The effect of hyperventilation upon cerebral blood flow and metabolism in patients with fulminant hepatic failure

Gitte Irene Strauss

This review has been accepted as a thesis together with seven previously published papers, by the University of Copenhagen, November 1, 2006, and defended on Marts 30, 2007.

Department of Hepatology, Rigshospitalet, University of Copenhagen, Denmark.

Correspondence: Gitte Irene Straus, Department of Hepatology A.2121, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, 2100 Copenhagen, Denmark.

E-mail: gstrauss@dadlnet.dk

Official opponents: Henrik Vilstrup and Martin Lauritsen.

Dan Med Bull 2007;54:99-111

1.0 INTRODUCTION AND AIM

"Over the oxygen supply of the body, carbon dioxide spreads its protecting wings- especially as it cares for the brain which, for unknown reasons, may not lack air in warm blooded animals whereas skin and muscle may tolerate ischemia of a tourniquet for more than half an hour." These poetic words of the virtues of carbon dioxide (CO_2) were extolled by Miescher-Rüsch [1] in 1855. Four years before Donders [2] had described the reactions of blood vessels to changes in respiratory activity. However, almost a century had to pass before the exquisite sensitivity of *intracranial* blood vessels to changes in arterial carbon dioxide tension ($PaCO_2$) was finally demonstrated [3], and the first quantitative measurements of cerebral blood flow (CBF) in relationship to changes in $PaCO_2$ were performed [4].

Fulminant hepatic failure (FHF) is a devastating condition elicited by an acute liver injury with the development of hepatic encephalopathy. The most feared complication in patients with FHF is the development of cerebral edema and intracranial hypertension arising in approximately 50 percent of the patients [5]. Despite extensive research, the pathophysiological background for the development of cerebral edema and intracranial hypertension is still not completely understood. The glutamine hypothesis, which was suggested in 1986 by Brusilow and Traystman [6], advocates that a shift of water into the astrocytes is a result of intracellular glutamine accumulation; osmotic changes that result in brain edema [7-9]. Also changes in CBF is assumed to be of importance for the development of cerebral edema in FHF, as CBF correlates to brain water content and subsequently intracranial pressure in experimental models [10-12].

Spontaneous hyperventilation is often observed in liver failure. Stanley et al [13], suggested that the ventilatory stimulation that arises in liver failure might be caused by stimulation of peripheral chemoreceptors. Also lactate acidosis, renal impairment, hepatic encephalopathy and a high sympathic tone are common complications in FHF, which all stimulate hyperventilation.

Hyperventilation lowers $PaCO_2$ (hypocapnia) and produces vasoconstriction and lowering of CBF (I). Patients with FHF with overt stages of hepatic encephalopathy are in some liver failure centres intubated and mechanical normoventilated, as hyperventilation is assumed to induce brain hypoxia [14]. It remains, however, unknown if hyperventilation in the early stage of FHF, well before brain edema has evolved, is beneficial or harmful to these patients.

Before a detailed presentation of the main findings in this study it may be helpful to review how $PaCO_2$ modulates CBF in healthy subjects.

1.1 CARBON DIOXIDE AND MECHANISM OF ACTION

In the normal brain, the constancy of CBF and cerebral blood volume relies upon the intrinsic ability of the cerebral resistance vessels to alter their diameter in response to variations in blood pressure (CBF autoregulation) and changes in metabolic demands. One of the major products of cerebral metabolism, CO_2, can alter cerebrovascular resistance and ultimately affect CBF [15]. CO_2 is freely diffusible between arterial blood and brain tissue. Thus it can diffuse into the vascular smooth muscle cell from either brain tissue or the vessel lumen, while hydrogen ions in the vessel lumen are prevented from reaching the smooth muscle cell by the blood-brain barrier [16]. Even though the chemical reaction between CO_2 and tissue water is simple:

$$CO_2 + H_2O \leftrightarrow H_2CO_2 \leftrightarrow HCO^-_3 + H^+$$

the physiologic mechanisms that underlie the responses of the cerebral vessels to changes in CO_2 have not been simple to ascertain. The main mechanism of this effect upon cerebral vessels appears to be perivascular pH [17, 18], but there may be additional, yet unknown, mechanisms. There is also evidence to support that factors such as prostanoids [19-21] and nitric oxide [22] are involved in the response, whereas the influence of peripheral/central [23] nerves is conflicting, at least during hypercapnia. The influence of these factors on the cerebral carbon dioxide reactivity has mostly been explored during *hypercapnia*, while only few studies have addressed their influences in *hypocapnic* responses.

1.1.1 Extracellular fluid [H^+]

In 1961, Gotoh et al suggested that the action of CO_2 was mediated by direct effect of [H^+] on cerebrovascular smooth muscles [24]. Indeed, the majority of experimental studies support the concept that CO_2 regulates the cerebral circulation primarily by changes in pH in the extracellular fluid surrounding the vessels [17, 18, 25-28]. Kontos et al, found that marked changes in $PaCO_2$ did not affect cerebral vessel diameter unless a change in extracellular fluid pH occurred [17, 18]. However, it is not certain whether the effects on cerebral vessels are due solely to changes in extracellular fluid pH, or whether and to what extent changes in intracellular pH also contribute [29]. There is experimental evidence to support that the extracellular pH influences the vasodilatory response to a greater extent than the intracellular pH, as Toda et al [30], found that hypercapnic vasodilation in dog cerebral artery strips was reversed by infusion of sodium-bicarbonate, i.e., pH was raised while PCO_2 remained stable. In other experimental studies, blockade of adenosine triphosphate (ATP)-sensitive potassium channels completely inhibits the hypercapnia induced vasodilation [31, 32] as well as the hypocapnia-induced vasoconstriction [33]. These findings suggest that opening and closure of these channels are of importance for the vascular responses to alterations in $PaCO_2$. Recently, Nakahata et al [34], showed that blockade of ATP-sensitive potassium channels with glibenclamide completely abolished hypercapnia-induced vasodilation only when pH was decreased. Thus, CO_2-induced alteration in pH appears to affect ATP-sensitive potassium channels either by opening (hypercapnia-induced acidosis) or closure (hypocapnia-induced alkalosis) of the potassium channels.

1.1.2 Prostaglandins

Prostaglandins appear to be mediators of the cerebral CO_2 response to *hypercapnia* [19]. Several studies have shown that *hypercapnia* elicits vasodilation that is accompanied by increased prostanoid synthesis, and that this vasodilation is inhibited by indomethacin, a cyclo-oxygenase inhibitor [19, 35, 36]. By contrast, other studies in

other species did not find an effect of indomethacin on CBF [37, 38]. Also, indomethacin had no effect on dilator or constrictor prostanoids concentration in cerebrospinal fluid of newborn pigs in response to *hypocapnia* [39]. Nor did indomethacin affect pial arteriolar constriction in response to *hypocapnia*, or vasodilation when $PaCO_2$ was raised from hypocapnia to normocapnia [39].

1.1.3 Nitric oxide

Nitric oxide plays an important role in the tonic regulation of cerebrovascular tone and contributes to the changes in CBF produced by hypercapnia [40-42]. It has been suggested that the extracellular acidosis associated with hypercapnia might activate nitric oxide synthetase and increase nitric oxide production [22]. However, other studies suggest that nitric oxide is not solely responsible for the hypercapnia-induced vasodilation [41, 42]. It is assumed that the vasodilator response to nitric oxide in the cerebral circulation is secondary to stimulation of guanylyl cyclase [40] and involves activation of potassium channels [43].

1.1.4 Neural pathways

Definitive evidence to support the participation of either central and/or peripheral neural reflex pathways in mediating effects of CO_2 on the cerebral vessels are lacking [44]. However, in newborn piglets, Moore et al [45] showed that the *hypocapnic* vasoconstriction was attenuated after alpha blockade in the forebrain and brainstem and after cord transection, whereas the *hypercapnic* reactivity was unaltered by these procedures.

1.2 AIMS

The aims of the present clinical studies were to evaluate CBF and oxidative and amino acid metabolism in patients with FHF with special focus on the effect of short-term mechanical hyperventilation upon these measures. Also the influence of hyperventilation upon arterial content and cerebral fluxes of certain biomarkers and neuropeptides is investigated and discussed.

2.0 PATIENTS AND METHODS

2.1 PATIENTS

From February 1996 until June 2000, patients with FHF referred to the department of Hepatology, Rigshospitalet, were enrolled consecutively. All patients were investigated within 24 hrs after the first appearance of stage III-IV hepatic encephalopathy, and after intubation and mechanical ventilation had been instituted. All patients had arterial lines, central venous catheters and a catheter placed retrogradely in the internal jugular bulb. They all received N-acetylcysteine and 20% glucose intravenously, and were all sedated with midazolam. No forms of extracorporeal renal or liver assist devices were used before or during the studies.

Patients included in paper I were not included in other studies, and no quantitative measurements of absolute CBF were performed. Data in paper II-VII were obtained from 16 consecutively enrolled patients with FHF. Except for the study of regional CBF (II) and the validation study of the transcranial doppler (III), metabolic data and CBF were obtained in all 16 patients. Data on some of the brain fluxes during hyperventilation were only available in 14 patients.

Healthy subjects in the study of regional CBF (II) only participated in that study, while the group of healthy subjects are the same in paper IV-VII. Likewise data on patients with cirrhosis of the liver without hepatic encephalopathy are obtained from the same 5 patients in paper IV-VII. Additionally arterial blood samples of biomarkers of brain cell damage (VI) were collected in *all* patients with FHF and patients with acute on chronic liver disease.

Consequently, many data were obtained in the same group of patients, and many comparisons were made between the groups. In order to adjust for these multiple comparisons, Bonferroni correction factor 5 was applied to P values between 2 unpaired/paired groups. When more groups were compared Kruskal-Wallis analysis of variance (ANOVA) using Dunn's method for multiple comparison was applied.

2.2 METHODS TO EVALUATE CBF AND CEREBRAL CO_2 REACTIVITY

The aims of the present clinical studies were to determine the effect of hyperventilation upon global and regional CBF as well as the cerebral metabolism of different substances. For this purpose tracer-kinetic methods were used. These methods are based upon the cerebral uptake and wash-out of a freely diffusible inert tracer administered either intravenously or by inhalation. Two different tracer kinetic methods were applied, i.e., the Kety-Schmidt technique and Single Photon Emission Computed Tomography (SPECT) (II-VII). As tracer substances, ^{133}Xenon and Technetium-99m Hexamethyl propyleneamine oxide (^{99m}Tc-HMPAO) were used. In the clinical setting of FHF where mechanical hyperventilation is necessary, these methods are, however, too time-consuming. Accordingly, Transcranial Doppler technique (TCD) and the arterio-venous oxygen difference ($AVDO_2$) technique were evaluated as bed-side methods to monitor CBF responses to hyperventilation in patients with FHF (III).

2.2.1 The Kety-Schmidt technique

The Kety-Schmidt technique is based on Fick's principle and is considered to be the "Gold Standard" for measurement of global CBF in man [46]. In our clinical studies (II-VII), we applied the modified Kety-Schmidt technique, i.e., the desaturation mode. In this setting the brain is saturated by infusion of ^{133}Xenon at a constant rate for 30 minutes, and followed by a desaturation period of 10 minutes, where blood samples are obtained pairwise from the radial artery and internal jugular vein [47]. The Kety-Schmidt technique is widely acknowledged as an accurate technique for determination of global CBF. However, one critical assumption of the Kety-Schmidt technique is that the average ^{133}Xenon tension of the brain is the same as the ^{133}Xenon tension of the cerebral venous blood. In the experimental setting with a wash-out period of 10 minutes this assumption is not usually met, due to heterogeneously perfused cerebral tissues, leading to under representation of low flow areas such as cerebral white matter and hence, a systematic overestimation of global CBF [47, 48]. By computer simulation Madsen et al [47], demonstrated that the modified Kety-Schmidt technique over-estimates global CBF by 10 to 15% in healthy subjects, and proposed a simple conversion algorithm of measured CBF to "ideal" CBF. However, since the experiments by Madsen et al [47] were performed on healthy subjects, the assumptions by which the correction procedure is based may be invalid when applied to patients with FHF. Accordingly, this correction algorithm was not applied in the present clinical studies.

2.2.2 Single Photon Emission Computed Tomography

SPECT was applied to evaluate the regional CBF distribution pattern (II) as well as the global and regional cerebral CO_2 reactivity to hypocapnia in patients with FHF (III). Quantitative measurements of CBF during normoventilation and hyperventilation were obtained with ^{133}Xenon as the tracer substance. The use of freely diffusible tracers for the tomographic CBF calculation method is more complex, but founded on the same physical assumptions as originally proposed and described by Kety and Smith [46]. 1) simultaneous arrival of the tracer to all parts of the brain following administration, 2) instantaneous equilibrium between brain and blood, and 3) cerebral blood flow remains constant during the period of measurement. CBF values derived from SPECT studies are prone to inaccuracies due to problems with attenuation correction, Compton scattered radiation, the partial volume effect, and estimation of the arterial input function from the lung curve input function [49, 50]. Apart from the methodological problems, the most critical comparison between the Kety-Schmidt technique and SPECT, is that SPECT studies derive their CBF-values from one representative

Table 1. Comparison of ^{133}Xe SPECT and the Key-Schmidt technique in patients with FHF.

	SPECT	Kety-Schmidt
Rest		
CBF (mL/100g/min)	43±9	41±4
$PaCO_2$ (kPa)	4.9±0.7	5.0±0.3
MAP (mmHg)	76±13	80±12
Hyperventilation		
CBF (mL/100g/min)	36±7	34±5
$PaCO_2$ (kPa)	3.8±0.4	3.9±0.3
MAP (mmHg)	74±14	73±12
CO_2 reactivity		
(%/mmHg)	1.9±0.7	2.0±0.9

CBF: cerebral blood flow; $PaCO_2$: arterial carbon dioxide tension; MAP: mean arterial blood pressure; CO_2 reactivity: cerebral carbon dioxide reactivity.

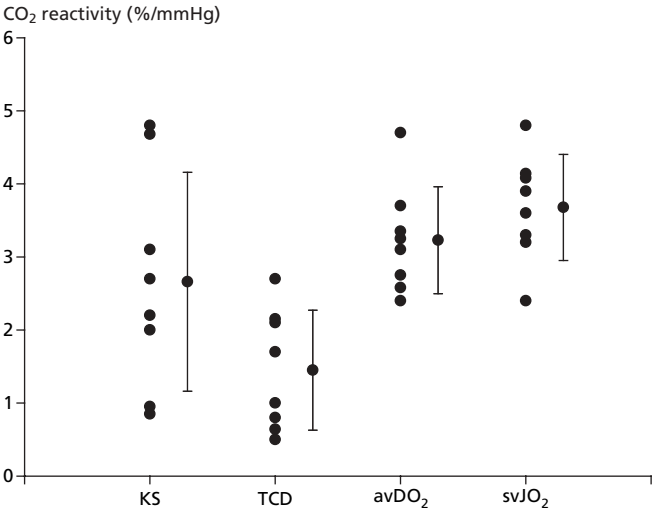

Figure 1. Cerebral CO_2 reactivity obtained by the Kety-Schmidt technique (KS), transcranial Doppler sonography (TCD), arterio-venous oxygen difference ($avDO_2$) and internal jugular bulb saturation ($svJO_2$) in patients with FHF. The TCD technique slightly underestimated the CO_2 reactivity compared to the other methods (ANOVA, p=0.047) (III). Reprinted with permission from publisher. (Strauss et al, Liver Transpl 2001).

whole brain slice, while CBF obtained by the Kety-Schmidt technique is from the entire brain volume. To assure that no major systematic error was applied in our studies (III-VII), we determined global CBF by both techniques in six patients with FHF, and found similar CBF values for the Kety-Schmidt technique and SPECT, i.e., 41 ± 4 vs. 43 ± 9 mL/(100g × min) (NS) during normoventilation, and 34 ± 5 vs. 36 ± 7 mL/(100g × min) (NS) during hyperventilation (unpublished data) (Table 1).

Since ^{133}Xenon only allows for evaluation of regional CBF with low-image resolution, ^{99m}Tc-HMPAO was used as tracer to obtain high-resolution SPECT images of regional CBF distribution pattern during hyperventilation (II). This tracer only yields relative CBF values. After intravenous administration of ^{99m}Tc-HMPAO approximately 70-80% of the primary complex reaching the brain crosses the blood brain barrier [51]. Inside the brain it is rapidly converted to its non-diffusible hydrophilic complex and retained for many hours [52]. Similar to the Kety-Schmidt technique, SPECT has a low time resolution and requires transportation of the patient to the tomograph. The advantage is that it allows for regional CBF determinations.

2.2.3 Transcranial Doppler Sonography.

The TCD method determines the mean flow velocity (V_{mean}) of flow in blood vessels. Measurements of V_{mean} were obtained from the middle cerebral artery through the temporal window with a 2 mHz probe and an insonation depth of 45-55 mm. The middle cerebral artery is the most extensively evaluated intracerebral vessel studied by TCD. The average V_{mean} in healthy adult subjects derived from previous studies is 62 (33-90) cm/s [53, 54], but may be affected by a variety of factors such as hematocrit, PaO2 and $PaCO_2$ in the same manner and direction as these factors affect CBF. The TCD technique has previously been evaluated as a reliable tool to evaluate relative changes in CBF during autoregulation studies where mean arterial pressure is raised by infusion of norepinephrine both in healthy man and in patients with FHF [55, 56]. Relative changes in V_{mean} however only reflect actual changes in CBF when the diameter of the insonated vessel and the perfusion territories remain unchanged during the study. In patients with FHF, it was found that the TCD technique correctly reflected changes in CBF during hyperventilation, but the numerical value of the CO_2 reactivity was slightly underestimated as compared to CO_2 reactivity obtained by the Kety-Schmidt technique and SPECT (Figure 1) (III). This slight discrepancy is probably caused by a decrease in the diameter of the middle cerebral artery produced during hyperventilation in patients with FHF, since the CBF/V_{mean} ratio decreased slightly from rest to hyperventilation, i.e., from 0.014 (range, 0.011 to 0.020) to 0.012 (range, 0.009 to 0.021) cm^2/g (p=0.047) (III). Although, the TCD method only provides relative CBF values, it has several advantages over both the Kety-Schmidt technique and SPECT: it is non-invasive, relatively inexpensive, less time consuming and has a high time resolution. Moreover, the technique can be applied bedside and provides on-line information on cerebral circulation, and alterations in this.

2.2.4 Arterio-venous oxygen difference method

This method is based on Fick's principle. As long as the cerebral metabolic rate of oxygen is constant, then changes in $AVDO_2$ will reflect variations in CBF, i.e., $AVDO_2$ varies inversely with CBF. However, it must be emphasised that this relationship only holds true as long as the normal relationship between CBF and metabolism is maintained [57]. The $AVDO_2$ method has most of the same advantages as the TCD technique, but requires placement of an internal jugular bulb catheter and an arterial line. The disadvantage is that blood samples are obtained from one internal jugular vein only, thus the possibility exists of overlooking severe ischemia in brain tissue drained by the opposite internal jugular vein, and although the catheter is correctly placed in the internal jugular bulb there will be an extracranial contamination of 2% to 3% [48]. In patients with FHF, we found that the $AVDO_2$ method yielded the same global CO_2 reactivity in response to hyperventilation as those obtained by the Kety-Schmidt technique (Figure 1) (III). Thus, the $AVDO_2$ method is a reliable and easy way to evaluate the effect of mechanical hyperventilation upon CBF in the clinical setting of FHF, as long as the normal relationship between CBF and metabolism is maintained.

3.0 MAIN FINDINGS AND DISCUSSION

3.1. CBF IN FHF

Wide variations in CBF have been reported in patients with FHF (Table 2). In the present work (III-VII), a reduced CBF was found in patients with FHF using both TCD technique (I) as well as tracer methods to measure absolute CBF (III-VII). This finding is in accordance with a number of previous studies [12, 14, 58-61]. Only, Ede et al [62] and Jalan et al [63-65] have reported significantly higher CBF values than that reported in other previous studies (Table 1). In the study by Ede et al [62] they report a normal value of 120 mL/(100g × min) using their technique. This is a much higher global CBF value than reported previously in healthy subjects [66], i.e., ~50 mL/(100g × min). The studies by Jalan et al [63-65] were, contrary to other previously published studies, performed during ongoing intracranial hypertension. In one of the clinical papers by Jalan et al [65], two groups of patients were included, one with intracranial hypertension and another without intracranial hypertension, demonstrating that only the group of patients with

Table 2. Published studies upon cerebral blood flow in patients with FHF.

Author (paper and year)	No. of patients	HE	CBF	Method
Ede (Gastroenterol Jpn, 1988) [62]	10	IV	195 (122-310)*	^{133}Xe injection
Almdal (Scand J Gastroenterol,1989) [59]	12	II-IV	31 ± 4	^{133}Xe injection
Aggarwal (Transpl Proc, 1991) [60]	9	IV	30 (4-60)	^{133}Xe-inj / Xe-CT
Aggarwal (Hepatology, 1994) [12]	33	IV	42 (16-95)	^{133}Xe-inj / Xe-CT
Wendon (Hepatology, 1994) [14]	30	IV	30 (14-71)	^{133}Xe injection
Durham (JCBF, 1995) [58]	24	IV	38 (18-66)	Xenon-CT
Larsen (Liver Transpl Surg, 1996) [61]	6	IV	34 (12-54)	^{133}Xe injection
Jalan (Lancet, 1999) [63]	7#	IV	103 (25-134)	N_2O inhalation
Jalan (Hepatology, 2001) [93]	9#	IV	111 (69-134)	N_2O inhalation
Strauss (Liver Transpl, 2001) (III)	8	IV	43 (36-60)	^{133}Xe injection
Strauss (Gastroenterol, 2001) (IV)	16	IV	39 ± 8	^{133}Xe injection
Jalan (J Hepatol, 2004) [65]	8#	IV	85 (24-134)	N_2O inhalation
	13	-do-	45 (23-56)	-do-
Jalan (Gastroent., 2004) [64]	14#	IV	78 ± 9.7§	N_2O inhalation

*) normal value for this study was reported to be 120 mL/100g/min
#) patients with increased intracranial pressure
§) mean ± SEM

intracranial hypertension had increased CBF, i.e., 85 (23-134) mL/(100g × min), whereas the patients without intracranial hypertension had a reduced CBF, i.e., 45 (23-56) mL/(100g × min), corresponding to other studies of patients with FHF without intracranial hypertension (Table 2). Thus, there seems to be evidence to support that CBF is low unless intracranial hypertension has evolved in patients with FHF. Whether this increase in CBF develops gradually or immediately before surges of intracranial hypertension in the clinical setting is not possible to unravel from the present studies in this thesis (I-VII) but studies of rats have shown that CBF gradually increases during the course of FHF [67].

The pathophysiological reason for the decreased CBF found in this thesis may rely on a number of different mechanisms:

3.1.1 Hepatic encephalopathy and sedation

Increased neural activity increases the energy expenditure for ion-pumping and transmitter synthesis, resulting in increased energy production due to increased oxidative glucose consumption, which is supplied by an increase in CBF. Conversely, CBF decreases during states with decreased neuronal activity, such as during coma [68, 69] and sleep [70]. Patients with FHF in the present study were in deep coma; hence the finding of a reduced CBF is most probable due to the decreased cerebral metabolism and neuronal activity (VII). Additionally, administration of midazolam may also have contributed to the reduced CBF in the present studies. In healthy volunteers intravenous administration of midazolam (0.15 mg/kg) given as a single bolus over 15 sec, resulted in a reduction in CBF of ~30% 6 minutes after the injection [71]. After a bolus injection of diazepam, Sari et al [72] showed that CBF was maximally reduced after 10 minutes, and reached normal values after 90-120 minutes. The studied patients in this thesis were administered midazolam continuously (~0.125 mg/kg/h). Thus, the percent reduction in CBF due to administration of bolus injection of midazolam is not directly comparable to the data in this thesis. During craniotomy of patients with cerebral tumors where midazolam was administered continuously (0.125 mg/kg/h vs. 0.250 mg/kg/h) Knudsen et al found reduced CBF values with no relationship to the dose administered [73]. However, CBF was not measured prior to administration of midazolam, thus it is not possible to determine to what extent CBF was reduced from normal values.

3.1.2 Hyperammonia and glutamine

To avoid the deleterious effects of ammonia, humans detoxify ammonia by incorporating it into urea. Urea is generated in the liver by the urea cycle, when the liver is failing blood ammonia levels increase. At high levels ammonia is neurotoxic, and leads to functional disturbances of the central nervous system [74], but it is conflicting whether ammonia per se affects CBF. Some studies have demonstrated that acute ammonia infusion dilate cerebral vessels and increases CBF [10], while others found that CBF decrease [75]. In the present thesis, there was no relationship between CBF and arterial ammonia levels in patients with FHF (Figure 2) (IV). Contrary, Jalan et al [65] found a positive correlation between CBF and arterial ammonia. This discrepancy may be due to time differences, as the patients in this thesis were investigated well before the development of cerebral edema and intracranial hypertension, while the patients in the study by Jalan et al [65] were investigated later during the cerebral illness of FHF.

Since the brain lacks urea cycle enzymes, ammonia removal from the brain relies on the formation of different amino acids, mostly glutamine and alanine, which are the main nitrogen carriers out of the brain (IV). A recent study shows that accumulation of glutamine per se only plays a limited role as a cause of cerebral edema in FHF, as mild hypothermia prevented cerebral edema in an animal model of FHF despite glutamine accumulation [76]. However, these findings do not preclude other important contributions of glutamine to the cerebral complications in FHF as hypothermia may have several other effects on the brain that may have contributed to the protective effect. In accordance with the experimental study by Master et al [77] we found that patients who subsequently died of intracranial hypertension had significantly higher cerebral ammonia uptake and cerebral glutamine efflux as compared to patients who survived (IV), suggesting that ammonia and glutamine plays an important role for the subsequent surges of intracranial hyper-

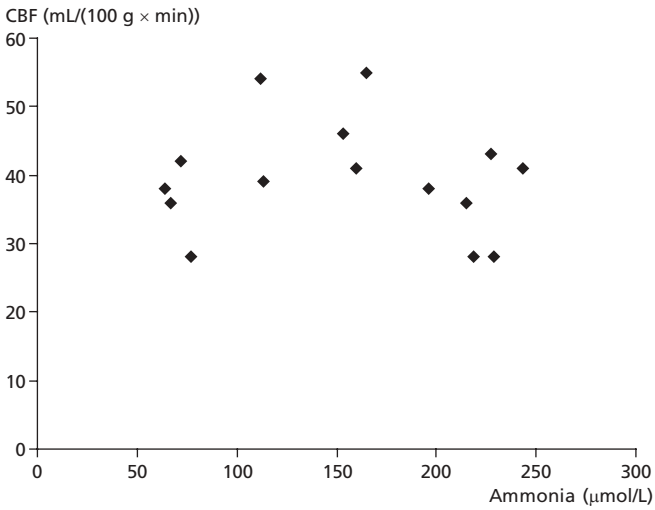

Figure 2. Arterial ammonia concentration plotted against CBF in patients with FHF.

tension. Whether or not this effect is cytotoxic, or is a combination of a cytotoxic and vasogenic effect is not possible to settle from the present study (IV).

Although the exact mechanism of ammonia toxicity is unresolved, hyperammonemia and cerebral glutamine accumulation appears to have several other effects on brain and cerebral metabolism that may contribute to or aggravate cerebral edema formation and induce cerebral vasodilation, including effects on cerebral energy metabolism [78], lactate/pyruvate production [79], astrocytic glutamate transport [80], brain ATP depletion by activation of NMDA receptors [81], nitrosactive/oxidative stress and induction of the mitochondrial permeability transition in cultured astrocytes [82]. The present clinical studies do not allow for any conclusions on the cellular effects of hyperammonemia and cerebral glutamine accumulation, nor do they allow for conclusion on their effects upon CBF later during the disease course of FHF. However, in the early stages of FHF hyperammonemia and glutamine accumulation did not affect CBF and cerebral oxidative metabolism (VII).

3.1.3 Acetaminophen and CBF

Recent studies have revealed evidence that acetaminophen inhibits prostaglandin E_2 production in rat cerebral endothelial cells possibly by acting against cyclooxygenase-2 [83]. Accordingly, inhibition of prostaglandin E_2 production could also to some extent have influenced CBF in patients with FHF, as prostaglandin E_2 is a vasodilator and acetaminophen intoxication was the reason for FHF in most of the patients (I-VII). Notwithstanding, CBF in patients without acetaminophen intoxication was similar to patients with acetaminophen intoxication, i.e., 38 (28-55) vs. 40 (28-54) mL/(100g × min) (NS). Patients with acetaminophen intoxication appears to have a better outcome in larger series of FHF, and it is possible that this inhibitory effect upon cyclooxygenase-2 may play a role in this setting by inhibiting the gradual increase in CBF that seems to evolve during the disease course.

In conclusion CBF is reduced within the first 24 h after development of stage III-IV hepatic encephalopathy. Increase in CBF seems to be a phenomenon that takes place later during the disease course, and only evolve in patients who subsequently develop intracranial hypertension. The low CBF values found in the studied patients in this thesis can be explained by the presence of hepatic encephalopathy and sedation by midazolam.

3.2 THE EFFECT OF HYPERVENTILATION ON CBF AND METABOLISM IN FHF

Cerebral CO_2 reactivity is the change in CBF per unit change in $PaCO_2$, defined as the % change in CBF divided by the $\Delta PaCO_2$ (in mmHg). At first the relationship between $PaCO_2$ and CBF was thought to be linear, however, later studies have shown that it is sigmoid, with a CO_2 reactivity that increases at high $PaCO_2$ levels and decreases at low $PaCO_2$ levels.

3.2.1 Global cerebral CO_2 reactivity

In this thesis global CO_2 reactivity was found normal in patients with FHF compared to controls. All clinical studies of cerebral CO_2 reactivity to hypocapnia performed on patients with FHF are displayed in Table 3 (III) [12, 14, 58, 84, 85]. All these studies reported almost similar cerebral CO_2 reactivity, except for one study where the hypocapnic CO_2 reactivity appeared much higher [85]. One explanation for this apparent discrepancy could be that by Sari et al [85], contained pooled data of patients with hepatic encephalopathy and septic encephalopathy. Thus, that study was not completely comparable with the other studies, which only contained patients with FHF. As can be seen from Table 3, values of CO_2 reactivity varied widely among patients with FHF. In two of the studies [12, 58] a paradox increase in CBF to hypocapnia, i.e., a negative CO_2 reactivity, was found in one patient (Table 3). Neither mean arterial blood pressure nor intracranial pressure was measured in these studies. Alteration of these pressures during the study period may have accounted for the apparent increase in CBF to hypocapnia. That is, if intracranial pressure was high before institution of hypocapnia, and subsequently was reduced during hypocapnia, then the resultant cerebral perfusion pressure is increased, and thereby also CBF. Likewise, if mean arterial pressure drops significantly during hyperventilation, then the resultant cerebral perfusion pressure is reduced and thereby CBF. Methodological problems should also be considered as well as time difference, as it cannot be excluded that cerebral CO_2 reactivity is completely lost later during the course of FHF [84].

The cerebral CO_2 reactivity is influenced both by the oxygen status and by the mean arterial blood pressure, as both hypoxia and hypotension induce vasodilation [86]. Thus, vasodilation induced by either hypoxia or hypotension may blunt the cerebral CO_2 reactivity during hypercapnia. In 1996, Larsen et al [84] explored the cerebral CO_2 reactivity in a prospective study including both patients with FHF and rats with thioacetamide-induced liver failure. It was found that patients with FHF had a reduced cerebral CO_2 reactivity during hypercapnia as compared to healthy subjects, ~2.2 vs. ~4.6% $mmHg^{-1}$, while it was normal during hypocapnia (Table 3) [84]. This finding was in accordance with a retrospective study of patients with FHF published by Durham et al a year before [58]. Accordingly, Larsen et al suggested that the cerebral CO_2 reactivity curve is left-shifted in FHF, i.e., CO_2 reactivity decreases during hypercapnia, while it is relatively preserved during hypocapnia (Figure 3) [84].

Animal studies have reported that cerebrovascular reactivity to hypercapnia is blunted following acute elevation of blood ammonia levels [87-89]. Thus, it could speculated that the increased blood

Table 3. Previous published studies on cerebral CO_2 reactivity to hypocapnia in healthy subjects and patients with FHF.

	Normoventilation			Hyperventilation			
	MAP mmHg	$PaCO_2$ mmHg	CBF ml (100 g min)$^{-1}$	MAP mmHg	$PaCO_2$ mmHg	CBF ml (100 g min)$^{-1}$	CO_2 reactivity %mmHg^{-1}
Healthy Subj							
Larsen (1996) [84]	---	39 (24-44)	66(38-88)§	---	20 (13-27)	35 (20-45)§	3.0 (1.7-5.0)
Moller (2002) [102]	---	42 (37-43)	71 (49-79)	---	25 (20-31)	47 (34-50)	2.1 (1.6-2.7)
FHF							
Sari (1990) [85]	86 ± 25	43 ± 5	52 ± 31	86 ± 25	35 ± 5	26 ± 7	~6.2[a]
Wendon (1994) [14]	---	37 (31-41)	36 (15-57)	---	28 (25-31)	28 (9-35)	~2.5*
Aggarwal (1994) [12]	---	32 (19-43)	47 (23-78)	---	$\Delta CO_2 \approx 8$	---	3.1 (-1.5–6.1)
Durham (1995) [58]	---	36 (15-45)	40 (28-57)	---	28 (10-32)	28 (14-50)	3.5 (-1–11)
Larsen (1996) [84]	72 (56-88)	36 (27-44)	61 (28-116)§	72 (56-88)	28 (23-39)	44 (23-100)§	4.0 (1.1-7.4)
Strauss (2001) (III)	80 (60-92)	37 (34-41)	43 (36-60)	75 (55-88)	28 (26-33)	32 (27-39)	2.5 (0.8-4.9)
			52 (39-66)§			43 (31-55)§	1.4 (0.6-2.7)[b]

a) this study contains patients with both septic and hepatic encephalopathy
*) calculated from the reported values
§) not an absolute measure of CBF, but a relative measure of CBF obtained by transcranial doppler (TCD) mean flow velocity (cm s^{-1})
b) significantly lower CO2 reactivity obtained by the TCD technique compared to the Kety Schmidt technique.

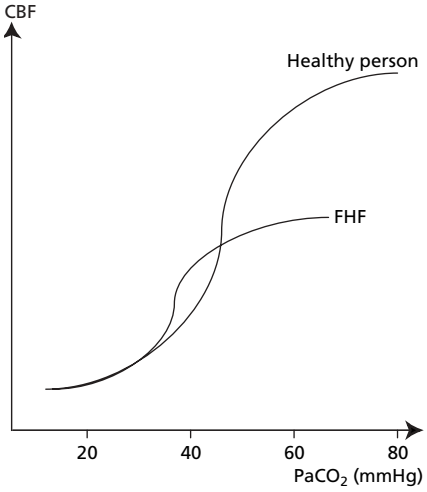

Figure 3. S-shaped cerebral CO_2 reactivity curve in normal healthy subjects, and the suggested cerebral CO_2 reactivity curve in patients with FHF.

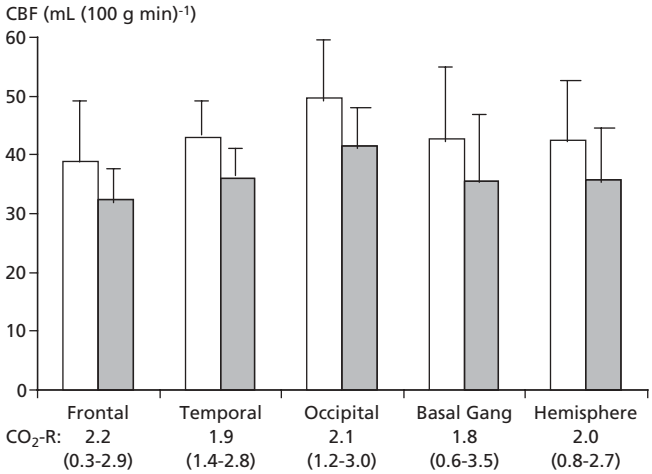

Figure 4. CBF responses to hyperventilation (dashed bars) in different brain regions in patients with fulminant hepatic failure. CBF decreased significantly in all brain regions (Signed Rank, $p<0.05$). CO_2 reactivity (CO_2-R) (% $mmHg^{-1}$) for all brain regions was similar between groups (ANOVA on the ranks).

ammonia levels could be responsible for the blunted CO_2 reactivity in patients with FHF, as arterial ammonia levels are significantly elevated (IV) [90].

In the brain, ammonia is detoxified by the formation of glutamine from glutamate. A recent study by Okada et al [91], have demonstrated that glutamine exerts a modulatory effect on the cerebrovascular reactivity to CO_2. They showed that a threefold increase in plasma glutamine concentration induced by infusion of glutamine blunted the cerebrovascular CO_2 reactivity in rats. Furthermore, they showed that co-infusion with arginine completely counteracted the effect of glutamine upon cerebrovascular reactivity, i.e., restored CO_2 reactivity. From these studies it is suggested that glutamine inhibits the recycling of citruline to arginine. Consequently the availability of arginine for nitric oxide synthesis is reduced [91]. In patients with FHF arterial (IV) and brain glutamine content is increased [92], thus glutamine could be responsible for the blunted CO_2 reactivity in FHF during hypercapnia.

Hypothermia appears to restore the hypercapnic cerebral CO_2 reactivity in patients with uncontrolled intracranial hypertension [93]. This may be explained by the vasoconstriction induced by hypothermia, which shifts the autoregulation curve to the left, thereby restoring at least some of the vasodilation capacity of the cerebral vessels.

In patients with FHF, we detected high circulating levels of two potent vasodilators, calcitonin gene-related peptide and vasoactive intestinal peptide (V). These neuropeptides exerts their effects on vascular smooth muscle receptors localised on the abluminal side of the blood-brain barrier and they do not seem to pass the blood-brain barrier to a greater extent. Thus, the circulating neuropeptides probably do not influence cerebrovascular tone to a major extent, unless the blood-brain barrier is disrupted [94, 95]. As they are stored in perivascular nerves, it cannot be excluded that high brain levels of one or both these two vasodilators play a role for the blunted cerebral CO_2 reactivity in FHF. Since, the effect of calcitonin gene-related peptide on cerebral vessels is mediated through opening of potassium channels, especially the calcium dependent but also to a minor degree the ATP dependent potassium channels [96], it could be speculated that the blunted CO_2 reactivity to hypercapnia in FHF is due to already opened ATP dependent potassium channels produced partly by high brain levels of calcitonin gene-related peptide.

3.2.2 Regional CO_2 reactivity

Even though global CO_2 reactivity is normal in patients with FHF there may be alterations of regional CBF and CO_2 reactivity, which may turn imminent regional cerebral ischemia into manifest ischemia. Furthermore, alterations of $PaCO_2$, may result in redistribution of blood flow from regions with a relatively high tissue pressure and low CO_2 reactivity to regions with a high CO_2 reactivity and relatively low tissue pressure, referred to as a Steal phenomenon. On the other hand hypocapnia may redistribute blood flow from regions with low tissue pressure and high CO_2 reactivity to regions with high tissue pressure and relatively low CO_2 reactivity, the so-called inverse steal phenomenon [97]. In a study addressing the regional CBF distribution pattern prior to and during hyperventilation in patients with FHF, we found neither steal nor inverse steal phenomena in patients with FHF (II). We also found preserved and similar CO_2 reactivity to hypocapnia in all brain regions (Figure 4) (II), which is in accordance with a retrospective study by Durham et al [58]. Thus, global as well as regional cerebral CO_2 reactivity to hypocapnia is preserved in patients with FHF.

3.2.3 Cerebral autoregulation and hyperventilation.

Cerebral autoregulation is impaired in patients with FHF [56], and is re-established shortly after recovery of liver function [98]. The reason for the impaired cerebral autoregulation in FHF is not completely settled, but has been suggested to result from gradual cerebral vasodilation [11]. This vasodilation hypothesis was supported by the observation where moderate hypocapnia ($PaCO_2$ ~3.0 kPa) restored cerebral autoregulation in five of seven patients with FHF (Figure 5) (I). On the other hand global CBF was found to be decreased compared to normal values in the subsequent studies (III-VII). This finding does not support the vasodilation theory, as responsible for impairment of autoregulation (III-VII). If vasodilation does not account for the impaired cerebral autoregulation in patients with FHF what could then be the explanation of the restoration of cerebral autoregulation following hyperventilation? Although the nature of the association between hypocapnia and cerebral autoregulation was not investigated in the present work, a number of possible explanations may be considered.

If patients prior to intubation had been hyperventilating spontaneously for some time, then the institution of mechanical normoventilation would render the patient relatively hypercapnic if mechanical ventilation was instituted at a level of $PaCO_2$ that was higher than the spontaneous $PaCO_2$. Hypercapnia shortens the autoregulatory plateau, i.e., increases the lower limit and decreases the upper limit of autoregulation, whereas hypocapnia widens the autoregulatory plateau (Figure 6). Hence, in a state of relative hypercapnia cerebral autoregulation will be abolished, while it would subsequently be restored by re-institution of hyperventilation. It cannot be excluded that relative hypercapnia was of importance for the impaired cerebral autoregulation, but in other conditions with impaired cerebral autoregulation where spontaneous hyperventilation is not a prominent feature, e.g. neurotrauma, autoregulation is also

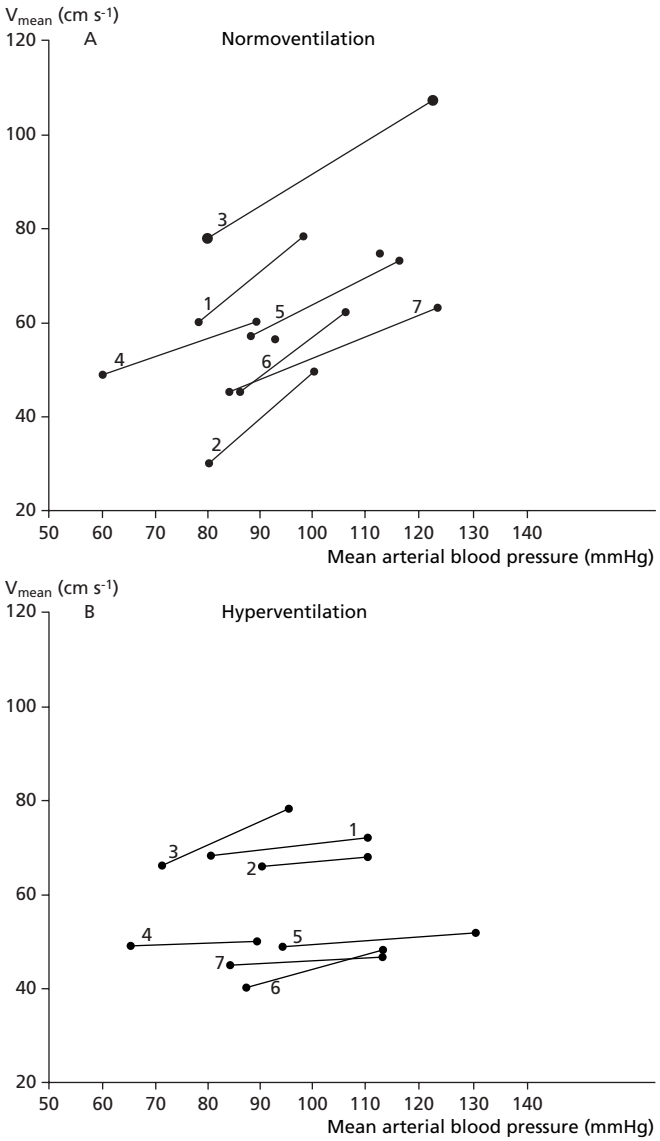

Figure 5. Cerebral autoregulation before (A) and during hyperventilation (B) in patients with FHF. Relative changes in CBF were evaluated by transcranial Doppler mean flow velocity (Vmean, cm/s) during a raise in mean arterial blood pressure (MAP). Each line represents one patient (I). Reprinted with permission from publisher. (Strauss et al, J Hepatol 1998).

restored by mechanical hyperventilation. Another explanation could be that the cerebral autoregulation curve is right-shifted, i.e., lower limit reached at a higher cerebral perfusion pressure, corresponding to a functionally impaired cerebral autoregulation. Thus, cerebral autoregulation is impaired in the "physiological" range of mean arterial blood pressures, and since hypocapnia widens the plateau of the cerebral autoregulation curve it may be restored during hyperventilation. Indeed, this could be an explanation as rats subjected to portacaval anastomosis demonstrated a right-shift of the autoregulation curve that was not affected by ammonia infusion [99]. In the study by Dethloff et al [99], the lower limit of autoregulation was ~25% right-shifted. In healthy subjects lower limit of autoregulation is ~60 mmHg, corresponding to a lower limit of ~75 mmHg if the lower limit of autoregulation is right-shifted to the same extent as in rats with portacaval anastomosis. Mean arterial blood pressure was > 75 mmHg in 6 of 7 patients with FHF (I), thus a right-shift of the autoregulation curve does not appear to be the only explanation, although it cannot be excluded that the lower limit of autoregulation is present at even higher blood pressure levels in FHF.

To conclude, loss of cerebral autoregulation and recovery during hyperventilation have been described in other diseases affecting the brain, such as acute bacterial meningitis [100-102], and acute head injury [103]. It is likely that a common denominator, which is influenced by alkalosis is responsible. Whether this common denominator is cerebrovascular tone, metabolic changes, low-grade cerebral edema, increased sympathetic tone, a right-shifted autoregulation curve or other yet undetermined factors remains to be explored.

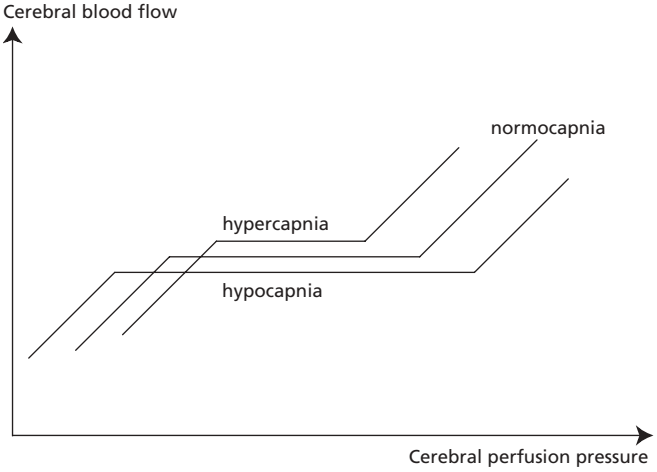

Figure 6. Influence of hypocapnia and hypercapnia on the cerebral autoregulation curve. Hypocapnia widdens the autoregulatory plateau, whereas hypercapnia shortens the autoregulatory plateau.

3.2.4 CBF and hyperventilation

The major concern of hyperventilation is that it may reduce CBF to critically low levels resulting in decreased oxygen delivery capacity. According to the data obtained in previous studies of FHF a change in $PaCO_2$ of ~1 kPa results in ~20-30% decrease in CBF (Table 3). Since the overall mean CBF reported in patients with FHF is 33-40 mL (100 g min)$^{-1}$ (IV) [12, 14, 58, 59, 104], this moderate decrease in $PaCO_2$ will reduce CBF to values ~25-32 mL (100 g min)$^{-1}$, which is well above the ischemic threshold, i.e., CBF > 18-20 mL (100 g min)$^{-1}$ reported in patients with head injury [105-107]. Furthermore, a recent study in patients with traumatic brain injury using positron emission tomography, demonstrated that although short-term hyperventilation produced large reductions in CBF, cerebral metabolism of oxygen ($CMRO_2$) remained unchanged due to the low metabolic demands and compensatory increases in oxygen extraction fraction [108]. Thus, the reduction in global CBF observed during hyperventilation in FHF is unlikely to have caused ischemia.

3.2.5 Regional CBF (rCBF) and hyperventilation

In a high resolution image study addressing regional CBF in patients with FHF (II), we found lower cerebral perfusion in frontal regions and basal ganglia as compared to the other brain regions and healthy subjects, respectively. This altered regional CBF distribution pattern is in accordance with another study of patients with FHF [58]. The regional CBF distribution pattern was not altered by moderate hyperventilation, i.e., $PaCO_2$ decreased from 4.8 (range, 3.8 to 5.5) kPa to 3.7 (range, 3.2 to 4.4) kPa (II). Thus, CBF decreased to the same extent in frontal brain regions and basal ganglia as compared to other brain regions (Figure 4), which may expose these regions to hypoperfusion during prolonged and pronounced hyperventilation. However, it is not possible from our study (II) to conclude that frontal hypoperfusion is present in FHF and that this is aggravated by moderate hyperventilation, as we did not have any measures of regional cerebral oxidative metabolism.

The presented data in this thesis have demonstrated that the frontal areas and the basal ganglia have relatively lower rCBF than the posterior regions. This distribution pattern has also been demonstrated in other conditions such as acute bacterial meningitis [109] suggesting that it is either the unconsciousness that results in this altered distribution pattern or the sedatives administered. Against administration of sedatives as the reason for the altered rCBF distribution pattern in patients with FHF speaks that the same rCBF distri-

Table 4. Previous published studies of cerebral oxidative metabolism in patients with FHF.

	Oxygen	Glucose	Lactate	OGI
Wendon (1991) [14]	0.8 (0.17-2.03)*	26.6 (1.3-102)	–2.94 (–66-3.3)	~1.4
	0.78 (0.46-1.6) *	18 (4-33)	–2.40 (–37.8-8.4)	~2.0
Aggarwal (1994) [12]	1.6 ± 0.4*	---	---	
Larsen (1996) [61]	1.4 (0.9-2.4)*	11 (4.8-20)	3.2 (0-8.9)	~5.9
Jalan (1999) [63]	0.4 (0.1-0.8)*	35.1 (7.8-88.6)	12.0 (–19.2-33.8)	~0.5
Strauss (2003) (VII)	90.7 ± 19.5	13.8 ± 3.2	3.70 ± 4.62	~7.6

All values are in μmol/100g/min except those with *) which are in mL/100 g/min.

Table 5. Previous published data on cerebral oxygen, glucose and lactate metabolism during normoventilation and hyperventilation in patients with FHF and healthy subjects.

	Moller et al (2002) [102] Healthy subjects	Wendon et al (1994) [14] FHF	Strauss et al (VII) FHF
Normoventilation			
Cerebral oxygen metabolism in μmol (100 g min)$^{-1}$	190 (140-210)	0.92 (0.11-2.23) mL (100 g min)$^{-1}$ ~43 μmol (100 g min)$^{-1}$	86 ± 18
Cerebral glucose metabolism in μmol (100 g min)$^{-1}$	37 (32-57)	27.2 (4.4-50)	11.8 ± 2.7
Cerebral lactate metabolism in μmol (100 g min)$^{-1}$	–2 (–5-4)	–1.6 (–13-7.6)	3.01 ± 3.78
Oxygen-glucose index	~5.1	~1.6	7.6 ± 1.9
Hyperventilation			
Cerebral oxygen metabolism in μmol (100 g min)$^{-1}$	190 (150-200)	0.65 (0.02-1.45) mL (100 g min)$^{-1}$ ~30 μmol (100 g min)$^{-1}$	93 ± 17
Cerebral glucose metabolism in μmol (100 g min)$^{-1}$	41 (31 – 43)	3.15 (–77-58)	11.7 ± 3.3
Cerebral lactate metabolism in μmol (100 g min)$^{-1}$	–12 (–15- –5)	–1.6 (–69-3.15)	1.85 ± 3.22
Oxygen-glucose index	~4.6	~9.5	8.0 ± 1.3

bution pattern has been found in patients with subclinical hepatic encephalopathy [110], where sedatives were not administered.

3.2.6 Cerebral oxidative metabolism and hyperventilation

In accordance with other studies on patients with FHF cerebral oxygen metabolism was reduced in patients with FHF investigated in this thesis (Table 4) (VII) [12, 14, 61, 63]. By contrast, the results on cerebral glucose metabolism in patients with FHF have ranged from reduced [14, 61] to normal [14], and even increased cerebral glucose metabolism [63]. The study with increased cerebral glucose metabolism [63] was contrary to the other studies performed on patients with ongoing intracranial hypertension. It has been suggested that ammonia may interfere with cerebral energy metabolism, including stimulation of certain glycolytic enzymes, inhibition of certain enzymes in the TCA cycle, and induction of the mitochondrial permeability transition in astrocytes leading to energy failure [78, 111]. The results on cerebral glucose metabolism in patients with FHF investigated in this thesis (VII) do not corroborate this hypothesis, as all glucose taken up by the brain was aerobically metabolized, i.e., the oxygen to glucose index was normal. It cannot be excluded that these metabolic changes takes place later during the disease course. Alterations in cerebral metabolism later during the disease course may be supported by the study by Jalan et al [63] which was performed during ongoing intracranial hypertension in patients with FHF, i.e., the oxygen to glucose index calculated from the reported cerebral metabolic rates is ~ 0.5 (Table 4). Notwithstanding there may be either a miscalculation in their reported metabolic rates or a misprint of the reported arterial and venous oxygen contents in their articles, i.e., $avDO_2$ is 3.1 mL/dL, and CBF is 103 mL/(100g min) corresponding to a $cmrO_2$ on ~ 3.2 mL/(100g min) [63]. If this recalculation of $cmrO_2$ is correct the oxygen to glucose index is ~4.2 in the patients studied by Jalan et al [63] which is indeed lower than required for aerobic glycolysis.

Only one other study has evaluated the effect of shortterm hyperventilation on cerebral oxidative metabolism in FHF (Table 5) (VII) [14]. Wendon et al [14], found a slight cerebral lactate efflux in patients with FHF and suggested that the reduction in $CMRO_2$ was inappropriate for cerebral metabolic requirements, and therefore a further reduction in CBF by hyperventilation was anticipated to be harmful for patients with FHF. In that study Wendon et al [14], reported a slight increase in cerebral lactate efflux during short-term mechanical hyperventilation in patients with FHF, i.e., the lactate-glucose index became more negative from –0.05 to – 0.5, indicating that the amount of cerebral glucose uptake, that was accounted for by lactate excretion, increased from 2.5% to 25%. In normal resting subjects, a slight cerebral efflux of lactate is normally present, and this cerebral lactate efflux increases during hyperventilation (Table 5) [102]. When indices of cerebral oxidative metabolism are calculated from the reported median values in the study by Wendon et al [14], it appears that anaerobic metabolism was present during normoventilation, i.e., the oxygen-glucose index was ≈ 1.6, while hyperventilation brought about aerobic metabolism, i.e., the oxygen-glucose index increased to ≈ 9.5 [14]. Furthermore, studies using brain microdialysis in patients with FHF [112], has demonstrated that the cerebral lactate concentration remains unchanged during moderate short-term hyperventilation. In our study (VII), cerebral oxidative metabolism was not compromised neither during normoventilation nor during hyperventilation as a striking net cerebral lactate uptake was demonstrated during normoventilation, which was only slightly decreased by hyperventilation, i.e., the lactate-oxygen index remained positive, and the cerebral oxygen to glucose index remained unchanged and normal (VII). Contrary to our findings, Wendon et al [14] found that hyperventilation produced a significant reduction both in $CMRO_2$ and cerebral glucose metabolism in patients with FHF. As can be seen from Table 5 both the $CMRO_2$ and cerebral glucose metabolism varied widely among patients with FHF in the study by Wendon et al. Since they also found negative values of cerebral glucose metabolism (Table 5) it is likely that not all of their patients were in a steady state condition at the time of their measurements that normally is required for accurate sampling of CBF and AV differences of various metabolites.

Measurements of markers of astroglial (S-100b) and neuronal (NSE) damage during hyperventilation in patients with FHF, may support that moderate hyperventilation is safe, as the arterial concentrations as well as net brain flux of these markers were unaltered (VI). A borderline ischemia that is turned into manifest ischemia will result in astroglial and neuronal cell death; thus, a cerebral efflux of these markers would have been expected to arise if hyperventilation induced severe ischemia. However, it cannot be excluded that efflux of biomarkers requires a longer time to arise. Thus, from the current studies performed in patients with FHF there is no convincing evidence that short-term hyperventilation, corresponding to a $PaCO_2$ of ~25-30 mmHg, is detrimental to global CBF and oxidative metabolism.

3.2.7 Cerebral nitrogen balance and hyperventilation

Critically ill patients are most often in a state of negative whole body nitrogen balance (IV) [113, 114] which is due in part to muscle protein wasting. In patients with FHF (IV) also the brain has a negative nitrogen balance that is primarily caused by a manifest cerebral glutamine efflux (IV). No other comparable human studies have been published on cerebral nitrogen balance in FHF or other conditions. Thus, it is not possible to conclude that this is a specific feature of FHF, but there is evidence to support that hyperammonemia causes cerebral protein breakdown, as experimental studies have shown that ammonia results in a reduced cerebral protein content [115], and loss of glial fibrillary acidic protein has been demonstrated in humans with hepatic encephalopathy [116], as well as in experimental liver failure [117].

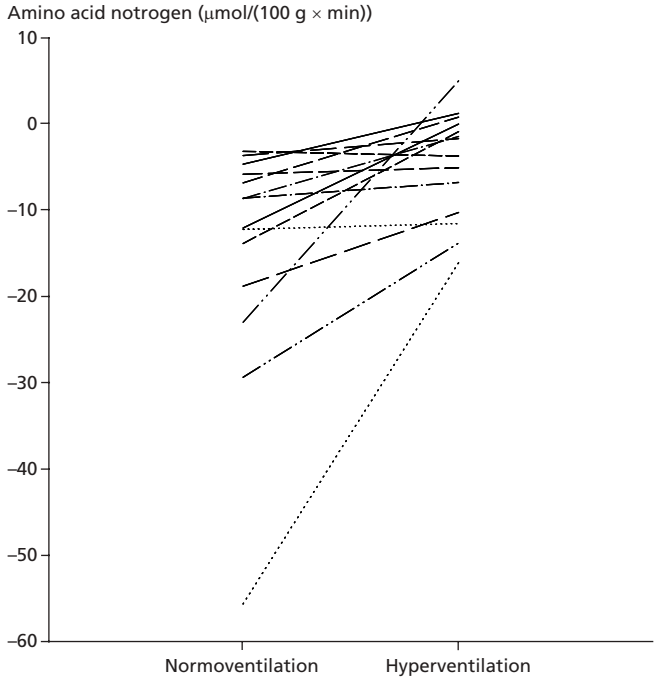

Figure 7. Cerebral amino acid nitrogen efflux in patients with FHF (n = 14) before and after short-term mechanical hyperventilation.

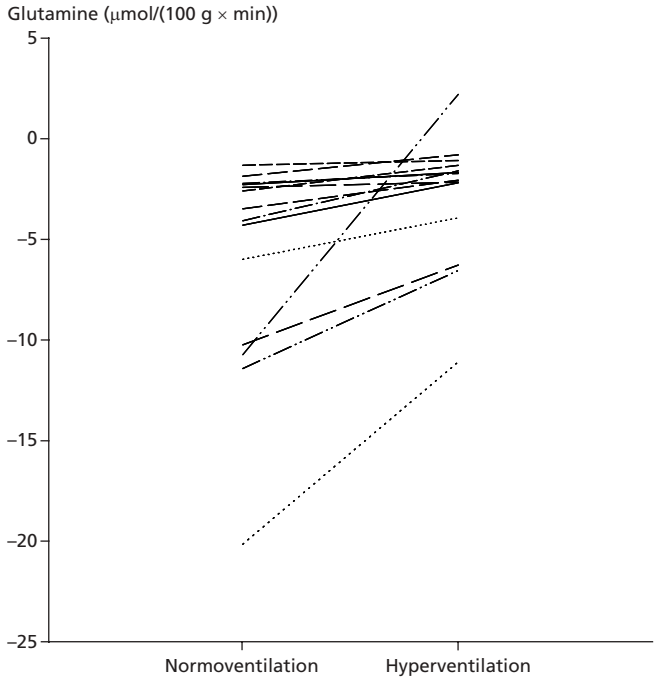

Figure 8. Cerebral glutamine efflux in patients with FHF (n = 14) before and after short-term mechanical hyperventilation.

Short-term mechanical hyperventilation significantly reduced the cerebral nitrogen release, i.e., it became less negative (from –14.81 ± 14.02 to – 4.69 ± 6.26 μmol (100g min)$^{-1}$) (Figure 7) (IV). The ameliorated cerebral nitrogen balance was primarily caused by a reduction of the cerebral glutamine efflux, i.e., from –6.11 ± 5.19 μmol (100g min)$^{-1}$ during normoventilation to – 2.91 ± 3.22 μmol (100g min)$^{-1}$ during hyperventilation (Figure 8) (IV). However, a decreased cerebral proline, alanine and tyrosine efflux, and increased cerebral uptake of branched chain amino acids, also contributed slightly to normalisation of the cerebral nitrogen balance during hyperventilation (IV). In that study, (IV) pH increased from 7.46 ± 0.05 to 7.54 ± 0.05 during hyperventilation. Since the pKa for glutamine is ~2.17 for the carboxyl group and ~9.28 for the amino group, respectively, this slight increase in pH cannot have influenced the amount of glutamine available for transport across the blood-brain barrier to a major extent, as only a small amount of glutamine will become ionised at that pH. Increased flux into the brain and/or decreased flux out of the brain could also account for the apparent reduction in glutamine efflux during hyperventilation. Recent studies have shown that active transport from brain to blood through sodium-dependent transport carrier systems is pH dependent, i.e., "flux" decreases with decreasing pH [118, 119]. Some of these transporter systems are also located on astrocytes and neurons. The astrocytic form being pH sensitive [120], while the neuronal form is not [121]. If the effect of pH on these carrier transport systems were linear, an increased transport out of brain as well as an increased astrocytic glutamine uptake would be expected to arise during alkalosis, with a resulting decrease of extracellular glutamine. We cannot exclude that pH may have influenced our results to some extent. However, alkalosis have been shown to stimulate muscle protein synthesis in critically ill patients [114], and in the perfused working heart [122]. During normoventilation we found evidence of cerebral protein degradation, as there was a net brain efflux of tyrosine and threonine, two essential amino acids. During hyperventilation this flux became zero, indicating that alkalosis may also inhibit protein degradation. A firmer conclusion on whether alkalosis stimulates protein synthesis or inhibits protein breakdown or a combination of both is not possible to determine from the data presented in this thesis.

4.0 CONCLUSIONS AND PERSPECTIVE

Only alteration of cerebral ammonia and glutamine metabolism is a prominent feature during the very early phase of the cerebral illness in patients with FHF. The cerebral oxidative metabolism was reduced in parallel with CBF, tightly matching the metabolic needs for oxidative metabolism. Although CBF is low during this early phase of FHF, our findings do not contradict that CBF gradually increases and plays a role for aggravation of cerebral edema and intracranial hypertension later during the course of FHF. Even a slight increase in CBF in patients with manifest cerebral edema can result in intracranial hypertension, as the brain is located in the rigid skull allowing for limited expansion only. Thus, the finding in the present thesis suggests that if an increase in CBF appears it is a secondary phenomenon that takes place later during the disease course, probably initiated by cerebral metabolic alterations during the early phase such as increased cerebral ammonia and glutamine fluxes.

From the present clinical studies performed in patients with FHF there was no evidence to support that short-term moderate mechanical hyperventilation is detrimental to the brain. Although global CBF was reduced further by hyperventilation, it did not compromise global cerebral oxidative metabolism. Using SPECT, we found that the perfusion in frontal brain regions and basal ganglia was lower as compared to the other brain regions. These regions had the same CO_2 reactivity to hypocapnia as the other brain regions. However, moderate hyperventilation did not reduce regional CBF under the ischemic level.

Hyperventilation restored cerebral autoregulation in most pa-

tients, which from a clinical point of view is important as it may protect the brain from fluctuations in cerebral perfusion pressure, and secondary brain damage. Also hyperventilation appeared to normalise the cerebral nitrogen balance, which indicates that hyperventilation protects the brain from cerebral protein breakdown. Although alterations in pH may have influenced the efflux of glutamine to some extent, we cannot extrapolate that alkalosis increases flux through this carrier system, since pH sensitivity of the carrier system has been performed during reduced pH only.

Thus, from the present clinical studies of FHF, institution of hyperventilation for short-term periods appears safe.

Although the present studies have addressed many of the effects of hyperventilation upon cerebral perfusion and metabolism in patients with FHF, many new questions have emerged.

In order to elucidate the chain of reactions that leads to recovery of autoregulation during hyperventilation, future studies should try to address possible factors responsible for the impairment of autoregulation, and also explore the value of therapeutic interventions such as selective cerebral vasoconstrictors, that would restore cerebral autoregulation as well as their clinical relevance. In experimental models the effect of glutamine infusion should be explored to address its effect upon cerebral autoregulation. Likewise, infusion of neuropeptides should be explored in animal models, to address whether high circulating levels of vasodilating neuropeptides can affect cerebral autoregulation. In patients with FHF, the effect of prolonged hyperventilation on cerebral autoregulation and outcome should be explored in large randomised controlled trials.

To elucidate if an exhausted vasodilatory capacity may account for the blunted cerebral hypercapnic CO_2 reactivity in FHF, the effect of infusion of vasodilating peptides, e.g., calcitonin gene-related peptide, on cerebral CO_2 reactivity should be explored in future studies. In order to address whether cerebral CO_2 reactivity to hypocapnia is lost later during the course of FHF, daily evaluation of CO_2 reactivity should be performed in patients with FHF.

To address whether short-term hyperventilation compromise regional cerebral oxidative metabolism in patients with FHF methods such as positron emission tomography and/or magnetic resonance imaging should be used. To obtain a better understanding of the metabolic effects of hyperventilation intracerebral microdialysis should be applied concomitant with arterio-venous differences of oxidative substrates and amino acids. Intracerebral microdialysis should also be applied to measure alterations in brain pH during short-term, and during prolonged hyperventilation to evaluate if and when adaptation arise.

5.0 SUMMARY IN ENGLISH

Patients with FHF have a high risk of cerebral edema and intracranial hypertension. The pathophysiological background for this phenomenon is not completely settled, but alteration in CBF as well as cerebral metabolism seems to be of importance. Mechanical hyperventilation has a prompt effect on intracranial pressure. This effect is assumed to be caused by the hypocapnia induced alkalosis which produces vasoconstriction and thereby a decrease in CBF and cerebral blood volume. It has been stated that hyperventilation may be harmful to patients with FHF, but only few studies have addressed the effect of hyperventilation upon cerebral metabolism. In the present clinical studies we evaluated the effect of short-term mechanical hyperventilation upon cerebral circulation and metabolism in patients with FHF. Although global CBF was reduced in patients with FHF it tightly matched the cerebral oxidative requirements. Already in the early phase of FHF there was a prominent cerebral efflux of glutamine that could not be accounted for by cerebral ammonia uptake. Moderate hyperventilation reduced global CBF without compromising cerebral oxidative metabolism. In addition, moderate hyperventilation restored cerebral autoregulation in most patients with FHF, and normalised the cerebral nitrogen balance during short-term interventions. Studies of global and regional cerebral carbon dioxide reactivity showed normal global as well as regional cerebral carbon dioxide reactivity in almost all patients with FHF. However, cerebral perfusion in frontal brain regions as well as basal ganglia is low in FHF as compared to healthy subjects, which may make these regions at risk of hypoperfusion during pronounced hyperventilation. It is concluded that moderate short-term hyperventilation does not compromise cerebral oxidative metabolism. Recommendation of its prolonged use in FHF awaits further studies. Furthermore, the data of this thesis demonstrates that alterations in cerebral glutamine and ammonia metabolism precedes increases of CBF, which seems to be a phenomenon that takes place later during the disease course, i.e., immediately before intracranial pressure is rising.

ABBREVIATIONS

ATP	adenosine triphosphate
$AVDO_2$	arterio-venous difference of oxygen
CO_2	carbon dioxide
CBF	cerebral blood flow
$CMRO_2$	cerebral metabolic rate of oxygen
FHF	fulminant hepatic failure
GMP	guanosine monophosphate
$PaCO_2$	arterial carbon dioxide tension
SPECT	single photon emission computed tomography
TCD	transcranial doppler sonography
V_{mean}	mean flow velocity
^{99}mTc-HMPAO	Technetium-99m Hexamethyl propyleneamine oxide

THIS THESIS IS BASED UPON THE FOLLOWING ORIGINAL PAPERS:

I Strauss G, Hansen BA, Knudsen GM, Larsen FS. Hyperventilation restores cerebral blood flow autoregulation in patients with acute liver failure. Journal of Hepatology 1998;28:199-203.

II Strauss G, Høgh P, Møller K, Knudsen GM, Hansen BA, Larsen FS. Regional cerebral blood flow during mechanical hyperventilation in patients with fulminant hepatic failure. Hepatology 1999;30:1368-73.

III Strauss GI, Møller K, Holm S, Sperling B, Knudsen GM, Larsen FS. Transcranial doppler sonography and internal jugular bulb saturation during hyperventilation in patients with fulminant hepatic failure. Liver Transplantation 2001;7(4): 352-8.

IV Strauss GI, Knudsen GM, Kondrup J, Møller K, Larsen FS. Cerebral Metabolism of Ammonia and Amino Acids in Patients with Fulminant Hepatic Failure. Gastroenterology 2001; 121(5):1109-19.

V Strauss GI, Edvinsson L, Larsen FS, Møller K, Knudsen GM. Circulating levels of Neuropeptides (CGRP, VIP, NPY) in patients with fulminant hepatic failure. Neuropeptides 2001; 35(3-4):174-80.

VI Strauss GI, Christiansen M, Møller K, Clemmesen JO, Larsen FS, Knudsen GM. S-100b and Neuron-Specific Enolase in patients with fulminant hepatic failure. Liver Transplantation 2001;7(11):964-70.

VII Strauss GI, Møller K, Larsen FS, Kondrup J, Knudsen GM. Cerebral glucose and oxygen metabolism decrease in parallel in patients with fulminant hepatic failure. Liver Transplantation 2003;9(12):1244-52.

REFERENCES

1. Miescher-Rüsch F. Bemerkungen zur lehre von den athembewegungen. Archs Anat Physiol, Physiol. 1855;355-61.
2. Donders FC. Die bewegungen des gehirns und die Veranderungen der gefassfullung dere pia mater. Schmid Fahrbucher. 1851;69:16-20.
3. Wolff HG, Lennox WG. The cerebral circulation: XII. The effects on pial

vessels of variations in the O_2 and CO_2 content of the blood. Arch Neurol Psychiatr (Chicago). 1930;23:1097-120.
4. Kety SS, Schmidt CF. The effects of arterial tensions of carbon dioxide and oxygen on the cerebral blood flow and carbon dioxide consumption of normal young men. J Clin Invest. 1948;27:484-92.
5. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 1989;97:439-45.
6. Brusilow SW, Traystman RJ. Letter to the Editor. N Engl J Med. 1986;314:786
7. Ganz R, Swain M, Traber P, DalCanto M, Butterworth RF, Blei AT. Ammonia-induced swelling of rat cerebral cortical slices: implications for the pathogenesis of brain edema in acute hepatic failure. Metab Brain Dis. 1989;4:213-23.
8. Swain M, Butterworth RF, Blei AT. Ammonia and related amino acids in the pathogenesis of brain edema in acute ischemic liver failure in rats. Hepatology. 1992;15:449-53.
9. Norenberg MD, Bender AS. Astrocyte swelling in liver failure: role of glutamine and benzodiazepines. Acta Neurochir Suppl (Wien). 1994;60:24-7.
10. Dempsey RJ, Kindt GW. Experimental acute hepatic encephalopathy: relationship of pathological cerebral vasodilation to increased intracranial pressure. Neurosurgery. 1982;10:737-41.
11. Larsen FS. Cerebral circulation in liver failure: Ohm's law in force. Semin Liver Dis. 1996;16:281-92.
12. Aggarwal S, Kramer D, Yonas H, et al. Cerebral hemodynamic and metabolic changes in fulminant hepatic failure: a retrospective study. Hepatology. 1994;19:80-7.
13. Stanley NN, Salisbury BG, McHenry LCJ, Cherniack NS. Effect of liver failure on the response of ventilation and cerebral criculation to carbon dioxide in man and in the goat. Clin Sci Mol Med. 1975;49:157-69.
14. Wendon JA, Harrison PM, Keays R, Williams R. Cerebral blood flow and metabolism in fulminant liver failure. Hepatology. 1994;19:1407-13.
15. Takano T, Nagatsuka K, Ohnishi Y, et al. Vascular response to carbon dioxide in areas with and without diaschisis in patients with small, deep hemispheric infarction. Stroke 1988;19:840-5.
16. Brubakk AM, Oh W, Stonestreet BS. Prolonged hypercarbia in the awake newborn piglet: effect on brain blood flow and cardiac output. Pediatr Res. 1987;21:29-33.
17. Kontos HA, Raper AJ, Patterson JL. Analysis of vasoactivity of local pH, PCO_2 and bicarbonate on pial vessels. Stroke 1977;8:358-60.
18. Kontos HA, Wei EP, Raper AJ, Patterson JLJ. Local mechanism of CO_2 action of cat pial arterioles. Stroke 1977;8:226-9.
19. Pickard JD, MacKenzie ET. Inhibition of prostaglandin synthesis and the response of baboon cerebral circulation to carbon dioxide. Nat New Biol. 1973;245:187-8.
20. Wang Q, Bryowsky J, Minshall RD, Pelligrino DA. Possible obligatory functions of cyclic nucleotides in hypercapnia-induced cerebral vasodilation in adult rats. Am J Physiol. 1999;276:H480-H487
21. Endoh H, Honda T, Komura N, Shibue C, Watanabe I, Shimoji K. Effects of nicardipine-, nitroglycerin-, and prostaglandin E1-induced hypotension on human cerebrovascular carbon dioxide reactivity during propofol-fentanyl anesthesia. J Clin Anesth. 1999;11:545-9.
22. Wang Q, Paulson OB, Lassen NA. Effect of nitric oxide blockade by NG-nitro-L-arginine on cerebral blood flow response to changes in carbon dioxide tension. J Cereb Blood Flow Metab. 1992;12:947-53.
23. Iadecola C. Nitric oxide participates in the cerebrovasodilation elicited from cerebellar fastigial nucleus. Am J Physiol. 1992;263:R1156-R1161
24. Gotoh F, Tazaki Y, Meyer JS. Transport of gases through brain and their extravascular vasomotor action. Exp Neurol. 1961;4:48-58.
25. Skinhoj E. Regulation of cerebral blood flow as a single function of the interstitial pH in the brain. A hypothesis. Acta Neurol Scand. 1966;42: 604-7.
26. Severinghaus JW, Lassen N. Step hypocapnia to separate arterial from tissue PCO_2 in the regulation of cerebral blood flow. Circ Res. 1967;20: 272-8.
27. Busija DW, Heistad DD. Factors involved in the physiological regulation of the cerebral circulation. Rev Physiol Biochem Pharmacol. 1984;101: 161-211.
28. Warner DS, Turner DM, Kassell NF. Time-dependent effects of prolonged hypercapnia on cerebrovascular parameters in dogs: acid-base chemistry. Stroke 1987;18:142-9.
29. Harder DR. Effect of H^+ and elevated PCO_2 on membrane electrical properties of rat cerebral arteries. Pflugers Arch. 1982;394:182-5.
30. Toda N, Hatano Y, Mori K. Mechanisms underlying response to hypercapnia and bicarbonate of isolated dog cerebral arteries. Am J Physiol. 1989;257:H141-H146
31. Kontos HA, Wei EP. Arginine analogues inhibit responses mediated by ATP-sensitive K+ channels. Am J Physiol. 1996;271:H1498-H1506
32. Faraci FM, Sobey CG. Role of potassium channels in regulation of cerebral vascular tone. J Cereb Blood Flow Metab. 1998;18:1047-63.
33. Wei EP, Kontos HA. Blockade of ATP-sensitive potassium channels in cerebral arterioles inhibits vasoconstriction from hypocapnic alkalosis in cats. Stroke 1999;30:851-3.
34. Nakahata K, Kinoshita H, Hirano Y, Kimoto Y, Iranami H, Hatano Y. Mild hypercapnia induces vasodilation via adenosine triphosphate-sensitive K+ channels in parenchymal microvessels of the rat cerebral cortex. Anesthesiology 2003;99:1333-9.
35. Sakabe T, Siesjo BK. The effect of indomethacin on the blood flow-metabolism couple in the brain under normal, hypercapnic and hypoxic conditions. Acta Physiol Scand. 1979;107:283-4.
36. Dahlgren N, Siesjo BK. Effects of indomethacin on cerebral blood flow and oxygen consumption in barbiturate-anesthetized Normocapnic and hypercapnic rats. J Cereb Blood Flow Metab. 1981;1:109-15.
37. Cuypers J, Cuevas A, Duisberg R. Effect of indomethacin on CO_2-induced hyperaemia (CO_2-response) in the rabbit brain. Neurochirurgia (Stuttg). 1978;21:62-6.
38. Wei EP, Ellis EF, Kontos HA. Role of prostaglandins in pial arteriolar response to CO_2 and hypoxia. Am J Physiol. 1980;238:H226-H230
39. Mirro R, Pharris LJ, Armstead WM, Shibata M, Leffler CW. Effects of indomethacin on newborn pig pial arteriolar responses to PCO_2. J Appl Physiol. 1993;75:1300-5.
40. Faraci FM, Heistad DD. Regulation of the cerebral circulation: role of endothelium and potassium channels. Physiol Rev. 1998;78:53-97.
41. Iadecola C, Zhang F. Nitric oxide-dependent and – independent components of cerebrovasodilation elicited by hypercapnia. Am J Physiol. 1994;266:R546-R552
42. Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. J Cereb Blood Flow Metab. 1994;14:175-92.
43. Paterno R, Faraci FM, Heistad DD. Role of Ca(2+)-dependent K+ channels in cerebral vasodilatation induced by increases in cyclic GMP and cyclic AMP in the rat. Stroke. 1996;27:1603-7.
44. Wolff HG. The cerebral circulation. Physiol Rev. 1936;16:545-96.
45. Moore LE, Kirsch JR, Helfaer MA, Greenberg RS, Traystman RJ. Hypercapnic blood flow reactivity not increased by alpha-blockade or cordotomy in piglets. Am J Physiol. 1992;262:H1884-H1890
46. Kety SS, Schmidt CF. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. J Clin Invest. 1948;27:476-83.
47. Madsen PL, Holm S, Herning M, Lassen NA. Average blood flow and oxygen uptake in the human brain during resting wakefulness: a critical appraisal of the Kety-Schmidt technique. J Cereb Blood Flow Metab. 1993;13:646-55.
48. Lassen NA. Cerebral blood flow and oxygen consumption in man. Physiol Rev. 1959;39:183-238.
49. Holm, S. Dynamic SPECT of the human brain, for measurement of regional cerebral blood flow, a critical appraisal. 1-174. 1988. Copenhagen University. Thesis.
50. Vorstrup S. Tomographic cerebral blood flow measurements in patients with ischemic cerebrovascular disease and evaluation of the vasodilatory capacity by the acetazolamide test. Acta Neurol Scand Suppl. 1988;77:5-48.
51. Andersen AR, Friberg H, Knudsen KB, et al. Extraction of [99mTc]-d,l-HM-PAO across the blood-brain barrier. J Cereb Blood Flow Metab. 1988;8:S44-S51
52. Andersen AR, Friberg H, Lassen NA, Kristensen K, Neirinckx RD. Serial studies of cerebral blood flow using 99Tcm-HMPAO: a comparison with 133Xe. Nucl Med Commun. 1987;8:549-57.
53. Aaslid R, Markwalder TM, Nornes H. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. J Neurosurg. 1982;57:769-74.
54. Lindegaard KF, Bakke SJ, Grolimund P, Aaslid R, Huber P, Nornes H. Assessment of intracranial hemodynamics in carotid artery disease by transcranial Doppler ultrasound. J Neurosurg. 1985;63:890-8.
55. Larsen FS, Olsen KS, Hansen BA, Paulson OB, Knudsen GM. Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. Stroke. 1994;25:1985-8.
56. Larsen FS, Ejlersen E, Hansen BA, Knudsen GM, Tygstrup N, Secher NH. Functional loss of cerebral blood flow autoregulation in patients with fulminant hepatic failure. J Hepatol. 1995;23:212-7.
57. Robertson CS, Narayan RK, Gokaslan ZL, et al. Cerebral arteriovenous oxygen difference as an estimate of cerebral blood flow in comatose patients. J Neurosurg. 1989;70:222-30.
58. Durham S, Yonas H, Aggarwal S, Darby J, Kramer D. Regional cerebral blood flow and CO_2 reactivity in fulminant hepatic failure. J Cereb Blood Flow Metab. 1995;15:329-35.
59. Almdal T, Schroeder T, Ranek L. Cerebral blood flow and liver function in patients with encephalopathy due to acute and chronic liver diseases. Scand J Gastroenterol. 1989;24:299-303.
60. Aggarwal S, Yonas H, Kang Y, et al. Relationship of cerebral blood flow and cerebral swelling to outcome in patients with acute fulminant hepatic failure. Transplant Proc. 1991;23:1978-9.
61. Larsen FS, Ejlersen E, Clemmesen JO, Kirkegaard P, Hansen BA. Preser-

vation of cerebral oxidative metabolism in fulminant hepatic failure: an autoregulation study. Liver Transpl Surg. 1996;2:348-53.
62. Ede R, Gimson AE, Cannalese J, Williams R. Cerebral oedema and monitoring of intracranial pressure in fulminant hepatic failure. Gastroenterol Jpn. 1982;17:163-76.
63. Jalan R, Damink SW, Deutz NE, Lee A, Hayes PC. Moderate hypothermia for uncontrolled intracranial hypertension in acute liver failure. Lancet. 1999;354:1164-8.
64. Jalan R, Olde Damink S, Deutz NE, Hayes PC, Lee A. Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. Gastroenterology. 2004;127:1338-46.
65. Jalan R, Olde Damink S, Hayes PC, Deutz NE, Lee A. Pathogenesis of intracranial hypertension in acute liver failure: inflammation, ammonia and cerebral blood flow. J Hepatol. 2004;41:613-20.
66. Madsen PL, Sperling BK, Warming T, et al. Middle cerebral artery blood velocity and cerebral blood flow and O2 uptake during dynamic exercise. J Appl Physiol. 1993;74:245-50.
67. Chung C, Gottstein J, Blei AT. Indomethacin prevents the development of experimental ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology. 2001;34:249-54.
68. Schmidt CF, Kety SS, Pennes HH. The gasseous metabolism of the brain of the monkey. Am J Physiol. 1945;143:33-52.
69. Deutsch G, Eisenberg HM. Frontal blood flow changes in recovery from coma. J Cereb Blood Flow Metab. 1987;7:29-34.
70. Madsen PL, Schmidt JF, Wildschiodtz G, et al. Cerebral O2 metabolism and cerebral blood flow in humans during deep and rapid-eye-movement sleep. J Appl Physiol. 1991;70:2597-601.
71. Forster A, Juge O, Morel D. Effects of Midazolam on cerebral blood flow in human volunteers. Anesthesiology. 1982;56:453-5.
72. Sari A, Fukuda Y, Sakabe T, Maekawa T, Ishikawa T. Effects of psychotropic drugs on canine cerebral metabolism and circulation related to EEG-diazepam, clomipramine, and chlorpromazine. J Neurol Neurosurg Psychiatry. 1975;38:838-44.
73. Knudsen L, Cold GE, Holdgard HO, Johansen UT, Jensen S. The effects of midazolam on cerebral blood flow and oxygen consumption. Anaesthesia. 1990;45:1016-9.
74. Hazell AS, Butterworth RF. Hepatic encephalopathy: An update of pathophysiologic mechanisms. Proc Soc Exp Biol Med. 1999;222:99-112.
75. Gjedde A, Lockwood AH, Duffy TE, Plum F. Cerebral blood flow and metabolism in chronically hyperammonemic rats: effect of an acute ammonia challenge. Ann Neurol. 1978;3:325-30.
76. Chatauret N, Zwingmann C, Rose C, Leibfritz D, Butterworth RF. Effects of Hypothermia on brain glucose metabolism in acute liver failure: a $^{1}H/^{13}C$-nuclear magnetic resonance study. Gastroenterology. 2003;125:815-24.
77. Master S, Gottstein J, Blei AT. Cerebral blood flow and the development of ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology. 1999;30:876-80.
78. Rao KV, Norenberg MD. Cerebral energy metabolism in hepatic encephalopathy and hyperammonemia. Metab Brain Dis. 2001;16:67-78.
79. Kala G, Hertz L. Ammonia effects on pyruvate/lactate production in astrocytes-Interaction with glutamate. Neurochem Int. 2005;47:4-12.
80. Knecht K, Michalak A, Rose C, Rothstein JD, Butterworth RF. Decreased glutamate transporter (GLT-1) expression in frontal cortex of rats with acute liver failure. Neurosci Lett. 1997;229:201-3.
81. Kosenko E, Kaminsky Y, Grau E, et al. Brain ATP depletion induced by acute ammonia intoxication in rats is mediated by activation of the NMDA receptor and Na+,K(+)-ATPase. J Neurochem. 1994;63:2172-8.
82. Rama Rao KV, Jayakumar AR, Norenberg MD. Induction of the mitochondrial permeability transition in cultured astrocytes by glutamine. Neurochem Int. 2003;43:517-23.
83. Kis B, Snipes JA, Simandle SA, Busija DW. Acetaminophen-sensitive prostaglandin production in rat cerebral endothelial cells. Am J Physiol Regul Integr Comp Physiol. 2005;288:R897-R902
84. Larsen FS, Adel HB, Pott F, et al. Dissociated cerebral vasoparalysis in acute liver failure. A hypothesis of gradual cerebral hyperaemia. J Hepatol. 1996;25:145-51.
85. Sari A, Yamashita S, Ohosita S, et al. Cerebrovascular reactivity to CO_2 in patients with hepatic or septic encephalopathy. Resuscitation. 1990;19:125-34.
86. McPherson RW, Eimerl D, Traystman RJ. Interaction of hypoxia and hypercapnia on cerebral hemodynamics and brain electrical activity in dogs. Am J Physiol. 1987;253:H890-H897.
87. Altenau LL, Kindt GW. Cerebral vasomotor paralysis produced by ammonia intoxication. Acta Neurol Scand Suppl. 1977;64:346-7.
88. Chodobski A, Szmydynger-Chodobska J, Skolasinska K. Effect of ammonia intoxication on cerebral blood flow, its autoregulation and responsiveness to carbon dioxide and papaverine. J Neurol Neurosurg Psychiatry. 1986;49:302-9.
89. Barzilay Z, Britten AG, Koehler RC, Dean JM, Traystman RJ. Interaction of CO_2 and ammonia on cerebral blood flow and O_2 consumption in dogs. Am J Physiol. 1985;248:H500-H507
90. Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P. Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology. 1999;29:648-53.
91. Okada T, Watanabe Y, Brusilow SW, Traystman RJ, Koehler RC. Interaction of glutamine and arginine on cerebrovascular reactivity to hypercapnia. Am J Physiol. 2000;278:H1584.
92. Tofteng F, Hauerberg J, Hansen BA, Pedersen CB, Jorgensen L, Larsen FS. Persistent arterial hyperammonemia increases the concentration of glutamine and alanine in the brain and correlates with intracranial pressure in patients with fulminant hepatic failure. J Cereb Blood Flow Metab. 2006;26:21-7.
93. Jalan R, Olde DS, Deutz NE, Hayes PC, Lee A. Restoration of cerebral blood flow autoregulation and reactivity to carbon dioxide in acute liver failure by moderate hypothermia. Hepatology. 2001;34:50-4.
94. McCulloch J, Edvinsson L. Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide. Am J Physiol. 1980;238:H449-H456
95. Beattie DT, McNeil DK, Connor HE. The influence of neurokinins and calcitonin gene-related peptide on cerebral blood flow in anaesthetized guinea-pigs. Neuropeptides. 1993;24:343-9.
96. Hong KW, Pyo KM, Lee WS, Yu SS, Rhim BY. Pharmacological evidence that calcitonin gene-related peptide is implicated in cerebral autoregulation. Am J Physiol. 1994;266:H11-H16
97. Darby JM, Yonas H, Marion DW, Latchaw RE. Local "inverse steal" induced by hyperventilation in head injury. Neurosurgery. 1988;23:84-8.
98. Strauss G, Adel HB, Kirkegaard P, Rasmussen A, Hjortrup A, Larsen FS. Liver function, cerebral blood flow autoregulation, and hepatic encephalopathy in fulminant hepatic failure. Hepatology. 1997;25:837-9.
99. Dethloff T, Knudsen GM, Hansen BA, Larsen FS. Effects of porta-systemic shunting and ammonia infusion on cerebral blood flow autoregulation in the rat. Neurocrit Care. 2005;3:86-90.
100. Paulson OB, Olesen J, Christensen MS. Restoration of autoregulation of cerebral blood flow by hypocapnia. Neurology. 1972;22:286-93.
101. Moller K, Skinhoj P, Knudsen GM, Larsen FS. Effect of short-term hyperventilation on cerebral blood flow autoregulation in patients with acute bacterial meningitis. Stroke. 2000;31:1116-22.
102. Moller K, Strauss GI, Thomsen G, et al. Cerebral blood flow, oxidative metabolism and cerebrovascular carbon dioxide reactivity in patients with acute bacterial meningitis. Acta Anaesthesiol Scand. 2002;46:567-78.
103. Ma X, Willumsen L, Hauerberg J, Pedersen DB, Juhler M. Effects of graded hyperventilation on cerebral blood flow autoregulation in experimental subarachnoid hemorrhage. J Cereb Blood Flow Metab. 2000;20:718-25.
104. Larsen FS, Hansen BA, Ejlersen E, et al. Cerebral blood flow, oxygen metabolism and transcranial Doppler sonography during high-volume plasmapheresis in fulminant hepatic failure. Eur J Gastroenterol Hepatol. 1996;8:261-5.
105. Yonas H, Sekhar L, Johnson DW, Gur D. Determination of irreversible ischemia by xenon-enhanced computed tomographic monitoring of cerebral blood flow in patients with symptomatic vasospasm. Neurosurgery. 1989;24:368-72.
106. Cold GE, Jensen FT. Cerebral blood flow in the acute phase after head injury. Part 1: Correlation to age of the patients, clinical outcome and localisation of the injured region. Acta Anaesthesiol Scand. 1980;24: 245-51.
107. Obrist WD, Langfitt TW, Jaggi JL, Cruz J, Gennarelli TA. Cerebral blood flow and metabolism in comatose patients with acute head injury. Relationship to intracranial hypertension. J Neurosurg. 1984;61:241-53.
108. Diringer MN, Videen TO, Yundt K, et al. Regional cerebrovascular and metabolic effects of hyperventilation after severe traumatic brain injury. J Neurosurg. 2002;96:103-8.
109. Moller K, Hogh P, Larsen FS, et al. Regional cerebral blood flow during hyperventilation in patients with acute bacterial meningitis. Clin Physiol. 2000;20:399-410.
110. Catafau AM, Kulisevsky J, Berna L, et al. Relationship between cerebral perfusion in frontal-limbic-basal ganglia circuits and neuropsychologic impairment in patients with subclinical hepatic encephalopathy. J Nucl Med. 2000;41:405-10.
111. Hindfelt B, Plum F, Duffy TE. Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J Clin Invest. 1977;59:386-96.
112. Tofteng F, Jorgensen L, Hansen BA, Ott P, Kondrup J, Larsen FS. Cerebral microdialysis in patients with fulminant hepatic failure. Hepatology. 2002;36:1333-40.
113. Clemmesen JO, Kondrup J, Ott P. Splanchnic and leg exchange of amino acids and ammonia in acute liver failure. Gastroenterology. 2000;118:1131-9.
114. Vosswinkel JA, Brathwaite CE, Smith TR, Ferber JM, Casella G, Garlick PJ. Hyperventilation increases muscle protein synthesis in critically ill trauma patients. J Surg Res. 2000;91:61-4.
115. Subrahmanyam K, Prasad MS, Rangavalli G, Muralidhar K, Sadasivudu B. Functional relationship of ammonia to DNA, RNA and protein in brain. Neuroscience. 1985;15:887-90.

116. Sobel RA, DeArmond SJ, Forno LS, Eng LF. Glial fibrillary acidic protein in hepatic encephalopathy. An immunohistochemical study. J Neuropathol Exp Neurol. 1981;40:625-32.
117. Neary JT, Whittemore SR, Zhu Q, Norenberg MD. Destabilization of glial fibrillary acidic protein mRNA in astrocytes by ammonia and protection by extracellular ATP. J Neurochem. 1994;63:2021-7.
118. Xiang J, Ennis SR, Abdelkarim GE, Fujisawa M, Kawai N, Keep RF. Glutamine transport at the blood-brain and blood-cerebrospinal fluid barriers. Neurochem Int. 2003;43:279-88.
119. Xiang J, Fowkes RL, Keep RF. Choroid plexus histidine transport. Brain Res. 1998;783:37-43.
120. Nagaraja TN, Brookes N. Glutamine transport in mouse cerebral astrocytes. J Neurochem. 1996;66:1665-74.
121. Tamarappoo BK, Raizada MK, Kilberg MS. Identification of a system N-like Na(+)-dependent glutamine transport activity in rat brain neurons. J Neurochem. 1997;68:954-60.
122. Fuller SJ, Gaitanaki J, Sugden PH. Effects of increasing extracellular pH on protein synthesis and protein degradation in the perfused working heart. Biochem J. 1989;259:173-9.