Cerebral metabolism in streptozotocin-diabetic rats: an in vivo magnetic resonance spectroscopy study

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Abstract

Aims/hypothesis. It is increasingly evident that the brain is another site of diabetic end-organ damage. The pathogenesis has not been fully explained, but seems to involve an interplay between aberrant glucose metabolism and vascular changes. Vascular changes, such as deficits in cerebral blood flow, could compromise cerebral energy metabolism. We therefore examined cerebral metabolism in streptozotocin-diabetic rats in vivo by means of localised ³¹P and ¹H magnetic resonance spectroscopy.

Methods. Rats were examined 2 weeks and 4 and 8 months after diabetes induction. A non-diabetic group was examined at baseline and after 8 months.

Results. In ³¹P spectra the phosphocreatine:ATP, phosphocreatine:inorganic phosphate and ATP:inorganic phosphate ratios and intracellular pH in diabetic rats were similar to controls at all time points. In ¹H spectra a lactate resonance was detected as frequently in controls as in diabetic rats. Compared with baseline and 8-month controls ¹H spectra did, however,

show a statistically significant decrease in *N*-acetylaspartate:total creatine (–14% and –23%) and *N*-acetylaspartate:choline (–21% and –17%) ratios after 2 weeks and 8 months of diabetes, respectively.

Conclusion/interpretation. No statistically significant alterations in cerebral energy metabolism were observed after up to 8 months of streptozotocin-diabetes. These findings indicate that cerebral blood flow disturbances in diabetic rats do not compromise the energy status of the brain to a level detectable by magnetic resonance spectroscopy. Reductions in *N*-acetylaspartate levels in the brain of STZ-diabetic rats were shown by ¹H spectroscopy, which could present a marker for early metabolic or functional abnormalities in cerebral neurones in diabetes. [Diabetologia (2001) 44: 346–353]

Keywords Magnetic resonance spectroscopy, energy metabolism, phosphocreatine, *N*-acetylaspartate, complications, brain, diabetes mellitus, pathophysiology, experimental.

It is becoming increasingly clear that the brain is another site of diabetic end-organ damage [1]. Some diabetic patients develop cognitive deficits, which are

generally moderate in young adults [2] but can be more pronounced in the elderly [3]. Recent epidemiological studies even report an association between diabetes and dementia [3, 4]. Given the prevalence of diabetes among the elderly and the effect of cognitive impairment and dementia on the quality of life of patients and health care resources, a better understanding of the effects of diabetes on the brain is needed.

Experimentally diabetic rodents develop cerebral deficits similar to those observed in diabetic patients. Streptozotocin (STZ)-diabetic rats have been found to have impaired learning and memory functions [5] and increases in the peak latencies of brainstem audi-

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Abbreviations: Cho, Choline-containing compounds; MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; PCr, phosphocreatine; Pi, inorganic phosphate; STZ, streptozotocin; tCr, total creatine; NMR, nuclear magnetic resonance.

tory and visual evoked potentials [6, 7] and morphological alterations [8]. The development of these deficits is related to diabetes duration and the severity of hyperglycaemia [6]. Because insulin treatment prevents the deficits [9], they must be due to hyperglycaemia and insulin deficiency, rather than a direct toxic effect of STZ on the brain. Moreover, STZ transport and cytotoxicity is dependent on the GLUT-2 glucose transporter [10], which is not expressed at the blood-brain barrier in rats [11].

The pathogenesis of cerebral disorders in diabetes is incompletely understood. It seems to be a multifactorial process involving the adverse effects of chronic hyperglycaemia, and probably also those of recurrent hypoglycaemia, on the brain [1]. Analogous to the pathogenesis of peripheral diabetic neuropathy, chronic hyperglycaemia can lead to both metabolic and vascular disturbances in the brain. Cerebrovascular changes can be found in both diabetic rodents and patients. Studies in STZ-diabetic rats have shown regional reductions in cerebral blood flow within weeks [12, 13] to months [14] after diabetes induction. Moreover, thickening of capillary basement membranes [15] and shortening of the length of the capillary network in the neocortex have been noted [8]. Studies of diabetic patients report regional decreases in cerebral blood flow and impaired cerebrovascular reactivity as well as structural alterations in the cerebral vasculature, including thickening of capillary basement membranes [16]. These functional and structural changes in the vasculature could impede the delivery of nutrients and oxygen to the brain, thus affecting cerebral energy metabolism. This issue has been addressed in a number of ex vivo biochemical studies in diabetic rats but the results have been inconsistent, possibly partially due to interference of post-mortem changes. Concentrations of lactate were found to be unaltered [17, 18] or slightly increased [19], those of ATP unaltered [17, 19] or slightly decreased [18] and those of phosphocreatine (PCr) unaltered [17, 18] or increased [19].

We examined cerebral metabolism in STZ-diabetic rats longitudinally in vivo using ^{31}P and ^1H magnetic resonance spectroscopy (MRS). With ^{31}P MRS the relative concentrations of PCr, ATP and inorganic phosphate (Pi) can be measured, reflecting the energy status of the brain [20]. In addition, the intracellular pH (pH_i) can be estimated [20]. With ^1H MRS increased lactate concentrations, as a consequence of increased anaerobic glycolysis, can be detected. In addition, ^1H MRS yields a measure of the levels of *N*-acetylaspartate (NAA), total creatine (tCr; the sum of creatine and phosphocreatine) and choline-containing compounds (Cho; including compounds such as choline, acetylcholine and phosphorylcholine) in the brain. The measurement of these metabolites provides information on neuronal integrity and membrane phospholipid metabolism [20, 21].

Materials and methods

Animals. Male Wistar rats (starting weight ~350 g, aged 3 months, UWU-CPD, Harlan, Utrecht, The Netherlands) were housed on sawdust, maintained on a 12 h-12 h light-dark cycle and given free access to food and water. The rats were weighed weekly. Diabetes was induced by a single intravenous injection of streptozotocin (Serva Feinbiochemica, Heidelberg, Germany) at a dose of 30 mg/kg body weight, dissolved in saline. Blood glucose was measured 4 days after the STZ injection in blood samples obtained by tail prick, by a strip-operated blood glucose sensor (Companion2, Medisense Ltd, Birmingham, UK). Blood glucose concentrations were higher than 15.0 mmol/l in all STZ-injected animals. We did not measure ketone bodies but previous studies in this model have shown that at STZ dosages up to 50 mg/kg animals become only mildly ketotic [19, 22, 23]. Given the relative abundance of glucose as an energy source in the brain of STZ-diabetic rats, these low concentrations of ketone bodies should not affect the cerebral metabolites that were the subject of our study. The principles of laboratory animal care were followed and the Utrecht University Committee for welfare of experimental animals approved the experiments.

Experimental design. We used two groups of rats. The first consisted of eight non-diabetic rats, which were examined at baseline and after 8 months. The second consisted of seven diabetic rats. In these rats nuclear magnetic resonance (NMR) measurements were done at 2 weeks, 4 and 8 months after induction of diabetes. The NMR protocol included T_2 weighted ^1H MR imaging, single voxel ^1H MRS and ^{31}P MRS. One control and one diabetic rat did not survive the 8 months of the experiment. Spare animals replaced these rats in the final measurements.

Nuclear magnetic resonance (NMR) protocol. Nuclear magnetic resonance experiments were done on a SIS Co spectrometer (Varian Associates, Palo Alto, Calif., USA) interfaced to a 4.7 T Oxford magnet (Oxford Instruments Ltd., Oxford, UK), equipped with actively shielded gradients with a maximum gradient strength of 32 mT/m. For ^1H NMR measurements, a saddle-shaped volume coil was used for radio frequency pulse transmission. An inductively coupled surface coil (2 cm diameter) was placed against the skull and used for signal detection. For ^{31}P MRS experiments, a two turn surface coil (2.5 cm diameter) was used for both signal transmission and detection.

The rats were anaesthetised by subcutaneous injection of a mixture of fluanisone (5.5 mg/kg), fentanyl citrate (0.17 mg/kg) and midazolam (2.75 mg/kg), followed by intubation and mechanical ventilation with 0.7–0.8% halothane in $\text{O}_2/\text{N}_2\text{O}$ (30/70%) throughout the NMR protocol, which lasted approximately 3 h. Body temperature was continuously measured and kept within the physiological range, using a heating pad with circulating water. Expiratory CO_2 was monitored (Datascopes, Paramus, N.J., USA) and used to assess and adjust the depth of anaesthesia. Average end-expiratory CO_2 levels were similar among the experimental groups at the start of the NMR protocol and did not change appreciably during the measurements. Respiratory volumes and rates were based on previous experiments in which arterial blood gas was sampled. We did not analyse blood gas or monitor arterial pressure in the present experiments because cannulation of the femoral arteries was incompatible with the longitudinal nature of the protocol. After the experiments, the animals were allowed to recover and returned to their cages.

Single voxel ^1H MRS. Multislice T_2 weighted spin-echo imaging experiments (repetition time, 2 s; echo time, 50 ms; number

Fig. 1. Coronal and transversal T_2 -weighted images of a control rat brain, illustrating the localisation of the 240 μl ^1H MRS PRESS box (A,C; black box) and the localisation of the two-dimensional ISIS ^{31}P MRS column (B, C; white lines/box)

of acquisitions, 2; 15 coronal slices of 1.2 mm thickness) was done to guide the positioning of the ^1H MRS volume of interest. A PRESS localisation technique [24] was used to define a 240 μl voxel ($8 \times 5 \times 6 \text{ mm}^3$) centrally in the brain, including parts of the cortex, caudate-putamen, thalamus, hippocampus, corpus callosum and the ventricular system (Fig. 1). Anatomical landmarks determined the placement of the voxel. As multiple coronal slices guided the lateral position and depth of the voxel, the precision of tissue sampling was mainly determined by its rostro-caudal position. Owing to the partial volume effect, the 1.2 mm slice thickness thus allowed for a maximum variation in voxel placement in rostro-caudal direction of 0.6 mm, leading to a maximum variation in tissue sampling of 10 % between animals (0.6 mm/6 mm). Given the relative limited variation of NAA, Cho and tCr concentrations in different brain areas, this 10 % variation should not affect peak ratios [25].

Water suppression was achieved using CHESS pulses [26] and BISTRO, consisting of 32 frequency-selective radio frequency pulses with increasing amplitude [27]. Fully relaxed spectra were obtained, taking 64 transients with a relaxation time of 6 s and a total echo time of 144 ms. At the final measurement, at 8 months, spectra were also acquired with four other echo times (40, 80, 120 and 180 ms, with a relaxation time of 3 s), to calculate the T_2 relaxation time of NAA, tCr and Cho. The free induction decay was zero filled to 4 k points, followed by exponential multiplication (4 Hz line-broadening). Spectra were obtained by Fourier transformation and were manually phased (zero and first order). The NAA resonance was assigned a chemical shift of 2.02 ppm. The relative amplitudes of the metabolite resonances were measured, using iterative fitting of the time domain signals with the variable projection method VARPRO [28]. Peak ratios of NAA:Cho,

NAA:tCr and Cho:tCr were calculated. T_2 relaxation times were calculated by fitting the decay of peak amplitudes at increasing echo time, assuming mono-exponential behaviour. Quantitative analysis of lactate concentrations was not feasible because normal cerebral lactate concentrations ($\sim 1 \text{ mmol/l}$) are close to the detection limit for the present ^1H MRS technique [25]. Therefore, lactate peaks were assessed qualitatively. Lactate was considered detectable if a negative resonance at 1.33 ppm clearly exceeded the noise level.

^{31}P Magnetic resonance spectroscopy. After the ^1H MRS experiments, the volume coil was detuned and the ^1H surface coil replaced by a ^{31}P coil. A transversal proton MR image was obtained to define a restricted column in the z (20 mm) and x (11 mm) direction, perpendicular to the transversal image (Fig. 1). After localised shimming on the ^1H MR signal of water, a two-dimensional ISIS ^{31}P MRS experiment [29] was carried out on the column defined earlier. Pilot experiments showed that with the sensitivity of our surface coil and this two-dimensional localisation procedure, the ^{31}P -spectrum is essentially derived from cerebral tissue and without contamination by phosphorous metabolites from extracranial (temporal and neck) muscles. For excitation an adiabatic half passage pulse was applied. Localisation was achieved by adiabatic full passage inversion pulses [25]. Transients ($n = 128$) were taken with a repetition time of 15 s to exclude differential saturation effects on the peak ratios. The free induction decay was zero filled to 4 k points followed by exponential multiplication (10 Hz line broadening). After baseline-correction, the peaks of PCr, Pi and α -ATP, β -ATP and γ -ATP were integrated in the frequency domain. The γ -ATP and β -ATP peaks were averaged. The PCr:Pi, PCr:ATP and ATP:Pi peak ratios were determined. Intracellular pH (pH_i) was calculated from the chemical shift of the Pi peak relative to the PCr peak [30].

Statistical analysis. Data are expressed as means $\pm$ standard error of the mean (SEM), unless indicated otherwise. To evaluate the effect of diabetes, diabetic rats at 2 weeks were compared with baseline controls and diabetic rats at 8 months were compared with 8-month controls. Where there was homogeneity of variances (Levene test, $p > 0.05$), group means

Fig. 2. Typical examples of single voxel PRESS ^1H MR spectra (upper panel) and ^{31}P MR spectra (lower panel) from baseline controls and diabetic rats 2 weeks and 4 months after streptozotocin injection. ^1H MR spectra were arbitrarily scaled on the total creatine (tCr) peaks. The decrease in NAA:tCr and NAA:Cho peak ratios in diabetic rats at both time points, compared with control animals, is visible. Note the increase in amplitude of the resonance complex between 3.5 to 4 ppm in spectra from diabetic rats. ^{31}P MR spectra were scaled on the PCr peak. The relative peak amplitudes of the PCr, Pi, and ATP resonances do not change

were compared with a two-tailed t test for independent samples. Because metabolite ratios in ^{31}P MRS were not distributed normally these data were analysed using Mann-Whitney U-test for nonparametric data. The effect of diabetes duration on ^1H MRS metabolite ratios and intracellular pH was tested with paired sample t tests. The effects of diabetes duration on ^{31}P MRS metabolite ratios were assessed with Wilcoxon signed rank tests for nonparametric data.

Results

Body weight and blood glucose concentrations of the animals are presented in Table 1. Diabetic animals had significantly reduced body weights, which re-

mained stable throughout the experiment. Blood glucose concentrations in diabetic animals were increased fourfold compared with controls.

^1H Magnetic resonance spectroscopy (Table 2, Figure 2, 3). Typical ^1H MR spectra are presented in Figure 2. Peak ratios are presented in Figure 3. After 2 weeks and 8 months of diabetes, the NAA:tCr ratio was reduced by 14 and 23 % compared with the corresponding controls ($p < 0.001$, $p < 0.005$). The NAA:Cho ratio was decreased by 21 and 17 % after 2 weeks and 8 months of diabetes compared with the respective control ratios ($p < 0.001$, $p < 0.005$). There was no statistically significant effect of diabetes duration on metabolite ratios. The tCr:Cho ratio was not affected by diabetes. A small lactate resonance was detected in four out of eight controls at baseline and in two out of seven controls at 8 months. In diabetic animals a lactate resonance was detected in four out of seven rats after 2 weeks of diabetes, in one out of seven rats after 4 months of diabetes and in one out of seven rats after 8 months of diabetes.

We found that T_2 relaxation times at 8 months were similar in control and diabetic rats for NAA and Cho (Table 2). The T_2 relaxation time of tCr was slightly but statistically significantly increased in the diabetic group (control 177 ± 3 ; diabetic 190 ± 4 ms; $p < 0.05$). This 7 % increase in relaxation time would at the most account for 6 % of the reduction in NAA:tCr after 8 months of diabetes. Therefore the observed NAA:Cho and NAA:tCr reductions are not due to changes in NMR relaxation properties of these compounds.

In diabetic rats an increase in the signal intensity from 3.5 to 4 ppm was observed (Fig. 2). Several compounds, including inositol, glucose, glutamine and glutamate, could contribute to this signal [31]. Assessment of the levels of the individual compounds contributing to this resonance complex requires adapted MR protocols involving high-field strength, short echo times and/or spectral editing techniques [25].

^{31}P Magnetic resonance spectroscopy (Figs. 2, 4). Typical ^{31}P MR spectra are presented in Figure 2. Peak ratios are presented in Figure 4. No statistically significant changes were observed in the PCr:ATP,

Table 1. Body weights, blood glucose

	Controls baseline	Controls 8 months	DM baseline	DM 2 weeks	DM 4 months	DM 8 months
Number of rats	8	7	7	7	7	7
Body weight (g)	356 ± 5	649 ± 23	353 ± 8	300 ± 8^a	309 ± 15	298 ± 17^a
Blood glucose (mmol/l)	5.5 ± 0.2	6.9 ± 0.2	5.9 ± 0.3	23.9 ± 0.7^a	18.8 ± 0.1	26.1 ± 1.4^a

Data are means $\pm$ SEM and were analysed by two-tailed t test for independent samples. Diabetic rats at 2 weeks were compared with baseline controls and diabetic rats at 8 months

were compared with 8-month controls (significant differences between the groups are indicated: $^a p < 0.05$). DM = diabetic

Fig. 3. ^1H MR metabolite ratios. Both the NAA:tCr and NAA:Cho ratio were significantly reduced after 2 weeks (t test; both $p < 0.001$) and 8 months (both $p < 0.005$) of diabetes (open bars), compared with the respective baseline and 8-month controls (hatched bars). There was no significant effect of diabetes duration on metabolite ratios (paired sample t test). Data are means $\pm$ SEM

PCr:Pi and ATP:Pi ratios after 2 weeks or 8 months of diabetes compared with the respective control values. There was no statistically significant effect of diabetes duration on peak ratios. The cerebral intracellular pH was similar in the three groups (control rats: baseline 7.18 ± 0.02 ; 8 months 7.13 ± 0.02 ; diabetic rats: 2 weeks 7.14 ± 0.01 ; 4 months 7.14 ± 0.02 ; 8 months 7.12 ± 0.01).

Table 2. ^1H MRS: calculated T_2 relaxation times for total creatine, choline and *N*-acetylaspartate at 8 months

	Controls	Diabetic
T_2 total creatine	177 ± 3	190 ± 4^a
T_2 choline	317 ± 11	303 ± 13
T_2 <i>N</i> -acetylaspartate	358 ± 16	358 ± 13

Data are means $\pm$ SEM and were analysed by two-tailed t test for independent samples. Diabetic rats at 8 months were compared with 8-month controls (significant differences between the groups are indicated: $^a p < 0.05$)

Discussion

Cerebral metabolism in diabetic rats was examined longitudinally with *in vivo* ^{31}P and ^1H MRS. No changes in cerebral high-energy phosphate metabolites were observed compared with controls up to 8 months after diabetes induction. Moreover, in the brain of diabetic rats lactate peaks were detected as frequently as in controls and the intracellular pH was unaffected. Diabetes seems not to have affected the energy status of the brain. It did lead to a statistically significant decrease in the NAA:tCr and NAA:Cho ratios at all time points studied. Notably, reduced cerebral NAA resonances have also been shown in a small group of diabetic patients [32]. Although in our study the amount of NAA was assessed relative to that of tCr and Cho, the data strongly suggest that diabetes reduces the concentration of NAA in the brain. The alternative explanation, that diabetes simultaneously increased the concentrations of tCr and of Cho, is unlikely. The tCr peak is believed to be relatively insensitive to tissue pathology, and is often used as a reference resonance for the measurement of relative changes in NAA or Cho or both [21]. The Cho peak reflects cerebral choline stores. Choline-containing compounds are involved in membrane synthesis and degradation [21], and increased concentrations of choline are associated with conditions such as brain tumours and demyelinating disease [20]. Even in peripheral nerves of STZ-diabetic rats, in which pathological changes are generally more pronounced than in the brain, demyelination does not occur as early as after 2 weeks of diabetes [33].

The metabolite, NAA, is found in high concentrations (8–10 mmol/l) only in the brain [21, 20]. Because it is present largely, if not entirely, in neurones [21], the NAA resonance is often used as a neuronal marker, although its physiological role is largely not known [21, 20]. A relative decrease in cerebral NAA levels has now been reported in such disorders as stroke, Alzheimer's disease and multiple sclerosis and has been attributed to neuronal loss [21, 20]. More recently reductions in cerebral NAA were, however, shown to be potentially reversible [34, 35], indicating that NAA levels not only reflect neuronal

Fig. 4. ^{31}P MR metabolite ratios. Boxes present quartile ranges with median. Whiskers indicate range. Extreme outliers (more than three times interquartile range) are indicated (●). Median values are printed below boxes. No significant differences were detected between 2-week diabetic rats and baseline controls and between 8-month diabetic rats and 8-month controls (Mann-Whitney U test). There was no significant effect of diabetes duration on metabolite ratios (Wilcoxon signed rank test)

density and viability but can also be used as a dynamic marker of neuronal metabolic (dys)function and integrity [34]. This latter interpretation would be in line with our observation that NAA ratios in the brain of STZ-diabetic rats were reduced well before

functional and structural abnormalities are known to occur in this model. Deficits in learning and memory and in signal transduction, for example, become detectable at 2 to 3 months after diabetes induction and develop gradually thereafter [9, 6, 7]. Whether reductions in NAA, as an early marker of neuronal dysfunction in STZ-diabetes, are causally related to the behavioural, neurophysiological and structural cerebral changes that occur later on is not known.

Phosphocreatine is a high-energy phosphorus compound that serves as an energy buffer in the creatine-kinase-mediated ATP:ADP equilibrium. Under (moderate) ischaemic or hypoxic conditions a fall in cerebral ATP is preceded by a reduction in PCr and a rise in lactate [36, 37]. In our study the PCr:ATP ratio was unaffected up to 8 months after diabetes induction. In addition, cerebral lactate was detected in a similar percentage of diabetic and control animals, indicating that diabetes did not lead to an increased dependence of the brain on anaerobic glycolysis. It should be noted, however, that with the present technique normal cerebral lactate concentrations are near the detection threshold for *in vivo* MRS in rats, which limits the assessment of subtle changes. Higher magnetic field strength and shorter echo times are required for quantitative measurements of normal or slightly increased cerebral lactate concentrations [38]. Our observations are in line with the baseline findings of an earlier *in vivo* ^{31}P MRS study, which examined the effects of acute ischaemia in BB/Wor diabetic rats [39]. In that study PCr concentrations in the brain of 4-month diabetic BB/Wor rats before induction of ischaemia were similar to controls.

Earlier studies on cerebral blood flow in diabetic rats have provided results that seem to be inconsistent [16]. It is likely, however, that differences in the techniques used for assessing blood flow are the major source of this inconsistency. For example, studies that assessed blood flow indirectly, by measuring erythrocyte velocity in cortical arterioles [40] or venous outflow [41] reported increases in cerebral blood flow. In contrast, studies that assessed blood flow at the tissue level, by measuring the cerebral uptake of tracers like [^{14}C]iodoantipyrine in awake or anaesthetized rats, consistently report flow reductions of 10 to 15% during the first month of diabetes [12, 13] and 10 to 30% after 4 months of diabetes [42, 14], with some degree of regional variation. Structural changes in the vasculature, including basement membrane thickening [15] could further impede energy delivery to the brain. Our data indicate that these vascular abnormalities are of insufficient magnitude to compromise the energy status of the brain to a level detectable with MRS. This does not rule out involvement of vascular disturbances in cerebral dysfunction in diabetes. The sensitivity of MRS might not be insufficient to detect subtle regional variations. More importantly, energy consumption is

known to be reduced in the brain of diabetic rats [14], which could counterbalance an impaired energy delivery, thus preserving the energy status of the brain. Moreover, studies on the effects of mild to moderate hypoxia in non-diabetic animals showed that changes in cognitive functions and the electroencephalogram can occur at oxygen levels at which the PCr and ATP concentrations in the brain are still in the normal range [37]. The functional deficits at these moderate levels of tissue hypoxia were suggested to be the consequence of oxygen-dependent changes in the metabolism of neurotransmitters rather than the effect of a failure in ATP and PCr production [37]. The threshold at which chronic reductions in cerebral blood flow lead to reductions in PCr and ATP is not exactly known but seems to exceed the 10–30 % flow reduction that can be expected to occur in the brain of STZ-diabetic rats [12, 13, 14, 42]. In comparison, in peripheral nerves of diabetic rats, where blood flow is reduced by approximately 50 % [43, 44], increased lactate and decreased PCr concentrations have been observed [22, 45, 46]. The reductions in PCr are still relatively small and have not been found consistently [47, 46]. In the superior cervical ganglion, which like the brain has a higher metabolic rate, blood flow is reduced by 45 % and PCr is reduced by 38 % in STZ-diabetic rats [48, 46]. Likewise, in the retina of diabetic rabbits ATP concentrations can also be reduced [49], but it should be noted that ATP reductions in the retina have not been found consistently [50] and much controversy still exists about the nature of blood flow changes in the retina [51].

The pathogenesis of cerebral dysfunction in diabetes seems to be a multifactorial process [1]. Therefore, in addition to cerebrovascular changes other factors, such as aberrant glucose metabolism in glia and neurones, are likely to affect the brain. Notably, increased glucose concentrations in the brain of STZ-diabetic rats were found to be associated with oxidative damage [52] and accumulation of sorbitol, fructose and advanced glycation end products [53, 54, 55], albeit to a lesser extent than in peripheral nerves. These metabolic changes are paralleled by a reduction in Na⁺, K⁺-ATPase activity in brain homogenates [56], which could account for a part of the reduced energy consumption in the brain of diabetic rats [14].

In conclusion, in vivo ³¹P and ¹H MRS of the brain of diabetic rats showed no alterations in either high-energy phosphate metabolite ratios or lactate resonances after a diabetes duration of 2 weeks to 8 months. Therefore, reductions of cerebral blood flow in STZ-diabetic rats do not seem to affect the energy status of the brain. In vivo ¹H MRS showed a relative reduction in NAA in the brain of STZ-diabetic rats, which could present a marker for early metabolic or functional abnormalities in cerebral neurones in diabetes.

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