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

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original publications:


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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 fulfill 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 lifestyle, 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 sources.
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).

Glucose is oxidized into free energy in two phases: first by glycolysis in the cytosol and second in the tricarboxylic 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 1** Glucose transport from blood to brain, basics of glucose metabolism, and the interplay between neurons and astrocytes. TCA = tricarboxylic acid, NADH = nicotinamide adenine dinucleotide, FADH2 = flavin adenine dinucleotide, PCr = creatine phosphate, Cr = creatine, ATP = adenosine triphosphate, ADP = adenosine diphosphate, H+ = proton, Gln = glutamine, Glu = glutamate, GLUT = glucose transporter.
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 GLUT4 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. ¹H MRS of Brain Metabolites

MRI can reveal brain structure, and proton magnetic resonance spectroscopy (¹H 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 ¹H 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 ¹H MRS, its clear advantage is that it allows study of endogenous brain metabolites and native glucose (Figure 3).

![Figure 3](image)

**Figure 3** The main metabolites in ¹H 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 (ml). 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 (B₀) and high-energy state antiparallel to the B₀. Since a lower energy state is present, slightly more spins are parallel to the B₀, forming a macroscopic net magnetization parallel to the main field.

The equilibrium of the spins in B₀ 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. T₁ relaxation describes the recovery of longitudinal and T₂ relaxation the recovery of transverse magnetization. T₁ depends on interactions of the spin with its surroundings, whereas T₂ depends on spin-spin interactions. T₂ relaxation time is never longer than T₁ 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 T₂. The actual observed decay of the FID is determined by T₂*, that, in addition to true T₂, 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 ¹H 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 T₁-weighted images, fat-containing tissues typically appear bright and water- and fluid-containing tissues dark. In T₂-weighted images, brightness is proportional to the mobility of water. T₂-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 T₂-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 equilibrium 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 Alzheimer’s 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 phosphatidylinositol 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 1H 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

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance, IGT, or diabetes + 2 other</td>
<td>Plasma insulin &gt; 75th percentile + 2 other</td>
<td>3 of the following</td>
<td>Abdominal obesity + 2 other</td>
<td>3 of the following</td>
<td>3 of the following</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>BMI &gt; 30 kg/m² and/or WHR M &gt; 0.90 F &gt; 0.85</td>
<td>Waist circumference M ≥ 94 cm F ≥ 80 cm</td>
<td>Waist circumference M ≥ 102 cm F ≥ 88 cm</td>
<td>Waist circumference for non-Asian M ≥ 94 cm F ≥ 88 cm</td>
<td>Waist circumference for non-Asian M ≥ 94 cm F ≥ 80 cm</td>
</tr>
<tr>
<td>Hypertension</td>
<td>≥ 140/90 mmHg</td>
<td>≥ 140/90 mmHg or treatment</td>
<td>≥ 130/85 mmHg or treatment</td>
<td>≥ 130/85 mmHg or treatment</td>
<td>≥ 130/85 mmHg or treatment</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>HDL M &lt; 0.9 mmol/l F &lt; 1.0 mmol/l and/or Tg ≥ 2.0 mmol/l or treatment</td>
<td>HDL &lt; 1.0 mmol/l and/or Tg ≥ 2.0 mmol/l or treatment</td>
<td>HDL M &lt; 1.0 mmol/l F &lt; 1.3 mmol/l or treatment</td>
<td>HDL M &lt; 1.03 mmol/l F &lt; 1.29 mmol/l or treatment</td>
<td>HDL M &lt; 1.0 mmol/l F &lt; 1.3 mmol/l or treatment</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tg ≥ 1.7 mmol/l</td>
<td>Tg ≥ 1.7 mmol/l or treatment</td>
<td>Tg ≥ 1.7 mmol/l or treatment</td>
<td>Tg ≥ 1.7 mmol/l or treatment</td>
<td></td>
</tr>
<tr>
<td>Impaired glucose regulation (fP-Glucose)</td>
<td>Insulin resistance, IGT, or diabetes, but not diabetes</td>
<td>≥ 6.1 mmol/l or IGT, but not diabetes</td>
<td>≥ 6.1 mmol/l or diabetes</td>
<td>≥ 5.6 mmol/l or diabetes</td>
<td>≥ 5.6 mmol/l or diabetes</td>
</tr>
<tr>
<td>UAER</td>
<td>≥ 20 µg/min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Study</th>
<th>Subject groups ((n = M / n = F))</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>Triglycerides; HDL cholesterol (mmol/)</th>
<th>Systolic/diastolic blood pressure (mmHg)</th>
<th>HbA1c (%)</th>
<th>duration (years)</th>
<th>Brain regions</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Catani et al. 2002</td>
<td>HT+ (5/5)</td>
<td>78</td>
<td>NA</td>
<td>NA</td>
<td>137/78</td>
<td>NA; 0</td>
<td></td>
<td>Paratrigonal white matter bilaterally</td>
<td>HT+ vs HT-: NAA/tCr ↓ (trend), ml/tCr ↑, Cho/tCr ↔</td>
</tr>
<tr>
<td>(206)</td>
<td>HT- (5/5)</td>
<td>79</td>
<td>NA</td>
<td>NA</td>
<td>130/74</td>
<td>NA; 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension and type 2 diabetes</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kario et al. 2005</td>
<td>T2DHT+ (7/13)</td>
<td>69</td>
<td>24.5</td>
<td>DL in ((n))</td>
<td>161/85</td>
<td>NA; 0</td>
<td></td>
<td>Left periventricular white matter</td>
<td>T2DHT vs HT-: NAA/H₂O↓, NAA/tCr↓ (trend)</td>
</tr>
<tr>
<td>(208)</td>
<td>HT+ (7/13)</td>
<td>69</td>
<td>23.5</td>
<td>7</td>
<td>158/84</td>
<td>NA; 0</td>
<td></td>
<td></td>
<td>T2DHT vs HT+ NAA/H₂O↓, NAA/tCr↓</td>
</tr>
<tr>
<td></td>
<td>HT- (4/8)</td>
<td>69</td>
<td>24.1</td>
<td>5</td>
<td>123/70</td>
<td>NA; 0</td>
<td></td>
<td></td>
<td>T2DHT vs HT- tCr/H₂O↔, Cho/H₂O↔</td>
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<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HT+ vs HT- NAA/H₂O↔, NAA/tCr↔</td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Sinha et al. 2004</td>
<td>DL+</td>
<td>33</td>
<td>Higher in DL+ than in DL-</td>
<td>3.7; 1.0</td>
<td>NA</td>
<td>NA; 0</td>
<td></td>
<td>Parieto-temporal region</td>
<td>DL+ vs DL-: NAA/tCr↔, Cho/tCr↔, NAA/Cho↔</td>
</tr>
<tr>
<td>(207)</td>
<td>DL+MED+ 13</td>
<td>30</td>
<td></td>
<td>2.6; 1.1</td>
<td>NA</td>
<td>NA; 0</td>
<td></td>
<td></td>
<td>DL+MED+: Tg↑ correlated with NAA/Cr↑ and Cho/Cr↑</td>
</tr>
<tr>
<td></td>
<td>DL-MED- 6</td>
<td>30</td>
<td></td>
<td>1.6; 1.1</td>
<td>NA</td>
<td>NA; 0</td>
<td></td>
<td></td>
<td>DL+MED+: HDL↓ correlated with Cho/Cr↑</td>
</tr>
<tr>
<td></td>
<td>DL- 21</td>
<td>30</td>
<td></td>
<td></td>
<td>NA</td>
<td>NA; 0</td>
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</tr>
<tr>
<td></td>
<td>Altogether: (34/6)</td>
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</tbody>
</table>
### Impaired glucose regulation

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Group</th>
<th>N</th>
<th>NAA/tCr</th>
<th>Cho/tCr</th>
<th>NAA/Cho</th>
<th>T2DHbA1c</th>
<th>Age</th>
<th>Fat%</th>
<th>HDL</th>
<th>TChol</th>
<th>Tg</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahin et al. 2008 (209)</td>
<td>T2DHbA1c&lt;10%</td>
<td>IGT (5/8)</td>
<td>45</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5.7; 0</td>
<td>46</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>7.9; 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2DHbA1c&gt;10%</td>
<td>(6/9) NGT (5/9)</td>
<td>54</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>13.6; 8</td>
<td>42</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>5.2; 0</td>
<td></td>
</tr>
</tbody>
</table>

**Occipital region**
- DL+ vs DL-: NAA/tCr ↔, Cho/tCr ↔, NAA/Cho ↔
- All: Fat%↑ and HDL↓ correlated with Cho/Cr↑
- All: TChol↑ and HDL↓ correlated with NAA/Cho↓
- DL+MED+: Age↑ correlated with NAA/Cr↓
- DL+MED-: Tg↑ and HDL↓ correlated with NAA/Cr↑

**Frontal cortex**
- T2DHbA1c>10% vs T2DHbA1c<10%: NAA/tCr↓, Cho/tCr↓
- IGT vs NGT: Cho/tCr↑
- T2D vs NGT: mI/tCr↑
- All: S-Insulin↑ correlated with NAA/tCr↓
- All: HbA1c↑ correlated with NAA/tCr↓ and Cho/tCr↓

**Parietal white matter**
- T2D vs NGT or IGT Cho/tCr↓
- T2DHbA1c<10% vs T2DHbA1c>10%: Cho/tCr↓
- All: HOMA-IR↑ and fP-Glc↑ correlated with NAA/tCr↓
- All: HbA1c↑ correlated with Cho/tCr↓

**Thalamus**
- No differences in metabolites
- All: HbA1c↑ 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 β-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, HbA1c 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 HbA1c that indicates the mean blood glucose in the past three months. An acceptable HbA1c can therefore be reached both with steady or with highly variable blood glucose concentrations (215). Although HbA1c 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\textsubscript{1c} < 7.4% (57 mmol/mol) performed better in tasks requiring motor speed and psychomotor efficiency than did patients with HbA\textsubscript{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 1H 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 hyperglycemia (278), but a 12% decreased glucose uptake during hyperglycemia (279). This is in line with a 1H 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 HbA1c 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 1H 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 HbA1c. 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

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject groups (n = M/n = F)</th>
<th>Age</th>
<th>HbA$_{1c}$ (%) duration (years)</th>
<th>Brain regions</th>
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<td><strong>Type 1 diabetes</strong></td>
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<td>Kreis et al. 1992 (254)</td>
<td>T1D (7/10)</td>
<td>43</td>
<td>NA</td>
<td>White matter in posterioromedial parietal cortex</td>
<td>▪ T1D vs non-DM: NAA/tCr↓, Cho/tCr↔, mI/Cr↑</td>
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<td></td>
<td>9 DKA</td>
<td>47</td>
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<td></td>
<td>non-DM (19/11)</td>
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<td>Perros et al. 1997 (233)</td>
<td>IDDM HG- 11</td>
<td>42</td>
<td>NA</td>
<td>Parietal and frontal lobes</td>
<td>▪ IDDM HG+ vs IDDM HG-: NAA/Cr↔, NAA/Cho↔</td>
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<td>IDDM HG+ 10</td>
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<td>Mäkimattila et al. 2004 (255)</td>
<td>T1D (10/0)</td>
<td>36</td>
<td>9.1; 28</td>
<td>Frontal cortical gray matter</td>
<td>▪ T1D vs non-DM: H$_2$O↑, NAA/H$_2$O↔, tCr/H$_2$O↔, Cho/H$_2$O↔, mI/H$_2$O↑</td>
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<td>retinopathy and peripheral</td>
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<td>neuropathy non-DM (10/0)</td>
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<td>Sarac et al. 2005 (282)</td>
<td>T1D 30 (NA)</td>
<td>13</td>
<td>11.9; NA</td>
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<td>▪ T1D vs non-DM: NAA/Cr↓, Cho/tCr↔</td>
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<td>12</td>
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<td>Study</td>
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<td>Sample Size</td>
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<tr>
<td>Wootton-Gorges et al. 2007</td>
<td>T1D+DKA 29</td>
<td>12</td>
<td>Occipital gray matter: NAA/Cr ↓ (trend), Cho/Cr ↔</td>
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<td>Periaqueductal gray matter: NAA/Cr ↓ (trend), Cho/Cr ↔</td>
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<td>Basal ganglia: NAA/Cr ↓ (trend), Cho/Cr ↔</td>
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<td>Neural dysfunction but not loss</td>
<td></td>
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<tr>
<td>Selvarajah et al. 2008</td>
<td>T1D DPN+ (10/0)</td>
<td>30</td>
<td>Right thalamus lobe: NAA/tCr ↔, NAA/Cho ↔, Cho/tCr ↔</td>
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<td>T1D DPN- (8/0)</td>
<td>26</td>
<td>NAA/tCr ↔, NAA/Cho ↓, Cho/tCr ↔</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>non-DM (6/0)</td>
<td>43</td>
<td>Neural dysfunction but not loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Severity of peripheral neuropathy correlated with brain metabolites†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 and 2 diabetes not separated</td>
<td></td>
<td></td>
<td>NAA, 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 hypoglycemia; TE, echo time; NAA, N-acetylaspartate; tCr, total creatine; Cho, choline; mI, myo-inositol; Glc, glucose.</td>
<td></td>
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<tr>
<td>Geissler et al. 2003</td>
<td>T1D (5/1) + T2D (14/10)</td>
<td>46</td>
<td>Parietal white matter: T1D+T2D vs non-DM: NAA/tCr ↔, Cho/tCr ↔, mI/tCr ↑</td>
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<tr>
<td></td>
<td>3 DKA</td>
<td>37</td>
<td>T1D vs T2D mI/tCr ↑</td>
<td></td>
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<tr>
<td></td>
<td>non-DM (15/15)</td>
<td></td>
<td>S-Osmolality↑ and S-Sodium↑ correlated with mI/tCr ↑</td>
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<td></td>
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<tr>
<td></td>
<td>T1D 2 + T2D 10</td>
<td>57</td>
<td>Dorsolateral prefrontal cortex: DM Pain- vs non-DM: NAA/H2O ↓, tCr/H2O ↓, Cho/H2O ↔</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DM Pain- (13/1)</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1D 6 + T2D 8</td>
<td></td>
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</table>
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).

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-β peptide that is secreted into the ECF with accelerative effect of insulin. In the ECF, amyloid-β 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-β 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-β1 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 fulfilled 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 fulfilled the main criterion concerning waist circumference and one the minor criteria concerning HDL cholesterol. 1H 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/m²). 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 HbA₁c. 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 arithmetic 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 C128 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 1H 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. HbA1c was analyzed by immunoturbidometry (normal range 4.0-6.0% / 20-42 mmol/mol).

Plasma creatinine, serum alanine aminotransferase, 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 immunoturbidimetric 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 1H 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 mm³ = 8.0 ml), the left frontal white matter (30 x 16 x 16 mm³ = 7.7 ml), the left thalamic lobe (20 x 20 x 20 mm³ = 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 mm³ = 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 zero-filled 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 H2O (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/H2O 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 MnCl2 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 mm³) 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 $\mu$g) and infusion (0.75 $\mu$g/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.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Risk group</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>36 ± 6</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>87 ± 8</td>
<td>104 ± 6**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 2.6</td>
<td>29.0 ± 2.5**</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126 ± 8</td>
<td>144 ± 13**</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76 ± 8</td>
<td>89 ± 5**</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>93 ± 7</td>
<td>107 ± 7***</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.7 ± 0.34</td>
<td>5.3 ± 0.5**</td>
</tr>
<tr>
<td>Fasting serum insulin (pmol/l)</td>
<td>28.4 ± 11.5</td>
<td>51.1 ± 22.9*</td>
</tr>
<tr>
<td>OGTT:</td>
<td></td>
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<tr>
<td>Plasma glucose at 120 min (mmol/l)</td>
<td>---</td>
<td>6.6 ± 1.8</td>
</tr>
<tr>
<td>Serum insulin at 120 min (pmol/l)</td>
<td>---</td>
<td>215.3 ± 142.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.5 ± 0.2</td>
<td>0.9 ± 0.4*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 ± 0.2</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.4 ± 1.1</td>
<td>4.7 ± 0.9</td>
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<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.4 ± 0.2</td>
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<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.5 ± 1.0</td>
<td>2.6 ± 0.6</td>
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<td>Triglycerides (mmol/l)</td>
<td>0.9 ± 0.3</td>
<td>1.7 ± 1.1</td>
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<td>CRP (mg/l)</td>
<td>0.4 ± 0.6</td>
<td>0.5 ± 0.4</td>
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<td>IL-6 (ng/l)</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>30.0 ± 15.23</td>
<td>41.4 ± 17.2</td>
</tr>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>7.3 ± 1.3</td>
<td>9.1 ± 4.1</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>217.6 ± 32.2</td>
<td>211.0 ± 37.7</td>
</tr>
</tbody>
</table>

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$_2$O in the thalamus than did the control group (Figure 7C). No difference existed in the thalamic glucose/H$_2$O, NAA/H$_2$O, Cho/H$_2$O, mI/H$_2$O, 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$_2$O (Figure 8A) and mI/H$_2$O (Figure 8B) correlated with thalamic glucose/H$_2$O. In the risk group, neither tCr/H$_2$O ($r = 0.19, p = 0.651$) nor mI/H$_2$O ($r = 0.43, p = 0.289$) correlated with thalamic glucose/H$_2$O. Instead, in the risk group, tCr/H$_2$O (Figure 9A) and mI/H$_2$O (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$_2$O ratios and H$_2$O 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/H2O) and B. myo-inositol (mI/H2O) in the risk group (●) and in the non-diabetic group (○).

Figure 9  Associations between fasting plasma glucose and A. thalamic total creatine (tCr/H2O) and B. myo-inositol (mI/H2O), and C. between the 2-h plasma glucose in the oral glucose tolerance test (OGTT) and thalamic tCr/H2O 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 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 ($9.2 \pm 3.0$ vs. $4.8 \pm 0.5$, $p < 0.001$), whereas the mean of serum insulin were at a similar level ($30.5 \pm 42.0$ vs. $20.8 \pm 6.5$, $p = 0.437$).

*Cortex vs. white matter vs. thalamus.* The non-diabetic subjects had a 47% higher glucose/H$_2$O ratio in the cortex than in the white matter (Figure 10A). The diabetic subjects showed no regional differences in cerebral glucose/H$_2$O ratio. The regional differences in H$_2$O 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$_2$O 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$_2$O in their white matter (Figure 10B) and 8% higher mI/H$_2$O in their cortices and 20% higher mI/H$_2$O in their white matter (Figure 10E). tCr/H$_2$O and Cho/H$_2$O showed no differences between study groups.

*Brain glucose vs. plasma glucose.* In the diabetic subjects, brain glucose/H$_2$O correlated with plasma glucose in all brain regions (Figure 12).
**Table 5.** Subject characteristics in Studies II, III, and IV.

<table>
<thead>
<tr>
<th></th>
<th>Study II: Cerebrum at baseline</th>
<th>Study IV: Cerebellum at baseline</th>
<th>Studies III and IV: Acute hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T1D</td>
<td>Control</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29 ± 6</td>
<td>28 ± 4</td>
<td>33 ± 7</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>83 ± 5</td>
<td>88 ± 8</td>
<td>92 ± 12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4 ± 2.1</td>
<td>24.7 ± 3.0*</td>
<td>25.1 ± 4.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 7</td>
<td>113 ± 7**</td>
<td>133 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 8</td>
<td>75 ± 7</td>
<td>80 ± 11</td>
</tr>
<tr>
<td>Pulse (beats per min)</td>
<td>57 ± 10</td>
<td>62 ± 6</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>Age at diabetes onset (years)</td>
<td>21 ± 6</td>
<td>21 ± 6</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>6.7 ± 5.2</td>
<td>7.0 ± 5.3</td>
<td>7.0 ± 5.3</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 ± 0.2</td>
<td>7.4 ± 1.1***</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>Insulin dose/weight (IU/kg)</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Urinary albumin excretion rate (mg/24h)</td>
<td>11 ± 8</td>
<td>12 ± 10</td>
<td>19 ± 19</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>79 ± 12</td>
<td>74 ± 13</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.2 ± 0.9</td>
<td>4.3 ± 0.9</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.5 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.4 ± 0.9</td>
<td>2.3 ± 0.9</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>0.7 ± 0.3</td>
<td>0.9 ± 0.4</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.3 ± 0.3</td>
<td>0.5 ± 0.9</td>
<td>1.9 ± 6.5</td>
</tr>
<tr>
<td>IL-6 (ng/l)</td>
<td>2.1 ± 0.6</td>
<td>2.0 ± 0.3</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>30.0 ± 14.0</td>
<td>41.3 ± 17.9</td>
<td>32.3 ± 16.3</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>216.1 ± 27.8</td>
<td>210.2 ± 46.5</td>
<td>218.3 ± 32.2</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>7.8 ± 1.9</td>
<td>6.5 ± 2.4</td>
<td>8.4 ± 3.0</td>
</tr>
</tbody>
</table>

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\(_2\)O ratios and H\(_2\)O 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$_2$O ratio (Figure 13), or in the glucose/H$_2$O ratio (Figure 14A) or H$_2$O (Figure 14B) between diabetic and non-diabetic subjects.

**Figure 13** Cerebellar metabolite/H$_2$O 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$_2$O ratio and B. H$_2$O 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\textsubscript{2}O 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\textsubscript{2}O was $77.1 \times 10^{-6} \pm 14.9 \times 10^{-6}$ in the cerebellum and $26.5 \times 10^{-6} \pm 6.0 \times 10^{-6}$ in the cerebral white matter ($p < 0.001$) in the non-diabetic participants. The H\textsubscript{2}O 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\textsubscript{2}O 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 ($11.8 \pm 2.9$ vs $12.3 \pm 1.5$ 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 $272 \pm 4$ to $277 \pm 3$ mOsm/kg ($p = 0.043$) in the diabetic and from $274 \pm 4$ to $279 \pm 2$ 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 $^3$H MRS data collection

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

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](image)  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 H2O and tCr. Both in diabetic and non-diabetic subjects, H2O 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, H2O 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 H2O 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 H2O or in tCr.

Brain glucose vs. plasma glucose. In both study groups, brain glucose/H2O correlated with plasma glucose in the cortex, white matter, and in the thalamus (Figure 19). In the cerebellum, glucose/H2O and glucose/tCr ratios correlated with plasma glucose only in the non-diabetic subjects (Figure 20).

Table 7. Change in brain tissue H2O during acute hyperglycemia

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>White matter</th>
<th>Thalamus</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1D</td>
<td>MRS 0</td>
<td>292 ± 11</td>
<td>279 ± 12</td>
<td>273 ± 15</td>
</tr>
<tr>
<td></td>
<td>MRS I</td>
<td>292 ± 12</td>
<td>278 ± 13</td>
<td>268 ± 14</td>
</tr>
<tr>
<td></td>
<td>MRS II</td>
<td>291 ± 14</td>
<td>277 ± 12</td>
<td>268 ± 15</td>
</tr>
<tr>
<td>p</td>
<td>0.989</td>
<td>0.989</td>
<td>0.812</td>
<td>0.028</td>
</tr>
<tr>
<td>Non-T1D</td>
<td>MRS 0</td>
<td>297 ± 10</td>
<td>274 ± 9</td>
<td>264 ± 8</td>
</tr>
<tr>
<td></td>
<td>MRS I</td>
<td>294 ± 11</td>
<td>274 ± 12</td>
<td>260 ± 8</td>
</tr>
<tr>
<td></td>
<td>MRS II</td>
<td>296 ± 13</td>
<td>274 ± 9</td>
<td>261 ± 8</td>
</tr>
<tr>
<td>p</td>
<td>0.750</td>
<td>0.998</td>
<td>0.543</td>
<td>0.011</td>
</tr>
</tbody>
</table>

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$_2$O and brain glucose/tCr ratios in the cerebellum in diabetic (●) and non-diabetic (○) subjects.

Steady state hyperglycemia. During MRS II, the glucose/H$_2$O 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$_2$O 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$_2$O ratio between the cerebellum and the other brain regions is impossible, because cerebellar H$_2$O 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$_2$O 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 fulfilled 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 1H 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. 1H 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 homocysteine 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 neglected.

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, 1H 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 (inhomogeneities 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/H2O (92, 339, 340) and metabolite/tCr (91, 93). The limitation of this approach was that the concentrations of H2O 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/H2O ratio, that the cortex contained 47% more glucose than the white matter. Because the H2O 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 1H 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 1H MRS research. We preferred “brain metabolites” or “markers of brain metabolism.” 1H MRS is not comparable to PET, a method dependent on tracers to assess glucose uptake and consumption. 1H 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/H2O and tCr/H2O 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 impaired 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, HbA1c, 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 1H 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
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 1H 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 yielded 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.
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