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Mol Neurobiol (2015) 51:843–852
DOI 10.1007/s12035-014-8808-4

Alteration of Interaction Between Astrocytes and Neurons in Different Stages of Diabetes: a Nuclear Magnetic Resonance Study Using [1-^{13}C]Glucose and [2-^{13}C]Acetate

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Received: 15 March 2014 / Accepted: 10 July 2014 / Published online: 22 July 2014

Abstract Increasing evidence has shown that the brain is a site of diabetic end-organ damage. This study investigates cerebral metabolism and the interactions between astrocytes and neurons at different stages of diabetes to identify the potential pathogenesis of diabetic encephalopathy. [1-^{13}C]glucose or [2-^{13}C]acetate is infused into 1- and 15-week diabetic rats, the brain extracts of which are analyzed by using ^{1}H and ^{13}C magnetic resonance spectroscopy. The ^{13}C-labeling pattern and enrichment of cerebral metabolites are also investigated. The increased ^{13}C incorporation in the glutamine, glutamate, and γ-aminobutyric acid carbons from [2-^{13}C]acetate suggests that the astrocytic mitochondrial metabolism is enhanced in 1-week diabetic rats. By contrast, the decreased labeling from [1-^{13}C]glucose reflected that the neuronal mitochondrial metabolism is impaired. As diabetes developed to 15 weeks, glutamine and glutamate concentrations significantly decreased. The increased labeling of glutamine C4 but unchanged labeling of glutamate C4 from [2-^{13}C]acetate suggests decreased astrocyte supply to the neurons. In addition, the enhanced pyruvate recycling pathway manifested by the increased lactate C2 enrichment in 1-week diabetic rats is weakened in 15-week diabetic rats. Our study demonstrates the overall metabolism disturbances, changes in specific metabolic pathways, and interaction between astrocytes and neurons during the onset and development of diabetes. These results contribute to the mechanistic understanding of diabetes pathogenesis and evolution.

Keywords Diabetic encephalopathy · Pathogenesis · ^{13}C magnetic resonance spectroscopy · Pyruvate recycling · Anaplerotic pathway

Abbreviations

DM	Diabetes mellitus
NAA	N-acetyl aspartate
m-Ins	Myo-inositol
^{1}H NMR	Proton nuclear magnetic resonance
Cho	Choline
LDH	Lactate dehydrogenase
PDH	Pyruvate dehydrogenase
PC	Pyruvate carboxylase
MCA	Monocarboxylic acid
GS	Glutamine synthetase
Glu	Glutamate
Gln	Glutamine
TCA	Tricarboxylic acid
ALAT	Alanine aminotransferase

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin resistance, or both [1]. DM can result in various complications, including retinopathy, nephropathy, and peripheral neuropathy. The relationship between diabetes and cognitive dysfunction has been first recognized in 1922 [2]. Such condition has been later designated as "diabetic

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encephalopathy" in 1950 [3]. Many studies have indicated that diabetes affects the brain [4, 5]. Pathological researches have observed significant progressive neuronal losses in the hippocampus in type 1 diabetic rats [6, 7]. Studies using magnetic resonance imaging techniques have identified abnormal white matter structure and function as well as reduced gray matter volume and density in diabetic patients [8, 9]. All these abnormalities are closely related to brain metabolic disorders [10]. For example, decreased N-acetyl aspartate (NAA) [11] and increased myo-inositol (m-Ins) [12] concentrations have been observed in the white matter of individuals with diabetes by using localized proton nuclear magnetic resonance (^{1}H NMR). In addition, streptozotocin (STZ)-induced diabetic rats exhibited increased cerebral lactate/choline (Lac/Cho) ratio [13]. Analysis on metabolic derangement-related enzymes have shown increased activity of lactate dehydrogenase (LDH) [14] but decreased activity of pyruvate dehydrogenase (PDH) and glutamine synthetase (GS) [15] in the brains of alloxan-induced diabetic rats. Most previous studies focused on determining the individual metabolites or metabolism-related enzymes, but only few facilitated a comprehensive understanding of DM-associated metabolic characteristics. In particular, the specific metabolic pathways directly related to the development of diabetic encephalopathy are poorly understood.

Astrocytes have crucial functions in the brain, including absorbing and disposing glutamate (Glu) from the synaptic cleft, maintaining pH and appropriate concentrations of K^{+} and other ions in the interstitial milieu, and providing energy substrates for neurons [16]. Increased astrocyte-specific glial fibrillary acidic protein (GFAP) expression has been found in the cortex and hippocampus of STZ-induced diabetic rats [17]. In addition, Glu uptake has been decreased in the brain [18], whereas neurotransmitter homeostasis has been impaired in the hippocampus [19]. These functions and changes in the neurotransmitter cycles between astrocytic and neuronal compartments in diabetic brain have not yet been reported.

The administration of ^{13}C-labeled substrates combined with ^{13}C magnetic resonance spectroscopy (MRS) is a powerful tool to detect ^{13}C incorporation into different metabolites. Sparse isotopic labeling using [1-^{13}C]glucose or [2-^{13}C]acetate results in specific carbon positions within the same metabolites and thus provides site-specific information. These positions include tricarboxylic acid (TCA) cycle intermediates and amino acids. As a result, the fate of ^{13}C label can be determined through multiple metabolic pathways in the brain under different physiological and pathophysiological conditions [20]. ^{13}C MRS has successfully revealed decreased Glu neurotransmission and impaired TCA cycle in patients with Alzheimer's disease [21]. Decreased glutamine (Gln) supply to gamma-aminobutyric acid (GABA)ergic neurons has been observed in the cortex and subcortex of young epileptic rats, whereas astrocytic metabolism has been increased in adult epileptic rats [22]. Mason et al. applied [2-^{13}C]acetate as a tracer to evaluate the hypothesis that patients with type 1 diabetes exhibit increased blood–brain transport and monocarboxylic acid (MCA) metabolism to sustain upregulated MCA transporters during hypoglycemia [23].

^{13}C MRS is a primary technique for studying brain metabolism. Glucose is metabolized primarily in the neurons, whereas acetate is a well-known astrocyte-specific substrate. The neuronal–glial metabolism and the interaction of their compartments in the state of diabetes can be investigated through the injection of ^{13}C-labeled glucose and acetate. In this study, the specific ^{13}C-labeled location and labeled abundance after substrate injection were measured in 1- and 15-week diabetic rats, as well as in their age-matched controls. The results demonstrated the comprehensive metabolism disturbances and the changes in specific metabolic pathways in the generation and development of diabetes, which provide deep insights of pathogenesis and evolution of diabetes.

Materials and Methods

Animal Preparation and Infusion Techniques

Male Sprague–Dawley rats weighing 220±15 g (8 weeks of age) are purchased from the SLAC Laboratory Animal Co. Ltd. Shanghai, China, and are kept in a specific pathogen-free colony of the Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China) with regulated temperature and humidity and a 12:12-h light–dark cycle with lights on at 8:00 a.m. During the whole experimental process, rats are fed with certified standard rat chow and tap water ad libitum. All animal treatments are strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Four groups of rats are used (n=20~28 for each group), including diabetic 1-week, diabetic 15-week, and two age-matched control groups. It should be mentioned that every group of rats at each time point were randomly divided into four subgroups. After a 12-h fast, rats are randomly selected and injected intraperitoneally with STZ (Sigma-Aldrich), which is prepared freshly in citrate buffer (0.1 M, pH 4.5) at a single dosage of 70 mg/kg body weight, whereas control rats are injected with the same volume of vehicle. Two days after STZ administration, the blood glucose concentrations are measured and the rats of which higher than 16.70 mmol/L are defined as diabetic rats.

Prior to each experiment, the rats are fasted overnight (12 h) but had free access to water. The animals are anesthetized, and the left jugular veins are dissected and cannulated for infusion of the labeled or unlabeled substrates. During the whole procedure, rats are kept under a glow lamp to maintain

rectal temperature in the range of 36.8~37.5 °C. As for unlabeled or [1-^{13}C]glucose (enriched at 99 %, 0.75 M), it is infused at a continuous rate of 0.11 mL/kg per min while unlabeled or [2-^{13}C]acetate (enriched at 99 %, 1.5 M) is infused at a constant rate of 0.18 mL/kg per min. The rats are sacrificed by decapitation at the end of the infusion, and the brains (whole brain minus cerebellum) are immediately removed from the skull within 15 s and frozen in liquid nitrogen.

Brain Metabolite Extraction

The frozen cerebral tissue is weighed and put into a centrifuge tube. Ice-cold methanol (4 mL/g) and distilled water (0.85 mL/g) are added into the tube, homogenized at 4 °C after thawing, and mixed by vortex. Chloroform (2 mL/g) and distilled water (2 mL/g) are added into the tube and mixed again. After the sample tube is kept on ice for 15 min, the homogenate is centrifuged at 1,000 *g* for 15 min at 4 °C. The supernatant is extracted and lyophilized for about 24 h. The obtained aqueous extracts were then dissolved in 550 μL of 99.5 % D_2O for NMR spectroscopy.

Acquisition of ^{1}H and ^{13}C NMR Spectra

High-resolution ^{1}H and ^{13}C NMR spectra of brain extracts are carried out at 298 K on a Bruker AVANCE III 600-MHz NMR spectrometer operating at 600.13 (H) and 150.92 (C) MHz, respectively. ^{1}H NMR spectra of brain extracts are acquired using a 90° flip angle with a spectral width of 9,615 Hz and 64 K data points. The number of scans is 64, and an additional 10 s relaxation delay is used to ensure full relaxation. The spectra are corrected manually for phase and baseline and referenced to the chemical shift of the Lac methyl peaks at 1.33 ppm. An inverse-gated decoupling sequence (INVGATE) is used for ^{13}C experiments to avoid the Nuclear Overhauser effect, and spectra are accumulated using a 30° flip angle with a spectral width of 33,333 Hz and 64 K data points. The acquisition time is 1 s, and an additional delay of 2 s is used to achieve fully relaxed conditions for each carbon resonance. A total of 32,768 scans are accumulated for each spectrum.

Data and Statistical Analysis

The specific ^{13}C-enrichment of metabolites is calculated as previously described [24, 25]. Metabolite contents (mmol/kg wet weight) are determined relative to the concentration of creatine (assumed to be 9 mmol/kg) from fully relaxed ^{1}H NMR spectra of brain extracts [26]. All values are expressed as mean±SEM. Statistical comparisons between the experimental groups are performed by independent samples *t* test. A calculated value of $p<0.05$ is considered to be statistically significant.

Results

^{1}H NMR Data of Cerebral Metabolite

The representative ^{1}H NMR spectra of the brain extracts obtained from the rats in the control and diabetic groups (1 week and 15 weeks of diabetes) are shown in Fig. 1a–c. The resonances in Fig. 1a are unambiguously assigned from our published work [27] and confirmed here in 2D ^{1}H-^{1}H COSY and TOCSY spectra (data not shown). Endogenous metabolites, such as Lac (δ1.33), alanine (Ala, δ1.46), NAA (δ2.01), GABA (δ2.28), Glu (δ2.34), succinate (Suc, δ2.40), Gln (δ2.46), aspartate (Asp, δ2.82), taurine (Tau, δ3.42), and m-Ins (δ3.53), were simultaneously measured through the ^{1}H NMR spectra of the brain tissue extracts.

The concentrations of the metabolites measured in the brain of 1- and 15-week diabetic rats and their age-matched controls are summarized in Table 1. No changes are observed in the metabolite concentrations in the brain of 1-week diabetic rats, except for a decrease in NAA. By contrast, most metabolites evidently changed in the 15-week diabetic group. Interestingly, the neurotransmitter concentrations of Glu and Gln are significantly decreased, whereas that of m-Ins is significantly increased. The concentration of NAA is remarkably decreased in the 15-week diabetic group.

^{13}C NMR Spectra of [2-^{13}C]Acetate Metabolism

The representative high-resolution ^{13}C NMR spectra of the brain extracts obtained from the rats infused with unlabeled acetate or [2-^{13}C]acetate are presented in Fig. 2a–c. The NMR peaks in Fig. 2b have been previously assigned [28]. The ^{13}C NMR spectra in Fig. 2a obtained from the rats infused with unlabeled acetate exhibited low peak sensitivity due to low natural abundance. ^{13}C NMR spectra with [2-^{13}C]acetate metabolism (Fig. 2b, c) show that the ^{13}C isotope has been successfully incorporated into the specific carbon sites of a given metabolite, such as Gln, Glu, GABA, and Lac.

Interaction between neurons and astrocytes can be revealed by analyzing the labeled patterns of the neurotransmitters. Figure 3a, b demonstrates the site-specific ^{13}C-enrichment of Gln, Glu, and GABA in the C4, C3, and C2 carbons derived from [2-^{13}C]acetate. Each metabolite in the brains of 1- or 15-week diabetic rats was evidently marked. The labeling abundance is higher in Gln than in Glu, suggesting that the exogenous acetate can be metabolized as a glial-specific substrate. For 1-week diabetic rats, the ^{13}C labeling percentage in Gln has increased, and all carbon positions are obtained compared with those in the control (C4, 32.15±1.53 vs 23.77±1.39, $p=0.007$; C3, 12.40±0.39 vs 9.35±0.60, $p=0.005$; and C2, 15.13±0.57 vs 11.63±0.63, $p=0.006$). ^{13}C-enrichment in the C4 position of Glu has significantly increased (14.60±0.63 vs 12.27±0.59, $p=0.035$). Moreover, the ^{13}C-label

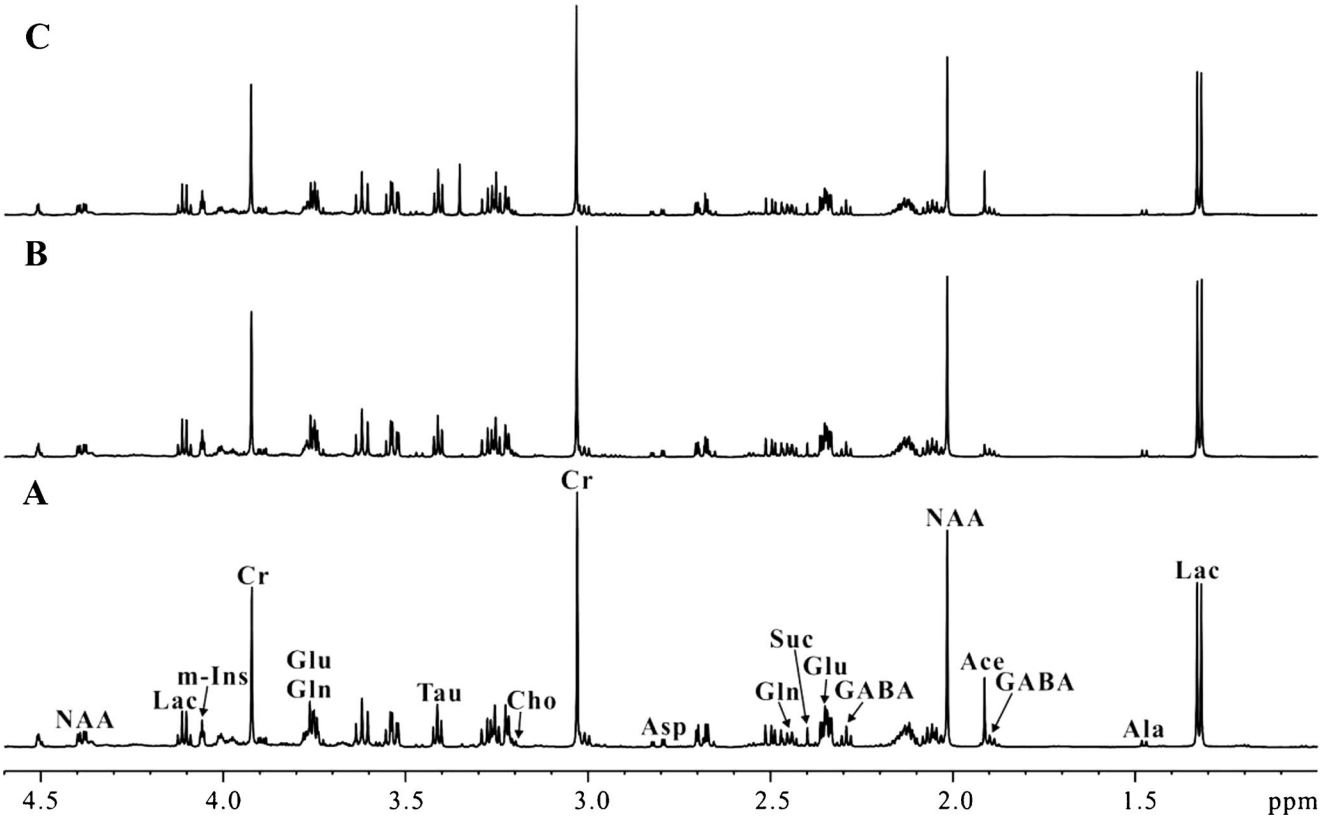

Fig. 1 Representative 600-MHz ^{1}H NMR spectra of the brain extracts obtained from a control rat (**a**), a 1-week diabetic rat (**b**), and a 15-week diabetic rat (**c**)

incorporation into the C2 and C3 positions showed a clear increasing trend compared with that of the age-matched control. The ^{13}C-enrichment of GABA has remarkably increased in the C2 position (12.89±0.50 vs 10.14±0.72, p=0.021) but remained constant in the C3 and C4 positions. As diabetes developed to 15 weeks, the ^{13}C-enrichment of Gln, Glu, and GABA derived from [2-^{13}C]acetate in diabetic rats remains unchanged at all carbon sites, and a decreasing trend is observed despite the increase in Gln C4.

Figure 3c shows the specific ^{13}C-enrichment of Asp and Lac in the C2 and C3 carbons, as well as Suc C2+3 derived from [2-^{13}C]acetate in the brain of the control and diabetic groups (1 and 15 weeks of diabetes). The specific ^{13}C-enrichment of these metabolites is significantly higher in 1-week

Table 1 Metabolite concentration (mmol/kg) of brain tissue extracts obtained from age-matched control and diabetic 1- and 15-week rats

δ ^{1}H (ppm)	Metabolites	1-week			15-week		
		CON	DM	DM vs CON (%)	CON	DM	DM vs CON (%)
1.33	Lactate	13.35±1.28	13.92±0.42	104.3	10.04±0.27	10.63±1.25	105.9
1.46	Alanine	0.73±0.05	0.63±0.03	87.21	0.52±0.02	0.60±0.03	115.4
2.01	NAA	7.42±0.06	6.93±0.06	93.35*	7.43±0.04	6.87±0.05	92.50***
2.28	GABA	2.78±0.13	2.53±0.07	91.13	2.78±0.04	2.53±0.12	91.10
2.34	Glutamate	10.37±0.37	10.88±0.12	104.9	10.74±0.12	9.63±0.19	89.74**
2.40	Succinate	1.07±0.11	0.92±0.04	86.06	0.63±0.05	0.68±0.03	108.2
2.46	Glutamine	5.42±0.20	5.18±0.16	95.51	5.97±0.12	5.01±0.09	83.96***
2.82	Aspartate	1.56±0.07	1.40±0.02	89.54	1.47±0.05	1.46±0.03	99.73
3.42	Taurine	5.73±0.15	6.02±0.12	105.1	5.75±0.09	5.83±0.87	101.6
3.53	Myo-inositol	6.26±0.10	6.74±0.19	107.5	5.92±0.07	7.28±0.22	122.9***

Values are expressed as mean±SEM (n=5~7 for each group)

*p<0.05; **p<0.01; and ***p<0.001 indicate significant differences

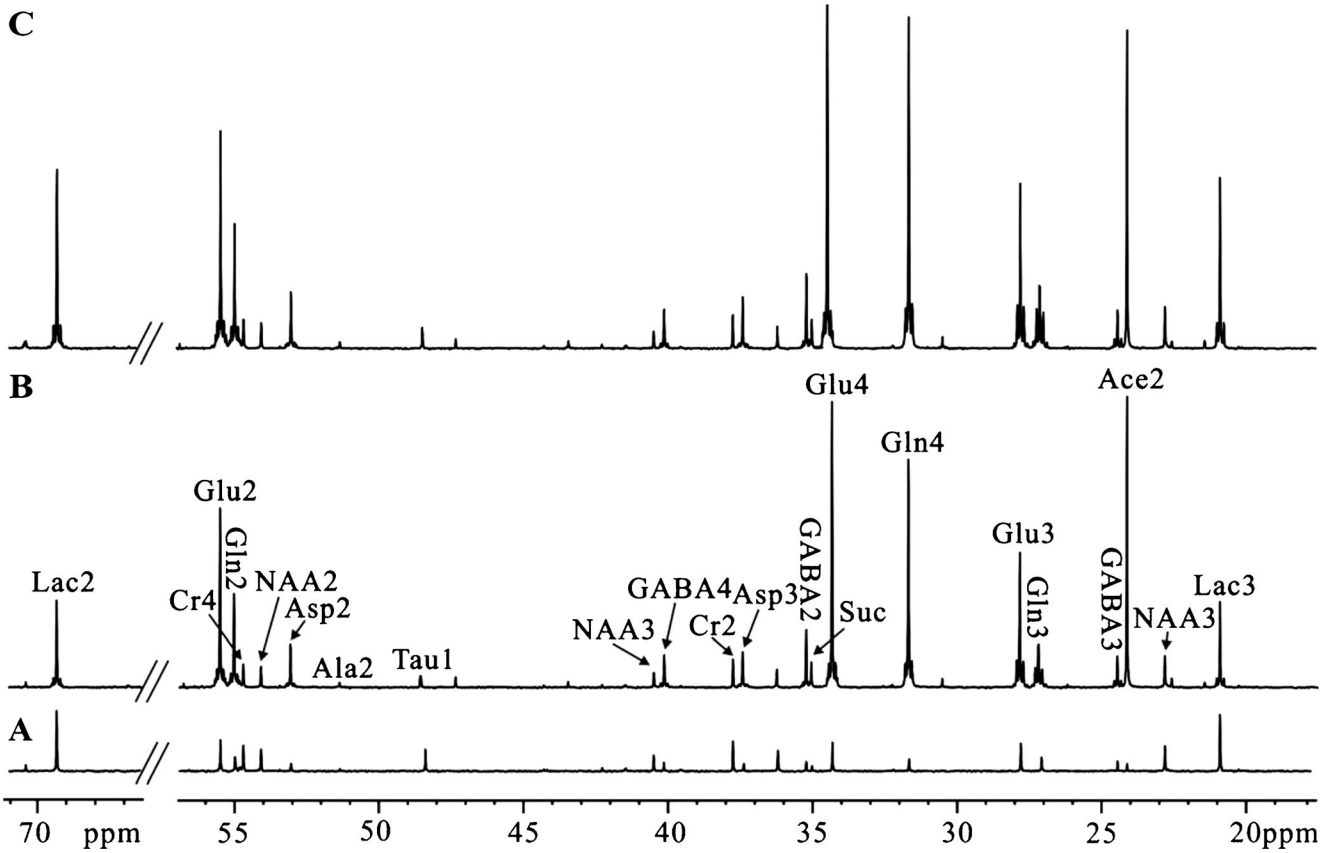

Fig. 2 Representative ^{13}C NMR spectra of the brain extracts obtained from the **a** control rat infused with unlabeled acetate, **b** control rat, and **c** 1-week diabetic rat infused with [2-^{13}C]acetate

diabetic rats than in the control. By contrast, a significant decrease in Lac C2 and C3, as well as Suc C2+3 enrichment, is observed in 15-week diabetic rats compared with the control. No significant changes in Asp C2 and C3 enrichments are found.

^{13}C NMR Spectra of [1-^{13}C]Glucose Metabolism

Figure 4a, b illustrates the specific ^{13}C-enrichment of Glu, Gln, and GABA in the C4, C3, and C2 carbons derived from [1-^{13}C]glucose. ^{13}C labeling of Glu is more enhanced than Gln, revealing the predominantly neuronal metabolism of [1-^{13}C]glucose. In 1-week diabetic rats, the ^{13}C-label incorporation into the C4, C3, and C2 positions has remarkably decreased in Glu and GABA and shown a decreasing trend in Gln compared with the control. As diabetes developed to 15 weeks, the labeling of Gln, Glu, and GABA in all carbon positions investigated from [1-^{13}C]glucose has apparently decreased. In addition, as shown in Fig. 4c, the enrichment of Asp C3, Lac C3, and Suc C2+3 from [1-^{13}C]glucose has largely decreased in 1-week diabetic rats and more dramatically reduced in 15-week diabetic rats. However, Lac at the C2 position is unchanged in 1- and 15-week diabetic rats.

The ratio between the carbon flux through pyruvate carboxylase (PC) and PDH activities is conventionally determined on the basis of the (C2-C3)/C4 ^{13}C ratios in Glu or Gln from [1-^{13}C]glucose [29]. As shown in Fig. 5, the Glu and Gln PC/PDH ratios calculated for the 1-week diabetic group are not apparently different from those obtained for the control group. However, the 15-week diabetic group exhibits a clear increase in the Gln PC/PDH ratio.

Discussion

Diabetes is a complex metabolic disorder that extensively affects multiple organs and tissues throughout the body. Its complications are the leading causes of morbidity and mortality among diabetic patients. The effect of diabetes on the central nervous system has gained considerable attention; this effect is characterized by cognitive dysfunction and cerebral neurophysiological changes [30]. Multiple studies have shown that the relative risk of developing dementia increases in diabetic patients [31]. Moreover, the possibility of comorbid depression is twice higher in the presence of diabetes [32]. In this study, the ^{1}H NMR-based metabonomics and ^{13}C

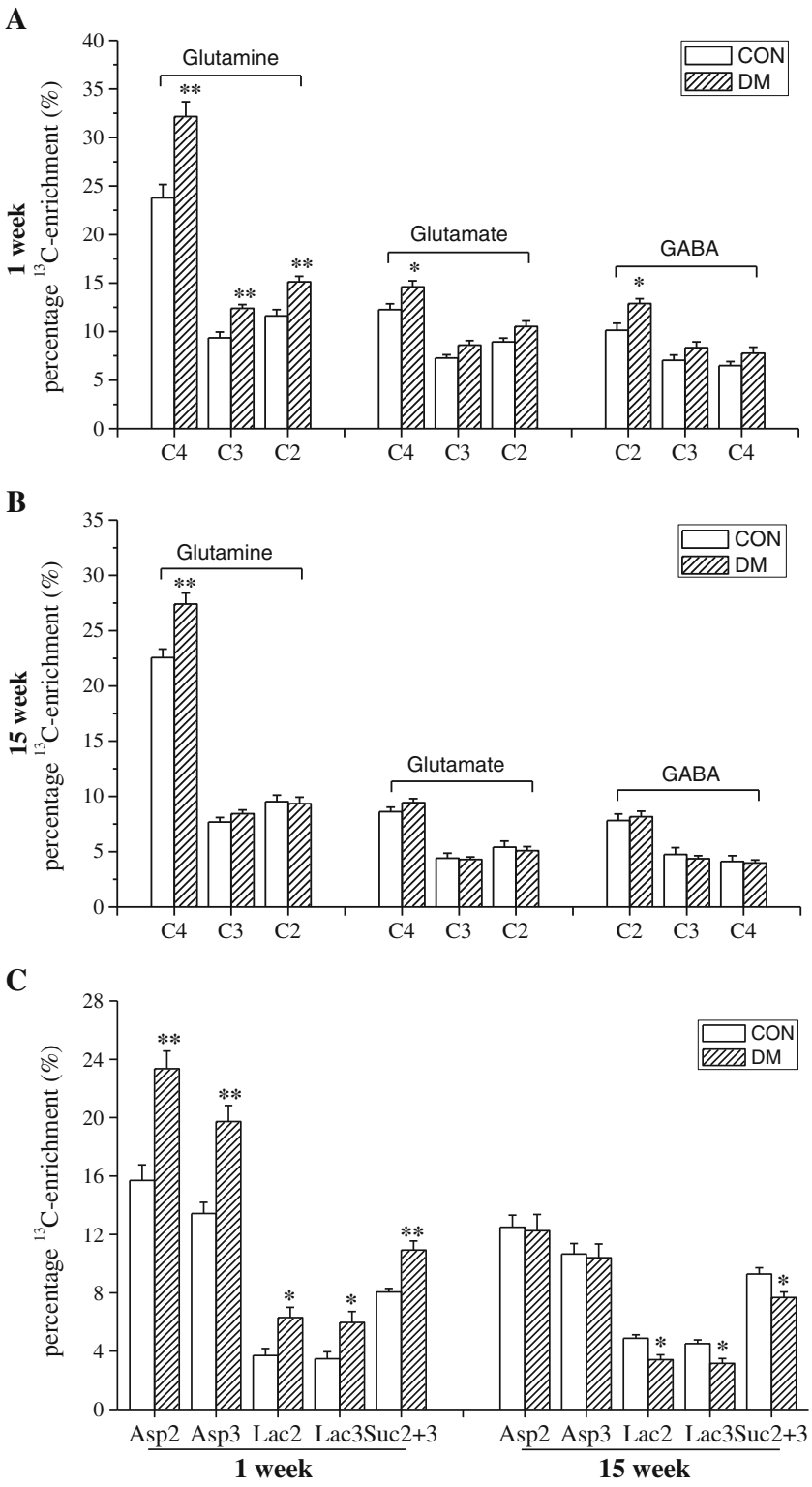

Fig. 3 **a–c** Changes in the percentages of the ^{13}C-enrichments in the specific carbon positions of the metabolites derived from [2-^{13}C]acetate. The percentages are calculated from the ^{13}C NMR spectra of the brain extracts from 1- and 15-week diabetic rats compared with that of the control rats. Significant differences are assigned as follows: *p<0.05; and **p<0.01

NMR with in vivo injection of ^{13}C-labeled substrates are applied to the brain extracts of 1- and 15-week diabetic rats. The global metabolic networks related to glycolysis, TCA cycle, and specific metabolic pathways, such as PC, pyruvate recycling, and Glu–Gln–GABA cycle, have been determined.

Effects of Diabetes on Cerebral Nutrient Transport

Glucose is the major energy substrate for brain function under normal conditions. [1-^{13}C]glucose is metabolized to [3-^{13}C]pyruvate via glycolysis and then converted to

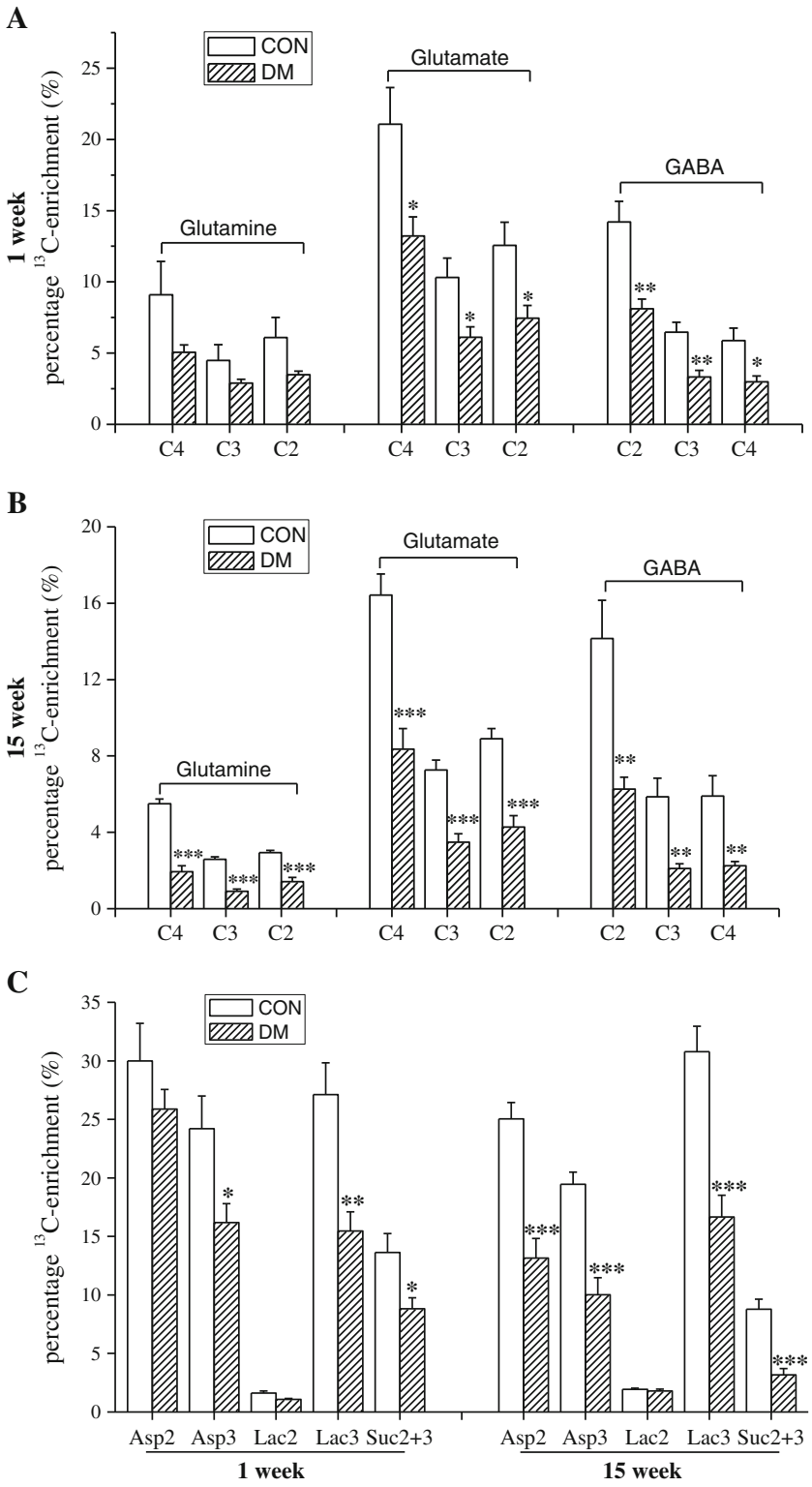

Fig. 4 **a–c** Changes in the percentages of the ^{13}C-enrichments in the specific carbon positions of the metabolites derived from [1-^{13}C]glucose. The percentages are calculated from the ^{13}C NMR spectra of the brain extracts from 1- and 15-week diabetic rats compared with that of the control rats. Significant differences are assigned as follows: *p<0.05; **p<0.01; and ***p<0.001

[3-^{13}C]lactate by LDH and [3-^{13}C]alanine by alanine aminotransferase or introduced to the TCA cycle via PC and PDH. The PC and PDH pathway, respectively, result in the specific labeling of TCA cycle-derived Glu and Gln. In this study, the ^{13}C-enrichment of [3-^{13}C]lactate has decreased in 1- and 15-week diabetic rats. This decrease agrees well with the decreased LDH activity reported in a previous study [15]. In addition, the lower ^{13}C incorporation into the cerebral Gln, Glu, GABA, and Asp after [1-^{13}C]glucose infusion indicates a decreased mitochondrial pyruvate utilization and neuronal

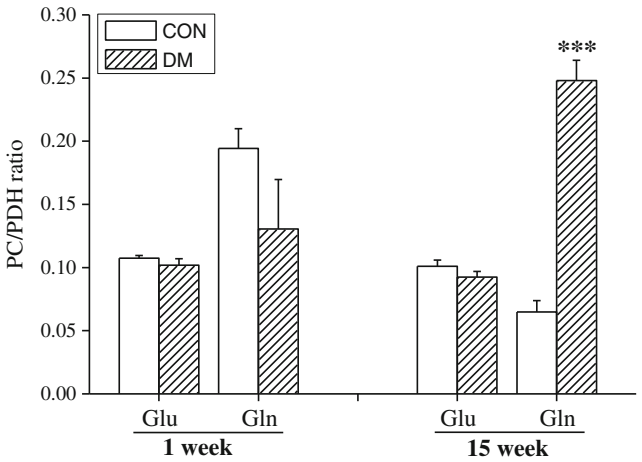

Fig. 5 PC/PDH ratios for Glu and Gln in the brains of 1- and 15-week diabetic rats compared with those of the age-matched rats. Significant differences are assigned as follows: ***$p<0.001$

TCA cycle activity in 1- and 15-week diabetic rats. Lapidot and Haber have showed that the lower enrichments of the cerebral glucose metabolites in diabetic fetal brain may be attributed not only to endogenous glucose dilution but also to the fetal brain utilization of a non-labeled carbon source β-hydroxybutyrate in severe diabetes cases [33]. A decrease in glucose metabolism may result from neuronal damage or increased utilization of other energy substrates, such as MCAs. Besides, such decrease may protect the brain against the effects of hyperglycemia [34].

In 1-week diabetic rats, the enrichment of Gln C4, Glu C4, GABA C2, Asp C2, and Asp C3 from [2-^{13}C]acetate has increased, implying more active astrocytic TCA cycle activity and acetate utilization. This result is consistent with a previous observation of the increased brain MCA transport activity in patients with well-controlled type 1 diabetes [23], which can possibly lead to decreased glucose utilization.

As an important intermediate product of the TCA cycle, Suc is an indicator of energy metabolism and mitochondrial function or activity. After [1-^{13}C]glucose infusion, the enrichment of Suc C2+3 decreases in 1- and 15-week diabetic rats, suggesting an impairment in neuronal mitochondrial function. This is consistent with another study that chronic hyperglycemia damages mitochondrial function, which can directly injure the neuronal cells in STZ-diabetic rats [35]. The ^{13}C-enrichment of Suc increases in 1-week diabetic rats but decreases in 15-week diabetic rats after [2-^{13}C]acetate infusion. This indicates that the astrocytic mitochondrial function is enhanced in the brain of 1-week diabetic rats but impaired in 15-week diabetic rats.

Alteration in Neuronal and Astrocytic Markers in Diabetic Rat Brain

The concentration of the neuronal marker NAA, which is synthesized primarily in the mitochondria [36], decreased in 1- and 15-week diabetic rats. This result is closely related to the neuronal dysfunction or loss in neuronal density in diabetic rat brains and consistent with a relative reduction of the NAA level in the gray matter of T1DM patients based on in vivo ^{1}H MRS [37]. M-Ins is localized in glial cells and is a membrane constituent that reflects the proliferation or activation of glia. This compound can possibly stabilize the intracellular environment [38]. The concomitant increases of m-Ins and Tau in 15-week diabetic rats suggest that glial proliferation is a cerebral pathological feature of 15-week diabetic rats. The high expression of GFAP, a structural and functional astroglial marker [39], results in an increase in astroglial reactivity, which is related to diabetic complications [40]. In addition, neurons are more susceptible to external stimulation and suppression than glial cells [12, 41]. Such glial proliferation is most likely an indicator of neuronal damage. This matches well to a study of human astrocytes express plasminogen activator inhibitor-1 and protease nexin-1, where astrocytic expression has been found to be dynamically regulated by injury-related factors [42].

Pyruvate Recycling in Diabetic Rat Brain

Pyruvate recycling is a metabolic pathway in the brain, which was first reported in 1990 using in vivo ^{13}C NMR method [43]. Subsequent metabolism studies have confirmed that the pyruvate recycling pathway primarily operates in the glial compartment [44]. Consistently, we have found that Lac C2 and Lac C3 carbons are labeled equally from [2-^{13}C]acetate (astrocyte-specific substrate) infusion. This labeling pattern indicates the presence of the pyruvate recycling pathway in glial cells. By contrast, [1-^{13}C]glucose heavily labels the Lac at C3 position, suggesting that the [1-^{13}C]glucose primarily metabolizes through glycolysis. The physiological significance of pyruvate recycling is not completely understood. Several studies have shown a neuroprotective effect of pyruvate or lactate administration [45, 46]. Taking together, we hypothesize that the synthesized pyruvate can transfer from astrocytes to neurons in the form of Lac, enter the neuronal TCA cycle, and provide the neurons with a substrate for energy metabolism. The present study proposes the enhanced pyruvate recycling pathway as a protective mechanism against metabolic impairments in 1-week diabetic rats.

Neuronal–Glial Interaction in Diabetic Rat Brain

Glu is released by neurons, dispersed from the synaptic cleft, absorbed by astrocytes via Glu transporters, and then converted into Gln by the astrocyte-specific enzyme GS. Thereafter, Gln may be released in the neurons and then re-converted to Glu by the enzyme phosphate-activated glutaminase. In GABAergic neurons, Glu can be converted to GABA by glutamic acid decarboxylase. These interactions between

neurons and astrocytes are referred as the Gln–Glu–GABA cycle, which are critical pathways for the neurotransmitters between astrocytes and neurons. The balance between the excitatory (Glu) and inhibitory (GABA) processes was altered in 1-week diabetic rats. The decreased ^{13}C-enrichments of Glu and GABA in all carbon positions and the increased ratio of Glu C4/GABA C2 and Glu C2/GABA C4 from [1-^{13}C]glucose infusion are possibly due to the decreased conversion of Glu to GABA or increased GABA degradation in neurons. Similar experiments using [2-^{13}C]acetate have shown an increase in the enrichments of Gln C4, Glu C4, and GABA C2 in 1-week diabetic rats. This pattern is consistent with the increased astrocytic mitochondrial metabolic activity and thus suggests a better preservation mechanism for the Glu formation from astrocytic Gln than neuronal Glu synthesis. A study in diabetic Wistar rats after 10 days of induction has revealed an enhanced astroglial metabolism to sustain glutamatergic and GABAergic neurotransmissions [47]. In addition, the relative ^{13}C-enrichments in Glu compared with Gln significantly decrease at the C4 and C2 positions. Moreover, the decrease in Glu C4/GABA C2 is presumably resulted from the relative decrease in the transport and utilization of Gln from astrocytes to glutamatergic and GABAergic neurons. The limited use of astrocytic precursors by glutamatergic or GABAergic neurons is indicative of energy metabolism disorders and neuronal death [48].

As diabetes developed to 15 weeks, the concentration of Glu and Gln has significantly decreased. The [1-^{13}C]glucose infusions decrease the ^{13}C incorporation of the cerebral Glu and Gln. The label in Gln C4 is the sum of the astrocytic de novo synthesis and Glu C4 from neurons. The increased ratios of Glu C4/Gln C4 can be attributed to the reduced de novo synthesis of Gln in glial cells and/or decreased Glu uptake by astrocytes. The increased labeling of Gln C4 but unchanged Glu C4 from [2-^{13}C]acetate in 15-week diabetic rats indicates a decrease in the transfer of Gln to neurons. The increase in the Gln PC/PDH ratios but unchanged Glu PC/PDH ratios in 15-week diabetic rats confirms that the transport of Gln to Glu neurons is inhibited. A study using in vivo microdialysis has reported a reduced neurotransmitter synthesis and a release in diabetic hippocampus [19]. Therefore, these results reveal the disordered metabolic trafficking between the astrocytes and glutamatergic neurons in 15-week diabetic rats.

Conclusions

We have successfully utilized ex vivo high-resolution NMR technology and [1-^{13}C]glucose and [2-^{13}C]acetate infusion to investigate the changes in cerebral energy metabolism during development of diabetes. The study uncovers altered metabolic pathways, such as the enhanced pyruvate recycling pathway as a protective mechanism against metabolic impairments in 1-week diabetic rats but weakened in 15-week diabetic rats. Our results reveal the changes in neuronal–glial interaction as diabetes developed and thus provide novel insights into the functions of astrocytes in maintaining normal neuronal activity at the early stage of diabetes. However, astrocytes could also damage the metabolic cycle with the development of diabetes. These results contribute to the understanding of the underlying mechanism that leads to brain damage in diabetes.

Acknowledgments We thank Huan Zhu and Jian-min Jia for their participation. This work was supported by the National Natural Science Foundation of China (No. 21175099), the Zhejiang Provincial Natural Science Foundation of China (No. LY14H090014), the Zhejiang Provincial Program for the Cultivation of Health talents, and the Science and Technology Foundation of Wenzhou (No. Y20100005).

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