determined in a small area that may

not reflect the whole brain. NIRS is not able to separate oxygenation in white and grey matter, and as white matter is the most vulnerable to ischemia [13], there is some risk that small but significant decreases in white matter are missed. Changes in arterio-venous ratio also influence NIRS outcomes, and a change in the arterio-venous ratio has been suggested as an explanation for an observed mismatch between dopamine-induced increases in CBF measured by LDF and NIRS in a piglet study [77]. We cannot rule out that such an effect may have influenced the estimates of COx in the dopamine group in study II. However, OI was less affected than PU in that piglet study [77] and therefore we would expect that if dopamine influenced the arterio-venous ratio in the same manner in the infants, then COx would have been underestimated in the dopamine group, and this would not have exerted influence on the overall conclusion that dopamine was associated with impaired CA.
In the animal experimental study [study III], LDF was used as a continuous measure of changes in CBF. LDF only reflects perfusion in a very small area of the cortex, and definitely does not reflect the entire CBF. It has been shown that LDF correlates well with several 'gold standard' methods for measuring cerebral blood flow in animals, including the microsphere technique [79,80]. Furthermore, LDF has been shown to be a reliable method for determining the lower limit of CA [81], which was the purpose of study III.
The experiment could possibly be improved by estimating CBF by microspheres at each MAP level. We would not be able to estimate vasoreactivity by CA capacity, but we would be able to plot CBF against MAP with and without dopamine, and we would also be able to detect regional differences in CBF.

Spontaneous or induced MAP changes when estimating CA?
Another challenge when estimating CA is that we need changes in MAP when estimating CA. At the recently held CARNet (Cerebral Autoregulation Research Network) meeting in June 2016, vivid debate occurred over whether CA was best described by spontaneous fluctuations in MAP or whether induced changes in MAP would give the best interpretation of CA.
The proponents for spontaneous fluctuations in MAP argued that CA needs to work in 'natural situations', and that induced MAP changes would trigger other factors that are known to influence CA. Hence, an estimate of CA based on induced MAP changes may not reflect the true CA. On the other side, the proponents for induced MAP changes argued that CA is most effective when we go to the extremes, and therefore we need to challenge CA to get an estimate of how good CA is at buffering the MAP changes. Also, this group argued that induced MAP changes are the only way to be sure that the fluctuations in CBF are caused by changes in MAP; otherwise it is possible that, for instance, fluctuations in sympathetic tone drive both fluctuations in MAP as well as in arterial and venous tones.
As a first step towards consensus we will need to determine whether estimates of CA based on spontaneous and induced MAP fluctuations correspond. Despite an incomplete knowledge of this area, I am convinced that the two ways of describing CA will not fully correspond. In my opinion, CA estimates based on spontaneous MAP fluctuation are best used to describe whether an infant is within its autoregulatory range; whereas induced MAP fluctuation is probably best used to determine how wide the autoregulatory range is for that particular infant.

FROM EXPERIMENTAL STUDIES TOWARDS AN UNDERSTANDING OF CIRCULATION IN NEWBORN INFANTS
The conclusion of this thesis draws upon work from a range of settings: clinical studies of preterm infants; animal experimental studies including research on mainly mature animals of different species and a few studies on fetal lambs; as well as studies of the cerebral arteries from adults, adult animals, and a few studies of preterm and term newborns.
We cannot just extrapolate results from animal experiments into clinical practice. On the other hand, important clinical questions cannot be answered without using animal models.
The first striking challenge is that most animal experimental research is performed on mature animals, whereas all the clinical studies are performed on preterm infants. Preterm infants differ fundamentally from term infants and we would expect the same to be true for animals. Fetal lambs have been used as a preterm model [121,122], but this model also has some drawbacks such as the issue of fetal circulation. So, why do we not use preterm animals in the required experiments? The answer is probably due to ethical and practical considerations. Term animals can be examined one-by-one at a specific age, whereas preterm animals may be delivered by caesarian section which for most animals means a whole litter will be delivered. This may raise ethical concerns, especially in those cases where only one or two animals can be examined. Also, for many species, the viability is low in preterm delivery, and intensive care may be needed if the animals are required to survive for several days.
The dopamine receptors have shown a maturational-dependent expression in the kidneys in both preterm and term newborn animals [138–140], but in newborn infants it seems as though the maturation and expression of the renal dopaminergic receptors are more advanced [58,141]. Again, we cannot just extrapolate results from animals to infants, and we need to understand if the receptors and physiological responses are alike in animals and humans – an understanding that may be even more important in a newborn model.
We tried to clarify whether the cerebral arteries from preterm and term newborn piglets differed when examined by wire myography. We did not find any differences in the active and passive mechanical characteristics or in the concentration-response to dopamine [study IV]. It has been shown that the myogenic response changes with gestational and postnatal age [135,142,143], and the fact that we did not find any differences may reflect that the difference in age was too small between our two groups. The preterm piglets were delivered at 90% of full gestational age and were four-to-five days old, whereas the term piglets were less than 48 hours old. 90% of full gestational age is not a very good preterm model. It would have been valuable to examine the preterm piglets at younger gestational and postnatal ages.

PRESSURE CURVES – THE CLOSEST IN VITRO MODEL OF CA
The myogenic response is a major factor in maintaining CA and therefore we intended to mimic the myogenic response to increased pressure in the pressure myograph. In the event, though, the myogenic tone prior to the pressure curves was lowered, possibly due to a persisting dopamine effect from the preceding part of the experimental protocol. Thus, we were not able to describe whether dopamine affected the pressure-induced myogenic response. To answer this question, the study design should therefore only include the pressure curves. One design could be that one pressure curve was examined per segment and that each segment was randomized to be exposed to either PSS, dopamine

or $Ca^{2+}$-free PSS. Preferably, the three segments used from one animal should be from the same 2nd order branch of MCA. In order to achieve this, the number of included segments would need to be tripled. The pressure myograph setup is time consuming, and it is tempting to optimize the experimental time by examining three pressure curves in each segment, as we did in study IV. In that case, the PSS curve may be examined prior to the dopamine curve to avoid low myogenic tone in the PSS curve. Also, the experiments should be run both with and without endothelium.

## 9. CONCLUSION

The primary hypothesis in this thesis was that dopamine induces a rightward shift of the CA curve caused by a vasoconstriction of the cerebral arteries.

Literature has shown that hypotension is associated with and can lead to impaired CA. Dopamine has also been associated with impaired CA, and this association could not be explained solely by lower MAP in infants treated with dopamine [study II]. However, initiation of dopamine therapy did not change CA, which might indicate that dopamine itself does not lead to impaired CA. No causal relationship between dopamine therapy and impaired CA was established in study III; in fact, dopamine did not affect CA, CBF, or cerebral venous saturation [study III]. In support of this, dopamine in therapeutic concentrations did not induce vasoconstriction in the studies of pial arteries [study IV].

Based on a review of the existing literature and the studies included in this thesis, the answer to the central question in this thesis is: "No, dopamine did not induce a rightward shift of the CA curve".

## 10. FUTURE STUDIES

The background for this study was the observed association between dopamine therapy and higher mortality and poorer neurodevelopmental outcomes.

Based on this thesis, I concluded that the observed association could not be explained by a dopamine-induced impairment of CA. So, we may speculate that the observed association may be explained either by a direct effect of dopamine on the developing brain or, more likely, that the association solely reflects that those infants who are treated with dopamine are those who are the most ill, and therefore have the highest *a priori* risk of an adverse outcome.

A direct deleterious effect of dopamine on the developing brain has not been investigated, and animal models may be vital in answering that question.

It may be difficult to determine if dopamine is merely an indicator of illness despite this being the most plausible explanation. The HIP trial will definitely provide new insight and raise the evidence level in this area. However, in the HIP trial both the placebo group and the dopamine group may receive additional vasopressors if they are still circulatory compromised after the initial treatment per protocol [57]. In this way, those infants who are the most ill will probably receive vasopressors anyway, and it may be difficult to describe causative relationship between dopamine therapy and outcomes.

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## 12. SUMMARY

Hypotension in critically ill newborn infants is associated with higher mortality and higher risk of cerebral injuries. Yet treating hypotension has never been shown to improve outcomes. In fact, some studies have found that hypotensive newborn infants who were treated with dopamine fared worse than a comparable group of newborn infants who were not. Therefore, a concern has been raised that dopamine might cause the observed adverse outcomes. Cerebral autoregulation is a protective mechanism that maintains a fairly constant cerebral blood flow despite fluctuations in blood pressure. We hypothesized that dopamine might impair the cerebral autoregulation by inducing a rightward shift of the cerebral autoregulation curve. An increased cerebrovascular resistance due to a dopaminergic stimulation of α-adrenoceptors might cause this effect.

The main focus of this thesis is to clarify whether dopamine induces a rightward shift of the cerebral autoregulatory pressure curve.

The thesis is based on four papers:

(I) A methodological study comparing the two most commonly used methods of estimating cerebral autoregulation: time-domain analysis and frequency-domain analysis. We found that the consistency between the two methods was poor, and that time-domain analysis appeared a more robust – and simpler – method for describing cerebral autoregulation when estimates of cerebral autoregulation are based on spontaneous changes in blood pressure.

(II) A retrospective study estimating cerebral autoregulation in very preterm infants by time domain analysis. This study found an association between dopamine therapy and impaired cerebral autoregulation.

(III) An experimental animal study examining whether dopamine affected cerebral autoregulation in newborn piglets with low blood pressure. We found that dopamine did not negatively affect cerebral venous saturation, cerebral blood flow, or cerebral autoregulation capacity in hypotensive newborn piglets.

(IV) An in vitro experiment where middle cerebral arteries from newborn piglets were examined by wire myography and pressure myography. In the wire myograph, increasing concentrations of dopamine caused a biphasic response: starting with vasodilation at low concentrations followed by vasoconstrictions at higher concentrations. In the pressure myograph, dopamine mainly induced vasodilation and the internal arterial diameter only tended to decrease at the highest concentrations.

In summary, dopamine has been associated with impaired cerebral autoregulation and our conclusions in study II accorded with this. However, other work has found that initiation of dopamine infusion does not affect cerebral autoregulation. This may indicate that dopamine itself does not lead to impaired cerebral autoregulation. In support of these counter-observations, we did not find any causal relationship between dopamine therapy and impaired cerebral autoregulation in newborn piglets in study III. Also, dopamine in therapeutic concentrations did not induce vasoconstriction in pial arteries in study IV.

Based on a review of the current literature, and on the studies included in this thesis, the answer to the central question in this thesis is: "No, dopamine did not induce a rightward shift of the cerebral autoregulation curve".

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