Department of Medicine
Division of Nephrology
Helsinki University Central Hospital
Helsinki, Finland

and

Folkhälsan Research Center
Folkhälsan Institute of Genetics
University of Helsinki
Helsinki, Finland

# Effects of Metabolic Risk Factors and Type 1 Diabetes on Brain Glucose and Brain Metabolites

Outi Susanne Heikkilä

ACADEMIC DISSERTATION

To be presented,
with permission of the Medical Faculty of the University of Helsinki,
for public examination in Biomedicum Helsinki, Auditorium 2,
on Saturday, October 23rd 2010, at 12 noon.

Helsinki 2010

Supervisors

Docent Sari Mäkimattila
Folkhälsan Research Center
University of Helsinki
Helsinki, Finland

Docent Per-Henrik Groop
Department of Medicine
Helsinki University Central Hospital
and
Folkhälsan Research Center
University of Helsinki
Helsinki, Finland

Reviewers

Docent Juhana Hakumäki
Department of Clinical Radiology
Kuopio University Hospital
Kuopio, Finland

Docent Lauri Soinne
Department of Neurology
Helsinki University Central Hospital
Helsinki, Finland

Official opponent

Professor Johan Eriksson
Department of General Practice
and Primary Health Care
University of Helsinki
Helsinki, Finland

ISBN 978-952-92-7881-7 (paperback)
ISBN 978-952-10-6437-1 (PDF)

Yliopistopaino
Helsinki 2010

# CONTENTS

## LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications:

I. Heikkilä O, Lundbom N, Timonen M, Groop P-H, Heikkinen S, Mäkimattila S. Risk for metabolic syndrome predisposes to alterations in the thalamic metabolism. Metabolic Brain Disease 23:315–324, 2008

II. Heikkilä O, Lundbom N, Timonen M, Groop P-H, Heikkinen S, Mäkimattila S. Hyperglycemia is associated with changes in the regional concentrations of glucose and *myo*-inositol within the brain. Diabetologia 52:534–540, 2009

III. Heikkilä O, Lundbom N, Timonen M, Groop P-H, Heikkinen S, Mäkimattila S. Evidence for abnormal glucose uptake or metabolism in thalamus during acute hyperglycemia in type 1 diabetes – a $^{1}$H MRS study. Metabolic Brain Disease 25:227-234, 2010

IV. Heikkilä O, Lundbom N, Timonen M, Groop P-H, Heikkinen S, Mäkimattila S. Cerebellar glucose during fasting and acute hyperglycemia in nondiabetic men and in men with type 1 diabetes. Cerebellum [Epub ahead of print] 2010 March 26th

# ABBREVIATIONS

| | |
|---|---|
| $^{1}H$ | Proton |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| BBB | blood-brain barrier |
| Cho | choline-containing compounds |
| CRP | C-reactive protein |
| DTI | diffusion-tensor imaging |
| DWI | diffusion-weighted imaging |
| ECF | extracellular fluid |
| FID | free induction decay |
| GLUT | glucose transporter |
| Glx | glutamate-containing compounds |
| $HbA_{1c}$ | glycosylated hemoglobin |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| IL-6 | interleukine 6 |
| MAGE | mean amplitude of glycemic excursions |
| mI | *myo*-inositol |
| MRI | magnetic resonance imaging |
| MRS | magnetic resonance spectroscopy |
| NAA | N-acetylaspartate |
| OGTT | oral glucose tolerance test |
| PET | positron emission tomography |
| ppm | parts per million |
| ROS | reactive oxygen species |
| sICAM | soluble intercellular adhesion molecule |
| SNR | signal-to-noise ratio |
| tCr | total creatine |
| TE | echo time |
| TR | repetition time |

# ABSTRACT

## Background and Aims

The metabolic syndrome and type 1 diabetes are associated with brain alterations such as cognitive decline, increased risk for infarction, atrophy, and white matter lesions. Despite the clinical importance of these alterations, their pathomechanism is still poorly understood. This study was conducted to investigate brain glucose and metabolites in healthy individuals with an accumulation of metabolic cardiovascular risk factors and in patients with type 1 diabetes in order to discover more information on the nature of the known brain alterations.

## Subjects and Methods

We studied 43 non-smoking 20- to 45-year-old men. Study I compared two groups of non-diabetic men, one with an accumulation of metabolic risk factors and another without. Studies II to IV compared patients with type 1 diabetes (duration of diabetes 6.7 ± 5.2 years, no microvascular complications) with non-diabetic participants. Brain glucose, N-acetylaspartate (NAA), total creatine (tCr), choline, and *myo*-inositol (mI) were quantified with proton magnetic resonance spectroscopy in the frontal cortex, frontal white matter, thalamus, and cerebellar white matter. Data collection was performed for all participants at baseline after an overnight fast and in subgroups of diabetic and non-diabetic participants (Studies III and IV), also twice during a 2-h hyperglycemic normoinsulinemic clamp that increased plasma glucose concentration by 12 mmol/l.

## Results

In the men with metabolic risk factors (Study I), the thalamic tCr was 17% higher than in the control group and correlated with fasting plasma glucose and with the 2 h plasma glucose in an oral glucose tolerance test. During basal fasting glycemia (Study II), regional variation in the brain glucose levels appeared in the non-diabetic subjects but not in those with type 1 diabetes. Excess glucose had accumulated predominantly in the white matter where the metabolite alterations were also the most pronounced. Compared to the controls' values, the white matter NAA was 6% lower and the mI 20% higher. During acute hyperglycemia (Studies III and IV), the increase in cerebral glucose content in the patients with type 1 diabetes was, dependent on brain region, between 1.1 and 2.0 mmol/l. While chronic hyperglycemia had led to accumulation of glucose in the white matter, acute hyperglycemia burdened predominantly the gray matter. Acute hyperglycemia also revealed alterations in glucose uptake or metabolism in the thalamus of the diabetic patients. Type 1 diabetes showed no effect on the glucose content at baseline or during acute hyperglycemia in the cerebellum. Unlike the cerebral white matter, the cerebellar white matter showed no alterations in brain metabolites.

## Conclusions

Risk factors of the metabolic syndrome, most importantly insulin resistance, may influence brain metabolism. Normal brain glucose concentration differs between brain regions. In type 1 diabetes, hyperglycemia raises the cerebral glucose concentration in various brain regions depending on its duration: chronically in the white matter and acutely in the cortical gray matter. In the cerebellum, glucose concentration is twice its concentration in the cerebrum, with type 1 diabetes having no effect. The metabolic brain alterations in type 1 diabetes appear before any peripheral microvascular complications are detectable. Hyperglycemia is therefore a potent risk factor for diabetic brain disease.

# 1 INTRODUCTION

The metabolic syndrome consists of a cluster of cardiovascular risk factors such as abdominal obesity, hypertension, dyslipidemia, and impaired glucose regulation. Its prevalence in the 39-year-old Finnish population has been assessed as ranging from 14 to 25% (1). The number of patients with type 1 diabetes in Finland is approximately 45 000, a number rapidly increasing (2, 3). The metabolic syndrome as well as type 1 diabetes have both been associated with various brain alterations.

The accumulation of metabolic risk factors in midlife predicts cognitive decline later in life. Hypertension alone causes a 28 to 36% additional risk for cognitive decline (4), and in keeping with this, the metabolic syndrome and its separate components also elevate the risk for asymptomatic brain infarctions and stroke (5-7). In addition, obesity, hypertension, and low-grade inflammation have been associated with brain atrophy and white matter lesions. Patients that fulfilll all criteria of the metabolic syndrome have decreased nerve fiber integrity in their brain white matter (8).

The harmful effect of diabetes on cognitive function was recognized as early as 1922. The most recent meta-analysis showed that memory and learning are spared, but that the patients may be less able to apply acquired knowledge flexibly in a new situation (9). Type 1 diabetes also elevates the risk for lacunar infarctions seven-fold and stroke five-fold (10). Brain atrophy and white matter lesions have associations with type 1 diabetes (11) although to a lesser degree than with the metabolic syndrome.

Despite their obvious importance, the mechanisms of these metabolic-syndrome- and diabetes-related brain abnormalities are poorly known. The general belief regarding diabetes-related cognitive decline has been that the most important risk factors are the repetitive episodes of hypoglycemia. However, recent studies have shown that chronic hyperglycemia may be an even stronger risk factor for normal brain function. The proposed mechanisms for metabolic risk factors and diabetes to harm brain tissue are macro- and microvascular disease, hyperinsulinemia, and toxic effects of glucose.

This study was conducted in order to investigate brain glucose and metabolites in healthy individuals with an accumulation of metabolic cardiovascular risk factors and in patients with type 1 diabetes, and in order to provide more information on the nature of the known brain alterations.

# 2 REVIEW OF THE LITERATURE

## 2.1. The Brain

The brain is composed of the cerebrum, the cerebellum, and the brain stem. Brain tissue consists of gray and white matter. The gray matter regions that are responsible for various cognitive functions consist mainly of neuronal cell bodies that are the executive units in nerve signaling. The white matter carries the nerve signals between the gray matter regions and contains mainly myelinated nerve fibers, the axons. Lipid-rich myelin sheets surround the neural axons and serve to accelerate the transduction of the electrochemical signals. Glial cells, i.e. oligodendrocytes, astrocytes, ependymal cells, and microglia, make up about half of the brain cells and play an important role in their structural and nutritive support, in waste disposal, and in neuronal repair and regeneration.

The brain evolves throughout life. Its maturation continues until the end of the second decade, and the process of aging starts during the fifth decade when cognitive deterioration (12) and morphological changes (13, 14) become more common. These changes are further accelerated by life style, smoking (15, 16), and alcohol consumption (17).

The normal age-related morphological changes include reduced brain weight and volume, and ventricular and sulcal expansion (14). The white matter declines in volume to a greater extent than does the gray matter (18). In the white matter, magnetic resonance imaging (MRI) reveals an increasing number and volume of T2-hyperintense foci, i.e. white matter lesions. These lesions are suggested to be benign, but they may also result from an alteration in the autoregulation of the cerebral blood flow that exposes the tissue to brief and repeated episodes of hypotension and hypoperfusion (19). Differential histopathology also includes vascular malformations, congenital diverticula of the ventricle, demyelination, and isolated white matter infarctions (20). The prevalence of white matter lesions has been estimated to be 40% in 30- to 40-year-old adults and increases rapidly after 50 years of age (21) so that in old age the majority are affected (22).

Aging proceeds with a clear anterior to posterior gradient, with frontal lobe volumes showing a greater decline than the more posterior ones (14, 23). In addition, the white matter lesion burden (23) and decreased nerve fiber integrity found by diffusion-tensor (DTI) and diffusion-weighted imaging (DWI) (24, 25) show a predilection for the frontal regions. Positron emission tomography (PET) studies suggest that the metabolic rate decreases more in the frontal than in the posterior parts of the brain (26-28).

### 2.1.1. Brain Glucose

Brain energy is generated mainly from glucose (29). During starvation, when glucose is insufficient (30), and during high-intensity exercise (31), the brain can use other energy

substrates such as ketone bodies and lactate. In addition, brain astrocytes contain a glycogen store that has been thought to act as a metabolic buffer during physiological activity (32). Even so, the diurnal energy requirement of 400 to 460 kcal in relation to an 8 kcal cerebral energy reserve makes a constant supply of glucose essential. The local energy requirement is highly variable and is tightly coupled with neuronal activity (33, 34). The availability of glucose to the brain tissue depends on cerebral blood flow (35) and glucose uptake (36).

### Cerebral Blood Flow

Regional differences exist in cerebral blood flow. In subjects who are awake but at rest, blood flow is greatest in the frontal brain regions (37). Cerebral blood flow is regulated by constriction and dilatation of large arteries in response to humoral, neural, and metabolic stimuli, most importantly to hypercapnia, hypoxia, and blood pressure, but also to hyperglycemia (38). Most importantly, neural brain activity enhances blood flow in the small arteries of the activated regions (39).

### Glucose Uptake and Metabolism

Brain tissue is isolated from the intravascular space by a specific structure, the blood-brain barrier (BBB) (40). Vascular endothelial cells are connected by tight junctions of high electrical resistance that provide an effective barrier against molecules and prevent any bulk flow. Due to the lipophilic nature of the BBB, hydrophilic substances cannot cross it. Notable exceptions are metabolically important components such as glucose (41), insulin, free fatty acids, lactate, vitamins, and amino acids that use energy-requiring facilitated diffusion. The brain extracts 10 to 15% of the circulating glucose, of which approximately one-third is surplus and later transported back into the venous system (40). The extracellular glucose concentration in the brain tissue is 20 to 30% that in the blood. In rodents, it ranges from 0.35 to 2.2 mmol/l and depends on the brain region and the age of the animal (42). Studies in humans have found brain glucose concentrations between 0.5 and 2.5 mmol/l (43, 44).

The facilitated diffusion of glucose from blood to brain is considered an insulin-independent phenomenon and is mediated by glucose transporter (GLUT) proteins. The 55-kDa isoform of GLUT1 proteins accounts for at least 95% of the transport from blood to brain (36). The GLUT1s are located in the luminal membranes and show a three- to four-fold higher abundance at the abluminal membranes (45-47). This ensures a low glucose concentration in the endothelial cell and creates a concentration gradient that facilitates glucose flow from the blood into the endothelial cells and further into the extracellular fluid (ECF). The process of glucose transport in the brain is saturable (48), but is not rate-limiting for the metabolic pathway (49).

GLUT1s are present throughout the brain but has a variable distribution. The highest densities in rat brain are in the regions with high capillary density and high energy demand (36, 50). Such regions are the frontal cortex, motor cortex, the hippocampus (51), basal ganglia, thalamus (52, 52), and cerebellum (53).

GLUT3 is a neuron-specific transporter widely distributed throughout the brain (54). Under basal conditions, glucose is taken up from the ECF directly into neurons by

GLUT3s (55) (Figure 1). During physiological activation, a large part of the glucose in the ECF is taken up by the 45-kDa isoforms of the GLUT1s into the astrocytes, the cells that support the neurons with energy in the form of lactate (56, 57). GLUT3s have an approximately 30-fold higher affinity for glucose than that of GLUT1s and are therefore able to secure the glucose supply to neurons even at low glucose concentrations (58).

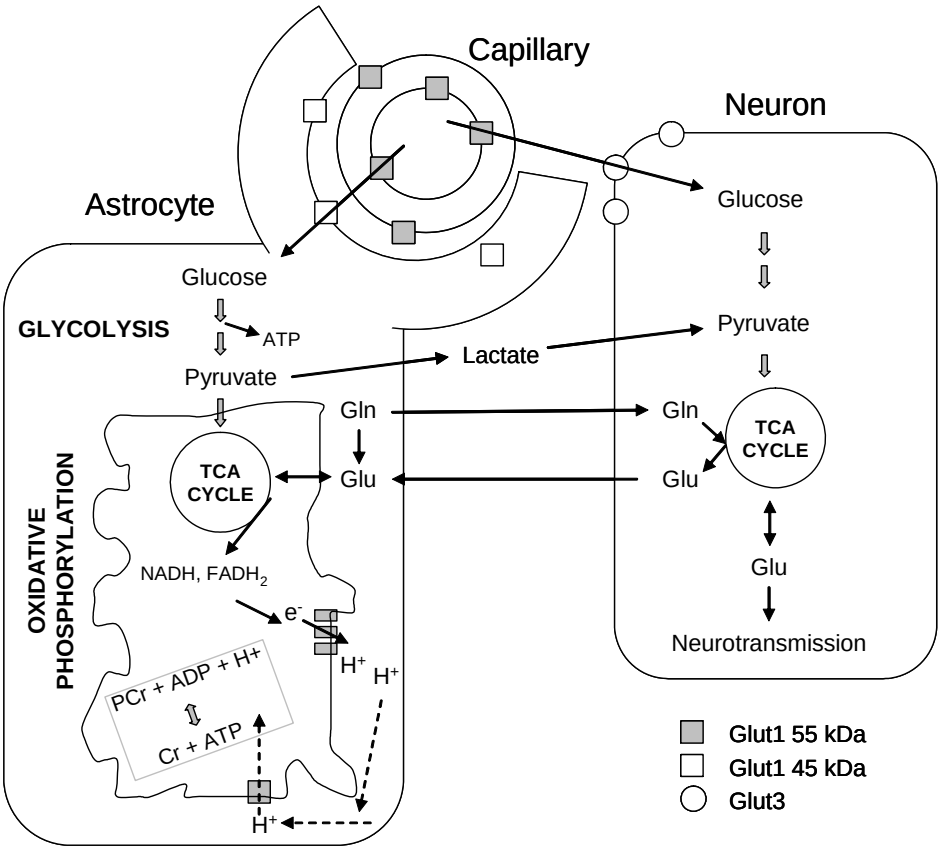


**Figure 1** Glucose transport from blood to brain, basics of glucose metabolism, and the interplay between neurons and astrocytes. TCA = tricarboxycyclic acid, NADH = nicotinamide adenine dinucleotide, $FADH_2$ = flavin adenine dinucleotide, PCr = creatine phosphate, Cr = creatine, ATP = adenosine triphosphate, ADP = adenosine diphosphate, H+ = proton, Gln = glutamine, Glu = glutamate, GLUT = glucose transporter.

Glucose is oxidized into free energy in two phases: first by glycolysis in the cytosol and second in the tricarboxycyclic acid (TCA) cycle in the mitochondria (Figure 1). The free energy produced is then trapped by the electron transport chain and transported in form of H+ to the site of phosphorylation. Creatine phosphate (PCr) delivers the high-energy phosphate to form adenosine triphosphate (ATP) (Figures 1 and 2). ATP is transported out of the mitochondrial matrix via the adenosine nucleotide transporter (ANT) (Figure 2). The PCr formed is shuttled through the cytosol by means of the creatine kinase (CK) towards the site of energy utilization (59). When the ATP generation is separated from consumption, creatine, by facilitating the transfer of the inorganic phosphate Pi, can act to buffer the ATP/adenosine diphosphate (ADP) ratio (60, 61).

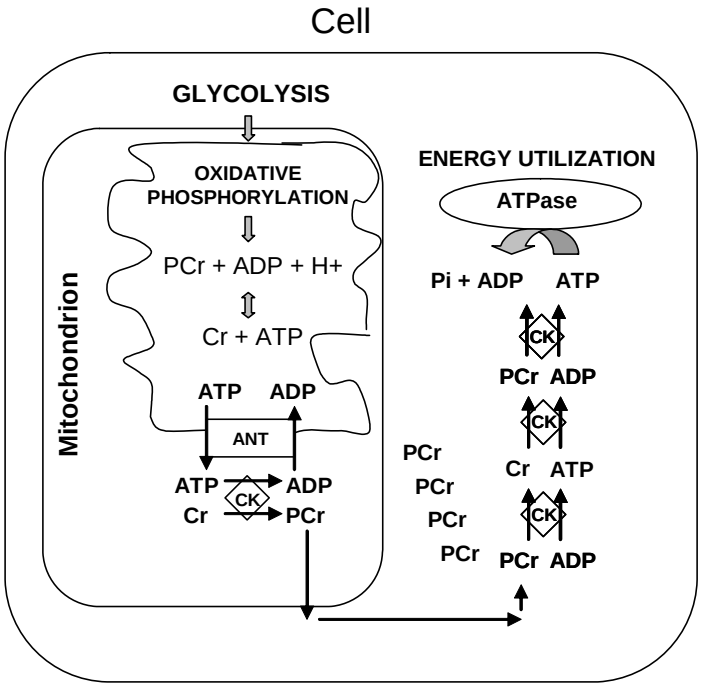


**Figure 2** Energy shuttle system between the site of energy production in the mitochondrion and its utilization in the cytosol. PCr = creatine phosphate, Cr = creatine, ADP = adenosine diphosphate, ATP = adenosine triphosphate, H+ = proton, ANT = adenosine nucleotide transporter, CK = creatine kinase, Pi = monophosphate, ATPase = adenosine triphosphatase.

The brain displays regional differences in its metabolic rate of glucose consumption. The metabolic rate detected with $^{1}H^{13}C$ magnetic resonance spectroscopy (MRS) has been as much as four-fold higher in the gray matter around the cingulate sulcus than in the white matter in the centrum semiovale (62). Consistently, PET has shown an up to two-fold higher metabolic rate in the cerebral cortex, thalamus, and cerebellum than in the cerebral white matter (63). The cortex has a higher metabolic rate than the cerebellum (63, 64).

### Brain Insulin

The brain was considered an insulin-insensitive organ until the 1970s, when insulin receptors were found widely distributed in the brain (65). Insulin is transported into the brain across the BBB by a saturable, insulin receptor-mediated transport process (66, 67). Brain regions with high concentration of insulin receptors (68) and insulin-dependent GLUTs (52, 69) are the hypothalamus, hippocampus, olfactory bulb, and cerebellum. Insulin-dependent glucose transporters found thus far are GLUT2, GLUT4, and GLUT8.

GLUT2 may be involved in glucose sensing also in the brain. It is abundant in the hypothalamus (70) where insulin function is essential in pathways that lead to such neuroendocrine events as suppression of hepatic glucose production (71) and suppression of food intake (72). GLUT2 expression is limited to astrocytes, and together with GLUT1, to highly specialized hypothalamic glial cells called tanycytes (73). The tanycytes may participate in these neuroendocrine events by bridging the gap between the central

nervous system and the portal blood (74). High concentrations of GLUT2 also occur in the hippocampus (75), where insulin enhances memory formation (76).

GLUT4 is expressed in the microvascular endothelium (77) and in the neurons (52). In cultured cerebellar neurons, insulin has caused translocation of GLUT4 to the cell surface and stimulated glucose uptake (78). In animals (79) or in humans (64, 80) physiologic concentrations of insulin have not led to an increased glucose uptake into the brain. In humans, high-dose insulin has also shown no effect (81). Yet physical exercise has in rats shown a similar effect on GLUT4s as does insulin in the cultured cells, and led to an acute increase in cerebellar glucose uptake (78). This suggests that at least during exercise, insulin may play a role in energy metabolism in the cerebellum.

### 2.1.2. $^{1}$H MRS of Brain Metabolites

MRI can reveal brain structure, and proton magnetic resonance spectroscopy ($^{1}$H MRS) can detect and quantify glucose and markers of brain metabolism without irradiation. Both methods rely on a physical phenomenon called nuclear magnetic resonance (NMR) (82, 83). The first $^{1}$H MRS study of the human brain was published in 1988 (84), and the method has been applied since both in basic research and clinical use (85). The variety of molecules visible with MRS is limited, and they are not always the ones of the greatest interest to neuroscientists. Despite the complex nature of $^{1}$H MRS, its clear advantage is that it allows study of endogenous brain metabolites and native glucose (Figure 3).

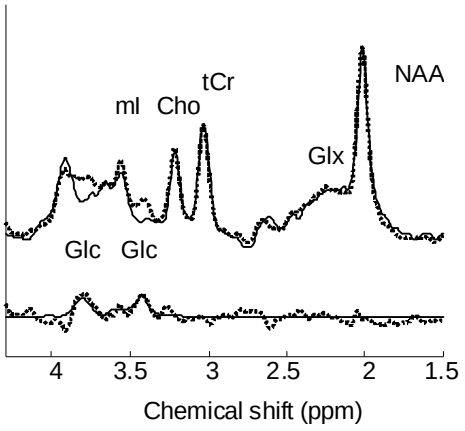


**Figure 3** The main metabolites in $^{1}$H MRS with TE/TR 30/3,000 ms are N-acetylaspartate (NAA), glutamine-containing compounds (Glx), total creatine (tCr), choline-containing compounds (Cho), glucose (Glc), and *myo*-inositol (mI). Increase in brain glucose during hyperglycemia: baseline in solid and hyperglycemia in dashed line.

### Physical Basis of Magnetic Resonance Studies

In NMR, the fundamental property of a nucleus is spin. Those nuclei containing odd numbers of protons or neutrons or both have a non-zero spin and thus a non-zero magnetic moment through which the nucleus can interact with an external magnetic field. In the external field, the magnetic moments orient with a low-energy state parallel to the external magnetic field ($B_0$) and high-energy state antiparallel to the $B_0$. Since a lower energy state is present, slightly more spins are parallel to the $B_0$, forming a macroscopic net magnetization parallel to the main field.

The equilibrium of the spins in $B_0$ can be modified by radiofrequency (RF) pulses. To achieve energy absorption (excitation), the nuclear spin system is exposed to energy of a frequency equal to the energy difference between the low and high energy states (Larmor frequency). An applied RF pulse flips the longitudinal magnetization onto the transverse plane, where it can be detected. Magnetization precesses in the xy-plane at Larmor frequency, and this oscillating magnetic field is detected by an RF coil. At the same time, the magnetization will return to its equilibrium value in a process called relaxation. The relaxation rate is characterized by two time constants. $T_1$ relaxation describes the recovery of longitudinal and $T_2$ relaxation the recovery of transverse magnetization. $T_1$ depends on interactions of the spin with its surroundings, whereas $T_2$ depends on spin-spin interactions. $T_2$ relaxation time is never longer than $T_1$ relaxation time. During the detection period, a decaying oscillation magnetic field induces a small current in the receiver coil. This signal is called free induction decay (FID). In idealized magnetic resonance procedures, the FID decays approximately exponentially with a time constant $T_2$. The actual observed decay of the FID is determined by $T_2$*, that, in addition to true $T_2$, also contains a contribution from magnetic field inhomogeneity to the decay rate. The actual spectrum is obtained via Fourier transformation of the time-domain data, FID.

### The Principles of MRI and $^1$H MRS

For medical applications, MR procedures require a homogenous and powerful magnetic field, usually 0.1 to 3.0 Tesla in strength.

MRI measures the NMR signal as a function of spatial location. Typically in MRI, the signal detected is from hydrogen nuclei, i.e. protons in water or fat molecules. The proton concentration of water in tissue is >80 mol/l. The spatial location is determined by causing the static magnetic field to vary linearly across the body (a field gradient), so that different spatial locations become associated with different precession frequencies. The contrast in images is based on relaxation properties of water in different tissue types. The contrast can be adjusted by a suitable choice of image acquisition parameters such as echo time (TE), repetition time (TR), and inversion time (TI). In $T_1$-weighted images, fat-containing tissues typically appear bright and water- and fluid-containing tissues dark. In $T_2$-weighted images, brightness is proportional to the mobility of water. $T_2$-weighted MR images show high sensitivity to edema and thereby also to pathology. By adding one RF pulse (inversion pulse) and by manipulation of the magnetic gradients, a $T_2$-weighted sequence can be transformed into a fluid-attenuated inversion-recovery (FLAIR) sequence where "free" water including cerebrospinal fluid is dark, but edematous tissue

becomes bright. FLAIR images therefore bring out periventricular or intraparenchymal lesions better than do pure T2 images.

In $^1$H MRS, the main focus is on detection of the signals from metabolites of relatively low concentrations (1 to 10 mmol/l) rather than detection of water. Compared to MRI, the low concentrations of compounds results in lower amplitude of MR signals, lower signal-to-noise ratio (SNR), lower spatial resolution, and to a longer acquisition time. A $^1$H MRS measurement results in a spectrum where metabolite protons resonate in characteristic frequencies depending on their chemical and physical vicinity in a molecule. The surroundings change the local magnetic field experienced by the proton (nuclear shielding) and thus its resonance frequency. Instead of frequencies, the resonance positions are expressed by a chemical shift scale with a unit of parts per million (ppm) of $B_0$. This unit is independent of the magnetic field strength used. Chemical shift and fine structure of the resonance allow characterization of the protons responsible for the resonance.

### $^1$H MRS Data Acquisition

What are called single voxel methods acquire the spectrum from a single selected cubical volume of typically ml to 10 ml (volume of interest or voxel). The size of the voxel is adjusted based on the anatomy of the organ in focus. Increasing the size of the voxel raises the SNR but reduces spatial resolution. Raising the number of excitations raises the SNR but prolongs data collection time (86).

The two most common methods are the stimulated echo acquisition mode (STEAM) (87) and point-resolved spectroscopy (PRESS) (88). Frequently, PRESS is preferred due to its better SNR and simplified signal quantitation. TR is the time between repetitions of the PRESS sequence. A relatively long TR is a perquisite for quantitation, since spins have enough time to relax towards equilibirium between repetitions. TE refers to the time between the first pulse and the start of the signal acquisition. Long TE (typically 144 ms or 270 ms) has the benefit of showing lactate but fails to show a number of metabolites that have short $T_2$ relaxation times. A short TE shows more metabolites and gives more SNR, but the analysis is more complex, since the baseline may become irregular and the peaks of different metabolites may overlap. In order to see the metabolites of interest, the signals of high concentration molecules such as water must be suppressed.

### Quantitation of the Spectrum

Although the spectroscopic peak area is proportional to the concentration of the metabolite, the acquired data are not unequivocally quantitative. In addition to the metabolite concentration, all factors that affect the SNR also affect the peak areas (89). In order to minimize the random effects from the instrument or the patient, the data must be quantitated (90).

*Peak ratios* can be used when one metabolite is not expected to have inter-subject variation or is not expected to vary between the brain regions in comparison. The body has, however, no constant metabolites able to serve as identical references. Total Creatine is one of the most stable and therefore often serves as an internal reference (91). Moreover, tissue water concentration in the brain varies only within narrow limits (92)

and an unsuppressed water signal can easily be obtained from the same voxels as those for the metabolites.

*Absolute quantification* with external standard solutions enables quantification of absolute concentrations of the metabolites, but using them requires a short TE and long TR, additional measurements, and certain corrections. Options are two: sameplace or sametime standards. The sameplace standard is positioned at the same location as the center of the brain, but its data collection occurs at a different time and is vulnerable to random fluctuations in the intensity levels originating from the instrument or from the patient. The sametime standard method has identical coil and receiver performance, but it requires corrections for temperature and for variation in RF uniformity.

### N-acetylaspartate (NAA)

Of the brain metabolites, N-acetylaspartate has been considered a neuronal marker (93). Its synthesis takes place primarily in the neuronal mitochondria and is dependent on the neuronal energy metabolism (94, 95). Its breakdown takes place in the oligodendrocytes (96) and at the surface of the astrocytes (97). NAA is therefore present in the neurons at relatively high concentrations but is absent from mature glial cells (98, 99).

N-acetylaspartate has functions in myelin synthesis (100), in cell-specific signaling, and in maintenance of the intracellular osmotic balance by removing large amounts of metabolic water generated by the neuronal glucose metabolism (101).

In the normal brain, NAA concentration is age-dependent. Its concentration is high during brain development and decreases during aging in the gray (102, 103) but not in the white matter (104, 105). Increased NAA has been found in Canavan's disease (106, 107) in which failure of NAA breakdown interferes with the normal myelin production. Decreased NAA has been observable in a wide variety of conditions causing neuronal dysfunction or loss including brain trauma, tumors, inflammation, infection, ischemia, Alzheimer's disease, and demyelinating diseases (86, 108). N-acetylaspartate may also recover during axonal recovery such as in multiple sclerosis (109, 109).

### Glutamate-containing Compounds (Glx)

Because $^{1}$H MRS cannot distinguish between glutamate (Glu) and glutamine (Gln), Glx is therefore the sum of both. In the brain, glutamate is the most important excitatory neurotransmitter. In the glutamine/glutamate cycle, glutamine is converted to glutamate in presynaptic neurons and then released into the synaptic cleft. It is taken up by astrocytes that convert it back to glutamine and transport it back to the neurons (110) (Figure 1). Glutamate is also involved in the redox cycle that governs lactate accumulation. According to the glutamine/glutamate cycle theory, Glx is present in all cell types. It is elevated in hyperosmolar (111, 112), hypoxic, and ischemic states and in hepatic encephalopathy (109).

### Total Creatine (tCr)

Creatine in the body is mainly of dietary origin (113) and actively transported into the brain from the circulating blood against its concentration gradient (114). The spectrum

peak of total Cr consists mainly of creatine (Cr) and phosphocreatine (PCr) and to a much lesser degree of γ-amino butyric acid, lysine, and glutathione. A Cr-to-PCr ratio of 2:1 is normal in healthy humans throughout the brain (91). Diabetes may elevate the proportion of PCr by reducing the activity of the enzyme creatine kinase (Figure 2) (115). The concentration of tCr is approximately 20% higher in the gray than in the white matter, and tCr has been related to both neurons and glial cells. The main function of creatine is to serve in an energy shuttle system at the mitochondrial inner membrane and in the cytosol (Figure 2).

In the normal brain, tCr concentration is relatively stable (91). It is thought to increase with age (102, 104), although findings are not entirely consistent (103, 116). Increased brain tCr level has occurred during increased oxidative metabolism and in hyperosmolar states (86). Conversely, decreased tCr has been observable in hypometabolic states concomitant with energy failure or cell death caused by such conditions as hypoxia, stroke or tumor, and in hypo-osmolar states (93, 93).

#### Choline-containing Compounds (Cho)

Choline is a precursor for acetylcholine, a neurotransmitter, and for phosphatidyl choline, a major constituent of all cell membranes and of sphingomyelin. The main determinants of Cho concentration in the tissue are the cell density and rate of phospholipid cell membrane turnover (117). Because of the abundance of cell membranes in myelin layers, Cho is higher in the white than in the gray matter (86).

Cho increases with age (102, 104). Increased Cho appears in areas with increased cell membrane synthesis or breakdown during chronic hypoxia, and during active demyelination or inflammation. It also is increased in tumors, in Alzheimers disease, and in hyperosmolar states. Decreased levels appear in hepatic encephalopathy and in subacute ischemia (86).

#### *Myo*-inositol (mI)

*Myo*-inositol has multiple cellular functions. It is a precursor for phospatidylinositol that constitutes 5 to 10% of the total cell membrane phospholipids and to inositol triphosphate and diacylglycerol that are intracellular second messengers (118). *Myo*-inositol is almost exclusively located in the astrocytes and is therefore considered an astrocyte marker whose concentration increases both during increased cell membrane synthesis (for example gliosis) or breakdown. It also is also one of the most important osmolytes in the brain cells (93).

*Myo*-inositol has been proposed to increase with age (102), although not all studies agree (104). Increased mI has been associated with Alzheimer's disease, brain tumors, hyperosmolar states, and inflammation (86).

#### Glucose

Glucose plays a central role in brain energy metabolism (see 2.1.1). Brain glucose content detected with $^{1}H$ MRS depends upon the cerebral blood flow that delivers glucose into the brain, upon glucose uptake, and also upon the metabolic rate of glucose.

#### Tissue Water

White matter contains approximately 78%, and cortical gray matter 82% water in proportion to their wet weight (119). Brain tissue water is compartmentalized into intracellular (85%) and extracellular (15%) space. $^{1}$H MRS detects only stationary water, which means that the circulating blood that accounts for about 10% of the water in the brain is not visible with this technique. $^{1}$H MRS cannot differentiate between intracellular and extracellular water compartments and thus represents a weighted average.

Brain water content increases during brain edema (120). Vasogenic edema due to BBB disruption results in extracellular water accumulation, whereas cytotoxic or cellular edema results in sustained intracellular water accumulation. A third type, osmotic brain edema, results from osmotic imbalances between blood and tissue. In all these types, gradients for osmotically active solutes are the driving forces for the movement of fluid between the three compartments: extracellular and intracellular fluids and blood. As mentioned, the markers of brain metabolism are osmolally active molecules that contribute to the maintenance of normal brain water content.

## 2.2. Metabolic Risk Factors and the Brain

### 2.2.1. Metabolic Syndrome

The clinical importance of the accumulation of metabolically related cardiovascular risk factors (called here metabolic risk factors) was recognized in the 1980s (121). Thereafter, several definitions of the metabolic syndrome have aided in predicting risk for cardiovascular and cerebrovascular morbidity (Table 1).

The prevalence of the metabolic syndrome varies according to the definition used. In the 1990s, the FINNRISK study on the 45- to 64-year-old Finnish population used the WHO definition and reported Finnish prevalences of 36% for men and 17% for women (128). The Cardiovascular Risk in Young Finns Study compared the different definitions and reported the following prevalences in 39-year-old men: National Cholesterol Education Program Adult Treatment Panel III (ATPIII) 20%, European Group for the study of Insulin Resistance (EGIR) 17%, International Diabetes Federation (IDF) 25% and women ATPIII 14%, EGIR 7%, and IDF 17% (1).

**Table 1.** Definitions of the metabolic syndrome

| | WHO 1999 | EGIR 1999 | ATPIII 2001 | IDF 2005 | AHA/NHLBI 2005 | IDF/NHBI/AHA/ WHF/IAS/IASO 2009 |
|---|---|---|---|---|---|---|
| | Insulin resistance, IGT, or diabetes + 2 other | Plasma insulin > 75th percentile + 2 other | 3 of the following | Abdominal obesity + 2 other | 3 of the following | 3 of the following |
| **Abdominal obesity** | BMI > 30 kg/m2 and/or WHR M > 0.90 F > 0.85 | Waist circumference M ≥ 94 cm F ≥ 80 cm | Waist circumference M > 102 cm F > 88 cm | Waist circumference M ≥ 94 cm F ≥ 80 cm | Waist circumference for non-Asian M ≥ 102 cm F ≥ 88 cm | Waist circumference for non-Asian M ≥ 94 cm F ≥ 80 cm |
| **Hypertension** | ≥ 140/90 mmHg | ≥ 140/90 mmHg or treatment | ≥ 130/85 mmHg | ≥ 130/85 mmHg or treatment | ≥ 130/85 mmHg or treatment | ≥ 130/85 mmHg or treatment |
| **Dyslipidemia** | HDL M < 0.9 mmol/l F < 1.0 mmol/l and/or Tg ≥ 1.7 mmol/l | HDL < 1.0 mmol/l and/or Tg ≥ 2.0 mmol/l or treatment | HDL M < 1.0 mmol/l F < 1.3 mmol/l | HDL M < 1.03 mmol/l F < 1.29 mmol/l or treatment | HDL M < 1.0 mmol/l F < 1.3 mmol/l or treatment | HDL M < 1.0 mmol/l F < 1.3 mmol/l or treatment |
| | | | Tg ≥ 1.7 mmol/l | Tg ≥ 1.7 mmol/l or treatment | Tg ≥ 1.7 mmol/l or treatment | Tg ≥ 1.7 mmol/l or treatment |
| **Impaired glucose regulation (fP-Glucose)** | Insulin resistance, IGT, or diabetes | ≥ 6.1 mmol/l or IGT, but not diabetes | ≥ 6.1 mmol/l or diabetes | ≥ 5.6 mmol/l or diabetes | ≥ 5.6 mmol/l or diabetes | ≥ 5.6 mmol/l or diabetes |
| **UAER** | ≥ 20 µg/min | - | - | - | - | - |

*WHO, World Health Organization (122); EGIR, European Group for the study of Insulin Resistance (123); ATPIII, National Cholesterol Education Program Adult Treatment Panel III (124); IDF, International Diabetes Federation (125); AHA/NHLBI, the American Heart Association/National Heart, Lung, and Blood Institute (126); IDF/NHBI/AHA/WHF/IAS/IASO, Joint Interim Statement has been published by the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity (127); IGT, impaired glucose tolerance; BMI, body mass index; WHR, waist-to-hip ratio; HDL, HDL cholesterol; Tg, triglycerides; UAER, urinary albumin excretion rate; M, males; F, females.*

The metabolic syndrome clusters in families (129-131), which suggests that genetic predisposition plays a central role. A family history of hypertension or of type 2 diabetes or both also predicts its occurrence (132). In 35- to 65-year-old individuals, the metabolic syndrome elevates risk for type 2 diabetes three- to six-fold in 3 to 7 years' time (133-135). The metabolic syndrome is therefore considered a prediabetic state. Approximately 75% of individuals with impaired glucose tolerance (128) and 87% of those with type 2 diabetes (136) have metabolic syndrome. Occurrence of metabolic syndrome is also promoted by some environmental factors: age, male gender, overnutrition, a sedentary lifestyle, smoking (137), and possibly also chronic stress (138) and fetal malnutrition (139).

The pathophysiology of the metabolic syndrome is multifactorial. Major contributors are abdominal fat (140), insulin resistance (141), abundance of free fatty acids (142), inflammation (143), and oxidative stress (144). Other contributors include disorders of the hypothalamus-pituitary-adrenal axis (145), altered glucocorticoid hormone action (146), and dysregulation of mitochondria (142). The metabolic syndrome associates with hyperuricemia and gout (147), sleep apnea (148), polycystic ovary syndrome (149), depression (150), and chronic schizophrenia (151).

### 2.2.2. Effects of Metabolic Risk Factors on the Brain

During the last two decades, metabolic syndrome has been associated with brain abnormalities that predispose to brain infarctions and cognitive decline. Very little is, however, known about the effect of metabolic syndrome or metabolic risk factors on brain glucose and insulin.

#### Morphological Abnormalities

Metabolic syndrome risk factors predispose to asymptomatic structural abnormalities in the brain. Increased body mass index (BMI) (152, 153), hypertension (154-156), and high concentration of the inflammatory marker interleukin 6 (IL-6) (157) and of homocysteine (159) have all been associated with decrease in total brain volume that is a sign of brain atrophy. Increased BMI has also been associated with decreased volume of particularly the cerebral cortex (153, 160). Hypertensive patients have shown a smaller volume of the thalamus and greater volumes of cerebrospinal fluid in the temporal lobes and the cerebellum than expected for their age (161).

Periventricular and subcortical white matter lesions are more common in patients with metabolic syndrome (162). Of the metabolic risk factors, an increased waist-to-hip ratio (163), hypertension (15, 155, 164, 165), and increased C-reactive protein (CRP) (166), increased intercellular adhesion molecule (ICAM) (167), and increased homocysteine (168) have been associated with a higher prevalence of white matter lesions. Increased anisotrophy that indicates decreased nerve fiber integrity in the white matter has appeared in patients with metabolic syndrome, mainly involving the frontal lobe (8).

### Brain Infarctions

Metabolic syndrome raises the risk for stroke two- to three-fold in 50- to 60-year-old individuals (5-7). Abdominal obesity (169), hypertension, dyslipidemia, hyperglycemia, and increased fasting plasma glucose have all been independent risk factors for stroke (170-173). The risk, not surprisingly, increases with the increasing number of metabolic syndrome risk factors (174-176).

Metabolic syndrome associates also with asymptomatic brain infarctions (162) and asymptomatic carotid atherosclerotic plaques (177, 178). Furthermore, metabolic syndrome as a whole as well as its components obesity, high low-density lipoprotein cholesterol, and high insulin level individually have predicted carotid intima media thickness progression even in 32-year-old adults (179).

### Cognitive Decline

Cardiovascular risk in midlife predicts worse cognitive function later in life (180-182). Obesity, dyslipidemia, and midlife diabetes all serve as independent risk factors for later cognitive decline (4, 180, 181). The most important risk factor for such decreased cognition in midlife appears to be hypertension, with a 28 to 36% additional risk at population level (4). Consistently, antihypertensive treatment has reduced the incidence of dementia (183).

In the elderly, cognitive decline has been associated with the metabolic syndrome in its entirety (184) as well as its components and features including obesity (185-187), hypertension (187-189), dyslipidemia (190), insulin resistance (191-193), type 2 diabetes (194), increased homocysteine (195), and increased CRP and IL-6 (184).

### Brain Glucose and Insulin

Glucose concentration in the brain tissue depends on cerebral blood flow as well as glucose uptake and metabolism. It is possible that the metabolic syndrome has an effect on both.

*Cerebral blood flow.* PET studies have shown reduced regional cerebral blood flow response during cognitive performance especially in the posterior parietal, thalamic, and the middle/posterior watershed areas in patients with hypertension (196). Blood flow in the middle cerebral arteries has been reduced in patients with obesity (197). Vascular autoregulation have been affected by hypertension, dyslipidemia, artherosclerosis, and hyperglycemia (38).

*Glucose uptake and metabolism.* In insulin-resistant mice, decreased GLUT1 density occurs in the thalamus, cerebellum, and hippocampus but not in the cerebral cortex or olfactory bulb (198). It is not known whether the metabolic syndrome has an effect on brain glucose uptake. In humans, higher cholesterol levels in late middle age have been associated with in precuneal, parietotemporal, and prefrontal hypometabolism (199). The effect of the other metabolic risk factors on brain metabolic rates remains unknown.

*Brain insulin.* In rats, insulin resistance gained with fructose feeding has led to a similar alteration in insulin-mediated post-receptor signaling in the brain (200, 201), as seen also

in the muscle (202) and the liver (203). Studies in humans and rats have suggested that insulin resistance and chronic peripheral hyperinsulinemia result in a reduction in insulin transport across the BBB, relative hypoinsulinemia, and reduced insulin signaling in the brain (204). Hyperinsulinemia has also been associated with cognitive decline and dementia (205, 205).

### 2.2.3. Effects of Metabolic Risk Factors on Brain Metabolites

Effects of metabolic syndrome risk factors on the brain have been studied with $^{1}$H MRS in several brain regions (Figure 4). Results (Table 2) are not always comparable, because they apply only to the specific brain region studied. Studies suggest that at least hypertension (206), dyslipidemia (207), impaired glucose tolerance, and type 2 diabetes (208, 209) may alter cerebral metabolism.

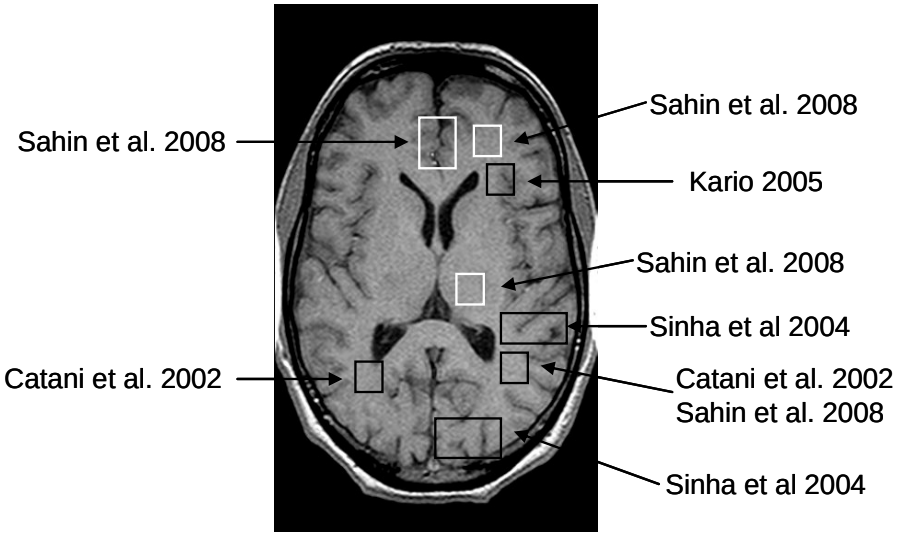


**Figure 4** Regions studied with $^{1}$H MRS in patients with metabolic risk factors.

**Table 2.** $^{1}$H MRS studies on brain metabolites in patients with metabolic syndrome risk factors

| Study | Subject groups ($n$ = M/$n$ = F) | Age (years) | BMI (kg/m$^2$) | Triglycerides; HDL cholesterol (mmol/) | Systolic/ diastolic blood pressure (mmHg) | $HbA_{1c}$ (%); duration (years) | Brain regions | Findings |
|---|---|---|---|---|---|---|---|---|
| **Hypertension** | | | | | | | | |
| Catani et al. 2002 (206) | HT+ (5/5)<br>HT- (5/5) | 78<br>79 | NA<br>NA | NA<br>NA | 137/78<br>130/74 | NA; 0<br>NA; 0 | Paratrigonal white matter bilaterally | ▪HT+ vs HT-: NAA/tCr↓ (trend), mI/tCr ↑, Cho/tCr ↔ |
| **Hypertension and type 2 diabetes** | | | | | | | | |
| Kario et al. 2005 (208) | T2DHT+ (7/13)<br>HT+ (7/13)<br>HT- (4/8) | 69<br>69<br>69 | 24.5<br>23.5<br>24.1 | DL in ($n$)<br>7<br>5<br>2 | 161/85<br>158/84<br>123/70 | NA; 0<br>NA; 0<br>NA; 0 | Left periventricular white matter | ▪T2DHT vs HT-: NAA/$H_2O$↓, NAA/tCr↓ (trend)<br>▪T2DHT vs HT+ NAA/$H_2O$↓, NAA/tCr↓<br>▪T2DHT vs HT- tCr/$H_2O$↔, Cho/$H_2O$↔<br>▪HT+ vs HT- NAA/$H_2O$↔, NAA/tCr↔ |
| **Dyslipidemia** | | | | | | | | |
| Sinha et al. 2004 (207) | DL+<br>DL+MED+ 13<br>DL-MED- 6<br>DL- 21<br>Altogether: (34/6) | 33<br>30<br>30 | Higher in DL+ than in DL- | 3.7; 1.0<br>2.6; 1.1<br>1.6; 1.1 | NA | NA; 0<br>NA; 0<br>NA; 0<br>NA; 0 | Parieto-temporal region | ▪DL+ vs DL-: NAA/tCr↔, Cho/tCr↔, NAA/Cho↔<br>▪DL+MED+: Tg↑ correlated with NAA/Cr↑ and Cho/Cr↑<br>▪DL+MED-: HDL↓ correlated with Cho/Cr↑ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Occipital region | ▪DL+ vs DL-: NAA/tCr↔, Cho/tCr↔, NAA/Cho↔<br>▪All: Fat%↑ and HDL↓ correlated with Cho/Cr↑<br>▪All: TChol↑ and HDL↓ correlated with NAA/Cho↓<br>▪DL+MED+: Age↑ correlated with NAA/Cr↓<br>▪DL+MED-: Tg↑ and HDL↓ correlated with NAA/Cr↑ |
| **Impaired glucose regulation** | | | | | | | | |
| Sahin et al. 2008 (209) | IGT (5/8)<br>T2DHbA$_{1c}$<10% (6/4)<br>T2DHbA$_{1c}$>10% (6/9)<br>NGT (5/9) | 45<br>46<br>54<br>42 | NA<br>NA<br>NA<br>NA | NA<br>NA<br>NA<br>NA | NA<br>NA<br>NA<br>NA | 5.7; 0<br>7.9; 9<br>13.6; 8<br>5.2; 0 | Frontal cortex | ▪T2DHbA$_{1c}$>10% vs T2DHbA$_{1c}$<10%: NAA/tCr↓, Cho/tCr↓<br>▪IGT vs NGT: Cho/tCr↑<br>▪T2D vs NGT: mI/tCr↑<br>▪All: S-Insulin↑ correlated with NAA/tCr↓<br>▪All: HbA$_{1c}$↑ correlated with NAA/tCr↓ and Cho/tCr↓ |
| | | | | | | | Parietal white matter | ▪T2D vs NGT or IGT Cho/tCr↓<br>▪T2DHbA$_{1c}$>10% vs T2DHbA$_{1c}$<10% Cho/tCr↓<br>▪All: HOMA-IR↑ and fP-Glc↑ correlated with NAA/tCr↓<br>▪All: HbA$_{1c}$↑ correlated with Cho/tCr↓ |
| | | | | | | | Thalamus | ▪No differences in metabolites<br>▪All: HbA$_{1c}$↑ correlated with mI/tCr↑ |

*NA, not applied; HT, hypertension; DHT, type 2 diabetes and hypertension; DL, dyslipidemia; TChol, total cholesterol; Tg, triglycerides; HDL, HDL cholesterol; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes; Fat%, body fat percent; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; fP-Glc, fasting plasma glucose; NAA, N-acetylaspartate; tCr, total creatine; Cho, choline; mI, myo-inositol; Glc, glucose.*

## 2.3. Type 1 Diabetes and the Brain

### 2.3.1. Type 1 Diabetes

Type 1 diabetes is an inflammatory autoimmune disease that leads to $\beta$-cell destruction in the pancreas and thereby to absolute insulin deficiency. Its etiology is not fully understood, but both genetic predisposition and environmental factors are important. The incidence of type 1 diabetes has doubled in the world over the past 10 years. In Finland, where there are approximately 45 000 patients, its incidence is the highest in the world and rapidly increasing (2, 3). Age of onset is often between 4 and 13 years (2).

The insulin deficiency in type 1 diabetes leads to hyperglycemia that is treated with insulin replacement therapy. In order to avoid microvascular complications, the aim is to achieve normoglycemia (3.9-6.1 mmol/l, $HbA_{1c}$ 6-7% / 42-53 mmol/mol). The limiting factor and the most common side effect in diabetes management is hypoglycemia. The hypoglycemic symptoms caused by adrenergic discharge (palpitation, sweating, hunger, tremor, and anxiety) and the blood glucose level at which these symptoms appear depend on the overall glycemic control and the frequency of hypoglycemic episodes. Lack of these symptoms is called hypoglycemia unawareness. The central symptoms (headache, confusion, optical illusions, and unusual behavior) appear at around 3.0 to 2.4 mmol/l, disorientation and drowsiness at around 2.3 to 1.6 mmol/l, and unconsciousness and convulsions at around 1.5 mmol/l (210). Brain damage occurs rather late and not until after prolonged severe hypoglycemia. The brain regions most vulnerable to hypoglycemic damage are the cortex, basal ganglia, thalamus, and hippocampus (211).

Good glycemic control is important because hyperglycemia is the most important risk factor for the diabetic microvascular complications retinopathy, nephropathy, and peripheral neuropathy (212). Incidence of the retinopathy that often is the first complication to occur, starts to increase after 5 years past diagnosis (213). With improved therapy over the last decades, however, the incidence of microvascular complications has decreased (214).

Glycemic control is estimated with glycated hemoglobin $HbA_{1c}$ that indicates the mean blood glucose in the past three months. An acceptable $HbA_{1c}$ can therefore be reached both with steady or with highly variable blood glucose concentrations (215). Although $HbA_{1c}$ variability does predict (216, 217), large daily glycemic variation may not predict (218) retinopathy or nephropathy. The peripheral nervous system may, however, be particularly vulnerable also to daily glycemic variation (219). Other important risk factors for microvascular complications are male gender, smoking (220), and metabolic risk factors including obesity, hypertension, and dyslipidemia (221-223). The prevalence of metabolic syndrome in Finnish patients with type 1 diabetes is 39% (224), higher than in an age-matched non-diabetic population (< 20%) (1).

## 2.3.2. Effects of Type 1 Diabetes on the Brain

The harmful effect of diabetes on brain function was already recognized in 1922 (225). The first attempt to describe cognitive impairment in diabetes was made in 1950 by Russel De Jong (226), who called the condition "diabetic encephalopathy." In the 1960s, an autopsy study first showed the widely distributed morphological brain changes typical of diabetes (227). Thereafter, diabetes-related brain changes did not enter the limelight until the 1980s, when imaging methods started to develop.

#### Morphological Abnormalities

Autopsy studies in young adults with type 1 diabetes and severe microvascular complications have shown several types of abnormalities (227, 228). The cortex has shown neuronal damage and gliosis and the white matter regional swelling and myelin damage. These same diffuse changes have also been seen in the basal ganglia, brain stem, and cerebellum. Blood vessels have thickened capillary basement membranes and decreased capillary density. These findings have differed from those previously seen in patients with hypertension or who had died due to hypoglycemia; the findings were regarded as consequences of a combination of primary diabetic abnormality and ischemia.

Type 1 diabetes elevates risk for cerebral atrophy, and possibly also for white matter lesions (11). Studies on atrophy are small and sometimes contradictory, but atrophy has been associated with onset of diabetes before age 7 (229), with hypoglycemic episodes and with poor glycemic control in children and adults (229, 230), and with retinopathy in adults (230, 231). An increased volume of white matter lesions has been reported in some (232-234) but not in all (235, 236) studies.

#### Brain Infarctions

Type 1 diabetes elevates risk for stroke (10) and cerebrovascular mortality five-fold (237), and the risk for lacunar infarctions seven-fold (10). Initial post-mortem studies have stressed the frequency of lacunes predominantly within the basal ganglia, paramedian basis pontis, and thalamus (238). The frequent hypertension in these patients, however, limits evaluation of the findings (239). Studies on stroke risk factors that include only patients with type 1 diabetes are sparse, but increased risk has been associated with low HDL cholesterol (240).

#### Cognitive Decline

Despite their long history, clinical features of cognitive decline and its causes in patients with type 1 diabetes all remain under debate. In a meta-analysis of 33 studies, memory and learning seemed to be spared, but modest yet highly significant slowing of mental speed and diminished mental flexibility were common findings (9).

*Hypoglycemia.* The general belief has been that repetitive episodes of hypoglycemia are the most important cause of diabetes-related cognitive decline. Although retrospective studies

have demonstrated this association (233, 241, 242), large prospective studies have been unable to repeat such findings (220, 243, 244). Furthermore, intensive insulin treatment that unavoidably leads to an increased number of hypoglycemic episodes has shown no effect on cognition in adults (230, 243-245). The effect of hypoglycemia on the developing brain may be more detrimental. Early onset of type 1 diabetes (246, 247) and repetitive episodes of hypoglycemia during childhood (248, 249) have repeatedly appeared as potent predictors of worse cognitive outcome.

*Chronic hyperglycemia.* Evidence is emerging regarding the deleterious effects of chronic hyperglycemia on the adult brain (250). In an 18-year follow-up, patients with $HbA_{1c}$ < 7.4% (57 mmol/mol) performed better in tasks requiring motor speed and psychomotor efficiency than did patients with $HbA_{1c}$ > 8.8% (73 mmol/mol) (244). A large variety of cognitive functions improved with better glycemic control. Cognitive decline has been associated with retinopathy and neuropathy (9).

*Acute hyperglycemia.* In children, acute hyperglycemia of > 22.2 mmol/l has been associated with cognitive deterioration to the same degree as for hypoglycemia < 3 mmol/l (251). Moreover, in adult patients with type 1 and 2 diabetes, acute hyperglycemia > 15 mmol/l has been associated with impairment in a variety of cognitive tests (252, 253).

### Brain Glucose

Brain glucose concentration has been 2 to 3 mmol/l higher in patients with type 1 diabetes than in non-diabetic individuals (254-256). Whether the brain is able to adjust its glucose uptake or metabolism in response to the altered glycemic conditions is unknown.

*Cerebral blood flow.* Early studies with PET suggest that patients with diabetes have more than a 20% higher local cerebral blood flow than do non-diabetic individuals, and that this difference is highest in the frontal brain regions (257, 258). The vasodilatation response to a rhythmic handgrip exercise has been increased (259).

In non-diabetic humans, repetitive hypoglycemia induced an increase in blood flow in one study (260) but had no effect in another (261). In type 1 diabetic patients both with hypoglycemia awareness (43, 258) and unawareness (258), hypoglycemia enhanced cerebral blood flow in the brains frontal regions. Some studies have suggested that the whole brain blood flow also increases but not before the blood glucose level is below 2.0 mmol/l (210, 262).

*Glucose uptake and metabolism.* Brain glucose uptake can be regulated by modulation of the expression of GLUT1s (263) and by changing their distribution between the luminal and abluminal membranes of the vasculatory endothelial cells (47).

Robust animal data have shown that the brain responds to hypoglycemia by upregulating glucose transporters (264-266). This hypothesis receives support from the early clinical studies that found increased glucose uptake by measuring the arteriovenous glucose differences in non-diabetic individuals during repetitive hypoglycemia (260) and in patients with type 1 diabetes with tight glycemic control (267). Later studies have presented more contradictory results. A PET study on repetitive hypoglycemia in non-

diabetic individuals showed no effect on their glucose uptake or on its metabolic rate (261). A $^{1}$H MRS study in turn found a 17% higher glucose concentration in the occipital cortex during acute hyperglycemia (16.6 mmol/l) in patients with hypoglycemia unawareness than in non-diabetic controls, suggesting that upregulation of glucose uptake may have occurred (256). The most recent studies suggest that during hypoglycemia, the glucose uptake and brain activity increase regionally, and that this response differs between hypoglycemia aware and unaware patients and in patients with and without microvascular disease (43, 268-270). These studies suggest that the metabolic rate increases in the regions that evoke hormonal and neural responses to hypoglycemia. The increased glucose utilization and the decreased densities of GLUT1 have also appeared to be similar at a local level (271).

The effects of chronic hyperglycemia on brain glucose uptake have been studied the most. Some studies in rats have reported that, as a result of chronic hyperglycemia, GLUT1 expression is decreased (272-274), while other studies reported GLUT1 expression to be unaltered (275, 276). Moreover, both down-regulated (271) and unchanged (277) glucose uptake have occurred. In PET studies, diabetic patients with poor glycemic control have shown an unchanged uptake of glucose during hypoglycemia (278), but a 12% decreased glucose uptake during hyperglycemia (279). This is in line with a $^{1}$H MRS study that assessed glucose uptake or metabolism during hyperglycemia (16.6 mmol/l) in type 1 and 2 diabetic patients with poor glycemic control (280). The diabetic patients had a 10% lower brain glucose concentration than did the non-diabetic participants, but this difference was non-significant. In the light of these studies, it is possible that chronic hyperglycemia reduces brain glucose uptake.

As mentioned, an acceptable $HbA_{1c}$ can be reached with steady or with variable blood glucose. In healthy individuals with undulating blood glucose concentrations, brain ECF glucose concentration parallels plasma glucose concentration at a time lag of 30 minutes (281). What is unknown is whether the brain is able to adjust its glucose metabolism in response to such blood glucose variation in type 1 diabetes.

### 2.3.3. Effects of Type 1 Diabetes on Brain Metabolites

Several regions in the cerebrum but none in the cerebellum have been studied with $^{1}$H MRS in patients with diabetes (Figure 5). The studies on the cerebrum provide conflicting data (Table 3) which may partly be explained by the following issues: First, in three of the nine studies, patients with type 1 and 2 diabetes have not been separated. Second, some variation has occurred in inclusion criteria, for example regarding age and gender of participants, duration of diabetes, and presence of diabetic complications and ketoacidosis. The studies that assessed the glycemic history of patients did it only with $HbA_{1c}$. Plasma glucose values during the study were reported in four studies only. For these reasons, drawing any general conclusions is impossible.

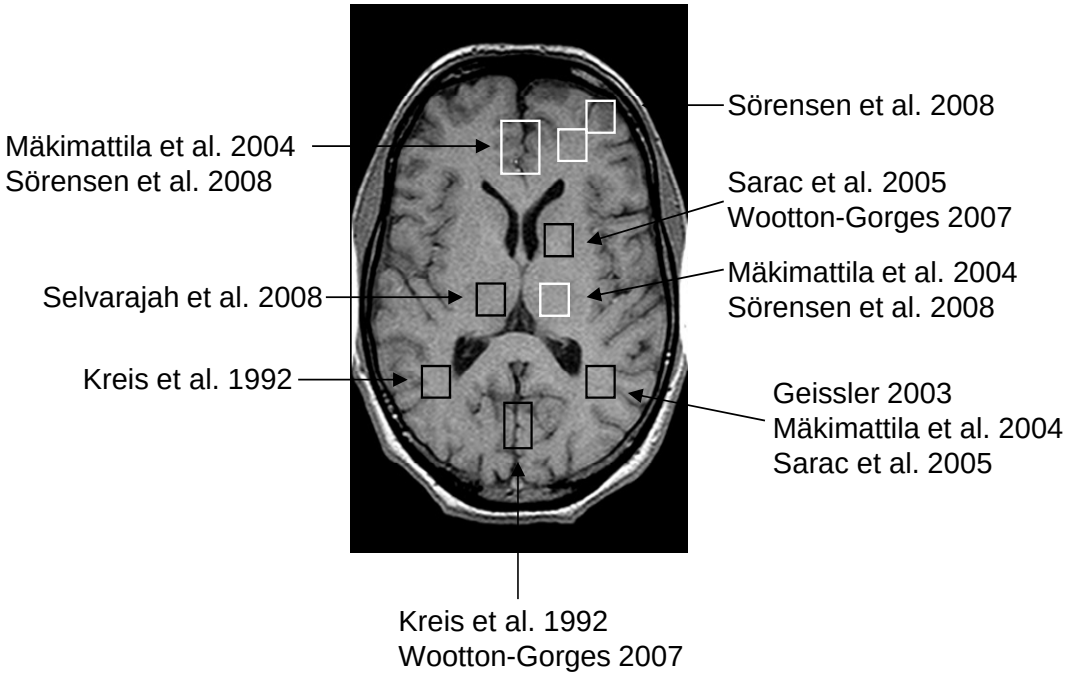


**Figure 5** Regions studied with $^{1}H$ MRS in patients with type 1 diabetes.

**Table 3.** $^{1}$H MRS studies on brain metabolites in patients with diabetes

| Study | Subject groups ($n$ = M/$n$ = F) | Age | $HbA_{1c}$ (%); duration (years) | Brain regions | Findings |
|---|---|---|---|---|---|
| **Type 1 diabetes** | | | | | |
| Kreis et al. 1992 (254) | T1D (7/10)<br>9 DKA<br>non-DM (19/11) | 43<br>47 | NA<br>NA | White matter in posteriomedial parietal cortex | ▪T1D vs non-DM: NAA/tCr↓, Cho/tCr↔, mI/Cr↑ |
| | | | | Gray matter in posterior occipital cortex | ▪T1D vs non-DM: NAA/tCr↔, Cho/tCr↔, mI/tCr↑ |
| Perros et al. 1997 (233) | IDDM HG- 11<br>IDDM HG+ 10 | 42<br>45 | NA | Parietal and frontal lobes | ▪IDDM HG+ vs IDDM HG-: NAA/Cr↔, NAA/Cho↔ |
| Mäkimattila et al. 2004 (255) | T1D (10/0)<br>retinopathy and peripheral neuropathy<br>non-DM (10/0) | 36<br>36 | 9.1; 28<br>5.4; 0 | Frontal cortical gray matter | ▪T1D vs non-DM: $H_2O$↑, NAA/$H_2O$↔, tCr/$H_2O$↔, Cho/$H_2O$↔, mI/$H_2O$↔ |
| | | | | Prosterior cortical white matter | ▪T1D vs non-DM: $H_2O$↑, NAA/$H_2O$↔, tCr/$H_2O$↔, Cho/$H_2O$↑, mI/$H_2O$↑<br>▪T1D: Cumlative $HbA_{1c}$ index↑ correlated with NAA/$H_2O$↓ and Cho/$H_2O$↓ |
| | | | | Thalamus | ▪T1D vs non-DM: $H_2O$↔, NAA/$H_2O$↔, tCr/$H_2O$↔, Cho/$H_2O$↑, mI/$H_2O$↔<br>▪T1D: Cumulative $HbA_{1c}$ index↑ correlated with Cho/$H_2O$↓ |
| Sarac et al. 2005 (282) | T1D 30 (NA)<br>14 retinopathy<br>non-DM 14 | 13<br>12 | 11.9; NA<br>NA; 0 | Posterior parietal white matter | ▪T1D vs non-DM: NAA/Cr↓, Cho/tCr↔ |

| | | | | | |
|---|---|---|---|---|---|
| Wootton-Gorges et al. 2007 (284) | T1D+DKA 29 | 12 | NA; 45% newly diagnosed | Occipital gray matter | ▪ During treatment vs after recovery: NAA/Cr↓ (trend), Cho/Cr↔ |
| | | | | Periaqueductal gray matter | ▪ During treatment vs after recovery: NAA/Cr↓ (trend), Cho/Cr↔ |
| | | | | Basal ganglia | ▪ During treatment vs after recovery: NAA/Cr↓ (trend), Cho/Cr↔ |
| Selvarajah et al. 2008 (285) | T1D DPN+ (10/0)<br>T1D DPN- (8/0)<br>non-DM (6/0) | 30<br>26<br>43 | 7.8; 22<br>9.1; 8 | Right thalamus lobe | ▪ Short TE: T1D DPN+ vs T1D DPN- vs non-DM: NAA/tCr↔, NAA/Cho↔, Cho/tCr↔<br>▪ Long TE: T1D DPN+ vs non-DM: NAA/tCr↓, NAA/Cho↓, Cho/tCr↔<br>→ Neural dysfunction but not loss<br>▪ Severity of peripheral neuropathy correlated with brain metabolites↑ |
| **Type 1 and 2 diabetes not separated** | | | | | |
| Geissler et al. 2003 (286) | T1D (5/1) + T2D (14/10)<br>3 DKA<br>non-DM (15/15) | 46<br>37 | 8.3; 17<br><6.0; 0 | Parietal white matter | ▪ T1D+T2D vs non-DM: NAA/tCr↔, Cho/tCr↔, mI/tCr↑<br>▪ T1D vs T2D mI/tCr↑<br>▪ S-Osmolality↑ and S-Sodium↑ correlated with mI/tCr↑ |
| Sörensen et al. 2008 (287) | Painful DPN+ (12/0)<br>T1D 2 + T2D 10<br>DM Pain- (13/1)<br>T1D 6 + T2D 8<br>non-DM (9/9) | 61<br>57<br>18 | 7.5; 15<br>7.7; 14<br>NA; 0 | Anterior cingulated cortex | ▪ Painful DPN+ vs DM Pain- vs non-DM: NAA/$H_2O$↔, tCr/$H_2O$↔, Cho/$H_2O$↔ |
| | | | | Dorsolateral prefrontal cortex | ▪ DM Pain- vs non-DM: NAA/$H_2O$↓, tCr/$H_2O$↓, Cho/$H_2O$↔ |

*NA, not applied; T1D, type 1 diabetes; T2D, type 2 diabetes; non-DM, no diabetes; IDDM, insulin-dependent diabetes; DKA, diabetic ketoacidosis; DPN, diabetic peripheral neuropathy; HG, severe hypoglycemias, TE, echo time; NAA, N-acetylaspartate; tCr, total creatine; Cho, choline; mI, myo-inositol; Glc, glucose.*

## 2.4. Mechanisms of Brain Damage in Metabolic Syndrome and Diabetes

Brain tissue in patients with metabolic risk factors or type 1 diabetes may be harmed by three presumed mechanisms atherosclerosis, hyperinsulinemia, and glucose toxicity (Figure 6) (205, 288).

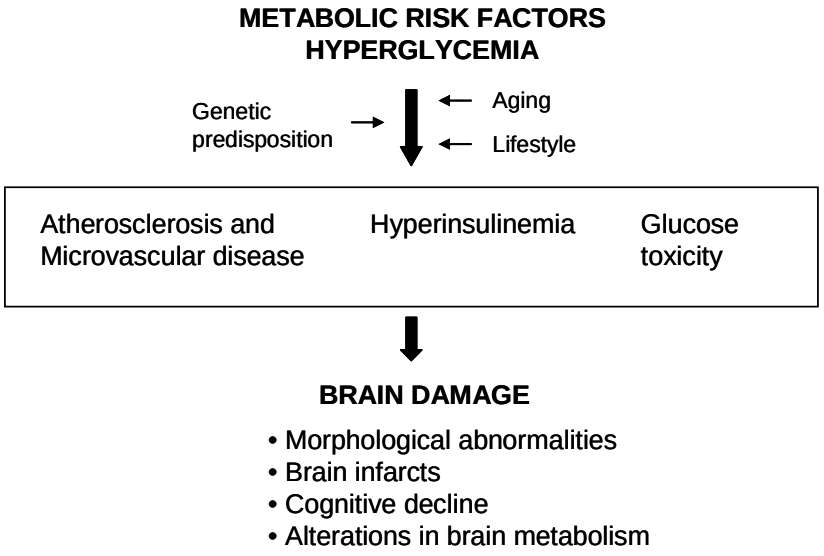


**Figure 6** Mechanisms that damage the brain in patients with metabolic syndrome and type 1 diabetes. Modified from Biessels et al. *Neurology* 2006;5:70 (205)

Atherosclerosis and microvascular disease may lead to impaired vascular reactivity, hypoperfusion, and in time to chronic cerebral ischemia.

Hyperinsulinemia in patients with impaired glucose regulation or type 2 diabetes has been associated with cognitive decline. Because insulin has vasoactive effects, some of this association may be mediated through vascular disease (289). Insulin has also been suggested to be involved in amyloid metabolism and in Alzheimer's disease (205). Amyloid precursor protein is a transmembrane glycoprotein of undetermined function. Its degradation produces amyloid-$\beta$ peptide that is secreted into the ECF with accelerative effect of insulin. In the ECF, amyloid-$\beta$ peptide can aggregate with other proteins to form the senile plaques of Alzheimer's disease or alternatively be cleaved. The cleavage processes are two: through LDL receptor-related protein-mediated endocytosis, or through direct extracellular proteolytic degradation. The latter involves an insulin-degrading enzyme with which insulin competes (290). Hyperinsulinemia may therefore inhibit the degradation of amyloid-$\beta$ peptide and favor the formation of senile plaques.

Tissue glucose may cause both microvascular damage and brain tissue damage by four different mechanisms (288, 291, 292). First, activation of the polyol pathway converts the excess glucose to sorbitol and fructose, which leads to an imbalance in nicotinamide adenine dinucleotide phosphate (NADPH) -redox and changes in signal transduction. Increased sorbitol has also been linked to alterations in metabolic pathways of phosphoinositide (including *myo*-inositol) and diacylglycerol which, together with alterations in calcium homeostasis, affect the activity of protein kinases. Second, the irreversible nonenzymatic glycation of proteins leads to formation of advanced glycation end-products (AGEs) that alter the function of proteins and cause generalized cellular dysfunction. Third, increased activation of protein kinase C leads to activation of growth factors and decreased endothelial nitric oxide synthase activation. Fourth, increased flux through the hexosamine pathway enhances expression of transforming growth factor-$\beta$1 and plasminogen activator inhibitor-1, both of which are harmful for diabetic blood vessels.

Each of these mechanisms results in production of reactive oxygen species (ROS) in the mitochondrial electron transport chain, which is reflected as an overall increase in cellular oxidative stress. In vitro work shows that in nerve cells, even short-term hyperglycemia leads to oxidative damage and apoptosis (293). The brains of hyperglycemic rats have shown increased sorbitol and fructose (294) protein kinase A and C (295), AGEs (296), and by-products of lipid peroxidation (297-299). Furthermore, activity occurs in the superoxide dismutase and catalase enzymes involved in antioxidant defence (297, 299).

# 3 AIMS OF THE STUDY

The present studies were undertaken in order to answer the following questions:

I. Do the risk factors of the metabolic syndrome influence various brain metabolites?

II. Are there tissue-specific regional differences in the glucose content of the cerebrum? Are the distributions of glucose or brain metabolites altered by type 1 diabetes?

III. How much does acute hyperglycemia raise cerebral glucose in type 1 diabetes, and is there regional variability? Do chronic fluctuations in blood glucose alter cerebral glucose uptake or metabolism in type 1 diabetes?

IV. Does the cerebellum have a glucose content different from that of the cerebrum? Does type 1 diabetes elevate glucose content or alter brain metabolites in the cerebellum? Do chronic fluctuations in the blood glucose concentration alter the cerebellar glucose uptake or metabolism in type 1 diabetes?

# 4 SUBJECTS AND STUDY DESIGN

This study is part of the nationwide Finnish Diabetic Nephropathy (FinnDiane) Study and was performed at the Department of Medicine, Division of Nephrology, and the Department of Radiology of the Helsinki University Central Hospital.

Forty-three men aged 20 to 45 years were recruited from the Helsinki metropolitan area. The patients with diabetes were selected from the database of the FinnDiane study or the outpatient clinics of the Helsinki University Central Hospital. The non-diabetic participants were recruited by advertisement from the University of Helsinki, the Taxi Drivers Union, and Health Care Centers located in the Helsinki metropolitan area. Exclusion criteria were head trauma, neurological or psychiatric diseases, smoking, cerebrovascular or cardiovascular diseases, known coagulopathy, chronic inflammatory disease, and cancer. Previous or present alcohol or drug abuse was not allowed, and contraindications for MRI also precluded participation.

None of the subjects had symptoms or signs of peripheral neuropathy (abnormal tendon reflexes or decreased fine touch or vibration perception threshold). Of the diabetic participants, two had very mild and two moderate background retinopathy, but the others had none. Microalbuminuria was observable in one non-diabetic (39 mg/24h) and three diabetic subjects (32, 38, and 115 mg/24h). The diabetic subject with the greatest albumin excretion was treated with 10 mg ramipril and 50 mg acetylsalicylic acid. He participated in the hyperglycemic clamp in Studies III and IV. Other subjects used no regular medication except for insulin for diabetes. Eleven used glargine insulin, two took detemir insulin, and five NPH insulin. None of the diabetic subjects had any history of unconsciousness due to hypoglycemia, and they reported experiencing hypoglycemic symptoms at a blood glucose < 3 mmol/l.

The non-diabetic control subjects for the diabetic patients were selected in each study based on age-matching. In Studies III and IV, BMI-matching was also used.

## 4.1. Study I

Brain glucose and metabolites were compared between nine men with risk factors for the metabolic syndrome (risk group) and nine men with no metabolic risk factors (control group). Associations were sought between brain metabolites, brain glucose, and metabolic risk factors.

Subjects in the risk group had at least one parent with type 2 diabetes, whereas those in the control group had no parent with type 2 diabetes. Risk-factor assessment was based on criteria of the IDF (Table 1). Subjects in the risk group fulfillled the main criterion and of the additional criteria, had one to four (four had one, three had two, one had three, and one had four). Type 2 diabetes was excluded with a 2-h oral glucose tolerance test (OGTT). Of the subjects in the control group, one fulfillled the main criterion concerning waist circumference and one the minor criteria concerning HDL cholesterol. $^{1}$H MRS was

performed after an overnight fast. The data from the voxels in the frontal cortex, the frontal white matter, and in the thalamus were included in analyses.

## 4.2. Study II

The glucose content and brain metabolites were compared on the one hand between brain regions and on the other hand between the group of non-diabetic subjects and the group of diabetic subjects. All the 17 patients with type 1 diabetes and 12 age-matched non-diabetic subjects were included. The $^{1}$H MRS study was performed after an overnight fast (fasting glycemia, MRS 0), and the analyses included data from the frontal cortex, frontal white matter, and the thalamus.

## 4.3. Study III

Changes in the brain glucose content during acute hyperglycemia were compared between brain regions in both non-diabetic and diabetic subjects. Absolute brain glucose content and brain metabolites during acute hyperglycemia were compared between the diabetic and the non-diabetic subjects. Seven patients with type 1 diabetes and 11 non-diabetic controls were selected based on age- and BMI-matching. The diabetic subjects had longer than 8 years of diabetes duration and large daily blood glucose variation defined by the mean amplitude of glycemic excursions (MAGE; see 5.2). After the collection of the baseline $^{1}$H MRS data during fasting glycemia (MRS 0), the subjects were studied with a normoinsulinemic hyperglycemic clamp (see 5.6.) that imitated a typical hyperglycemic episode in diabetic patients with a large daily blood glucose variation. During acute hyperglycemia, the spectroscopy data were collected twice (MRS I and II). The study included brain glucose and metabolite data from the frontal cortex, frontal white matter, and the thalamus.

## 4.4. Study IV

The glucose content and brain metabolites were compared on the one hand between the cerebellum and the cerebrum, and on the other hand between men with type 1 diabetes and healthy non-diabetic men during fasting glycemia and during acute hyperglycemia. Baseline data (MRS 0) on 18 subjects with type 1 diabetes and 20 control subjects as well as data during hyperglycemia (MRS I and II) of 7 subjects with type 1 diabetes and 11 control subjects were included in the analyses.

## 4.5. Ethical Aspects and Informed Consent

The research project was approved by the ethics committee of the Hospital District of Helsinki and Uusimaa, and given research licenses by the Helsinki Health Center, the Department of Medicine, and the Department of Radiology of Helsinki University Central Hospital. Each subject provided written informed consent prior to participation.

# 5 METHODS

## 5.1. Questionnaires and Anthropometric Measurements

The subjects answered the same questionnaires used in the FinnDiane study, questionnaires covering medical history, hypoglycemia awareness, symptoms of peripheral neuropathy, alcohol consumption, smoking habits, and the medical history of siblings and parents. Waist circumference was measured from midway between the lowest rib and the iliac crest. Body mass index was calculated by dividing weight in kilograms by height in meters squared ($kg/m^2$). Blood pressure was measured after 10 minutes of rest in a sitting position with an automated standardized blood pressure monitor. The mean of at least two measurements was used in each analysis.

## 5.2. Tests of Glucose Metabolism and Glycemic Control

In Study I, a 2-h 75-g glucose tolerance test was performed according to WHO criteria (122, 122). Blood samples were drawn at 0, 30, 60, and 120 minutes for the determination of plasma glucose and serum insulin concentrations. The homeostatic model Assessment of Insulin Resistance (HOMA-IR) (300) value was calculated (Diabetes Trial Unit, The Oxford Centre for Diabetes, UK; a site which is available at www.dtu.ox.ac.uk, last accessed in September 2010).

In the patients with diabetes, glycemic control was assessed based on one $HbA_{1c}$. In Study III, a variable blood glucose profile in the diabetic patients was verified with a continuous glucose monitoring system (CGMS; Medtronic MiniMed, Northridge, CA, USA). The glucose monitor recorded the interstitial fluid glucose every fifth minute for 3 to 5 days prior to the MR data collection. The data were downloaded via the Com_Station to the MiniMed Solutions Software version 2.0b (Medtronic MiniMed). To assess glucose variability, the daily curves were first assessed manually, and then the MAGE (301) was calculated with an in-house script in the Matlab programming environment (MathWorks Inc, Natick, MA, USA). The script calculated the arithmetric mean of the differences between consecutive peaks and nadirs.

## 5.3. Assessment of Microvascular Complications

Microvascular complications were assessed in all patients with diabetes.

Symptoms of *peripheral neuropathy* such as muscle weakness or cramps, prickle or tingling and numbness of the feet were gauged with a standardized questionnaire. Achilles and patellar tendon reflexes were tested in a sitting position by use of a standard

triangular rubber-headed reflex hammer. Any detectable reflexive response was considered an intact reflex. Fine touch was tested from the first toe, the second metatarsal bone and from the heel with a 2-g monofilament. Vibration perception threshold was tested in the first metatarsal bone and in medial malleoli with a $C_{128}$ tuning fork.

*Retinopathy* was assessed from fundus photographs by an ophthalmologist. The photographs were scored based on the Early Treatment Diabetic Retinopathy Study scale (302).

The presence of *nephropathy* was assessed by urinary albumin excretion rate (UAER) from a 24-hour urine collection. When necessary, either additional overnight urine collections were performed, or renal status was verified from medical files. Normoalbuminuria was defined as an UAER < 30 mg/24 h or in an overnight urine sample < 20 μg/min. Microalbuminuria was defined as an UAER 30 to 300 mg/24 h or 20 – 200 μg/min. Renal function was assessed as creatinine clearance.

## 5.4. Laboratory Assays

Blood samples were drawn from the antecubital vein during a fasting state on the study morning or within 2 weeks before the $^1$H MRS data collection.

Blood glucose was measured bedside with a Beta-glucose analyzer (HemoCue Glucose 201+; HemoCue, Ängelholm, Sweden). Plasma glucose was analyzed by the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA, USA), serum insulin with a time-resolved fluoroimmunoassay (PerkinElmer, Turku, Finland). A coefficient of 6.945 served to convert the plasma insulin values from mU/l to nmol/l. $HbA_{1c}$ was analyzed by immunoturbidometry (normal range 4.0-6.0% / 20-42 mmol/mol).

Plasma creatinine, serum alanine aminotranferase, urea, serum total cholesterol, HDL cholesterol, and triglycerides were analyzed with enzymatic photometric assays (Roche Diagnostics, Basel, Switzerland). Serum LDL cholesterol was calculated by Friedewald's formula (303). Serum sodium was analyzed by an ion selective electrode method (Roche Diagnostics). Osmolality was calculated by the equation 1.86 × S-sodium (mmol/l) + P-glucose (mmol/l) + S-urea (mmol/l) + 9.

Serum IL-6 (Bayer, Tarrytown, NY, USA), and urinary albumin concentration and plasma CRP (Orion Diagnostica, Espoo, Finland) were analyzed by immunoturbidometric methods. Plasma homocysteine was analyzed by an enzymatic assay (Axis-Shield Diagnostics, Dundee, UK) and serum soluble endothelial selectin and sICAM1 by commercial immunoassays (R&D Systems, Minneapolis, MN, USA).

## 5.5. MRI and $^1$H MRS

*Preparations for the study*. Participants were instructed to avoid physical exercise, heavy meals, medication, and alcohol on the day previous to the study. They were furthermore

instructed not to stay awake late on the previous night and to fast 12 hours before the study. The diabetic patients were also asked to avoid hypoglycemic episodes during the previous 24 hours. Patients using NPH insulin therefore reduced their normal morning dose by 50%, but for others, their dose of glargine or detemir insulin remained normal. Hypoglycemic symptoms or verified hypoglycemia (< 2.9 mmol/l) led to rescheduling of the study visit.

*Study design.* Baseline data (MRS 0) were collected from all subjects. A catheter was inserted into the right antecubital vein for drawing of basal blood samples and for a 0.9% saline infusion (50 ml/h). Those diabetic subjects with large daily glycemic variation and the non-diabetic control subjects continued with a hyperglycemic clamp (see 5.6.) during which time, data were collected twice (MRS I and II).

*Data acquisition.* The magnetic resonance procedures were performed with a 1.5 T magnetic resonance imager equipped with a standard head coil (Siemens Magnetom Sonata, Erlangen, Germany). T1-weighted sagittal, T2-weighted coronal, and fast FLAIR images were acquired to ensure normal brain structure and to position the voxels from which the metabolite data were collected. The voxels were in the midline of the frontal cortex (25 x 16 x 20 $mm^3$ = 8.0 ml), the left frontal white matter (30 x 16 x 16 $mm^3$ = 7.7 ml), the left thalamic lobe (20 x 20 x 20 $mm^3$ = 8.0 ml, shown as white squares in Figures 4 and 5), and in the middle of the right cerebellar hemisphere (20 x 20 x 20 $mm^3$ = 8.0 ml). Single voxel $^1H$ MRS was performed with PRESS sequence and chemical-shift selective (CHESS) water suppression (TE = 30 ms, TR = 3,000 ms, 64 acquisitions). For determining tissue water, non-water-suppressed spectra were collected from the same voxels (four acquisitions).

*Quantitation.* The MRS data were processed and analyzed with an in-house written script running on a Matlab 7.2 platform (MathWorks). The FIDs were apodized with a Gaussian function (2.5 Hz broadening factor) and zerofilled up to 2,048 complex points prior to Fourier transformation. The peak areas, i.e. metabolite signal intensities, were determined for NAA (1.98-2.06 ppm), Glx (3.40-3.45 ppm), tCr (3.00-3.08 ppm), Cho (3.18-3.27 ppm), mI (3.52-3.60 ppm) glucose (3.40-3.46 ppm), and $H_2O$ (4.2-5.2 ppm) by integration. The receiver gain was constant, and the signal intensities were automatically corrected for coil-loading and voxel size. The integral data were analyzed as peak ratios of metabolite/$H_2O$ or metabolite/tCr.

The increase in glucose concentration in each voxel was analyzed with difference spectra (Figure 3). The baseline spectrum was subtracted from the second and third spectra (MRS I minus 0 and II minus 0), and a spectrum recorded from 100 mmol/l glucose solution was fitted to the difference spectrum (all spectra corrected for voxel size and coil-loading effects). For measurement of the glucose phantom spectrum, a 100 mmol/l glucose solution was prepared in potassium phosphate buffer at pH 7.05. A 50-ml round-bottomed flask was filled with glucose solution and was positioned within a 4-l plastic spherical container filled with 0.1 mmol/l $MnCl_2$ solution and NaCl (0.4%). This phantom setup corresponded to approximately the coil loading obtained with in vivo studies; thus the required coil loading correction factor was not large. Single voxel $^1H$ MRS was performed (voxel size 20 x 20 x 20 $mm^3$) with a PRESS sequence and CHESS water suppression scheme (TE = 30 ms, TR = 6,000 ms, 64 acquisitions, spectral width of 1000 Hz, 1024 acquired complex points). The FID was apodized with a Gaussian

function (2.5 Hz broadening factor) and zerofilled up to 2,048 complex points prior to Fourier transformation.

A neuroradiologist blinded to the clinical data evaluated the MR images and quality of acquired spectra before the analysis. Of all the 316 spectra, 37 were disqualified.

## 5.6. Hyperglycemic Normoinsulinemic Clamp (III, IV)

A hyperglycemic normoinsulinemic clamp was performed to increase blood glucose concentration to 12 mmol/l above basal level. Acute hyperglycemia was achieved with a bolus of 50% glucose solution (0.5 ml/kg body weight) and maintained with a variable infusion of 20% glucose solution (50-300 ml/h) into the right antecubital vein. The left hand dorsum with a retrograde catheter was kept warm with heat packs and towels in order to obtain arterialized venous blood for measurements of blood glucose (304). Samples were drawn every 10 minutes, and blood glucose was analyzed bedside in order to adjust the 20% glucose infusion.

To reduce endogenous insulin secretion during the clamp and to avoid reactive hypoglycemia, the non-diabetic control subjects also received a bolus (25 μg) and infusion (0.75 μg/min) of a somatostatin analogue (Sandostatin; Novartis, Helsinki, Finland) into the left antecubital vein.

Samples for plasma glucose and serum insulin were drawn before and after each $^{1}$H MRS (0, I and II) data collection. Means of these values served in the analyses.

## 5.7. Statistical Methods

Power calculations were performed and sample size determined based on results of the pilot study (255).

In Studies I, III, and IV, more robust non-parametric tests were chosen, due to the small sample size and the non-normal distributions of the variables. Differences between study groups and brain regions were examined by the Mann-Whitney U-test. Correlations between $^{1}$H MRS data and clinical variables were tested with Spearman's pair-wise rank-order correlation test. Differences between three repeated measures during the clamp were assessed with the Wilcoxon signed rank test. Multiple testing was taken into consideration only in Study I, in which we performed the Bonferroni correction.

In Study II, in which the number of subjects was higher, parametric tests were used: Student's t-test for differences between study groups and brain regions and the Pearson correlation coefficient for correlations. The results were confirmed with non-parametric tests.

All analyses were performed with Sigma Stat Statistical Software (SPSS 15.0, Chicago, IL, USA). The data are given as mean ± SD, and *p*-values of 0.05 or less were considered significant.

# 6 RESULTS

## 6.1. Metabolic Risk Factors (Study I)

### Subjects

Subject characteristics are presented in Table 4. The risk group had abdominal obesity and increased blood pressure. Markers of insulin resistance were higher in the risk group than in the control group, although in the normal range.

**Table 4.** Subject characteristics in Study I.

| | Control group | Risk group |
|---|---|---|
| Age (years) | 36 ± 6 | 36 ± 7 |
| Waist (cm) | 87 ± 8 | 104 ± 6** |
| BMI (kg/m$^2$) | 23.0 ± 2.6 | 29.0 ± 2.5** |
| Systolic blood pressure (mmHg) | 126 ± 8 | 144 ± 13** |
| Diastolic blood pressure (mmHg) | 76 ± 8 | 89 ± 5** |
| Mean arterial pressure (mmHg) | 93 ± 7 | 107 ± 7*** |
| Fasting plasma glucose (mmol/l) | 4.7 ± 0.34 | 5.3 ± 0.5** |
| Fasting serum insulin (pmol/l) | 28.4 ± 11.5 | 51.1 ± 22.9* |
| OGTT: | | |
| Plasma glucose at 120 min (mmol/l) | --- | 6.6 ± 1.8 |
| Serum insulin at 120 min (pmol/l) | --- | 215.3 ± 142.0 |
| HOMA-IR | 0.5 ± 0.2 | 0.9 ± 0.4* |
| $HbA_{1c}$ (%) | 5.5 ± 0.2 | 5.3 ± 0.2 |
| Total cholesterol (mmol/l) | 4.4 ± 1.1 | 4.7 ± 0.9 |
| HDL cholesterol (mmol/l) | 1.4 ± 0.3 | 1.4 ± 0.2 |
| LDL cholesterol (mmol/l) | 2.5 ± 1.0 | 2.6 ± 0.6 |
| Triglycerides (mmol/l) | 0.9 ± 0.3 | 1.7 ± 1.1 |
| CRP (mg/l) | 0.4 ± 0.6 | 0.5 ± 0.4 |
| IL-6 (ng/l) | 1.9 ± 0.1 | 2.0 ± 0.2 |
| sE-selectin (ng/ml) | 30.0 ± 15.23 | 41.4 ± 17.2 |
| Homocysteine (µmol/l) | 7.3 ± 1.3 | 9.1 ± 4.1 |
| sICAM-1 (ng/ml) | 217.6 ± 32.2 | 211.0 ± 37.7 |

*OGTT, oral glucose tolerance test; CRP, C-reactive protein; IL-6, interleukin 6; sE-selectin, soluble endothelial selectin; sICAM-1, soluble inter-cellular adhesion molecule 1; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

### Brain MRI

Subjects in the control group had normal MR images with no white matter changes. Three subjects in the risk group had white matter T2-hyperintensities. One had a mild patchy signal increase in the periventricular white matter and had periventricular rims of increased signal intensity. Two had a mild patchy signal increase in the peritrigonal white matter and mildly widened lateral ventricles.

**Brain $^{1}$H MRS**

*Risk group vs. control group*. The risk group had higher tCr/$H_2O$ in the thalamus than did the control group (Figure 7C). No difference existed in the thalamic glucose/$H_2O$, NAA/$H_2O$, Cho/$H_2O$, mI/$H_2O$, or tissue water, between study groups (Figure 7). The cortex and white matter showed no difference in any of the metabolites between groups.

*Brain metabolites vs. brain glucose vs. metabolic risk factors*. In the control group, thalamic tCr/$H_2O$ (Figure 8A) and mI/$H_2O$ (Figure 8B) correlated with thalamic glucose/$H_2O$. In the risk group, neither tCr/$H_2O$ ($r$ = 0.19, $p$ = 0.651) nor mI/$H_2O$ ($r$ = 0.43, $p$ = 0.289) correlated with thalamic glucose/$H_2O$. Instead, in the risk group, tCr/$H_2O$ (Figure 9A) and mI/$H_2O$ (Figure 9B) correlated with fasting plasma glucose and tCr also with 2-h plasma glucose in the OGTT (Figure 9C).

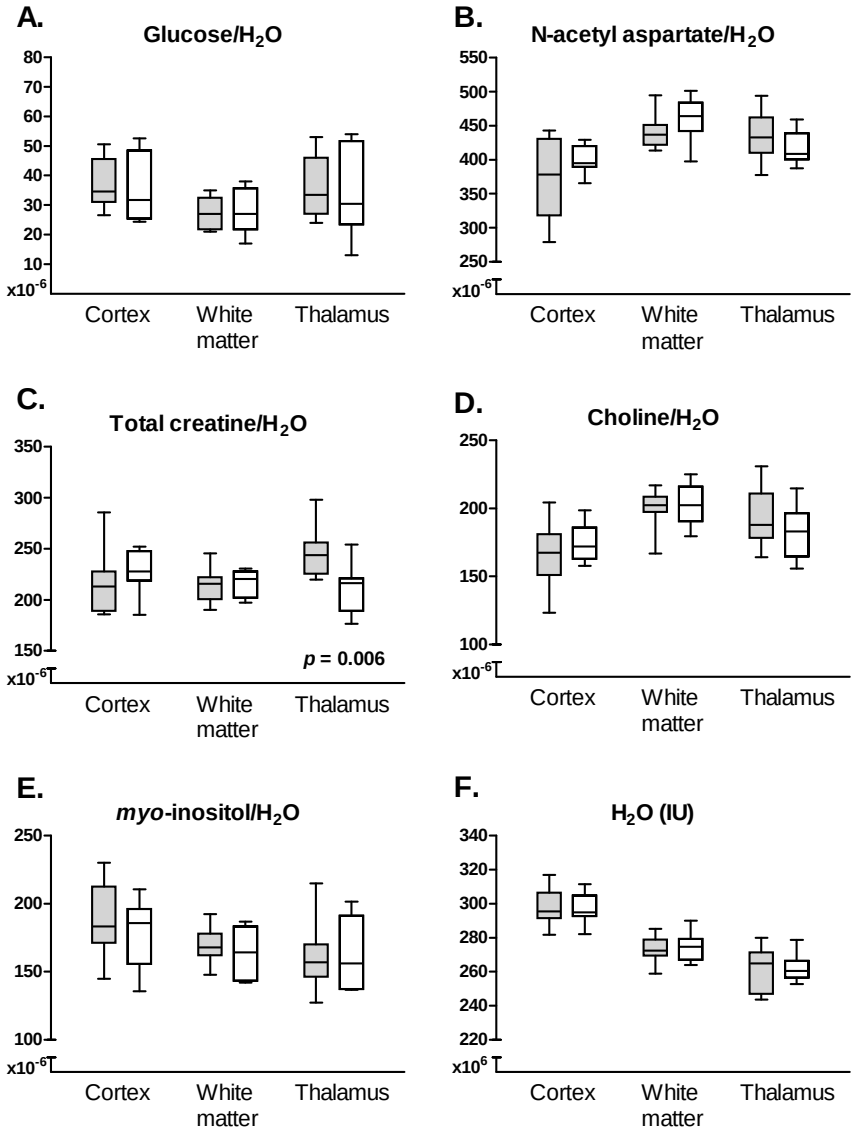


**Figure 7** Brain metabolite/$H_2O$ ratios and $H_2O$ in the risk group (gray) and in the non-diabetic group (white). Data are mean, SD and minimum and maximum.

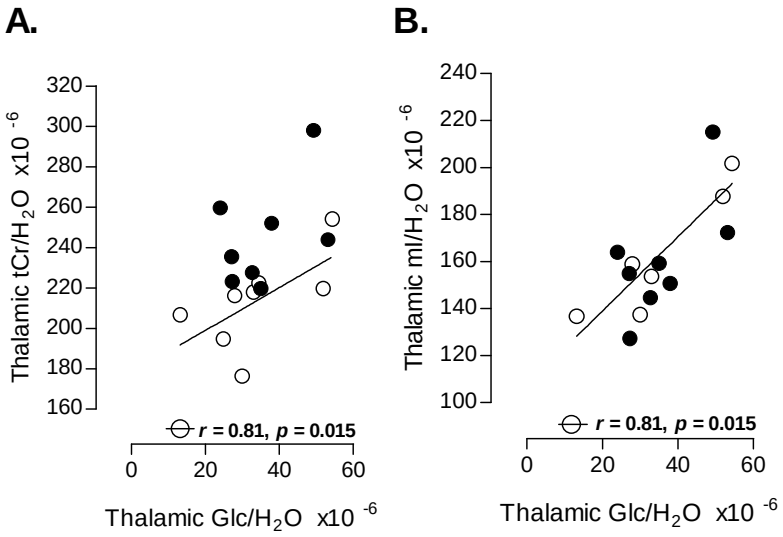


**Figure 8** Associations between thalamic glucose and **A.** thalamic total creatine ($tCr/H_2O$) and **B.** *myo*-inositol ($mI/H_2O$) in the risk group (●) and in the non-diabetic group (○).

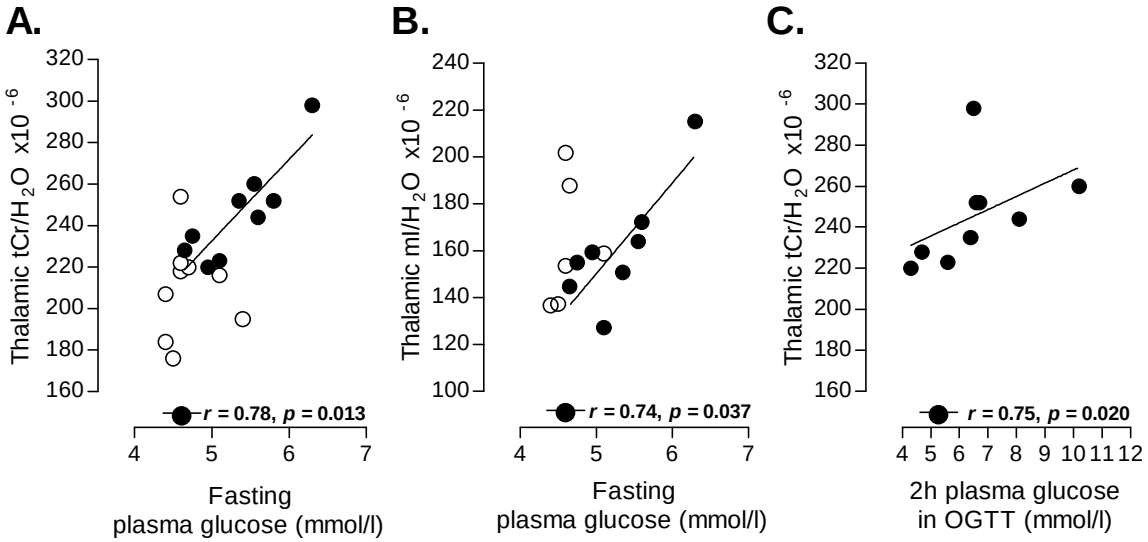


**Figure 9** Associations between fasting plasma glucose and **A.** thalamic total creatine ($tCr/H_2O$) and **B.** *myo*-inositol ($mI/H_2O$), and **C.** between the 2-h plasma glucose in the oral glucose tolerance test (OGTT) and thalamic $tCr/H_2O$ in the risk group (●) and in the non-diabetic group (○).

## 6.2. Type 1 Diabetes

Subject characteristics are presented in Table 5, where all diabetic and non-diabetic subjects have MR images in the normal range.

### 6.2.1. Cerebrum at Baseline (Study II)

Plasma glucose and and serum insulin remained stable during the study in the diabetic ($p$ = 0.361 and $p$ = 0.905) and non-diabetic ($p$ = 0.628 and $p$ = 0.083) subjects. The mean of plasma glucose was higher in the diabetic subjects (9.2 ± 3.0 vs. 4.8 ± 0.5, $p < 0.001$), whereas the mean of serum insulin were at a similar level (30.5 ± 42.0 vs. 20.8 ± 6.5, $p$ = 0.437).

*Cortex vs. white matter vs. thalamus.* The non-diabetic subjects had a 47% higher glucose/$H_2O$ ratio in the cortex than in the white matter (Figure 10A). The diabetic subjects showed no regional differences in cerebral glucose/$H_2O$ ratio. The regional differences in $H_2O$ are presented in Figure 10F.

*Type 1 diabetes vs. non-diabetes.* Compared to the non-diabetic subjects, the diabetic subjects had 64% higher glucose/$H_2O$ ratio in the white matter and 25% higher in the cortex (Figure 10A). Excess glucose in those diabetic subjects was the greatest in their white matter (Figure 11).

The diabetic subjects also had 6% lower NAA/$H_2O$ in their white matter (Figure 10B) and 8% higher mI/$H_2O$ in their cortices and 20% higher mI/$H_2O$ in their white matter (Figure 10E). tCr/$H_2O$ and Cho/$H_2O$ showed no differences between study groups.

*Brain glucose vs. plasma glucose.* In the diabetic subjects, brain glucose/$H_2O$ correlated with plasma glucose in all brain regions (Figure 12).

**Table 5.** Subject characteristics in Studies II, III, and IV.

| | Study II: Cerebrum at baseline | | Study IV: Cerebellum at baseline | | Studies III and IV: Acute hyperglycemia | |
|---|---|---|---|---|---|---|
| | **Control** | **T1D** | **Control** | **T1D** | **Control** | **T1D** |
| Age (years) | 29 ± 6 | 28 ± 4 | 33 ± 7 | 28 ± 4 | 29 ± 4 | 31 ± 7 |
| Waist (cm) | 83 ± 5 | 88 ± 8 | 92 ± 12 | 89 ± 9 | 90 ± 13 | 84 ± 7 |
| BMI (kg/m$^2$) | 22.4 ± 2.1 | 24.7 ± 3.0* | 25.1 ± 4.2 | 25.0 ± 3.2 | 25.1 ± 4.0 | 22.4 ± 2.3 |
| Systolic blood pressure (mmHg) | 125 ± 7 | 113 ± 7** | 133 ± 14 | 134 ± 7 | 134 ± 6 | 125 ± 7** |
| Diastolic blood pressure (mmHg) | 73 ± 8 | 75 ± 7 | 80 ± 11 | 76 ± 8 | 76 ± 9 | 75 ± 7 |
| Pulse (beats per min) | 57 ± 10 | 62 ± 6 | 60 ± 10 | 64 ± 10 | 66 ± 15 | 59 ± 9 |
| Age at diabetes onset (years) | | 21 ± 6 | | 21 ± 6 | | 17 ± 4 |
| Diabetes duration (years) | | 6.7 ± 5.2 | | 7.0 ± 5.3 | | 12.4 ± 2.8 |
| $HbA_{1c}$ (%) | 5.5 ± 0.2 | 7.4 ± 1.1*** | 5.4 ± 0.2 | 7.4 ± 1.1*** | 7.6 ± 0.8 | 5.5 ± 0.2*** |
| Insulin dose/weight (IU/kg) | | 0.7 ± 0.3 | | 0.7 ± 0.3 | 0.8 ± 0.3 | |
| Urinary albumin excretion rate (mg/24h) | 11 ± 8 | 12 ± 10 | 19 ± 19 | 19 ± 28 | 26 ± 44 | 15 ± 10 |
| Creatinine (µmol/l) | 79 ± 12 | 74 ± 13 | 80 ± 12 | 74 ± 13 | 78 ± 17 | 78 ± 12 |
| Total cholesterol (mmol/l) | 4.2 ± 0.9 | 4.3 ± 0.9 | 4.4 ± 1.0 | 4.3 ± 0.9 | 4.4 ± 0.8 | 4.4 ± 1.1 |
| HDL cholesterol (mmol/l) | 1.5 ± 0.3 | 1.6 ± 0.3 | 1.4 ± 0.3 | 1.6 ± 0.3 | 1.6 ± 0.5 | 1.4 ± 0.3 |
| LDL cholesterol (mmol/l) | 2.4 ± 0.9 | 2.3 ± 0.9 | 2.4 ± 0.8 | 2.3 ± 0.9 | 2.3 ± 0.9 | 2.7 ± 1.0 |
| Triacylglycerol (mmol/l) | 0.7 ± 0.3 | 0.9 ± 0.4 | 1.1 ± 0.9 | 1.0 ± 0.6 | 1.1 ± 0.7 | 0.8 ± 0.4 |
| CRP (mg/l) | 0.3 ± 0.3 | 0.5 ± 0.9 | 1.9 ± 6.5 | 0.8 ± 1.4 | 1.0 ± 1.8 | 0.5 ± 0.5 |
| IL-6 (ng/l) | 2.1 ± 0.6 | 2.0 ± 0.3 | 2.3 ± 1.0 | 2.1 ± 0.4 | 2.2 ± 0.5 | 2.2 ± 0.6 |
| sE-selectin (ng/ml) | 30.0 ± 14.0 | 41.3 ± 17.9 | 32.3 ± 16.3 | 41.0 ± 17.4 | 35.4 ± 12.4 | 29.1 ± 14.5 |
| sICAM-1 (ng/ml) | 216.1 ± 27.8 | 210.2 ± 46.5 | 218.3 ± 32.2 | 219.8 ± 60.8 | 245.7 ± 66.1 | 226.4 ± 26.7 |
| Homocysteine (µmol/l) | 7.8 ± 1.9 | 6.5 ± 2.4 | 8.4 ± 3.0 | 6.6 ± 2.3 | 6.9 ± 2.9 | 7.9 ± 1.9 |

*T1D, type 1 diabetes; CRP, C-reactive protein; IL-6, interleukin 6; sE-selectin, soluble endothelial selectin; sICAM-1, soluble inter-cellular adhesion molecule 1. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.*

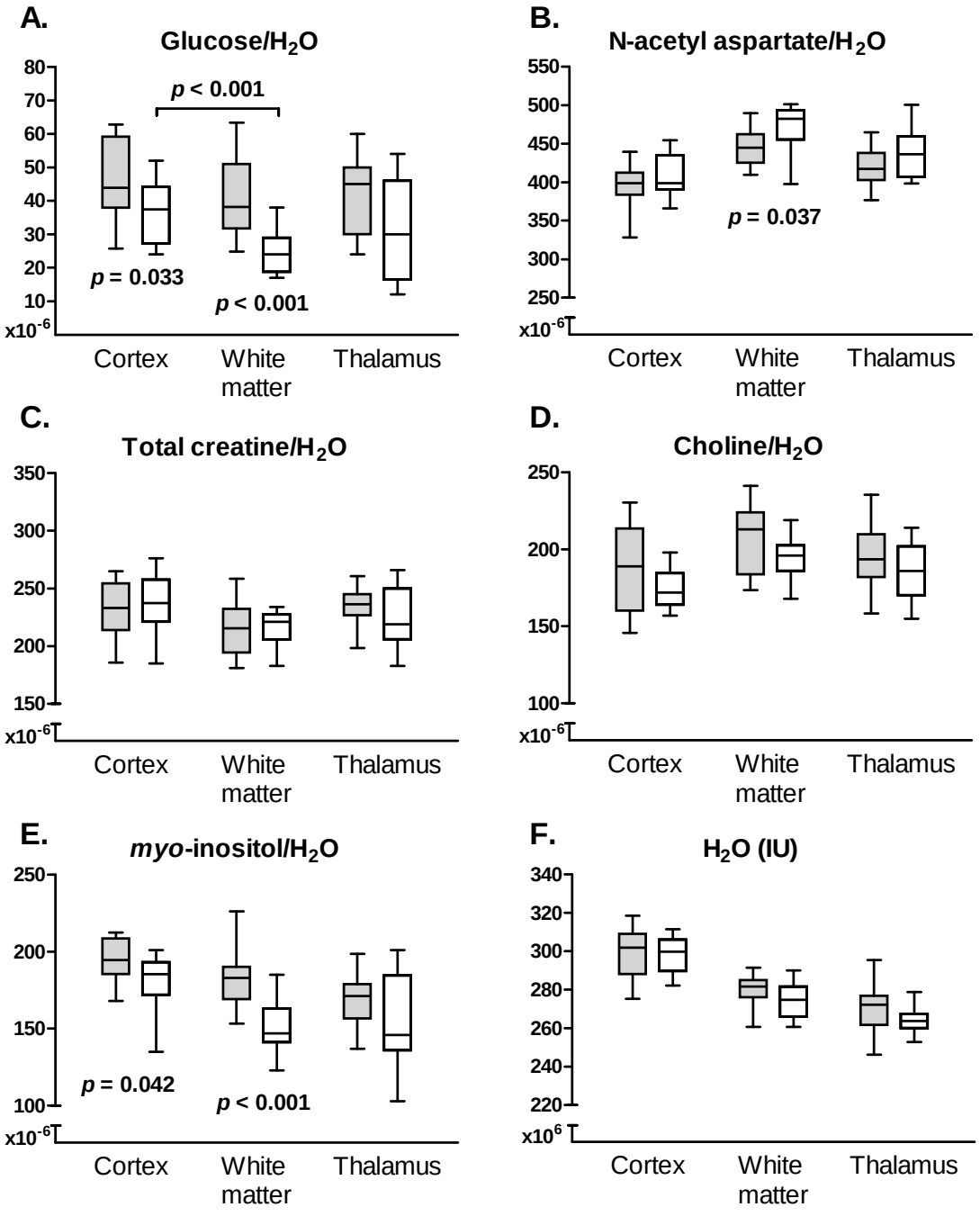


**Figure 10** Brain metabolite/$H_2O$ ratios and $H_2O$ in diabetic (gray) and non-diabetic (white) subjects. Data are mean, SD, and minimum and maximum.

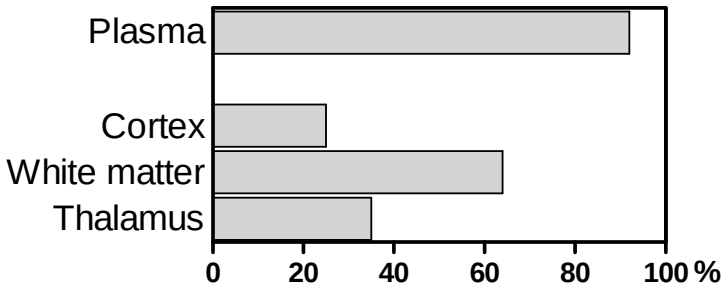


**Figure 11** Relative amounts of excess glucose in the plasma and brain in diabetic vs. non-diabetic subjects.

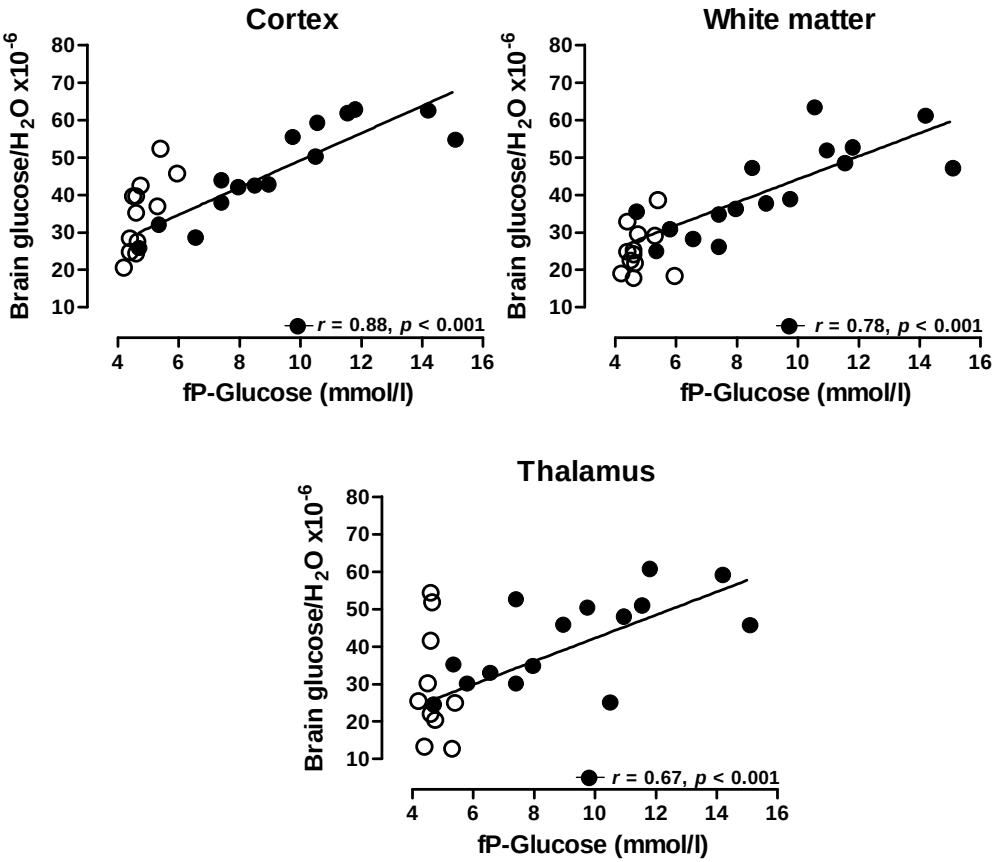


**Figure 12** Associations between brain and plasma glucose during fasting in diabetic (●) and non-diabetic subjects (○).

### 6.2.2. Cerebellum at Baseline (Study IV)

In the diabetic and non-diabetic subjects, plasma glucose concentrations were 9.0 ± 3.0 mmol/l and 5.0 ± 0.6 mmol/l ($p < 0.001$) and plasma insulin concentrations 33.8 ± 47.6 and 35.4 ± 20.7 ($p = 0.024$).

*Type 1 diabetes vs. non-diabetes.* No difference appeared in the cerebellar metabolite/$H_2O$ ratio (Figure 13), or in the glucose/$H_2O$ ratio (Figure 14A) or $H_2O$ (Figure 14B) between diabetic and non-diabetic subjects.

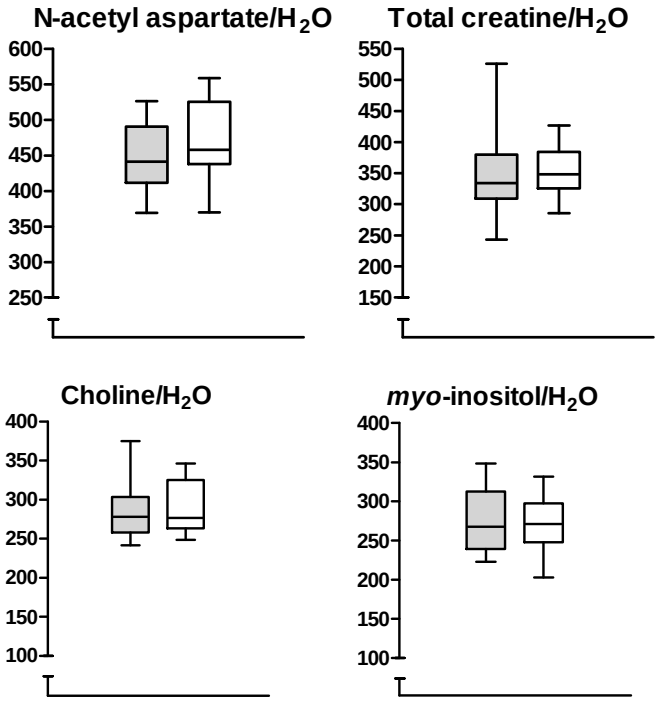


**Figure 13** Cerebellar metabolite/$H_2O$ ratios in diabetic subjects (gray) and in the non-diabetic subjects (white). Data are mean, SD, and minimum and maximum.

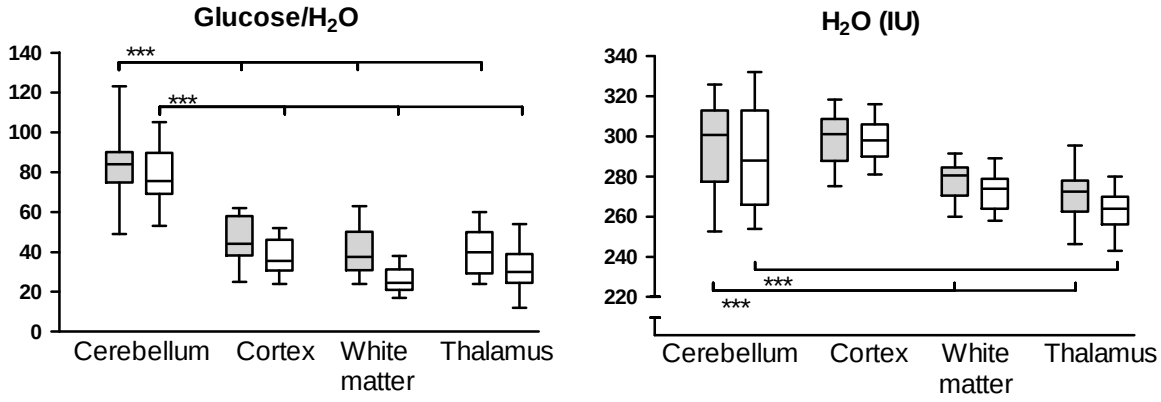


**Figure 14** Cerebellar **A.** glucose/$H_2O$ ratio and **B.** $H_2O$ in the diabetic (gray) and non-diabetic subjects (white). Data are mean, SD, and minimum and maximum. ***$p < 0.001$

*Cerebellum vs. cerebral cortex, white matter, and thalamus.* The glucose/$H_2O$ ratio was higher in the cerebellum than in the thalamus, cerebral cortex, or the cerebral white matter in both study groups (Figure 14A). As shown in Figure 14A, the glucose/$H_2O$ was 77.1 x $10^{-6}$ ± 14.9 x $10^{-6}$ in the cerebellum and 26.5 x $10^{-6}$ ± 6.0 x$10^{-6}$ in the cerebral white matter ($p$ < 0.001) in the non-diabetic participants. The $H_2O$ was higher in the cerebellum than in the white matter in the diabetic subjects and higher in the cerebellum than in the thalamus in both study groups (Figure 14B).

*Non-published data: brain glucose vs. plasma glucose.* During baseline, cerebellar glucose/$H_2O$ showed no correlation with plasma glucose in the diabetic ($r$ = 0.48, $p$ = 0.073) nor in the non-diabetic ($r$ = 0.08, $p$ = 0.709) subjects. Glucose/tCr did correlate with plasma glucose in the diabetic ($r$ = 0.69, $p$ = 0.04), but not in the non-diabetic ($r$ = 0.22, $p$ = 0.351) subjects.

### 6.2.3. Cerebrum and Cerebellum during Acute Hyperglycemia (Studies III-IV)

*Hyperglycemic clamp.* The clamp raised plasma glucose in the diabetic and non-diabetic subjects similarly (11.8 ± 2.9 vs 12.3 ± 1.5 mmol/l, $p$ = 0.892; Figure 15). Mean plasma glucose and serum insulin concentrations during the MRS 0, I and II are presented in Table 6. Serum osmolality increased from 272 ± 4 to 277 ± 3 mOsm/kg ($p$ = 0.043) in the diabetic and from 274 ± 4 to 279 ± 2 mOsm/kg ($p$ = 0.005) in the non-diabetic participants. No difference existed between these groups ($p$ = 0.526).

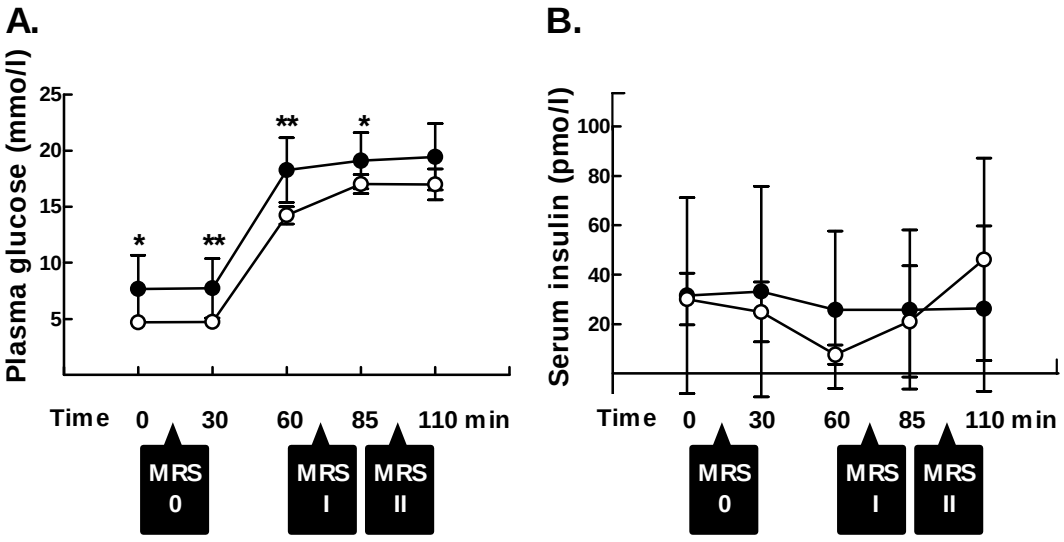


**Figure 15** **A.** Plasma glucose and **B.** serum insulin concentrations during the hyperglycemic normoinsulinemic clamp in diabetic (●) and non-diabetic subjects (○). MRS 0, I, and II, magnetic resonance spectroscopy data collections. *$p$ < 0.05, **$p$ < 0.01.

**Table 6.** Plasma glucose and serum insulin concentrations during $^{1}$H MRS data collection

| | MRS 0 | MRS I | MRS II |
|---|---|---|---|
| Plasma glucose (mmol/l) | | | |
| T1D | 7.7 ± 2.8 | 18.7 ± 2.2 | 19.3 ± 2.4** |
| Non-T1D | 4.7 ± 0.4 | 15.6 ± 0.7 | 16.8 ± 0.8*** |
| *p* T1D vs. Non-T1D | 0.005 | 0.004 | 0.011 |
| Serum insulin (pmol/l) | | | |
| T1D | 32.4 ± 41.1 | 24.9 ± 32.0 | 26.1 ± 32.8* |
| Non-T1D | 27.5 ± 10.7 | 14.4 ± 13.0 | 33.7 ± 23.3** |
| *p* T1D vs. Non-T1D | 0.297 | 0.683 | 0.220 |

*T1D, type 1 diabetes; Non-DM, no diabetes. MRS 0, I, and II, magnetic resonance spectroscopy data collections. *p < 0.05, **p < 0.01, ***p < 0.001.*

*Change in brain glucose.* Brain glucose increased significantly in all brain regions ($p < 0.05$). The increase observed in the difference spectra was similar in the diabetic and the non-diabetic subjects in the cerebellum (3.0 ± 0.9 vs. 3.1 ± 0.7 mmol/l, $p = 0.739$), cortex (2.0 ± 0.7 vs. 2.7 ± 0.9 mmol/l, $p = 0.093$), and in the white matter (1.3 ± 0.7 vs. 1.7 ± 0.7 mmol/l, $p = 0.306$). In the thalamus, glucose increased less in the diabetic than in the non-diabetic subjects (1.1 ± 0.4 vs. 2.3 ± 0.7 mmol/l, $p = 0.011$; Figure 16).

In the non-diabetic group, brain glucose increased more in the cerebellum than in the thalamus or in the white matter, and more in the cortex than in the white matter (Figure 17). In the diabetic subjects, brain glucose increased more in the cerebellum than in the white matter or the thalamus, and more in the cortex than in the thalamus.

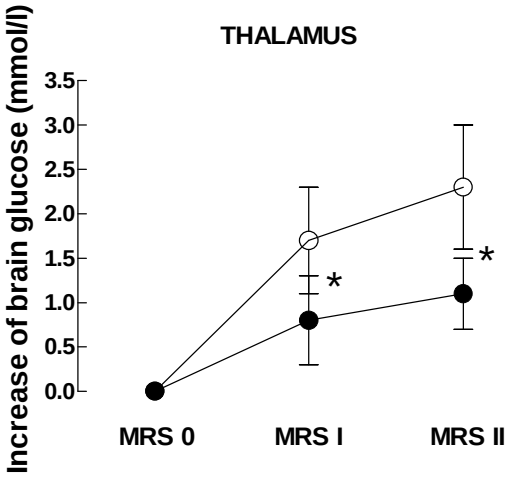


**Figure 16** Increase in thalamic glucose during acute hyperglycemia in diabetic (●) and non-diabetic (○) subjects. MRS 0, I, and II, magnetic resonance spectroscopy data collections. *$p <$ 0.05.

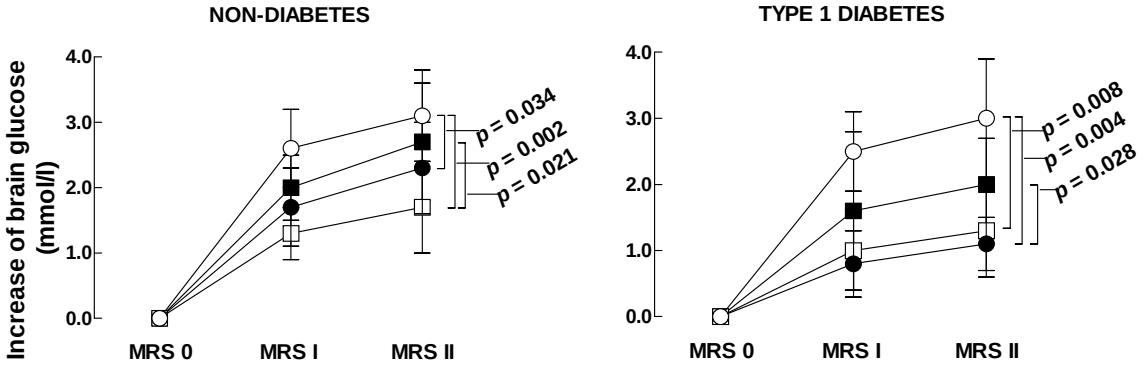


**Figure 17** Increase in cerebellar and cerebral glucose during acute hyperglycemia in the cerebellum (○), cerebral cortex (■), thalamus (●), and in the cerebral white matter (□). MRS 0, I, and II, magnetic resonance spectroscopy data collections.

*Internal references: brain tissue $H_2O$ and tCr.* Both in diabetic and non-diabetic subjects, $H_2O$ had a smaller coefficient of variation than did tCr (0.047 ± 0.008 vs. 0.126 ± 0.048, $p <$ 0.001 for diabetic and 0.102 ± 0.020 vs. 0.036 ± 0.005, $p < 0.001$ for non-diabetic subjects). During acute hyperglycemia, $H_2O$ was stable in the cortex, white matter, and in the thalamus, but increased 14% in the cerebellum of non-diabetic participants and 11% in the cerebellum of diabetic participants (Figure 18A). While $H_2O$ increased, tCr was stable during the clamp also in the cerebellum (Figure 18B) and therefore served as a reference in the analyses that included the cerebellum. No difference existed between the study groups either in $H_2O$ or in tCr.

*Brain glucose vs. plasma glucose.* In both study groups, brain glucose/$H_2O$ correlated with plasma glucose in the cortex, white matter, and in the thalamus (Figure 19). In the cerebellum, glucose/$H_2O$ and glucose/tCr ratios correlated with plasma glucose only in the non-diabetic subjects (Figure 20).

**Table 7.** Change in brain tissue $H_2O$ during acute hyperglycemia

| | | Cortex | White matter | Thalamus | Cerebellum |
|---|---|---|---|---|---|
| T1D | MRS 0 | 292 ± 11 | 279 ± 12 | 273 ± 15 | 276 ± 17 |
| | MRS I | 292 ± 12 | 278 ± 13 | 268 ± 14 | 308 ± 10 |
| | MRS II | 291 ± 14 | 277 ± 12 | 268 ± 15 | 305 ± 15 |
| *p* | | 0.989 | 0.989 | 0.812 | 0.028 |
| Non-T1D | MRS 0 | 297 ± 10 | 274 ± 9 | 264 ± 8 | 267 ± 9 |
| | MRS I | 294 ± 11 | 274 ± 12 | 260 ± 8 | 303 ± 13 |
| | MRS II | 296 ± 13 | 274 ± 9 | 261 ± 8 | 304 ± 13 |
| *p* | | 0.750 | 0.998 | 0.543 | 0.011 |

*T1D, type 1 diabetes; Non-T1D, no diabetes; MRS, magnetic resonance spectroscopy. MRS 0, I, and II, magnetic resonance spectroscopy data collections.*

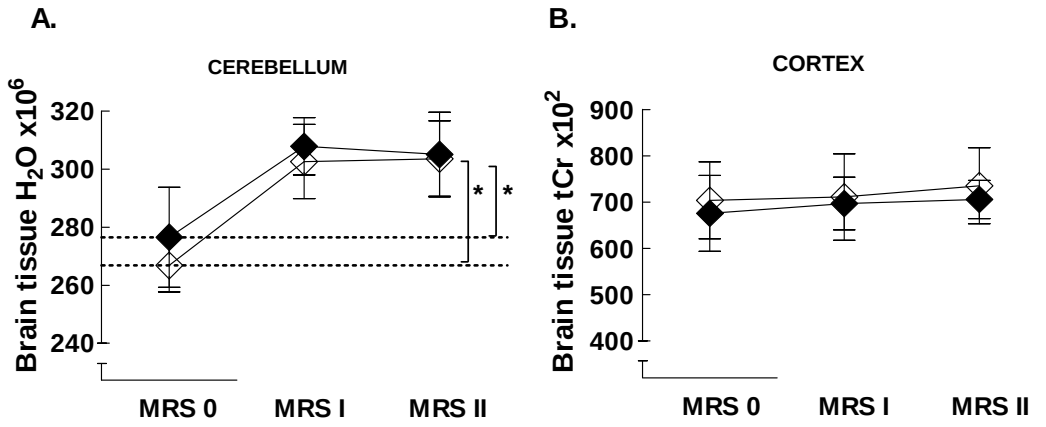


**Figure 18** **A.** Cerebellar $H_2O$ and **B.** total creatine (tCr) during acute hyperglycemia in diabetic (◆) and non-diabetic subjects (◇). MRS 0, I, and II, magnetic resonance spectroscopy data collections. *$p < 0.05$

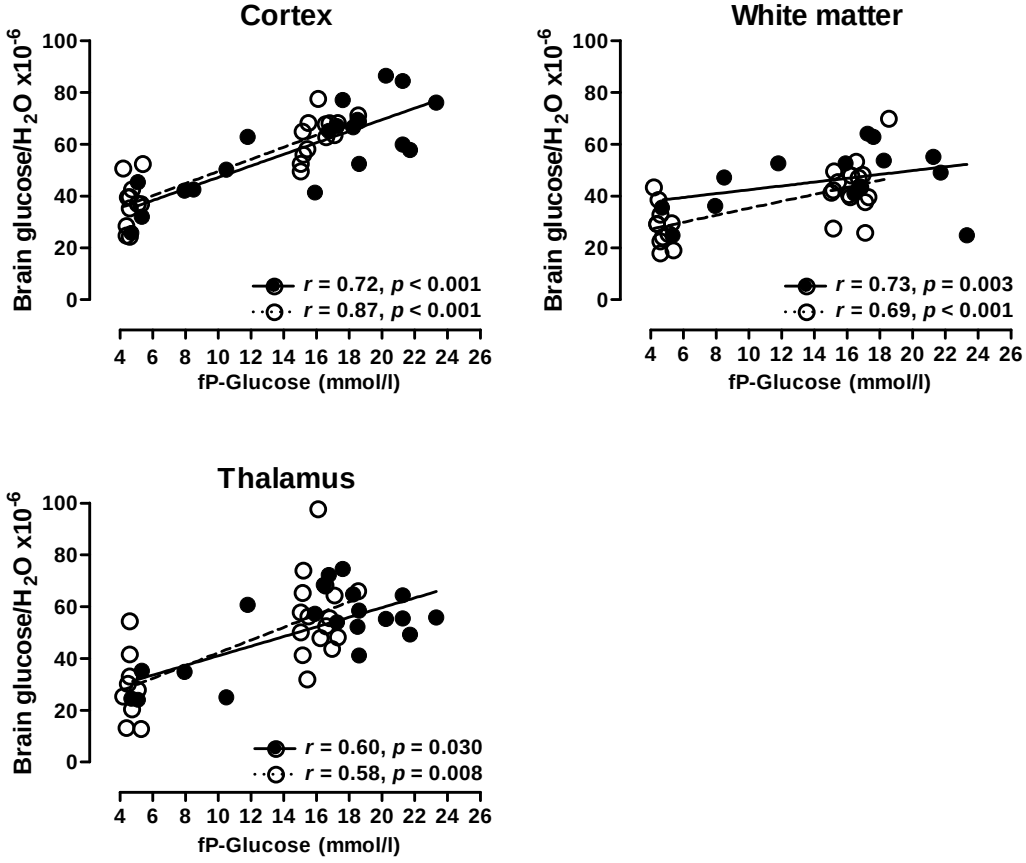


**Figure 19** Correlations between plasma glucose and brain glucose/$H_2O$ during acute hyperglycemia in diabetic (●) and non-diabetic (○) subjects.

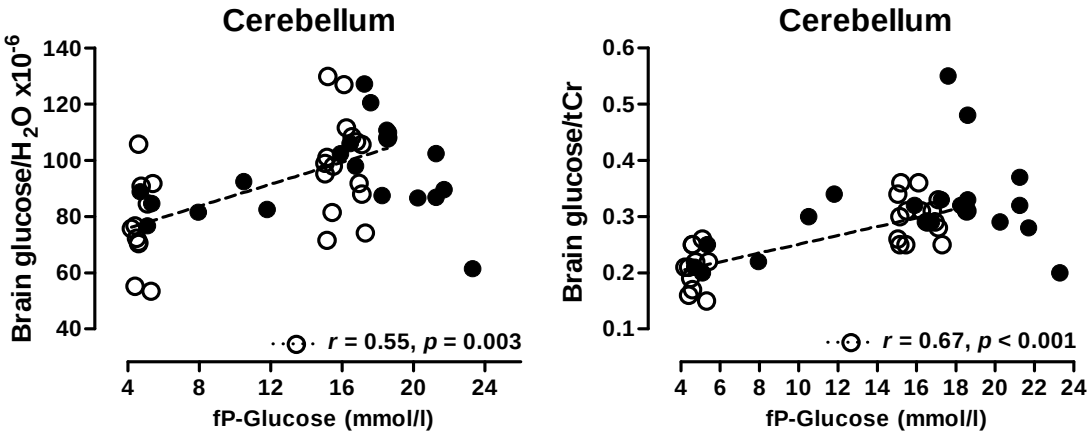


**Figure 20** Correlations between plasma glucose and brain glucose/$H_2O$ and brain glucose/tCr ratios in the cerebellum in diabetic (●) and non-diabetic (○) subjects.

*Steady state hyperglycemia.* During MRS II, the glucose/$H_2O$ was higher in the cortex than in the white matter both in the diabetic and in the non-diabetic subjects (Figure 21). In the diabetic subjects, the glucose/$H_2O$ was higher in the cortex than in the thalamus and in the non-diabetic subjects, higher in the thalamus than in the white matter. Comparison of the glucose/$H_2O$ ratio between the cerebellum and the other brain regions is impossible, because cerebellar $H_2O$ levels changed during acute hyperglycemia. In the cerebellum, no difference in brain glucose/tCr existed between the diabetic and non-diabetic subjects. Excluding the one patient taking medication for microalbuminuria did not affect results in Study II.

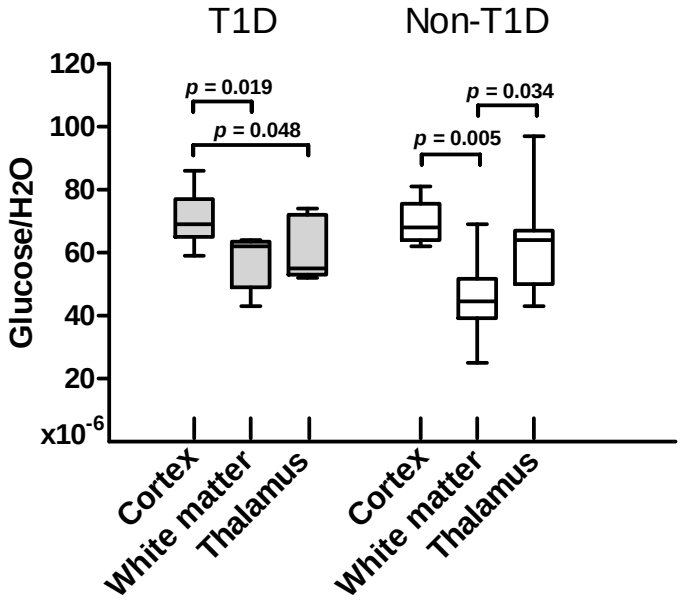


**Figure 21** Brain glucose/$H_2O$ ratio in the cortex, white matter, and thalamus during steady state hyperglycemia in diabetic (T1D) and non-diabetic subjects (Non-T1D). Data are mean, SD, and minimum and maximum. *$p < 0.05$, **$p < 0.01$.

# 7 DISCUSSION

## 7.1. Study Subjects

Participant selection was particularly important in this cross-sectional study since it comprised a relatively small number of subjects. The entry criteria were tight and aimed at homogenous study groups and at the exclusion of confounding factors that would affect the markers of brain metabolism. Conditions known to alter brain metabolites are aging (see 2.1.2.), psychiatric diseases (305), epilepsy (306), sleep apnea (307), sleep deprivation (308, 309), alcohol consumption (310), and smoking (311).

Only men were studied, since gender differences exist in brain structure and in glucose metabolism. A higher metabolic rate has been reported in men in the orbital frontal lobes (312), the medial frontal lobes (313), the temporal lobes (312), and the hippocampus (312, 314). In other studies, a lower metabolic rate has been reported in men in the orbital frontal lobes, the medial frontal lobes (315-317), the temporal lobes (315, 316, 318, 319), the thalamus (315, 316, 319), and the cerebellum (318, 319). These highly contradictory results may in fact reflect the overall difficulty in measuring glucose metabolism. In addition, in women, the NAA/tCr ratio in the prefrontal cortex has been shown to decline significantly from their follicular to their luteal phase (320). Optimal timing of the experiment for one particular phase of a menstrual cycle would have been challenging. Further, at which phase of the female cycle the brain metabolites are comparable with those of men is unknown.

#### Study I

Few studies have covered effect of metabolic risk factors on brain metabolism. At the time of our data collection, the most recent criteria for the metabolic syndrome were defined by the IDF (125). These criteria were the most rigorous and therefore most suitable for screening individuals at a low but still existing risk (1). Thereafter the Joint Interim Statement criteria of IDF/NHBI/AHA/WHF/IAS/IASO have been published (Table 1) (127). These criteria differ from those of the IDF by suggesting that waist circumference should, instead of being the main criterion, be valued as equal to the other criteria. These new criteria also suggest that the waist circumference cut-off should differ for different populations. For European populations, until more data become available, they recommend the IDF (M ≥ 94 cm, F ≥ 80 cm) or the AHA/NHLBI (M ≥ 102 cm, F ≥ 88 cm) cut-off values.

All subjects in the risk group fulfillled the IDF criterion concerning waist circumference and one to four of the other criteria concerning blood pressure, fasting plasma glucose, serum triglycerides, and serum HDL cholesterol. According to both the IDF and the Joint Interim Statement, five of our nine participants had metabolic syndrome. This corresponds well with the aim of studying participants with mild cardiovascular disease.

Hypertension and increased BMI, IL-6, ICAM, and homocysteine have been associated with brain abnormalities such as decreased brain volume or white matter lesions or both (see 2.2.2). Participants in the present study had increased waist circumference and hypertension, but their inflammatory and vascular markers were comparable to those of the control group. Although the participants had no atrophy in the MR images, three of the nine in the risk group had white matter lesions, suggesting that our subject selection was optimal. The white matter lesions imply possible hypoperfusion or chronic ischemia.

#### Studies II-IV

It has been suggested that insulin plays no role in brain glucose concentration in healthy individuals (321), but possible effects of insulin resistance and hyperinsulinemia on brain glucose metabolism are far from being understood. To limit variables to hyperglycemia alone, we studied men with only type 1 diabetes. Our rather narrow age-range aimed to minimize the effects of both brain maturation and aging. As the majority of $^{1}$H MRS studies have involved patients with a long diabetes duration, how early brain metabolism starts to change is unknown. We selected patients with a rather short duration of diabetes and aimed to study which brain regions are the first to be affected if any. $^{1}$H MRS was our choice because of its ability to detect early changes (322).

Assessment of cerebrovascular pathology by non-invasive methods is challenging. Risk for cerebral vascular disease in the diabetic patients was minimized by selection of relatively young non-smoking patients, and by exclusion of patients with metabolic risk factors or with modest or severe microvascular complications. Vascular or endothelial markers, inflammation markers, and homocystein in the diabetic and non-diabetic participants were similar. Retinal status was carefully evaluated because retinal vessels share common anatomic, embryologic, and physiologic characteristics with the cerebral microvasculature (323), and the eyes have therefore been considered a window to the microvasculature in the brain. Two patients had mild and two moderate background retinopathy, whereas the others had none. Although patients had no signs of microvascular disease such as white matter lesions in the MRI, its existence cannot be totally excluded. A recent study has suggested that the increased aortic pulse wave velocity that indicates increased aortic stiffness is associated with cerebral small vessel disease (324). Tonometry could therefore in future be an easy additional method to assess cerebral small-vessel disease.

According to recent results (see 2.3.2.), hypoglycemia in childhood may have a greater effect on the brain than does hypoglycemia in adulthood. We therefore studied patients with a late age of onset. They had had no diabetes during brain maturation.

## 7.2. Methodological Aspects

### Hyperglycemic Normoinsulinemic Clamp

Because we studied an acute increase in brain glucose, the most important target for the hyperglycemic normoinsulinemic clamp was to achieve a comparable increase between the diabetic and non-diabetic groups in blood glucose concentration. That was successfully achieved. Since the basal blood glucose concentrations were higher in the diabetic participants, they also were expected to show higher glucose concentrations during steady state hyperglycemia.

The second target was to maintain low blood insulin concentrations, comparable between study groups. Infusion of the somatostatin analogue ocreotide is an accurate, safe, and widely used method to inhibit insulin secretion (325-327). Without somatostatin, induction of acute hyperglycemia would in non-diabetic subjects cause a rapid physiologic increase in insulin secretion from the pancreatic $\beta$-cells. Because a high serum insulin concentration would have had marked effects on glucose metabolism perhaps also in the brain, we chose to use a concomitant somatostatin infusion in the non-diabetic participants. An important question concerning a possible systematic error in the present study is whether somatostatin has an effect on brain glucose concentration on its own. In rats with middle cerebral artery occlusion, intracerebral administration of somatostatin has led to an increased infarction volume (328). Somatostatin has also caused vasoconstriction in the rat brain when administered intrathecally (329). In patients with acromegaly, treatment with a long-acting somatostatin analogue for six months has reduced arterial stiffness in peripheral large arteries (330). These results are contradictory. More data are available on the effects of somatostatin in the cerebellum, where it plays a role in embryonic development (331, 332). In the adult rat cerebellum, however, no somatostatin-binding sites have been identified (333, 334). It is therefore unlikely that somatostatin would have had any immediate effects on glucose concentration in the cerebrum or cerebellum or have influenced the results.

### $^{1}$H MRS

Voxel locations were chosen according to previous research findings (Figures 4 and 5). The cortical and white matter voxels were placed in the frontal lobe because aging starts frontally and proceeds along an anteroposterior gradient. We hypothesized that the changes associated with metabolic risk factors and diabetes would behave similarly. The white matter watershed area is interesting because it is an easily compromised region of the circulation. Like the frontal white matter, the thalamus is also susceptible to microvascular disease. Its metabolism has also been shown to change in patients with severe neuropathic pain (285). The cerebellum was another focus of our interest because it tolerates hypoglycemia better than does the cerebrum (335), but its tolerance to hyperglycemia has not been negleted.

The gray matter in the frontal lobes is involved in problem-solving, constituting associations, spontaneity, initiation, memory, judgement, impulse control, and social and sexual behavior. The white matter carries nerve signals between the gray matter regions.

The thalamus is a subcortical gray matter nucleus of the cerebrum that serves as a relay station for vision, hearing, taste, and motor signals. It also contributes to regulation of arousal, attention, and sleep, and plays a role in the regulation of the higher-level brain functions such as learning and memory. The cerebellum, containing both gray matter cortex and white matter, is responsible for fine motor control, coordination, and postural regulation as well as being associated with many cognitive aspects of behavior (336).

Although its spectrum peak heights are proportional to the concentrations of brain metabolites and of glucose, $^{1}$H MRS does not provide quantitative data without quantitation (see 2.1.2). Each quantitation method has its strengths and limitations (90). Using peak ratios has the advantage of requiring no extra imaging time and time-consuming post-processing. Additionally, the partial volume effects of CSF, and random fluctuations in the intensity levels originating from the instrument (inhomogenities of magnetic field, instability of receiver gain, temperature of the MRI room) or the participant (size and composition of the head) can be avoided. It does, however, provide no absolute concentrations for the metabolites. Alteration of the peak ratio can originate from a change in the numerator, denominator, or both, and the direction of change remains unknown (86). Absolute quantification, on the other hand, lies within an estimated 10 mmol/g wet weight concentration for cortical tCr (256, 280, 321, 337) or estimated 9.9 mmol/l and 10.5 mmol/l concentrations for NAA in the gray and in the white matter (338). These are based on in vitro studies in animals, figures not always particularly accurate.

The aim of the present study was to compare diabetic patients and controls and on the other hand compare brain regions, which makes the absolute concentrations unnecessary. We used peak ratios, the metabolite/$H_2O$ (92, 339, 340) and metabolite/tCr (91, 93). The limitation of this approach was that the concentrations of $H_2O$ or tCr may have affected the results. We observed some regional differences in tissue water content (Figure 10F) that may have affected the regional comparison of glucose. We reported, based on the higher glucose/$H_2O$ ratio, that the cortex contained 47% more glucose than the white matter. Because the $H_2O$ was higher in the cortex than in the white matter, the difference in glucose was even larger. When comparing subject and control groups, one has to be aware that the concentration of tissue water may be higher in the patients with diabetes (255). In the present data, however, we saw no such difference. Tissue water did increase in the cerebellum during hyperglycemia in the diabetic and non-diabetic groups, whereas the tCr remained stable and served as an adequate reference for analyzing the change in cerebellar brain metabolites during the clamp.

Detection of glucose with 1.5T $^{1}$H MRS is not unambiguous, and the terminology requires clarification. The term "brain metabolism" normally is used in the context of PET, and no established terminology exists for $^{1}$H MRS research. We preferred "brain metabolites" or "markers of brain metabolism." $^{1}$H MRS is not comparable to PET, a method dependent on tracers to assess glucose uptake and consumption. $^{1}$H MRS makes it possible to detect the level of native glucose at a certain time point. The glucose level observed depends on the glucose supply via the circulation, glucose uptake into the brain tissue via the BBB, and the rate of glucose consumption in the tissue (see 2.1.1).

The glucose signal can be detected at two locations within the spectrum, at 5.23 ppm and 3.43 ppm. In theory, the signal at 5.23 ppm (anomeric proton of glucose) would be the most favorable (256, 280, 321, 337, 341). However, its detection would require a magnet with a 4T field. At the 1.5T field, 5.23 ppm is so close to the water signal (4.77 ppm) that water suppression will reduce its intensity or even suppress this small glucose signal. In the 1.5 T field: 5.23 ppm (glucose) minus 4.70 ppm (water) = 0.53 ppm, i.e. approximately 34 Hz. Because the band width of the water suppression pulse in our work was about 35 Hz i.e. +/- 17.5 Hz, the water suppression would extend to only about 17 Hzs distance from the glucose signal and would in practice suppress it. Moreover, if the glucose signal at 5.23 ppm is not wholly suppressed, depending on the quality of the water suppression, the glucose signal may partly overlap with the residual water, which would substantially complicate assessment of intensity.

In our data, the glucose signal at 5.23 ppm was not visible, so we chose to use the signal at 3.43 ppm. This has been done successfully (254, 255, 340). In addition to glucose, also mI resonates at several ppm including 3.43 ppm. Therefore a small portion of the brain glucose increase seen in patients with type 1 diabetes may in fact be an increase in mI, but its contribution to the results was estimated to be inconsequential.

The data representing the increase in glucose concentration during the clamp was referred to an external standard of 100 mmol/l glucose solution (see 5.5). As to possible sources of error concerning the line shape fitting, the in vivo glucose spectrum and the brain spectra may differ in their line shapes and chemical shifts; spectrum line shapes can vary due to the shimming (i.e. homogenization of the magnetic field) that occurs each time a new patient is positioned in the magnet. These differences can be diminished by use of a broader line width, but because of uniform quality data, we were able to use constant line widths. The chemical shift may theoretically vary due to differences in pH between the data collections. In the human brain this seems unlikely. In addition, heavy overlap of metabolites in the spectrum complicates their analysis. What is possible but unlikely is a change in concentrations of the metabolites or macromolecules underlying glucose during a hyperglycemic clamp. We also fitted the phantom spectrum to the difference spectrum which deletes the effect of macromolecules. Concerning water increase in the cerebellum during acute hyperglycemia, T2 relaxation may have affected the result, but the relatively short TE used in these studies should minimize such effects.

For technical reasons, 37 spectra were disqualified. This may have affected the results because pathological brain tissue may be more likely to produce poor quality spectra than normal tissue would. In such cases, the present results would show an underestimation of differences between subject and control groups. In Study III, brain metabolites were measured repeatedly during the hyperglycemic clamp, although no change was expected. The variation in NAA/$H_2O$ and tCr/$H_2O$ was between -3.4% and +4.7%, in line with previous findings (342).

## 7.3. Metabolic Risk Factors and Brain Glucose and Metabolites

In Study I, we sought any association between risk factors for metabolic syndrome and brain metabolism. We found that in 36-year-old men with an accumulation of metabolic

risk factors, thalamic tCr was increased by 17%. The correct interpretation of the increased tCr in the risk group can only be speculated. Total creatine plays a role in osmolar regulation. Osmolar regulation in this case, however, seems unlikely, because the other osmolar regulators, NAA, mI, and Cho, were unchanged, a finding that also suggests that neuronal density as well as metabolism and glial cell membrane turnover were intact. Creatine has at least three different neuroprotective mechanisms: First, it serves as a reservoir for the high-energy phosphates required for ATP production (see 2.1.1). Second, creatine inhibits the mitochondrial permeability transition, a process involved in apoptosis (343). Third, increased activation of the ADP re-cycling cycle reduces production of ROS (344). The protective effects have been evident in mice in which dietary creatine supplementation reduced the infarction volume after transient focal cerebral ischemia by 40% (345). In the present study any or all of these mechanisms were possible.

The risk group comprised men with a family history of type 2 diabetes, hypertension, abdominal obesity, and mild insulin resistance. The role of family history in altered thalamic metabolism cannot be excluded, because the first step in the pathogenesis has not been identified. It is also unclear whether brain metabolism changes due to metabolic risk factors or the reverse. Because the thalamus is susceptible to microvascular disease, hypertension may play a role. Hypertension occurred in one (206) but not in another (208) study associated with decreased NAA in the white matter, implying decreased neuronal metabolism (Table 2), but its possible effects on the thalamus seem to be unknown. The role of impaired glucose regulation for brain metabolites was examined in only one study (209) able to show that the cerebral metabolic disorder progresses when the impared glucose tolerance progresses to type 2 diabetes. Total Cr served as the internal reference, and therefore no information was available on the tCr itself. However, the metabolites in the frontal cortex, the parietal white matter, and the thalamus correlated with markers of insulin resistance such as serum insulin and plasma glucose concentrations, $HbA_{1c}$, and HOMA-IR (209).

The brain glucose concentration in healthy individuals correlates linearly with plasma glucose (321, 338). Here, the risk group had a 13% higher plasma glucose concentration than the control group, but with no difference in thalamic glucose content. The risk group thus had an approximately 13% lower thalamic glucose content than expected based on plasma glucose. Whether the metabolic syndrome affects glucose uptake or its consumption (see 2.2.2.) is unknown, but in the control group, tCr correlated with thalamic glucose content, although in the risk group, tCr metabolism and thalamic glucose content were uncoupled. Instead, tCr correlated with markers of insulin resistance, i.e. fasting plasma glucose and two-hour plasma glucose concentration in the OGTT. It can be hypothesized that in the risk group, the association between tCr and impaired glucose regulation indicates an increased need for energy to buffer the thalamic cells. This would be in accordance with the known neuroprotective mechanisms of tCr.

## 7.4. Type 1 Diabetes and Brain Glucose and Metabolites

### 7.4.1. The Cerebrum

In Study II, cerebral glucose and metabolites were detectable after an overnight fast when blood glucose was in the non-diabetic group 4.8 mmol/l and in the diabetic group 9.2 mmol/l.

The non-diabetic participants had a 47% higher glucose content in the cortex than in the white matter. In the diabetic participants, the differences in regional distribution had vanished. Compared to the non-diabetic participants, the diabetic patients had a 64% higher glucose level in the white matter and a 25% higher glucose level in the cortex. The difference was the greatest in the white matter, which suggests that in diabetes, the white matter contains an excess of glucose exceeding that of the other regions studied. A higher glucose content has previously been identified in the parietal than in the occipital cortex in diabetic patients (254), but no comparisons between the different tissue types are available.

As for glucose concentration in the tissue, it depends on the one hand on glucose supply and uptake and on the other hand on its consumption. It is of note that all these are higher in the gray than in the white matter (62, 63, 346). That glucose in the diabetic patients accumulated in their white matter suggests that the supply of glucose in relation to its oxidation is greater in the white matter than in the cortex.

In Study III, acute hyperglycemia was brought on by raising plasma concentration by 12 mmol/l for both the diabetic and the non-diabetic group. The resultant increase in cerebral glucose in those with type 1 diabetes depended on brain region and ranged from 1.1 to 2.0 mmol/l. Others report that the brain glucose concentration in healthy human subjects is between 0.5 and 2.5 mmol/l (43, 44), but is about 2 to 3 mmol/l higher in diabetic patients (254-256). An every-day hyperglycemic episode in a diabetic patient may therefore as much as double brain glucose concentration. During acute hyperglycemia, cerebral glucose correlated linearly with the plasma glucose concentration in our diabetic and non-diabetic groups. A similar correlation has appeared in healthy human subjects (280, 338), but to our knowledge we were the first to perform repeated $^{1}$H MRS in patients with diabetes (Study III). Plasma glucose concentration appears to determine brain glucose also in patients with diabetes.

Acute and chronic hypoglycemia had differing effects on brain glucose distribution. The Study II finding reveals the effect of chronic hyperglycemia and type 1 diabetes, because it was performed after an overnight fast. Exposure to excess glucose was highest in the white matter. Study III shows that in diabetic patients accustomed to high blood glucose excursions, acute hyperglycemia elevates glucose concentration most in the cortical gray matter. These are the first studies to show the differing effects on brain glucose distribution of acute and chronic hyperglycemia. Because our diabetic and the non-diabetic participants showed an equal increase in glucose in their white matter during acute hyperglycemia, diabetes seems not to have impaired their glucose supply into the

white matter. A possible explanation for this accumulation of glucose in the white matter may therefore be the abundant lipid layers of the myelin sheets that hinder the processing of water-soluble glucose. The greater exposure of the cortex to excess glucose during acute hyperglycemia may, in turn, reflect the greater capillary density and the higher density of GLUTs (36, 50, 51), and the higher metabolic rate of glucose (62, 63).

Thalamic glucose content increased less in diabetic patients than in the non-diabetic participants during acute hyperglycemia. It is interesting that the thalamus came into focus also in Study I, in which men with metabolic risk factors showed increased tCr and an unexpectedly low glucose content, particularly in the thalamus. The thalamus is joined to the blood via long, non-branching arteries and is thus susceptible to vascular disease. A reduced blood flow response (196) and chronic hyperglycemia with impaired vasodilatation (347) in the thalamic arteries has been associated with hypertension. In addition, reduced gray matter density has been observable in the thalamus in patients with type 1 diabetes (348). In theory, these results for the thalamus suggest that the risk group in Study I and the diabetic participants in Study III may in fact actually have had mild microvascular disease. Another possible explanation for our finding is that in both the risk group and in the patients with type 1 diabetes, chronic exposure to glucose may have down-regulated the number of GLUT1s in the thalamus and thereby limited glucose transport.

Study II shows that in type 1 diabetes, the metabolic brain alterations may appear earlier than do peripheral microvascular complications. The diabetic participants had an 8% higher mI in the frontal cortical gray matter, and a 6% lower NAA and 20% higher mI in the frontal white matter than did the non-diabetic participants.

Interpretation of the decreased NAA is unambiguous, since it is a neuronal marker, and its decrease signifies neuronal dysfunction or loss. The interpretation of the increased *myo*-inositol is, however, more complex. *Myo*-inositol is derived from glucose, and its level may, during chronic hyperglycemia, be increased because of the abundance of its precursor. *Myo*-inositol also has multiple functions in the cell (see 2.1.2). Because Glx, Cho, and tCr were normal, altered osmolality seems unlikely. A normal Cho would suggest that glial cell membrane turnover is intact. The most probable explanation would be an increased need for intracellular messaging due to increased astrocyte activity.

The frontal white matter has not been studied earlier with $^{1}$H MRS in diabetic patients (Table 3). Of the available reports on the posterior white matter, only one concerned adult patients with type 1 diabetes and is therefore comparable to our study (255). The present study in patients with type 1 diabetes but without microvascular complications showed that they had decreased white matter NAA and increased mI, whereas the previous one in patients with complications showed increased mI, increased Cho, and increased tissue water. The findings of increased Cho and tissue water may relate to longer-duration diabetes or to further advanced microvascular disease.

Brain metabolism in the frontal cortex in adult diabetic patients has been the topic of two studies (255, 287) (Table 2). One, a study in patients with type 1 diabetes of long duration and severe microvascular complications showed increased tissue water but no other metabolite alterations (255). In that study, tissue water served as an internal reference for the other metabolites, but because it was higher in the diabetic group, it may

have masked a possible metabolite finding. The other study used tissue water as a reference but did not include any comparison of levels between study groups (287). The authors found no alteration in the metabolites in the frontal cortex, but in the dorsolateral prefrontal cortex, NAA and tCr were decreased. These results are not congruent with ours probably due to the different management of the internal reference. The second study had also included a mix of patients with type 1 and 2 diabetes, quite large proportion of whom had newly diagnosed disease.

As thus hypothesized, metabolic brain damage may be a progressive disorder. One case report followed the brain metabolites in two patients suffering from carbon monoxide poisoning (349). In the frontal white matter of the patient with the milder coexposure, NAA decreased in the acute phase and increased to its normal level during the three-month recovery phase. Cho behaved conversely: increased first, and then fell to normal. In the more severe case, NAA decreased and remained low, and Cho increased and remained high. A similar pattern for NAA and Cho has emerged in patients with type 1 diabetes and severe diabetic complications (255). Both NAA and Cho correlated inversely with duration and severity of glucose exposure. NAA may first increase, indicating increased neuronal activity, and then at a certain point, start to decrease, indicating neuronal damage and loss. It is possible that the levels of brain metabolites depend on the phase and severity of the disease; prospective studies in patients with diabetes are therefore needed. Based on our data, hyperglycemia is a potent risk factor for diabetic brain disease, and frontal white matter may be the first region to show altered metabolism.

### 7.4.2. The Cerebellum

In Study IV, the cerebellum responded very differently from the cerebrum, in that it revealed absolutely no difference between diabetic and non-diabetic participants, nor did it reveal any difference in their glucose or in their brain metabolites.

In non-diabetic participants, their glucose level was more than twice as high in the cerebellum than in the cerebrum. The only available study that has compared glucose concentrations between the cerebellum and the cerebrum involves rats (335). The glucose content was 12 to 33% higher in the rat cerebellum than in the cerebral gray matter. Because in our study the cerebellar voxel contained mostly white matter, this comparison between cerebellum and cerebral white matter voxel seemed reasonable. The white matter glucose content was almost three-fold higher in the cerebellum than in the cerebrum.

In the present model of acute hyperglycemia and low serum insulin concentrations in non-diabetic individuals, the increase in cerebellar glucose concentration was almost 1.8-fold above that in the cerebral white matter. A model of normoglycemia and low serum insulin in healthy 50-year-old men has yelded interesting results. Insulin infusion facilitated glucose uptake in the cerebrum but not in the cerebellum. It was suggested that whereas the cerebrum needs a higher concentration of insulin than does the cerebellum, the abundant insulin receptors in the cerebellum (51, 53, 350) may be saturated even at low insulin concentrations. It has therefore been suggested that the regional differences in

the effect of insulin (351) and the low insulin effect (352) may be the mechanisms which protect vital brain regions such as the cerebellum from hypoglycemia.

In the cerebellum of patients with type 1 diabetes, chronic hyperglycemia had no effect on either glucose content or glucose uptake or metabolism. No such correlation as in the cerebrum appeared in the cerebellum between plasma and brain glucose concentrations. It seems that, unlike the cerebrum, the cerebellum reveals no association between systemic glucose metabolism and brain glucose metabolism.

During acute hyperglycemia in both the diabetic and the non-diabetic groups, the cerebellar white matter also showed a 14% increase in its tissue water. Decreased serum osmolality is known to raise brain water content, but here the osmolality increased less than 2% in both groups. Thus, the excess glucose in the cerebellum may become diluted by an increase in its water content. This finding provides no support for a dilution effect, but it may still be one factor that protects the cerebellum from the harmful effects of glucose. The cerebellar white matter also showed no metabolite alterations and is therefore definitely not among the first regions in which brain metabolism is altered in patients with diabetes. The cerebellum has in rats maintained its normal energy metabolism, i.e. adequate ATP, creatine, and lactate levels, longer than in the cerebrum during hypoglycemia (335). Compared to the cerebral white matter, cerebellar white matter seems to be more resistant also to the effects of hyperglycemia.

## 7.5. Future Perspectives

This research field concerning effects of insulin resistance (Study I) on the brain is new and intriguing. The results of the present study suggest that insulin resistance may have an effect on glucose metabolism in the thalamus. In obese individuals, human insulin does not affect brain functions as effectively as in lean individuals, indicating the existence of central insulin resistance (353). New data show that such central insulin resistance can be overcome by use of an insulin analogue that may have enhanced access through the BBB such as insulin detemir, which has a better cerebral effect than does human insulin despite similar peripheral effects (354, 355). Knowledge of insulin resistance and brain energy metabolism is sparse and may deserve further study with special emphasis on the role of the thalamus.

These data (Studies II-IV) emphasize the fact that the role of hyperglycemia as a risk factor for diabetic brain disease cannot be underestimated. The brain may be a target organ for diabetic microvascular complications, and tight glycemic control definitely is important in order to achieve normal cerebral function. Yet prospective studies are needed to document the progressive nature of diabetes-related metabolic brain disease.

Cognitive function in patients with diabetes has been the focus of active research for decades. Still, researchers have not achieved a consensus concerning the cognitive decline profile. The present study showed that depending on its duration, hyperglycemia elevates glucose concentration in the different brain regions, chronically in the white matter and acutely in the cortical gray matter. Anatomically variable glucose concentrations over the

course of time may be one source of variance in cognitive testing. It may therefore be worth studying whether and how acute and chronic hyperglycemia influences various brain functions.

# 8 SUMMARY AND CONCLUSIONS

I. Risk factors for the metabolic syndrome may influence brain metabolism. Insulin resistance may be associated with a decreased relative glucose concentration and an increased need for energy buffering in the thalamic cells.

II. Healthy individuals have tissue-specific regional differences in the glucose content of the cerebrum. In type 1 diabetes, these regional differences disappear, and glucose content becomes even. During chronic hyperglycemia, more excess glucose accumulates in the white matter than in the gray matter. Type 1 diabetes also alters brain metabolism earlier in the white matter than in the cortex or thalamus.

III. An everyday hyperglycemic episode in a diabetic patient may as much as double the brain glucose concentration. During acute hyperglycemia, the frontal cortex, the frontal white matter, and the thalamus are exposed to differing amounts of excess glucose, and increase of glucose appears highest in the frontal cortex region (regardless of diabetes). In type 1 diabetes, chronic fluctuation in blood glucose may be associated with alterations in glucose uptake or in metabolism in the thalamus.

IV. The healthy cerebellum contains twice as much glucose as the cerebrum, and type 1 diabetes alters neither its glucose content nor the brain metabolites. Chronic fluctuation in blood glucose concentration has no effect on cerebellar glucose uptake or metabolism. The cerebellar white matter is therefore more resistant to the effects of hyperglycemia than is the cerebral white matter.

# 9 ACKNOWLEDGEMENTS

This study was carried out at the Folkhälsan Institute of Genetics of the Folkhälsan Research Center at the University of Helsinki, and at the Division of Nephrology, Department of Medicine, Helsinki Universal Central Hospital, during 2004 to 2010. I am grateful to the former and present heads of both institutes, Docent Per-Henrik Groop, Professor Anna-Elina Lehesjoki, Docent Eero Honkanen, and Professor Carola Grönhagen-Riska, for providing excellent research facilities.

My warmest gratitude goes to

my supervisors Docents Sari Mäkimattila and Per-Henrik Groop. Docent Mäkimattila has taught me to honor both science and life. Without her irreplaceable expertise, contributions, support, and those numerous cups of cappuccino, this work would not have been possible. Docent Groop for giving me an opportunity to be a member of his esteemed research group and for constant support and enthusiasm. He has reminded me over the years that a study begins from a clinical observation that awakens one's curiosity.

my coauthors from the field of radiology and physics: Docent Nina Lundbom for being my third, unofficial, supervisor and for contributing elegant ideas and positive criticism throughout the study. Docent Sami Heikkinen and Dr. Marjut Timonen for irreplaceable input and for guidance in the challenging world of spectroscopy.

the official reviewers of this thesis, Docents Juhana Hakumäki and Lauri Soinne, for advice and constructive comments that considerably improved the thesis.

Dr. Carol Norris for author-editing.

Radiology technician Marja Piitulainen for those hard-working weekends at Meilahti hospital.

the FinnDiane study group that comprises talented researchers who also manage to create a warm and pleasant atmosphere at work: Carol Forsblom, Daniel Gordin, Kustaa Hietala, Riika Kilpikari, Janne Kytö, Ville Mäkinen, Maikki Parkkonen, Kim Pettersson-Fernholm, Mats Rönnback, Markku Saraheimo, Jenny Söderlund, Lena Thorn, Johan Wadén, and all the others for their support over the years. Laboratory technicians Tarja Vesisenaho, Anna Sandelin, and Sinikka Lindh for their professional work.

the patients and participants who volunteered for this study. You made this work possible.

my dear friends Riitta Sallinen, Milla Rosengård-Bärlund, Laura Salovaara, and Minna Takkunen for sharing opinions and experiences with research and other aspects of life. Annika Aalto-Partanen, Susanna Valve, Kristiina Oksava, Henna Mikkonen, Hanna Pesonen, Outi Kähkönen, Eevastiina Parikka, Mari Knuuttila, Piritta Simola and Mari

Aho for many joyful moments most often during walks and runs. The diving friends for the hilarious and relaxing weekends above and below the surface.

my family for their support. My mom Birgit, and Esko for the 24-hour computer aid and for all the house-calls. My grandmother Helena and my dad Jouko for their encouragement. Our soon-to-become neighbors, my sister Sanna and her husband Jani, for all the help with the house-building project. My lovely goddaughter Erika and her brother Lucas for all the joy you have brought into my life.

Heikki, my chosen one, for love and for sharing many moments of joy and despair during this project.

This work has been financially supported by grants from the Folkhälsan Research Foundation, the Finnish Medical Society (Finska Läkaresällskapet), the Diabetes Research Foundation, Waldemar von Frenckell Foundation, the Wilhelm and Else Stockmann Foundation, the Finnish Neurological Society, and from a special governmental grant for health sciences research (no. 5103).

Outi Heikkilä

# 10 REFERENCES


1. Mattsson N, Rönnemaa T, Juonala M, Viikari JS, Raitakari OT: The prevalence of the metabolic syndrome in young adults. The Cardiovascular Risk in Young Finns Study. *J Intern Med.* 261:159-169, 2007
2. Harjutsalo V, Sjöberg L, Tuomilehto J: Time trends in the incidence of type 1 diabetes in Finnish children: A cohort study. *Lancet.* 371:1777-1782, 2008
3. Lammi N, Blomstedt PA, Moltchanova E, Eriksson JG, Tuomilehto J, Karvonen M: Marked temporal increase in the incidence of type 1 and type 2 diabetes among young adults in finland. *Diabetologia.* 51:897-899, 2008
4. Kloppenborg RP, van den Berg E, Kappelle LJ, Biessels GJ: Diabetes and other vascular risk factors for dementia: Which factor matters most? A systematic review. *Eur J Pharmacol.* 585:97-108, 2008
5. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care.* 24:683-689, 2001
6. Najarian RM, Sullivan LM, Kannel WB, Wilson PW, D'Agostino RB, Wolf PA: Metabolic syndrome compared with type 2 diabetes mellitus as a risk factor for stroke: The Framingham Offspring Study. *Arch Intern Med.* 166:106-111, 2006
7. Kurl S, Laukkanen JA, Niskanen L, Laaksonen D, Sivenius J, Nyyssönen K, Salonen JT: Metabolic syndrome and the risk of stroke in middle-aged men. *Stroke.* 37:806-811, 2006
8. Segura B, Jurado MA, Freixenet N, Falcon C, Junque C, Arboix A: Microstructural white matter changes in metabolic syndrome: A diffusion tensor imaging study. *Neurology.* 73:438-444, 2009
9. Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP: The effects of type 1 diabetes on cognitive performance: A meta-analysis. *Diabetes Care.* 28:726-735, 2005
10. Janghorbani M, Hu FB, Willett WC, Li TY, Manson JE, Logroscino G, Rexrode KM: Prospective study of type 1 and type 2 diabetes and risk of stroke subtypes: The Nurses' Health Study. *Diabetes Care.* 30:1730-1735, 2007
11. van Harten B, de Leeuw FE, Weinstein HC, Scheltens P, Biessels GJ: Brain imaging in patients with diabetes: A systematic review. *Diabetes Care.* 29:2539-2548, 2006
12. Schaie KW: The course of adult intellectual development. *Am Psychol.* 49:304-313, 1994
13. Salonen O, Autti T, Raininko R, Ylikoski A, Erkinjuntti T: MRI of the brain in neurologically healthy middle-aged and elderly individuals. *Neuroradiology.* 39:537-545, 1997
14. DeCarli C, Massaro J, Harvey D, Hald J, Tullberg M, Au R, Beiser A, D'Agostino R, Wolf PA: Measures of brain morphology and infarction in the Framingham Heart Study: Establishing what is normal. *Neurobiol Aging.* 26:491-510, 2005
15. van Dijk EJ, Prins ND, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM: Progression of cerebral small vessel disease in relation to risk factors and cognitive consequences: Rotterdam Scan Study. *Stroke.* 39:2712-2719, 2008
16. Ruis C, Biessels GJ, Gorter KJ, van den Donk M, Kappelle LJ, Rutten GE: Cognition in the early stage of type 2 diabetes. *Diabetes Care.* 32:1261-1265, 2009
17. Mukamal KJ, Longstreth WT Jr, Mittleman MA, Crum RM, Siscovick DS: Alcohol consumption and subclinical findings on magnetic resonance imaging of the brain in older adults: The cardiovascular health study. *Stroke.* 32:1939-1946, 2001
18. Love S: Neuropathology of aging. In Pathy MSJ, Sinclair AJ, Morley JE: *Principles and Practice of Geriatric Medicine,* 4th ed. John Wiley & Sons Ltd., England, 2006, p.69-84
19. Pantoni L, Garcia JH, Gutierrez JA: Cerebral white matter is highly vulnerable to ischemia. *Stroke.* 27:1641-1646, 1996
20. Kirkpatrick JB, Hayman LA: White-matter lesions in MR imaging of clinically healthy brains of elderly subjects: Possible pathologic basis. *Radiology.* 162:509-511, 1987
21. Hopkins RO, Beck CJ, Burnett DL, Weaver LK, Victoroff J, Bigler ED: Prevalence of white matter hyperintensities in a young healthy population. *J Neuroimaging.* 16:243-251, 2006
22. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, Hofman A, Jolles J, van Gijn J, Breteler MM: Prevalence of cerebral white matter lesions in elderly people: A population

based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry.* 70:9-14, 2001
23. Raz N, Rodrigue KM: Differential aging of the brain: Patterns, cognitive correlates and modifiers. *Neurosci Biobehav Rev.* 30:730-748, 2006
24. Head D, Buckner RL, Shimony JS, Williams LE, Akbudak E, Conturo TE, McAvoy M, Morris JC, Snyder AZ: Differential vulnerability of anterior white matter in nondemented aging with minimal acceleration in dementia of the Alzheimer type: Evidence from diffusion tensor imaging. *Cereb Cortex.* 14:410-423, 2004
25. Pfefferbaum A, Adalsteinsson E, Sullivan EV: Frontal circuitry degradation marks healthy adult aging: Evidence from diffusion tensor imaging. *Neuroimage.* 26:891-899, 2005
26. Kuhl DE, Metter EJ, Riege WH, Hawkins RA: The effect of normal aging on patterns of local cerebral glucose utilization. *Ann Neurol.* 15 Suppl:S133-7, 1984
27. Salmon E, Maquet P, Sadzot B, Degueldre C, Lemaire C, Franck G: Decrease of frontal metabolism demonstrated by positron emission tomography in a population of healthy elderly volunteers. *Acta Neurol Belg.* 91:288-295, 1991
28. Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Mandel F, Alexander GE, Grady C, Pietrini P, Eidelberg D: The metabolic topography of normal aging. *J Cereb Blood Flow Metab.* 16:385-398, 1996
29. Gibbs EL, Lennox WG, Nims LF, Gibbs FA: Arterial and cerebral venous blood arterial-venous difference in man. *J Biol Chem.* 144:325-332, 1942
30. Williamson DH: Brain substrates and the effects of nutrition. *Proc Nutr Soc.* 46:81-87, 1987
31. Kemppainen J, Aalto S, Fujimoto T, Kalliokoski KK, Långsjö J, Oikonen V, Rinne J, Nuutila P, Knuuti J: High intensity exercise decreases global brain glucose uptake in humans. *J Physiol.* 568:323-332, 2005
32. Gruetter R: Glycogen: The forgotten cerebral energy store. *J Neurosci Res.* 74:179-183, 2003
33. Sokoloff L: Relation between physiological function and energy metabolism in the central nervous system. *J Neurochem.* 29:13-26, 1977
34. Hawkins RA, Mans AM, Davis DW, Hibbard LS, Lu DM: Glucose availability to individual cerebral structures is correlated to glucose metabolism. *J Neurochem.* 40:1013-1018, 1983
35. Kuschinsky W: Coupling of function, metabolism, and blood flow in the brain. *Neurosurg Rev.* 14:163-168, 1991
36. Duelli R, Kuschinsky W: Brain glucose transporters: Relationship to local energy demand. *News Physiol Sci.* 16:71-76, 2001
37. Ganong WF: Circulation. In Foltin J, Lebowitz H, Brown RY: *Review of Medical Physiology*, 22nd ed. The McGraw-Hill Companies, United States of America, 2005, p.515
38. Faraci FM, Heistad DD: Regulation of the cerebral circulation: Role of endothelium and potassium channels. *Physiol Rev.* 78:53-97, 1998
39. Lassen NA, Ingvar DH, Skinhoj E: Brain function and blood flow. *Sci Am.* 239:62-71, 1978
40. Paulson OB: Blood-brain barrier, brain metabolism and cerebral blood flow. *Eur Neuropsychopharmacol.* 12:495-501, 2002
41. Boado RJ, Pardridge WM: The brain-type glucose transporter mRNA is specifically expressed at the blood-brain barrier. *Biochem Biophys Res Commun.* 166:174-179, 1990
42. Messier C: Glucose improvement of memory: A review. *Eur J Pharmacol.* 490:33-57, 2004
43. Bingham EM, Dunn JT, Smith D, Sutcliffe-Goulden J, Reed LJ, Marsden PK, Amiel SA: Differential changes in brain glucose metabolism during hypoglycaemia accompany loss of hypoglycaemia awareness in men with type 1 diabetes mellitus. An $^{11}$C-3-*O*-methyl-D-glucose PET study. *Diabetologia.* 48:2080-2089, 2005
44. Schlenk F, Nagel A, Graetz D, Sarrafzadeh AS: Hyperglycemia and cerebral glucose in aneurysmal subarachnoid hemorrhage. *Intensive Care Med.* 34:1200-1207, 2008
45. Kalaria RN, Gravina SA, Schmidley JW, Perry G, Harik SI: The glucose transporter of the human brain and blood-brain barrier. *Ann Neurol.* 24:757-764, 1988
46. Pardridge WM, Boado RJ, Farrell CR: Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. *J Biol Chem.* 265:18035-18040, 1990

47. Farrell CL, Pardridge WM: Blood-brain barrier glucose transporter is asymmetrically distributed on brain capillary endothelial lumenal and ablumenal membranes: An electron microscopic immunogold study. *Proc Natl Acad Sci U S A.* 88:5779-5783, 1991
48. Crone C: Facilitated transfer of glucose from blood into brain tissue. *J Physiol.* 181:103-113, 1965
49. Betz AL, Gilboe DD, Drewes LR: The characteristics of glucose transport across the blood brain barrier and its relation to cerebral glucose metabolism. *Adv Exp Med Biol.* 69:133-149, 1976
50. Vannucci SJ, Clark RR, Koehler-Stec E, Li K, Smith CB, Davies P, Maher F, Simpson IA: Glucose transporter expression in brain: Relationship to cerebral glucose utilization. *Dev Neurosci.* 20:369-379, 1998
51. Choeiri C, Staines W, Messier C: Immunohistochemical localization and quantification of glucose transporters in the mouse brain. *Neuroscience.* 111:19-34, 2002
52. Brant AM, Jess TJ, Milligan G, Brown CM, Gould GW: Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochem Biophys Res Commun.* 192:1297-1302, 1993
53. LaManna JC, Harik SI: Regional comparisons of brain glucose influx. *Brain Res.* 326:299-305, 1985
54. McEwen BS, Reagan LP: Glucose transporter expression in the central nervous system: Relationship to synaptic function. *Eur J Pharmacol.* 490:13-24, 2004
55. Pellerin L, Magistretti PJ: Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A.* 91:10625-10629, 1994
56. Hyder F, Patel AB, Gjedde A, Rothman DL, Behar KL, Shulman RG: Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. *J Cereb Blood Flow Metab.* 26:865-877, 2006
57. Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ: Activity-dependent regulation of energy metabolism by astrocytes: An update. *Glia.* 55:1251-1262, 2007
58. Maher F, Davies-Hill TM, Simpson IA: Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons. *Biochem J.* 315(Pt 3):827-831, 1996
59. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM: Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem J.* 281(Pt 1):21-40, 1992
60. Brustovetsky N, Brustovetsky T, Dubinsky JM: On the mechanisms of neuroprotection by creatine and phosphocreatine. *J Neurochem.* 76:425-434, 2001
61. Ames A 3rd: CNS energy metabolism as related to function. *Brain Res Brain Res Rev.* 34:42-68, 2000
62. Mason GF, Pan JW, Chu WJ, Newcomer BR, Zhang Y, Orr R, Hetherington HP: Measurement of the tricarboxylic acid cycle rate in human grey and white matter in vivo by $^{1}H$-$^{13}C$ magnetic resonance spectroscopy at 4.1T. *J Cereb Blood Flow Metab.* 19:1179-1188, 1999
63. Heiss WD, Habedank B, Klein JC, Herholz K, Wienhard K, Lenox M, Nutt R: Metabolic rates in small brain nuclei determined by high-resolution PET. *J Nucl Med.* 45:1811-1815, 2004
64. Cranston I, Marsden P, Matyka K, Evans M, Lomas J, Sonksen P, Maisey M, Amiel SA: Regional differences in cerebral blood flow and glucose utilization in diabetic man: The effect of insulin. *J Cereb Blood Flow Metab.* 18:130-140, 1998
65. Havrankova J, Roth J, Brownstein M: Insulin receptors are widely distributed in the central nervous system of the rat. *Nature.* 272:827-829, 1978
66. Margolis RU, Altszuler N: Insulin in the cerebrospinal fluid. *Nature.* 215:1375-1376, 1967
67. Schwartz MW, Bergman RN, Kahn SE, Taborsky GJ Jr, Fisher LD, Sipols AJ, Woods SC, Steil GM, Porte D Jr: Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. Quantitative aspects and implications for transport. *J Clin Invest.* 88:1272-1281, 1991
68. Kar S, Chabot JG, Quirion R: Quantitative autoradiographic localization of $^{125}I$ insulin-like growth factor I, $^{125}I$ insulin-like growth factor II, and $^{125}I$ insulin receptor binding sites in developing and adult rat brain. *J Comp Neurol.* 333:375-397, 1993
69. Marks JL, Porte D Jr, Stahl WL, Baskin DG: Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology.* 127:3234-3236, 1990
70. Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing-Jallat P, Ferre P, Penicaud L: Glucose transporter 2 (GLUT 2): Expression in specific brain nuclei. *Brain Res.* 638:221-226, 1994

71. Obici S, Zhang BB, Karkanias G, Rossetti L: Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med.* 8:1376-1382, 2002
72. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. *Nature.* 404:661-671, 2000
73. Garcia MA, Millan C, Balmaceda-Aguilera C, Castro T, Pastor P, Montecinos H, Reinicke K, Zuniga F, Vera JC, Onate SA, Nualart F: Hypothalamic ependymal-glial cells express the glucose transporter GLUT2, a protein involved in glucose sensing. *J Neurochem.* 86:709-724, 2003
74. Rodriguez EM, Blazquez JL, Pastor FE, Pelaez B, Pena P, Peruzzo B, Amat P: Hypothalamic tanycytes: A key component of brain-endocrine interaction. *Int Rev Cytol.* 247:89-164, 2005
75. Ibberson M, Uldry M, Thorens B: GLUTX1, a novel mammalian glucose transporter expressed in the central nervous system and insulin-sensitive tissues. *J Biol Chem.* 275:4607-4612, 2000
76. Park CR: Cognitive effects of insulin in the central nervous system. *Neurosci Biobehav Rev.* 25:311-323, 2001
77. McCall AL, van Bueren AM, Huang L, Stenbit A, Celnik E, Charron MJ: Forebrain endothelium expresses GLUT4, the insulin-responsive glucose transporter. *Brain Res.* 744:318-326, 1997
78. Bakirtzi K, Belfort G, Lopez-Coviella I, Kuruppu D, Cao L, Abel ED, Brownell AL, Kandror KV: Cerebellar neurons possess a vesicular compartment structurally and functionally similar to Glut4-storage vesicles from peripheral insulin-sensitive tissues. *J Neurosci.* 29:5193-5201, 2009
79. Hom FG, Goodner CJ, Berrie MA: A $^{3}$H-2-deoxyglucose method for comparing rates of glucose metabolism and insulin responses among rat tissues in vivo. Validation of the model and the absence of an insulin effect on brain. *Diabetes.* 33:141-152, 1984
80. Hasselbalch SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, Paulson OB: No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes.* 48:1915-1921, 1999
81. Knudsen GM, Hasselbalch SG, Hertz MM, Paulson OB: High dose insulin does not increase glucose transfer across the blood-brain barrier in humans: A re-evaluation. *Eur J Clin Invest.* 29:687-691, 1999
82. Bloch F, Hansen WW, Packard ME: Physical review. *Phys Rev.* 70:460-473, 1946
83. Purcell EM, Torrey HC, Pound CV: Resonance absorption by nuclear magnetic moments in a solid. *Phys Rev.* 69:37-38, 1946
84. Hanstock CC, Rothman DL, Prichard JW, Jue T, Shulman RG: Spatially localized $^{1}$H NMR spectra of metabolites in the human brain. *Proc Natl Acad Sci U S A.* 85:1821-1825, 1988
85. Lin A, Ross BD, Harris K, Wong W: Efficacy of proton magnetic resonance spectroscopy in neurological diagnosis and neurotherapeutic decision making. *NeuroRx.* 2:197-214, 2005
86. Tofts PS and Waldman A.D: Spectroscopy: $^{1}$H metabolite concentrations. In Tofts PS: *Quantitative MRI of the Brain: Measuring Changes Caused by Diseases.* John Wiley & Sons Ltd., England, 2003, p.299
87. Frahm J, Merboldt KD, Häniche W: Localized proton spectroscopy using stimulated echoes. *J Magn Reson.* 72:502-508, 1987
88. Bottomley PA: Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci.* 508:333-348, 1987
89. Danielsen ER, Henriksen O: Absolute quantitative proton NMR spectroscopy based on the amplitude of the local water suppression pulse. Quantification of brain water and metabolites. *NMR Biomed.* 7:311-318, 1994
90. Jansen JF, Backes WH, Nicolay K, Kooi ME: $^{1}$H MR spectroscopy of the brain: Absolute quantification of metabolites. *Radiology.* 240:318-332, 2006
91. Pouwels PJ, Brockmann K, Kruse B, Wilken B, Wick M, Hanefeld F, Frahm J: Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. *Pediatr Res.* 46:474-485, 1999
92. Kreis R, Arcinue E, Ernst T, Shonk TK, Flores R, Ross BD: Hypoxic encephalopathy after near-drowning studied by quantitative $^{1}$H-magnetic resonance spectroscopy. *J Clin Invest.* 97:1142-1154, 1996
93. Ross B and Bluml S: Magnetic resonance spectroscopy of the human brain. *Anat Rec.* 265:54-84, 2001
94. Bates TE, Strangward M, Keelan J, Davey GP, Munro PM, Clark JB: Inhibition of N-acetylaspartate production: Implications for $^{1}$H MRS studies in vivo. *Neuroreport.* 7:1397-1400, 1996
95. Moreno A, Ross BD, Bluml S: Direct determination of the N-acetyl-L-aspartate synthesis rate in the human brain by $^{13}$C MRS and 1-$^{13}$C-glucose infusion. *J Neurochem.* 77:347-350, 2001

96. Baslow MH, Suckow RF, Sapirstein V, Hungund BL: Expression of aspartoacylase activity in cultured rat macroglial cells is limited to oligodendrocytes. *J Mol Neurosci.* 13:47-53, 1999
97. Berger UV, Luthi-Carter R, Passani LA, Elkabes S, Black I, Konradi C, Coyle JT: Glutamate carboxypeptidase II is expressed by astrocytes in the adult rat nervous system. *J Comp Neurol.* 415:52-64, 1999
98. Urenjak J, Williams SR, Gadian DG, Noble M: Specific expression of N-acetylaspartate in neurons, oligodendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes in vitro. *J Neurochem.* 59:55-61, 1992
99. Simmons ML, Frondoza CG, Coyle JT: Immunocytochemical localization of N-acetyl-aspartate with monoclonal antibodies. *Neuroscience.* 45:37-45, 1991
100. Chakraborty G, Mekala P, Yahya D, Wu G, Ledeen RW: Intraneuronal N-acetylaspartate supplies acetyl groups for myelin lipid synthesis: Evidence for myelin-associated aspartoacylase. *J Neurochem.* 78:736-745, 2001
101. Baslow MH, Suckow RF, Gaynor K, Bhakoo KK, Marks N, Saito M, Saito M, Duff K, Matsuoka Y, Berg MJ: Brain damage results in down-regulation of N-acetylaspartate as a neuronal osmolyte. *Neuromolecular Med.* 3:95-104, 2003
102. Chang L, Ernst T, Poland RE, Jenden DJ: In vivo proton magnetic resonance spectroscopy of the normal aging human brain. *Life Sci.* 58:2049-2056, 1996
103. Brooks JC, Roberts N, Kemp GJ, Gosney MA, Lye M, Whitehouse GH: A proton magnetic resonance spectroscopy study of age-related changes in frontal lobe metabolite concentrations. *Cereb Cortex.* 11:598-605, 2001
104. Leary SM, Brex PA, MacManus DG, Parker GJ, Barker GJ, Miller DH, Thompson AJ: A $^{1}$H magnetic resonance spectroscopy study of aging in parietal white matter: Implications for trials in multiple sclerosis. *Magn Reson Imaging.* 18:455-459, 2000
105. Chang L, Lee PL, Yiannoutsos CT, Ernst T, Marra CM, Richards T, Kolson D, Schifitto G, Jarvik JG, Miller EN, Lenkinski R, Gonzalez G, Navia BA, HIV MRS Consortium: A multicenter in vivo proton-MRS study of HIV-associated dementia and its relationship to age. *Neuroimage.* 23:1336-1347, 2004
106. Canavan MM: Schilder's encephalitis periaxialis diffusa. *Arch Neurol Psychiatr.* 25:299-308, 1931
107. Matalon R, Michals K, Sebesta D, Deanching M, Gashkoff P, Casanova J: Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. *Am J Med Genet.* 29:463-471, 1988
108. Moffett JR, Ross B, Arun P, Madhavarao CN, Namboodiri AM: N-acetylaspartate in the CNS. From neurodiagnostics to neurobiology. *Prog Neurobiol.* 81:89-131, 2007
109. Danielsen ER and Ross B: Magnetic resonance spectroscopy diagnosis of neurological disease. Marcel Dekker, United States of America, 1999
110. Hertz L, Dringen R, Schousboe A, Robinson SR: Astrocytes: Glutamate producers for neurons. *J Neurosci Res.* 57:417-428, 1999
111. Schulman M: Organic osmolytes in the brain of an infant with hypernatremia. *N Engl J Med.* 331:1776-1777, 1994
112. Lee JH, Arcinue E, Ross BD: Brief report: Organic osmolytes in the brain of an infant with hypernatremia. *N Engl J Med.* 331:439-442, 1994
113. Brosnan JT, Brosnan ME: Creatine: Endogenous metabolite, dietary, and therapeutic supplement. *Annu Rev Nutr.* 27:241-261, 2007
114. Ohtsuki S, Tachikawa M, Takanaga H, Shimizu H, Watanabe M, Hosoya K, Terasaki T: The blood-brain barrier creatine transporter is a major pathway for supplying creatine to the brain. *J Cereb Blood Flow Metab.* 22:1327-1335, 2002
115. Zhao X, Bassirat M, Zeinab K, Helme RD: Effects of diabetes on creatine kinase activity in streptozotocin-diabetic rats. *Chin Med J (Engl).* 112:1028-1031, 1999
116. Saunders DE, Howe FA, van den Boogaart A, Griffiths JR, Brown MM: Aging of the adult human brain: In vivo quantitation of metabolite content with proton magnetic resonance spectroscopy. *J Magn Reson Imaging.* 9:711-716, 1999
117. Klein J: Membrane breakdown in acute and chronic neurodegeneration: Focus on choline-containing phospholipids. *J Neural Transm.* 107:1027-1063, 2000
118. Holub BJ: Metabolism and function of myo-inositol and inositol phospholipids. *Annu Rev Nutr.* 6:563-597, 1986

119. Norton WT: The nervous system. Raven Press, New York, 1975
120. Levin DL: Cerebral edema in diabetic ketoacidosis. *Pediatr Crit Care Med.* 9:320-329, 2008
121. Reaven GM: Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes.* 37:1595-1607, 1988
122. World Health Organization: Definition, diagnosis, and classification of diabetes mellitus and its complications. *Report of a WHO consultation.* Geneva: WHO 1999.
123. Balkau B and Charles MA: Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med.* 16:442-443, 1999
124. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA.* 285:2486-2497, 2001
125. Alberti KG, Zimmet P, Shaw J: Metabolic syndrome - a new world-wide definition. A consensus statement from the international diabetes federation. *Diabet Med.* 23:469-480, 2006
126. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Fernando C: Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement: Executive summary. *Crit Pathw Cardiol.* 4:198-203, 2005
127. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr: Harmonizing the metabolic syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 120:1640-1645, 2009
128. Ilanne-Parikka P, Eriksson JG, Lindstrom J, Hämäläinen H, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Rastas M, Salminen V, Aunola S, Sundvall J, Valle T, Lahtela J, Uusitupa M, Tuomilehto J, Finnish Diabetes Prevention Study Group: Prevalence of the metabolic syndrome and its components: Findings from a Finnish general population sample and the Diabetes Prevention Study cohort. *Diabetes Care.* 27:2135-2140, 2004
129. Hanson RL, Imperatore G, Narayan KM, Roumain J, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC: Family and genetic studies of indices of insulin sensitivity and insulin secretion in Pima Indians. *Diabetes Metab Res Rev.* 17:296-303, 2001
130. Austin MA, Edwards KL, McNeely MJ, Chandler WL, Leonetti DL, Talmud PJ, Humphries SE, Fujimoto WY: Heritability of multivariate factors of the metabolic syndrome in nondiabetic Japanese Americans. *Diabetes.* 53:1166-1169, 2004
131. Henkin L, Bergman RN, Bowden DW, Ellsworth DL, Haffner SM, Langefeld CD, Mitchell BD, Norris JM, Rewers M, Saad MF, Stamm E, Wagenknecht LE, Rich SS: Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS family study design and methods. *Ann Epidemiol.* 13:211-217, 2003
132. Mattsson N, Rönnemaa T, Juonala M, Viikari JS, Raitakari OT: Childhood predictors of the metabolic syndrome in adulthood. The cardiovascular risk in Young Finns Study. *Ann Med.* 40:542-552, 2008
133. Ford ES: Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: A summary of the evidence. *Diabetes Care.* 28:1769-1778, 2005
134. Ford ES, Schulze MB, Pischon T, Bergmann MM, Joost HG, Boeing H: Metabolic syndrome and risk of incident diabetes: Findings from the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. *Cardiovasc Diabetol.* 7:35, 2008
135. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M, Finnish Diabetes Prevention Study Group: Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med.* 344:1343-1350, 2001
136. Kahn SE, Zinman B, Haffner SM, O'Neill MC, Kravitz BG, Yu D, Freed MI, Herman WH, Holman RR, Jones NP, Lachin JM, Viberti GC, ADOPT Study Group: Obesity is a major determinant of the association of C-reactive protein levels and the metabolic syndrome in type 2 diabetes. *Diabetes.* 55:2357-2364, 2006

137. Eliasson B, Attvall S, Taskinen MR, Smith U: The insulin resistance syndrome in smokers is related to smoking habits. *Arterioscler Thromb.* 14:1946-1950, 1994
138. Vitaliano PP, Scanlan JM, Zhang J, Savage MV, Hirsch IB, Siegler IC: A path model of chronic stress, the metabolic syndrome, and coronary heart disease. *Psychosom Med.* 64:418-435, 2002
139. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM: Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): Relation to reduced fetal growth. *Diabetologia.* 36:62-67, 1993
140. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox KA, Smith SC Jr, Barter P, Tan CE, Van Gaal L, Wittchen HU, Massien C, Haffner SM: International day for the evaluation of abdominal obesity (IDEA): A study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. *Circulation.* 116:1942-1951, 2007
141. Mykkänen L, Haffner SM, Rönnemaa T, Bergman RN, Laakso M: Low insulin sensitivity is associated with clustering of cardiovascular disease risk factors. *Am J Epidemiol.* 146:315-321, 1997
142. Eckel RH, Grundy SM, Zimmet PZ: The metabolic syndrome. *Lancet.* 365:1415-1428, 2005
143. Salmenniemi U, Ruotsalainen E, Pihlajamäki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E, Laakso M: Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation.* 110:3842-3848, 2004
144. Ando K, Fujita T: Metabolic syndrome and oxidative stress. *Free Radic Biol Med.* 47:213-218, 2009
145. Björntorp P, Rosmond R: The metabolic syndrome - a neuroendocrine disorder? *Br J Nutr.* 83 Suppl 1:S49-57, 2000
146. Whorwood CB, Donovan SJ, Flanagan D, Phillips DI, Byrne CD: Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. *Diabetes.* 51:1066-1075, 2002
147. Facchini F, Chen YD, Hollenbeck CB, Reaven GM: Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA.* 266:3008-3011, 1991
148. Svatikova A, Wolk R, Gami AS, Pohanka M, Somers VK: Interactions between obstructive sleep apnea and the metabolic syndrome. *Curr Diab Rep.* 5:53-58, 2005
149. Dunaif A, Segal KR, Futterweit W, Dobrjansky A: Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes.* 38:1165-1174, 1989
150. Laudisio A, Marzetti E, Pagano F, Pozzi G, Bernabei R, Zuccala G: Depressive symptoms and metabolic syndrome: Selective association in older women. *J Geriatr Psychiatry Neurol.* 22:215-222, 2009
151. Suvisaari JM, Saarni SI, Perälä J, Suvisaari JV, Härkanen T, Lönnqvist J, Reunanen A: Metabolic syndrome among persons with schizophrenia and other psychotic disorders in a general population survey. *J Clin Psychiatry.* 68:1045-1055, 2007
152. Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC: The effect of body mass index on global brain volume in middle-aged adults: A cross sectional study. *BMC Neurol.* 5:23, 2005
153. Gunstad J, Paul RH, Cohen RA, Tate DF, Spitznagel MB, Grieve S, Gordon E: Relationship between body mass index and brain volume in healthy adults. *Int J Neurosci.* 118:1582-1593, 2008
154. Salerno JA, Murphy DG, Horwitz B, DeCarli C, Haxby JV, Rapoport SI, Schapiro MB: Brain atrophy in hypertension. A volumetric magnetic resonance imaging study. *Hypertension.* 20:340-348, 1992
155. DeCarli C, Miller BL, Swan GE, Reed T, Wolf PA, Garner J, Jack L, Carmelli D: Predictors of brain morphology for the men of the NHLBI twin study. *Stroke.* 30:529-536, 1999
156. Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S, Schmidt R: Risk factors for progression of brain atrophy in aging: Six-year follow-up of normal subjects. *Neurology.* 64:1704-1711, 2005
157. Jefferson AL, Massaro JM, Wolf PA, Seshadri S, Au R, Vasan RS, Larson MG, Meigs JB, Keaney JF Jr, Lipinska I, Kathiresan S, Benjamin EJ, DeCarli C: Inflammatory biomarkers are associated with total brain volume: The Framingham Heart Study. *Neurology.* 68:1032-1038, 2007
158. den Heijer T, Vermeer SE, Clarke R, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MM: Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain.* 126:170-175, 2003
159. Sachdev PS, Valenzuela M, Wang XL, Looi JC, Brodaty H: Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. *Neurology.* 58:1539-1541, 2002

160. Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H: Relationship between body mass index and gray matter volume in 1,428 healthy individuals. *Obesity (Silver Spring).* 16:119-124, 2008
161. Strassburger TL, Lee HC, Daly EM, Szczepanik J, Krasuski JS, Mentis MJ, Salerno JA, DeCarli C, Schapiro MB, Alexander GE: Interactive effects of age and hypertension on volumes of brain structures. *Stroke.* 28:1410-1417, 1997
162. Bokura H, Yamaguchi S, Iijima K, Nagai A, Oguro H: Metabolic syndrome is associated with silent ischemic brain lesions. *Stroke.* 39:1607-1609, 2008
163. Jagust W, Harvey D, Mungas D, Haan M: Central obesity and the aging brain. *Arch Neurol.* 62:1545-1548, 2005
164. Liao D, Cooper L, Cai J, Toole J, Bryan N, Burke G, Shahar E, Nieto J, Mosley T, Heiss G: The prevalence and severity of white matter lesions, their relationship with age, ethnicity, gender, and cardiovascular disease risk factors: The ARIC study. *Neuroepidemiology.* 16:149-162, 1997
165. de Leeuw FE, de Groot JC, Oudkerk M, Witteman JC, Hofman A, van Gijn J, Breteler MM: Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain.* 125:765-772, 2002
166. van Dijk EJ, Prins ND, Vermeer SE, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM: C-reactive protein and cerebral small-vessel disease: The Rotterdam Scan Study. *Circulation.* 112:900-905, 2005
167. Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R: Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: Longitudinal results of the Austrian Stroke Prevention Study. *Stroke.* 36:1410-1414, 2005
168. Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, Breteler MM: Homocysteine, silent brain infarcts, and white matter lesions: The Rotterdam Scan Study. *Ann Neurol.* 51:285-289, 2002
169. Hu G, Tuomilehto J, Silventoinen K, Sarti C, Männistö S, Jousilahti P: Body mass index, waist circumference, and waist-hip ratio on the risk of total and type-specific stroke. *Arch Intern Med.* 167:1420-1427, 2007
170. Pyorälä M, Miettinen H, Laakso M, Pyorälä K: Hyperinsulinemia and the risk of stroke in healthy middle-aged men: The 22-year follow-up results of the Helsinki Policemen Study. *Stroke.* 29:1860-1866, 1998
171. Suk SH, Sacco RL, Boden-Albala B, Cheun JF, Pittman JG, Elkind MS, Paik MC, Northern Manhattan Stroke Study: Abdominal obesity and risk of ischemic stroke: The Northern Manhattan Stroke Study. *Stroke.* 34:1586-1592, 2003
172. Milionis HJ, Rizos E, Goudevenos J, Seferiadis K, Mikhailidis DP, Elisaf MS: Components of the metabolic syndrome and risk for first-ever acute ischemic nonembolic stroke in elderly subjects. *Stroke.* 36:1372-1376, 2005
173. McNeill AM, Rosamond WD, Girman CJ, Golden SH, Schmidt MI, East HE, Ballantyne CM, Heiss G: The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. *Diabetes Care.* 28:385-390, 2005
174. Dekker JM, Girman C, Rhodes T, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ: Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. *Circulation.* 112:666-673, 2005
175. Qiao Q, Laatikainen T, Zethelius B, Stegmayr B, Eliasson M, Jousilahti P, Tuomilehto J: Comparison of definitions of metabolic syndrome in relation to the risk of developing stroke and coronary heart disease in Finnish and Swedish cohorts. *Stroke.* 40:337-343, 2009
176. Kwon HM, Kim BJ, Lee SH, Choi SH, Oh BH, Yoon BW: Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke.* 37:466-470, 2006
177. Golden SH, Folsom AR, Coresh J, Sharrett AR, Szklo M, Brancati F: Risk factor groupings related to insulin resistance and their synergistic effects on subclinical atherosclerosis: The atherosclerosis risk in communities study. *Diabetes.* 51:3069-3076, 2002
178. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC, Muggeo M, Bruneck study: Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: Prospective data from the Bruneck Study. *Diabetes Care.* 26:1251-1257, 2003
179. Koskinen J, Kähönen M, Viikari JS, Taittonen L, Laitinen T, Rönnemaa T, Lehtimäki T, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Helenius H, Mattsson N, Raitakari OT, Juonala M:

Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: The Cardiovascular Risk in Young Finns Study. *Circulation.* 120:229-236, 2009
180. Kivipelto M, Helkala EL, Hänninen T, Laakso MP, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A: Midlife vascular risk factors and late-life mild cognitive impairment: A population-based study. *Neurology.* 56:1683-1689, 2001
181. Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H, Nissinen A: Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol.* 62:1556-1560, 2005
182. Rantanen K, Ylikoski R, Happola O, Strandberg TE: Higher cardiovascular risk in midlife is associated with worse cognitive function 29 years later, in old age. *J Am Geriatr Soc.* 56:2152-2153, 2008
183. Forette F, Seux ML, Staessen JA, Thijs L, Birkenhager WH, Babarskiene MR, Babeanu S, Bossini A, Gil-Extremera B, Girerd X, Laks T, Lilov E, Moisseyev V, Tuomilehto J, Vanhanen H, Webster J, Yodfat Y, Fagard R: Prevention of dementia in randomised double-blind placebo-controlled systolic hypertension in Europe (Syst-Eur) Trial. *Lancet.* 352:1347-1351, 1998
184. Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, Tylavsky FA, Newman AB: The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA.* 292:2237-2242, 2004
185. Sorensen TI, Sonne-Holm S, Christensen U: Cognitive deficiency in obesity independent of social origin. *Lancet.* 1:1105-1106, 1983
186. Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB: Lower cognitive function in the presence of obesity and hypertension: The Framingham Heart Study. *Int J Obes Relat Metab Disord.* 27:260-268, 2003
187. Wolf PA, Beiser A, Elias MF, Au R, Vasan RS, Seshadri S: Relation of obesity to cognitive function: Importance of central obesity and synergistic influence of concomitant hypertension. The Framingham Heart Study. *Curr Alzheimer Res.* 4:111-116, 2007
188. Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ: The association between midlife blood pressure levels and late-life cognitive function. The Tonolulu-Asia Aging Study. *JAMA.* 274:1846-1851, 1995
189. Kilander L, Nyman H, Boberg M, Hansson L, Lithell H: Hypertension is related to cognitive impairment: A 20-year follow-up of 999 men. *Hypertension.* 31:780-786, 1998
190. Yaffe K, Barrett-Connor E, Lin F, Grady D: Serum lipoprotein levels, statin use, and cognitive function in older women. *Arch Neurol.* 59:378-384, 2002
191. Kalmijn S, Feskens EJ, Launer LJ, Stijnen T, Kromhout D: Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men. *Diabetologia.* 38:1096-1102, 1995
192. Vanhanen M, Koivisto K, Karjalainen L, Helkala EL, Laakso M, Soininen H, Riekkinen PS: Risk for non-insulin-dependent diabetes in the normoglycaemic elderly is associated with impaired cognitive function. *Neuroreport.* 8:1527-1530, 1997
193. Young SE, Mainous AG 3rd, Carnemolla M: Hyperinsulinemia and cognitive decline in a middle-aged cohort. *Diabetes Care.* 29:2688-2693, 2006
194. Manschot SM, Biessels GJ, de Valk H, Algra A, Rutten GE, van der Grond J, Kappelle LJ, on behalf of the Utrecht Diabetic Encephalopathy Study Group: Metabolic and vascular determinants of impaired cognitive performance and abnormalities on brain magnetic resonance imaging in patients with type 2 diabetes. *Diabetologia.* 50:2388-2397, 2007
195. Di Bonito P, Di Fraia L, Di Gennaro L, Vitale A, Lapenta M, Scala A, Iardino MR, Cusati B, Attino L, Capaldo B: Impact of impaired fasting glucose and other metabolic factors on cognitive function in elderly people. *Nutr Metab Cardiovasc Dis.* 17:203-208, 2007
196. Jennings JR, Muldoon MF, Ryan C, Price JC, Greer P, Sutton-Tyrrell K, van der Veen FM, Meltzer CC: Reduced cerebral blood flow response and compensation among patients with untreated hypertension. *Neurology.* 64:1358-1365, 2005
197. Selim M, Jones R, Novak P, Zhao P, Novak V: The effects of body mass index on cerebral blood flow velocity. *Clin Auton Res.* 18:331-338, 2008
198. Vorbrodt AW, Dobrogowska DH, Tarnawski M, Meeker HC, Carp RI: Quantitative immunogold study of glucose transporter (GLUT-1) in five brain regions of scrapie-infected mice showing obesity and reduced glucose tolerance. *Acta Neuropathol.* 102:278-284, 2001

199. Reiman EM, Chen K, Langbaum JB, Lee W, Reschke C, Bandy D, Alexander GE, Caselli RJ: Higher serum total cholesterol levels in late middle age are associated with glucose hypometabolism in brain regions affected by Alzheimer's disease and normal aging. *Neuroimage.* 49:169-176, 2010
200. Carvalheira JB, Ribeiro EB, Araujo EP, Guimaraes RB, Telles MM, Torsoni M, Gontijo JA, Velloso LA, Saad MJ: Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats. *Diabetologia.* 46:1629-1640, 2003
201. Mielke JG, Taghibiglou C, Liu L, Zhang Y, Jia Z, Adeli K, Wang YT: A biochemical and functional characterization of diet-induced brain insulin resistance. *J Neurochem.* 93:1568-1578, 2005
202. Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ: A high fructose diet affects the early steps of insulin action in muscle and liver of rats. *J Nutr.* 130:1531-1535, 2000
203. Taghibiglou C, Rashid-Kolvear F, Van Iderstine SC, Le-Tien H, Fantus IG, Lewis GF, Adeli K: Hepatic very low density lipoprotein-ApoB overproduction is associated with attenuated hepatic insulin signaling and overexpression of protein-tyrosine phosphatase 1B in a fructose-fed hamster model of insulin resistance. *J Biol Chem.* 277:793-803, 2002
204. Kern W, Benedict C, Schultes B, Plohr F, Moser A, Born J, Fehm HL, Hallschmid M: Low cerebrospinal fluid insulin levels in obese humans. *Diabetologia.* 49:2790-2792, 2006
205. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P: Risk of dementia in diabetes mellitus: A systematic review. *Lancet Neurol.* 5:64-74, 2006
206. Catani M, Mecocci P, Tarducci R, Howard R, Pelliccioli GP, Mariani E, Metastasio A, Benedetti C, Senin U, Cherubini A: Proton magnetic resonance spectroscopy reveals similar white matter biochemical changes in patients with chronic hypertension and early Alzheimer's disease. *J Am Geriatr Soc.* 50:1707-1710, 2002
207. Sinha S, Misra A, Kumar V, Jagannathan NR, Bal CS, Pandey RM, Singhania R, Deepak: Proton magnetic resonance spectroscopy and single photon emission computed tomography study of the brain in asymptomatic young hyperlipidaemic Asian Indians in North India show early abnormalities. *Clin Endocrinol (Oxf).* 61:182-189, 2004
208. Kario K, Ishikawa J, Hoshide S, Matsui Y, Morinari M, Eguchi K, Ishikawa S, Shimada K: Diabetic brain damage in hypertension: Role of renin-angiotensin system. *Hypertension.* 45:887-893, 2005
209. Sahin I, Alkan A, Keskin L, Cikim A, Karakas HM, Firat AK, Sigirci A: Evaluation of in vivo cerebral metabolism on proton magnetic resonance spectroscopy in patients with impaired glucose tolerance and type 2 diabetes mellitus. *J Diabetes Complications.* 22:254-260, 2008
210. Mitrakou A, Ryan C, Veneman T, Mokan M, Jenssen T, Kiss I, Durrant J, Cryer P, Gerich J: Hierarchy of glycemic thresholds for counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol.* 260:E67-74, 1991
211. Fujioka M, Okuchi K, Hiramatsu KI, Sakaki T, Sakaguchi S, Ishii Y: Specific changes in human brain after hypoglycemic injury. *Stroke.* 28:584-587, 1997
212. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med.* 329:977-986, 1993
213. Henricsson M, Nilsson A, Groop L, Heijl A, Janzon L: Prevalence of diabetic retinopathy in relation to age at onset of the diabetes, treatment, duration and glycemic control. *Acta Ophthalmol Scand.* 74:523-527, 1996
214. Nathan DM, Zinman B, Cleary PA, Backlund JY, Genuth S, Miller R, Orchard TJ: Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications and Pittsburgh Epidemiology of Diabetes Complications Experience (1983-2005). *Arch Intern Med.* 169:1307-1316, 2009
215. Nathan DM, Turgeon H, Regan S: Relationship between glycated haemoglobin levels and mean glucose levels over time. *Diabetologia.* 50:2239-2244, 2007
216. Kilpatrick ES, Rigby AS, Atkin SL: A1C variability and the risk of microvascular complications in type 1 diabetes: Data from The Diabetes Control and Complications Trial. *Diabetes Care.* 31:2198-2202, 2008
217. Waden J, Forsblom C, Thorn LM, Gordin D, Saraheimo M, Groop PH, Finnish Diabetic Nephropathy Study Group: A1C variability predicts incident cardiovascular events,

microalbuminuria, and overt diabetic nephropathy in patients with type 1 diabetes. *Diabetes.* 58:2649-2655, 2009
218. Kilpatrick ES, Rigby AS, Atkin SL: The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. *Diabetes Care.* 29:1486-1490, 2006
219. Bragd J, Adamson U, Backlund LB, Lins PE, Moberg E, Oskarsson P: Can glycaemic variability, as calculated from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? *Diabetes Metab.* 34:612-616, 2008
220. Reichard P, Berglund B, Britz A, Cars I, Nilsson BY, Rosenqvist U: Intensified conventional insulin treatment retards the microvascular complications of insulin-dependent diabetes mellitus (IDDM): The Stockholm Diabetes Intervention study (SDIS) after 5 years. *J Intern Med.* 230:101-108, 1991
221. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: An epidemiological study. *Diabetologia.* 25:496-501, 1983
222. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL: The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmol.* 102:520-526, 1984
223. Thorn LM, Forsblom C, Waden J, Söderlund J, Rosengård-Bärlund M, Saraheimo M, Heikkilä O, Hietala K, Pettersson-Fernholm K, Ilonen J, Groop PH, FinnDiane Study Group: Effect of parental type 2 diabetes on offspring with type 1 diabetes. *Diabetes Care.* 32:63-68, 2009
224. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, Waden J, Rönnback M, Rosengård-Bärlund M, Björkesten CG, Taskinen MR, Groop PH, FinnDiane Study Group: Metabolic syndrome in type 1 diabetes: Association with diabetic nephropathy and glycemic control (The FinnDiane Study). *Diabetes Care.* 28:2019-2024, 2005
225. Miles WR and Root HF: Psychologic tests applied in diabetic patients. *Arch Intern Med.* 30:767-777, 1922
226. De Jong RN: The nervous system complications in diabetes mellitus with special reference to cerebrovascular changes. *J Nerv Ment Dis.* 111:181-206, 1950
227. Reske-Nielsen E, Lundbaed K, Rafeisen QJ: Pathological changes in the cerebral and peripheral nervous system of young long-term diabetics. I. Diabetic encephalopathy. *Diabetologia.* 1:233-241, 1965
228. Johnson PC, Brendel K, Meezan E: Thickened cerebral cortical capillary basement membranes in diabetics. *Arch Pathol Lab Med.* 106:214-217, 1982
229. Perantie DC, Wu J, Koller JM, Lim A, Warren SL, Black KJ, Sadler M, White NH, Hershey T: Regional brain volume differences associated with hyperglycemia and severe hypoglycemia in youth with type 1 diabetes. *Diabetes Care.* 30:2331-2337, 2007
230. Musen G, Jacobson AM, Ryan CM, Cleary PA, Waberski BH, Weinger K, Dahms W, Bayless M, Silvers N, Harth J, White N, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: Impact of diabetes and its treatment on cognitive function among adolescents who participated in The Diabetes Control and Complications Trial. *Diabetes Care.* 31:1933-1938, 2008
231. Wessels AM, Simsek S, Remijnse PL, Veltman DJ, Biessels GJ, Barkhof F, Scheltens P, Snoek FJ, Heine RJ, Rombouts SA: Voxel-based morphometry demonstrates reduced grey matter density on brain MRI in patients with diabetic retinopathy. *Diabetologia.* 49:2474-2480, 2006
232. Dejgaard A, Gade A, Larsson H, Balle V, Parving A, Parving HH: Evidence for diabetic encephalopathy. *Diabet Med.* 8:162-167, 1991
233. Perros P, Deary IJ, Sellar RJ, Best JJ, Frier BM: Brain abnormalities demonstrated by magnetic resonance imaging in adult IDDM patients with and without a history of recurrent severe hypoglycemia. *Diabetes Care.* 20:1013-1018, 1997
234. Kodl CT, Franc DT, Rao JP, Anderson FS, Thomas W, Mueller BA, Lim KO, Seaquist ER: Diffusion tensor imaging (DTI) identifies deficits in white matter microstructure in subjects with type 1 diabetes mellitus that correlate with reduced neurocognitive function. *Diabetes.* 57:3083-3089, 2008
235. Yousem DM, Tasman WS, Grossman RI: Proliferative retinopathy: Absence of white matter lesions at MR imaging. *Radiology.* 179:229-230, 1991

236. Brands AM, Kessels RP, Hoogma RP, Henselmans JM, van der Beek Boter JW, Kappelle LJ, de Haan EH, Biessels GJ: Cognitive performance, psychological well-being, and brain magnetic resonance imaging in older patients with type 1 diabetes. *Diabetes.* 55:1800-1806, 2006
237. Laing SP, Swerdlow AJ, Carpenter LM, Slater SD, Burden AC, Botha JL, Morris AD, Waugh NR, Gatling W, Gale EA, Patterson CC, Qiao Z, Keen H: Mortality from cerebrovascular disease in a cohort of 23 000 patients with insulin-treated diabetes. *Stroke.* 34:418-421, 2003
238. Peress NS, Kane WC, Aronson SM: Central nervous system findings in a tenth decade autopsy population. *Prog Brain Res.* 40:473-483, 1973
239. Mast H, Thompson JL, Lee SH, Mohr JP, Sacco RL: Hypertension and diabetes mellitus as determinants of multiple lacunar infarcts. *Stroke.* 26:30-33, 1995
240. Davis TM, Bruce DG, Davis WA: Predictors of first stroke in type 1 diabetes: The Fremantle Diabetes Study. *Diabet Med.* 22:551-553, 2005
241. Langan SJ, Deary IJ, Hepburn DA, Frier BM: Cumulative cognitive impairment following recurrent severe hypoglycaemia in adult patients with insulin-treated diabetes mellitus. *Diabetologia.* 34:337-344, 1991
242. Deary IJ, Crawford JR, Hepburn DA, Langan SJ, Blackmore LM, Frier BM: Severe hypoglycemia and intelligence in adult patients with insulin-treated diabetes. *Diabetes.* 42:341-344, 1993
243. Austin EJ, Deary IJ: Effects of repeated hypoglycemia on cognitive function: A psychometrically validated reanalysis of The Diabetes Control and Complications Trial data. *Diabetes Care.* 22:1273-1277, 1999
244. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group, Jacobson AM, Musen G, Ryan CM, Silvers N, Cleary P, Waberski B, Burwood A, Weinger K, Bayless M, Dahms W, Harth J: Long-term effect of diabetes and its treatment on cognitive function. *N Engl J Med.* 356:1842-1852, 2007
245. Effects of intensive diabetes therapy on neuropsychological function in adults in The Diabetes Control and Complications Trial. *Ann Intern Med.* 124:379-388, 1996
246. Northam EA, Rankins D, Cameron FJ: Therapy insight: The impact of type 1 diabetes on brain development and function. *Nat Clin Pract Neurol.* 2:78-86, 2006
247. Schoenle EJ, Schoenle D, Molinari L, Largo RH: Impaired intellectual development in children with type I diabetes: Association with $HbA^{1c}$, age at diagnosis and sex. *Diabetologia.* 45:108-114, 2002
248. Hershey T, Bhargava N, Sadler M, White NH, Craft S: Conventional versus intensive diabetes therapy in children with type 1 diabetes: Effects on memory and motor speed. *Diabetes Care.* 22:1318-1324, 1999
249. Northam EA, Anderson PJ, Jacobs R, Hughes M, Warne GL, Werther GA: Neuropsychological profiles of children with type 1 diabetes 6 years after disease onset. *Diabetes Care.* 24:1541-1546, 2001
250. Ryan CM, Williams TM, Orchard TJ, Finegold DN: Psychomotor slowing is associated with distal symmetrical polyneuropathy in adults with diabetes mellitus. *Diabetes.* 41:107-113, 1992
251. Gonder-Frederick LA, Zrebiec JF, Bauchowitz AU, Ritterband LM, Magee JC, Cox DJ, Clarke WL: Cognitive function is disrupted by both hypo- and hyperglycemia in school-aged children with type 1 diabetes: A Field Study. *Diabetes Care.* 32:1001-1006, 2009
252. Cox DJ, Kovatchev BP, Gonder-Frederick LA, Summers KH, McCall A, Grimm KJ, Clarke WL: Relationships between hyperglycemia and cognitive performance among adults with type 1 and type 2 diabetes. *Diabetes Care.* 28:71-77, 2005
253. Sommerfield AJ, Deary IJ, Frier BM: Acute hyperglycemia alters mood state and impairs cognitive performance in people with type 2 diabetes. *Diabetes Care.* 27:2335-2340, 2004
254. Kreis R, Ross BD: Cerebral metabolic disturbances in patients with subacute and chronic diabetes mellitus: Detection with proton MR spectroscopy. *Radiology.* 184:123-130, 1992
255. Mäkimattila S, Malmberg-Ceder K, Häkkinen AM, Vuori K, Salonen O, Summanen P, Yki-Järvinen H, Kaste M, Heikkinen S, Lundbom N, Roine RO: Brain metabolic alterations in patients with type 1 diabetes-hyperglycemia-induced injury. *J Cereb Blood Flow Metab.* 24:1393-1399, 2004
256. Criego AB, Tkac I, Kumar A, Thomas W, Gruetter R, Seaquist ER: Brain glucose concentrations in patients with type 1 diabetes and hypoglycemia unawareness. *J Neurosci Res.* 79:42-47, 2005
257. Grill V, Gutniak M, Björkman O, Lindqvist M, Stone-Elander S, Seitz RJ, Blomqvist G, Reichard P, Widen L: Cerebral blood flow and substrate utilization in insulin-treated diabetic subjects. *Am J Physiol.* 258:E813-20, 1990

258. MacLeod KM, Hepburn DA, Deary IJ, Goodwin GM, Dougall N, Ebmeier KP, Frier BM: Regional cerebral blood flow in IDDM patients: Effects of diabetes and of recurrent severe hypoglycaemia. *Diabetologia.* 37:257-263, 1994
259. Kim YS, Krogh-Madsen R, Rasmussen P, Plomgaard P, Ogoh S, Secher NH, van Lieshout JJ: Effects of hyperglycemia on the cerebrovascular response to rhythmic handgrip exercise. *Am J Physiol Heart Circ Physiol.* 293:H467-73, 2007
260. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C: Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci U S A.* 91:9352-9356, 1994
261. Segel SA, Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Powers WJ, Cryer PE: Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. *Diabetes.* 50:1911-1917, 2001
262. Neil HA, Gale EA, Hamilton SJ, Lopez-Espinoza I, Kaura R, McCarthy ST: Cerebral blood flow increases during insulin-induced hypoglycaemia in type 1 (insulin-dependent) diabetic patients and control subjects. *Diabetologia.* 30:305-309, 1987
263. Hou WK, Xian YX, Zhang L, Lai H, Hou XG, Xu YX, Yu T, Xu FY, Song J, Fu CL, Zhang WW, Chen L: Influence of blood glucose on the expression of glucose transporter proteins 1 and 3 in the brain of diabetic rats. *Chin Med J (Engl).* 120:1704-1709, 2007
264. McCall AL, Fixman LB, Fleming N, Tornheim K, Chick W, Ruderman NB: Chronic hypoglycemia increases brain glucose transport. *Am J Physiol.* 251:E442-7, 1986
265. Pelligrino DA, Segil LJ, Albrecht RF: Brain glucose utilization and transport and cortical function in chronic vs. acute hypoglycemia. *Am J Physiol.* 259:E729-35, 1990
266. Kumagai AK: Diabetes and the blood-brain barrier. *Lancet Neurol.* 2:209, 2003
267. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ: Brain glucose uptake and unawareness of hypoglycemia in patients with insulin-dependent diabetes mellitus. *N Engl J Med.* 333:1726-1731, 1995
268. Wessels AM, Rombouts SA, Simsek S, Kuijer JP, Kostense PJ, Barkhof F, Scheltens P, Snoek FJ, Heine RJ: Microvascular disease in type 1 diabetes alters brain activation: A functional magnetic resonance imaging study. *Diabetes.* 55:334-340, 2006
269. Dunn JT, Cranston I, Marsden PK, Amiel SA, Reed LJ: Attenuation of amydgala and frontal cortical responses to low blood glucose concentration in asymptomatic hypoglycemia in type 1 diabetes: A new player in hypoglycemia unawareness? *Diabetes.* 56:2766-2773, 2007
270. Arbelaez AM, Powers WJ, Videen TO, Price JL, Cryer PE: Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition: A mechanism for hypoglycemia-associated autonomic failure. *Diabetes.* 57:470-475, 2008
271. Duelli R, Maurer MH, Staudt R, Heiland S, Duembgen L, Kuschinsky W: Increased cerebral glucose utilization and decreased glucose transporter Glut1 during chronic hyperglycemia in rat brain. *Brain Res.* 858:338-347, 2000
272. Choi TB, Boado RJ, Pardridge WM: Blood-brain barrier glucose transporter mRNA is increased in experimental diabetes mellitus. *Biochem Biophys Res Commun.* 164:375-380, 1989
273. Pardridge WM, Triguero D, Farrell CR: Downregulation of blood-brain barrier glucose transporter in experimental diabetes. *Diabetes.* 39:1040-1044, 1990
274. Lutz AJ, Pardridge WM: Insulin therapy normalizes GLUT1 glucose transporter mRNA but not immunoreactive transporter protein in streptozocin-diabetic rats. *Metabolism.* 42:939-944, 1993
275. Harik SI, Gravina SA, Kalaria RN: Glucose transporter of the blood-brain barrier and brain in chronic hyperglycemia. *J Neurochem.* 51:1930-1934, 1988
276. Kainulainen H, Schurmann A, Vilja P, Joost HG: In-vivo glucose uptake and glucose transporter proteins GLUT1 and GLUT3 in brain tissue from streptozotocin-diabetic rats. *Acta Physiol Scand.* 149:221-225, 1993
277. Simpson IA, Appel NM, Hokari M, Oki J, Holman GD, Maher F, Koehler-Stec EM, Vannucci SJ, Smith QR: Blood-brain barrier glucose transporter: Effects of hypo- and hyperglycemia revisited. *J Neurochem.* 72:238-247, 1999
278. Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Cryer PE, Powers WJ: Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. *Diabetes.* 47:1444-1450, 1998

279. Brooks DJ, Gibbs JS, Sharp P, Herold S, Turton DR, Luthra SK, Kohner EM, Bloom SR, Jones T: Regional cerebral glucose transport in insulin-dependent diabetic patients studied using $^{11}$C-3-*O*-methyl-D-glucose and positron emission tomography. *J Cereb Blood Flow Metab.* 6:240-244, 1986
280. Seaquist ER, Tkac I, Damberg G, Thomas W, Gruetter R: Brain glucose concentrations in poorly controlled diabetes mellitus as measured by high-field magnetic resonance spectroscopy. *Metabolism.* 54:1008-1013, 2005
281. Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ, Sherwin RS: Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: Effects of hyperglycemia and hypoglycemia. *J Cereb Blood Flow Metab.* 22:271-279, 2002
282. Sarac K, Akinci A, Alkan A, Aslan M, Baysal T, Ozcan C: Brain metabolite changes on proton magnetic resonance spectroscopy in children with poorly controlled type 1 diabetes mellitus. *Neuroradiology.* 47:562-565, 2005
283. Cameron FJ, Kean MJ, Wellard RM, Werther GA, Neil JJ, Inder TE: Insights into the acute cerebral metabolic changes associated with childhood diabetes. *Diabet Med.* 22:648-653, 2005
284. Wootton-Gorges SL, Glaser NS: Imaging of the brain in children with type I diabetes mellitus. *Pediatr Radiol.* 37:863-869, 2007
285. Selvarajah D, Wilkinson ID, Emery CJ, Shaw PJ, Griffiths PD, Gandhi R, Tesfaye S: Thalamic neuronal dysfunction and chronic sensorimotor distal symmetrical polyneuropathy in patients with type 1 diabetes mellitus. *Diabetologia.* 51:2088-2092, 2008
286. Geissler A, Frund R, Scholmerich J, Feuerbach S, Zietz B: Alterations of cerebral metabolism in patients with diabetes mellitus studied by proton magnetic resonance spectroscopy. *Exp Clin Endocrinol Diabetes.* 111:421-427, 2003
287. Sorensen L, Siddall PJ, Trenell MI, Yue DK: Differences in metabolites in pain-processing brain regions in patients with diabetes and painful neuropathy. *Diabetes Care.* 31:980-981, 2008
288. Brands AM, Kessels RP, de Haan EH, Kappelle LJ, Biessels GJ: Cerebral dysfunction in type 1 diabetes: Effects of insulin, vascular risk factors and blood-glucose levels. *Eur J Pharmacol.* 490:159-168, 2004
289. Baron AD: Hemodynamic actions of insulin. *Am J Physiol.* 267:E187-202, 1994
290. Farris W, Mansourian S, Chang Y, Lindsley L, Eckman EA, Frosch MP, Eckman CB, Tanzi RE, Selkoe DJ, Guenette S: Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proc Natl Acad Sci U S A.* 100:4162-4167, 2003
291. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature.* 414:813-820, 2001
292. Gispen WH, Biessels GJ: Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci.* 23:542-549, 2000
293. Vincent AM, McLean LL, Backus C, Feldman EL: Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. *FASEB J.* 19:638-640, 2005
294. Knudsen GM, Jakobsen J, Barry DI, Compton AM, Tomlinson DR: Myo-inositol normalizes decreased sodium permeability of the blood-brain barrier in streptozotocin diabetes. *Neuroscience.* 29:773-777, 1989
295. Bhardwaj SK, Sandhu SK, Sharma P, Kaur G: Impact of diabetes on CNS: Role of signal transduction cascade. *Brain Res Bull.* 49:155-162, 1999
296. Vlassara H, Brownlee M, Cerami A: Excessive nonenzymatic glycosylation of peripheral and central nervous system myelin components in diabetic rats. *Diabetes.* 32:670-674, 1983
297. Makar TK, Hungund BL, Cook GA, Kashfi K, Cooper AJ: Lipid metabolism and membrane composition are altered in the brains of type II diabetic mice. *J Neurochem.* 64:2159-2168, 1995
298. Mooradian AD: The antioxidative potential of cerebral microvessels in experimental diabetes mellitus. *Brain Res.* 671:164-169, 1995
299. Kumar JS, Menon VP: Effect of diabetes on levels of lipid peroxides and glycolipids in rat brain. *Metabolism.* 42:1435-1439, 1993
300. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling. *Diabetes Care.* 27:1487-1495, 2004

301. Service FJ, Molnar GD, Rosevear JW, Ackerman E, Gatewood LC, Taylor WF: Mean amplitude of glycemic excursions, a measure of diabetic instability. *Diabetes.* 19:644-655, 1970
302. Davis MD, Fisher MR, Gangnon RE, Barton F, Aiello LM, Chew EY, Ferris FL 3rd, Knatterud GL: Risk factors for high-risk proliferative diabetic retinopathy and severe visual loss: Early treatment diabetic retinopathy study report #18. *Invest Ophthalmol Vis Sci.* 39:233-252, 1998
303. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 18:499-502, 1972
304. McGuire EA, Helderman JH, Tobin JD, Andres R, Berman M: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol.* 41:565-573, 1976
305. Yildiz-Yesiloglu A, Ankerst DP: Review of $^{1}$H magnetic resonance spectroscopy findings in major depressive disorder: A meta-analysis. *Psychiatry Res.* 147:1-25, 2006
306. Bernasconi A, Bernasconi N, Natsume J, Antel SB, Andermann F, Arnold DL: Magnetic resonance spectroscopy and imaging of the thalamus in idiopathic generalized epilepsy. *Brain.* 126:2447-2454, 2003
307. Kamba M, Inoue Y, Higami S, Suto Y, Ogawa T, Chen W: Cerebral metabolic impairment in patients with obstructive sleep apnoea: An independent association of obstructive sleep apnoea with white matter change. *J Neurol Neurosurg Psychiatry.* 71:334-339, 2001
308. Urrila AS, Hakkarainen A, Heikkinen S, Vuori K, Stenberg D, Häkkinen AM, Lundbom N, Porkka-Heiskanen T: Stimulus-induced brain lactate: Effects of aging and prolonged wakefulness. *J Sleep Res.* 13:111-119, 2004
309. Urrila AS, Hakkarainen A, Heikkinen S, Huhdankoski O, Kuusi T, Stenberg D, Häkkinen AM, Porkka-Heiskanen T, Lundbom N: Preliminary findings of proton magnetic resonance spectroscopy in occipital cortex during sleep deprivation. *Psychiatry Res.* 147:41-46, 2006
310. Meyerhoff DJ, Blumenfeld R, Truran D, Lindgren J, Flenniken D, Cardenas V, Chao LL, Rothlind J, Studholme C, Weiner MW: Effects of heavy drinking, binge drinking, and family history of alcoholism on regional brain metabolites. *Alcohol Clin Exp Res.* 28:650-661, 2004
311. Domino EF: Tobacco smoking and MRI/MRS brain abnormalities compared to nonsmokers. *Prog Neuropsychopharmacol Biol Psychiatry.* 32:1778-1781, 2008
312. Gur RC, Gunning-Dixon F, Bilker WB, Gur RE: Sex differences in temporo-limbic and frontal brain volumes of healthy adults. *Cereb Cortex.* 12:998-1003, 2002
313. Kawachi T, Ishii K, Sakamoto S, Matsui M, Mori T, Sasaki M: Gender differences in cerebral glucose metabolism: A PET study. *J Neurol Sci.* 199:79-83, 2002
314. Murphy DG, DeCarli C, McIntosh AR, Daly E, Mentis MJ, Pietrini P, Szczepanik J, Schapiro MB, Grady CL, Horwitz B, Rapoport SI: Sex differences in human brain morphometry and metabolism: An in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging. *Arch Gen Psychiatry.* 53:585-594, 1996
315. Baxter LR Jr, Mazziotta JC, Phelps ME, Selin CE, Guze BH, Fairbanks L: Cerebral glucose metabolic rates in normal human females versus normal males. *Psychiatry Res.* 21:237-245, 1987
316. Yoshii F, Barker WW, Chang JY, Loewenstein D, Apicella A, Smith D, Boothe T, Ginsberg MD, Pascal S, Duara R: Sensitivity of cerebral glucose metabolism to age, gender, brain volume, brain atrophy, and cerebrovascular risk factors. *J Cereb Blood Flow Metab.* 8:654-661, 1988
317. Andreason PJ, Zametkin AJ, Guo AC, Baldwin P, Cohen RM: Gender-related differences in regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res.* 51:175-183, 1994
318. Volkow ND, Wang GJ, Fowler JS, Hitzemann R, Pappas N, Pascani K, Wong C: Gender differences in cerebellar metabolism: Test-retest reproducibility. *Am J Psychiatry.* 154:119-121, 1997
319. Willis MW, Ketter TA, Kimbrell TA, George MS, Herscovitch P, Danielson AL, Benson BE, Post RM: Age, sex and laterality effects on cerebral glucose metabolism in healthy adults. *Psychiatry Res.* 114:23-37, 2002
320. Rasgon NL, Thomas MA, Guze BH, Fairbanks LA, Yue K, Curran JG, Rapkin AJ: Menstrual cycle-related brain metabolite changes using $^{1}$H magnetic resonance spectroscopy in premenopausal women: A pilot study. *Psychiatry Res.* 106:47-57, 2001
321. Seaquist ER, Damberg GS, Tkac I, Gruetter R: The effect of insulin on in vivo cerebral glucose concentrations and rates of glucose transport/metabolism in humans. *Diabetes.* 50:2203-2209, 2001

322. Vrenken H, Barkhof F, Uitdehaag BM, Castelijns JA, Polman CH, Pouwels PJ: MR spectroscopic evidence for glial increase but not for neuro-axonal damage in MS normal-appearing white matter. *Magn Reson Med.* 53:256-266, 2005
323. Wong TY, Klein R, Klein BE, Tielsch JM, Hubbard L, Nieto FJ: Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Surv Ophthalmol.* 46:59-80, 2001
324. van Elderen SG, Brandts A, Westenberg JJ, van der Grond J, Tamsma JT, van Buchem MA, Romijn JA, Kroft LJ, Smit JW, de Roos A: Aortic stiffness is associated with cardiac function and cerebral small vessel disease in patients with type 1 diabetes mellitus: Assessment by magnetic resonance imaging. *Eur Radiol.* 20:1132-1138, 2010
325. Marfella R, Nappo F, De Angelis L, Paolisso G, Tagliamonte MR, Giugliano D: Hemodynamic effects of acute hyperglycemia in type 2 diabetic patients. *Diabetes Care.* 23:658-663, 2000
326. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D: The effect of acute hyperglycaemia on QTc duration in healthy man. *Diabetologia.* 43:571-575, 2000
327. Clowes JA, Allen HC, Prentis DM, Eastell R, Blumsohn A: Octreotide abolishes the acute decrease in bone turnover in response to oral glucose. *J Clin Endocrinol Metab.* 88:4867-4873, 2003
328. Rauca C, Schafer K, Hollt V: Effects of somatostatin, octreotide and cortistatin on ischaemic neuronal damage following permanent middle cerebral artery occlusion in the rat. *Naunyn Schmiedebergs Arch Pharmacol.* 360:633-638, 1999
329. Long JB, Rigamonti DD, Dosaka K, Kraimer JM, Martinez-Arizala A: Somatostatin causes vasoconstriction, reduces blood flow and increases vascular permeability in the rat central nervous system. *J Pharmacol Exp Ther.* 260:1425-1432, 1992
330. Smith JC, Lane H, Davies N, Evans LM, Cockcroft J, Scanlon MF, Davies JS: The effects of depot long-acting somatostatin analog on central aortic pressure and arterial stiffness in acromegaly. *J Clin Endocrinol Metab.* 88:2556-2561, 2003
331. Viollet C, Bodenant C, Prunotto C, Roosterman D, Schaefer J, Meyerhof W, Epelbaum J, Vaudry H, Leroux P: Differential expression of multiple somatostatin receptors in the rat cerebellum during development. *J Neurochem.* 68:2263-2272, 1997
332. Yacubova E, Komuro H: Cellular and molecular mechanisms of cerebellar granule cell migration. *Cell Biochem Biophys.* 37:213-234, 2003
333. Reubi JC, Maurer R: Autoradiographic mapping of somatostatin receptors in the rat central nervous system and pituitary. *Neuroscience.* 15:1183-1193, 1985
334. Martin JL, Chesselet MF, Raynor K, Gonzales C, Reisine T: Differential distribution of somatostatin receptor subtypes in rat brain revealed by newly developed somatostatin analogs. *Neuroscience.* 41:581-593, 1991
335. Ratcheson RA, Blank AC, Ferrendelli JA: Regionally selective metabolic effects of hypoglycemia in brain. *J Neurochem.* 36:1952-1958, 1981
336. Strick PL, Dum RP, Fiez JA: Cerebellum and nonmotor function. *Annu Rev Neurosci.* 32:413-434, 2009
337. Gruetter R, Ugurbil K, Seaquist ER: Steady-state cerebral glucose concentrations and transport in the human brain. *J Neurochem.* 70:397-408, 1998
338. de Graaf RA, Pan JW, Telang F, Lee JH, Brown P, Novotny EJ, Hetherington HP, Rothman DL: Differentiation of glucose transport in human brain gray and white matter. *J Cereb Blood Flow Metab.* 21:483-492, 2001
339. Soher BJ, Hurd RE, Sailasuta N, Barker PB: Quantitation of automated single-voxel proton MRS using cerebral water as an internal reference. *Magn Reson Med.* 36:335-339, 1996
340. Biller A, Bartsch AJ, Homola G, Solymosi L, Bendszus M: The effect of ethanol on human brain metabolites longitudinally characterized by proton MR spectroscopy. *J Cereb Blood Flow Metab.* 29:891-902, 2009
341. Gruetter R, Garwood M, Ugurbil K, Seaquist ER: Observation of resolved glucose signals in $^{1}$H NMR spectra of the human brain at 4 tesla. *Magn Reson Med.* 36:1-6, 1996
342. Geurts JJ, Barkhof F, Castelijns JA, Uitdehaag BM, Polman CH, Pouwels PJ: Quantitative $^{1}$H-MRS of healthy human cortex, hippocampus, and thalamus: Metabolite concentrations, quantification precision, and reproducibility. *J Magn Reson Imaging.* 20:366-371, 2004

343. Dolder M, Walzel B, Speer O, Schlattner U, Wallimann T: Inhibition of the mitochondrial permeability transition by creatine kinase substrates. Requirement for microcompartmentation. *J Biol Chem.* 278:17760-17766, 2003
344. Meyer LE, Machado LB, Santiago AP, da-Silva WS, De Felice FG, Holub O, Oliveira MF, Galina A: Mitochondrial creatine kinase activity prevents reactive oxygen species generation: Antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity. *J Biol Chem.* 281:37361-37371, 2006
345. Prass K, Royl G, Lindauer U, Freyer D, Megow D, Dirnagl U, Stockler-Ipsiroglu G, Wallimann T, Priller J: Improved reperfusion and neuroprotection by creatine in a mouse model of stroke. *J Cereb Blood Flow Metab.* 27:452-459, 2007
346. Keymeulen B, Jacobs A, de Metz K, de Sadeleer C, Bossuyt A, Somers G: Regional cerebral hypoperfusion in long-term type 1 (insulin-dependent) diabetic patients: Relation to hypoglycaemic events. *Nucl Med Commun.* 16:10-16, 1995
347. Oizumi XS, Akisaki T, Kouta Y, Song XZ, Takata T, Kondoh T, Umetani K, Hirano M, Yamasaki K, Kohmura E, Yokono K, Sakurai T: Impaired response of perforating arteries to hypercapnia in chronic hyperglycemia. *Kobe J Med Sci.* 52:27-35, 2006
348. Musen G, Lyoo IK, Sparks CR, Weinger K, Hwang J, Ryan CM, Jimerson DC, Hennen J, Renshaw PF, Jacobson AM: Effects of type 1 diabetes on gray matter density as measured by voxel-based morphometry. *Diabetes.* 55:326-333, 2006
349. Kado H, Kimura H, Murata T, Itoh H, Shimosegawa E: Carbon monoxide poisoning: Two cases of assessment by magnetization transfer ratios and $^{1}$H-MRS for brain damage. *Radiat Med.* 22:190-194, 2004
350. Hopkins DF, Williams G: Insulin receptors are widely distributed in human brain and bind human and porcine insulin with equal affinity. *Diabet Med.* 14:1044-1050, 1997
351. Wozniak M, Rydzewski B, Baker SP, Raizada MK: The cellular and physiological actions of insulin in the central nervous system. *Neurochem Int.* 22:1-10, 1993
352. Bingham EM, Hopkins D, Smith D, Pernet A, Hallett W, Reed L, Marsden PK, Amiel SA: The role of insulin in human brain glucose metabolism: An $^{18}$fluoro-deoxyglucose positron emission tomography study. *Diabetes.* 51:3384-3390, 2002
353. Tschritter O, Preissl H, Hennige AM, Stumvoll M, Porubska K, Frost R, Marx H, Klosel B, Lutzenberger W, Birbaumer N, Haring HU, Fritsche A: The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: A magnetoencephalographic study. *Proc Natl Acad Sci U S A.* 103:12103-12108, 2006
354. Tschritter O, Hennige AM, Preissl H, Porubska K, Schafer SA, Lutzenberger W, Machicao F, Birbaumer N, Fritsche A, Haring HU: Cerebrocortical beta activity in overweight humans responds to insulin detemir. *PLoS One.* 2:e1196, 2007
355. Hallschmid M, Jauch-Chara K, Korn O, Molle M, Rasch B, Born J, Schultes B, Kern W: Euglycemic infusion of insulin detemir compared with human insulin appears to increase direct current brain potential response and reduces food intake while inducing similar systemic effects. *Diabetes.* 59:1101-1107, 2010