RESEARCH ARTICLE

Expression of the Human Isoform of Glutamate Dehydrogenase, hGDH2, Augments TCA Cycle Capacity and Oxidative Metabolism of Glutamate During Glucose Deprivation in Astrocytes

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A key enzyme in brain glutamate homeostasis is glutamate dehydrogenase (GDH) which links carbohydrate and amino acid metabolism mediating glutamate degradation to CO_2 and expanding tricarboxylic acid (TCA) cycle capacity with intermediates, i.e. anaplerosis. Humans express two GDH isoforms, GDH1 and 2, whereas most other mammals express only GDH1. hGDH1 is widely expressed in human brain while hGDH2 is confined to astrocytes. The two isoforms display different enzymatic properties and the nature of these supports that hGDH2 expression in astrocytes potentially increases glutamate oxidation and supports the TCA cycle during energy-demanding processes such as high intensity glutamatergic signaling. However, little is known about how expression of hGDH2 affects the handling of glutamate and TCA cycle metabolism in astrocytes. Therefore, we cultured astrocytes from cerebral cortical tissue of hGDH2-expressing transgenic mice. We measured glutamate uptake and metabolism using [^{3}H]glutamate, while the effect on metabolic pathways of glutamate and glucose was evaluated by use of ^{13}C and ^{14}C substrates and analysis by mass spectrometry and determination of radioactively labeled metabolites including CO_2, respectively. We conclude that hGDH2 expression increases capacity for uptake and oxidative metabolism of glutamate, particularly during increased workload and aglycemia. Additionally, hGDH2 expression increased utilization of branched-chain amino acids (BCAA) during aglycemia and caused a general decrease in oxidative glucose metabolism. We speculate, that expression of hGDH2 allows astrocytes to spare glucose and utilize BCAAs during substrate shortages. These findings support the proposed role of hGDH2 in astrocytes as an important fail-safe during situations of intense glutamatergic activity.

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Introduction

Maintenance of glutamate homeostasis is essential to brain function and to avoid excitotoxicity (Anderson and Swanson, 2000). Astrocytes take up the major part of neurotransmitter glutamate from the synapse in which glutamate may be converted to glutamine or enter the mitochondria for

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oxidative metabolism (Dienel and Hertz, 2005; Sonnewald et al., 1997). Glutamate dehydrogenase (GDH) catalyzes the initial oxidative step deaminating glutamate to the tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate, using $NAD(P)^+$ as cofactor (McKenna et al., 2012). We have shown that GDH is important to sustain the catalytic capacity of the TCA cycle in mouse astrocytes by mediating the net formation of intermediates and that reduced GDH expression induces the usage of alternative substrates such as isoleucine, one of the branched-chain amino acids (BCAA) (Nissen et al., 2015). Most mammals, including rodents, express only one GDH isoform, GDH1 (hGDH1 in humans), encoded in humans by the *GLUD1* gene, but humans and apes also express a second isoform, GDH2, encoded by the *GLUD2* gene (Plaitakis et al., 1984). hGDH1 is widely expressed in human tissue, while hGDH2 expression has been localized to Sertoli cells in the testis, astrocytes in the brain (Spanaki et al., 2010), and epithelial cells in the kidney (Spanaki and Plaitakis, 2012). The two isoforms display different enzymatic properties, allosteric regulation, pH optimum, and localization in the brain (Plaitakis et al., 2000). While in the mammalian brain GDH1 is expressed in both neurons and astrocytes (Drejer et al., 1985; Larsson et al., 1985; McKenna et al., 2000; Schousboe et al., 1977), hGDH2 is expressed in astrocytes rather than neurons (Plaitakis et al., 2003; Spanaki et al., 2010).

GDH found across different species is allosterically regulated by a wide range of substances including ADP and GTP (Frieden, 1965; Wolff, 1962), and further affected by several others, e.g. L-leucine (Li et al., 2011 and references therein). However, the two human isozymes differ very significantly in their allosteric regulation (reviewed in Spanaki et al., 2012), the most important difference being their respective activity in the presence of ADP and GTP. hGDH1 and hGDH2 are both activated by ADP, they exhibit basal activities in the absence of ADP at 35 and 6% of maximal activity, respectively, with their maximal specific activity being close to identical (Mastorodemos et al., 2005; Shashidharan et al., 1997). hGDH2 is almost insensitive to inhibition by GTP in contrast to hGDH1 which is strongly inhibited by the presence of GTP (Plaitakis et al., 2000). Furthermore, hGDH2 displays optimum in activity in a slightly more acidic milieu (pH 7.5) compared to hGDH1 (pH 7.75–8.0) (Kanavouras et al., 2007). These factors, astrocyte-specific expression in brain, low basal activity, more acidic optimum, and large increase in activity with ADP stimulation places hGDH2 in an important position to potentially increase glutamate oxidation during energy-demanding processes such as high intensity glutamatergic signaling as speculated by Plaitakis et al. (2000, 2003). To test the effects of hGDH2, Li et al. recently generated mice transgenic for *GLUD2* (Li et al., 2016). They found that hGDH2 expression affects the concentration of transcripts and metabolites involved in glycolysis and the TCA cycle.

These findings are in agreement with the role of GDH as a key enzyme in glutamate homeostasis mediating glutamate degradation to CO_2 and supporting anaplerosis by a net production of the TCA cycle intermediate α-ketoglutarate (McKenna et al., 2012). However, it is still unknown how the expression of hGDH2 affects uptake and metabolism of glutamate in astrocytes. The present study was therefore designed to study the significance of hGDH2 for astrocytic uptake of glutamate and specific metabolic pathways important for energy metabolism. Astrocytes were cultured from cerebral cortical tissue of hGDH2-expressing transgenic mice and their wild-type littermates (Li et al., 2016). We find that the expression of hGDH2 has consequences for carbon metabolism particularly during augmented workload and hypoglycemic conditions. In addition, hGDH2 expression reduces anaplerotic input from glucose while increasing that from glutamate and the BCAAs.

Materials and Methods

Materials

Plastic ware for culturing of astrocytes was obtained from NUNC A/S (Roskilde, Denmark) or Becton, Dickinson and Company (Franklin Lakes, NJ). Fetal calf serum (FCS) and OptiMEM were from Gibco, Life Technologies (Carlsbad, CA). Dulbecco's modified Eagle medium (DMEM, D5030) powder (without glucose, glutamine, glutamic acid, and aspartic acid but containing 0.8 mM of each BCAA), dBcAMP (N6,2′-*O*-dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt), *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA), and *N*,*N*-dimethylformamide (DMF) were purchased from Sigma–Aldrich (St. Louis, MO). L-[U-^{13}C]Glutamic acid and D-[U-^{13}C]glucose (both 99% [^{13}C] enriched) were produced by Cambridge Isotopes Laboratories Inc (Andover, MA). L-[U-^{14}C]Glutamic acid (0.1 mCi mL^{-1} or 278.0 mCi mmol^{-1}) and L-[3,4-^{3}H]glutamic acid (1 mCi mL^{-1} or 51.1 Ci mmol^{-1}) were from Perkin Elmer Inc (Waltham, MA). Ecoscint A was from National Diagnostics (Hessle Hull, UK). Chemicals for gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) were acquired from Phenomenex (Torrance, CA). Chromatography columns were purchased from Agilent Technologies (Santa Clara, CA). Micro bicinchoninic acid (BCA) protein assay kit was from Thermo Fisher Scientific (Rockford, IL). The remaining chemicals used were of the purest grade available from commercial suppliers.

The following definitions are used in the methods section: Basic DMEM and culture medium. Basic DMEM was made from reconstituted DMEM powder supplemented with 26.2 mM sodium bicarbonate while culture medium consisted of basic DMEM supplemented with 2.5 mM glutamine, 6 mM glucose, 100 i.u. (international unit)/ml penicillin and FCS.

Methods

GLUD2 transgenic mice (hGDH2). GLUD2 transgenic mice (hGDH2) were generated by random insertion of a bacterial artificial chromosome (BAC) carrying a 176 kb fragment from the human X chromosome containing the *GLUD2* gene into C57BL/6 mice by pronuclear injection (Sparwasser et al., 2004). From 108 potentially transgenic mice, one transgenic line with mRNA Glud2 expression levels 24 times that of humans was maintained and used for the present study. Transgenic lineage was preserved by crossings with wild-type C57BL/6 mice to allow for comparisons with wild-type litter mates. A prerequisite for this was genotyping of all litters by tail DNA extraction from pups and polymerase chain reaction with a BAC-specific primer. The primer used had the following forward and backward sequences, respectively: 5′-TGCGTTC CTTATGCTGTAGT-3′ and 5′-TGGTCACTCTTGTCATACCC-3′. The mice were recently characterized in a paper by Li et al. (2016) and previously described by Bryk (2009).

Primary cultures of murine astrocytes. Cortical astrocytes were prepared and cultured in accordance with Hertz et al. (1989) and (Walls et al., 2014). In short, cerebral cortices were dissected from 7-day-old mice. Single cell suspensions were obtained by mechanical dissociation of the tissue by squeezing it through a nylon sieve (80 μm pore size) into a cell culture medium containing 20% FCS. Subsequently, the cell suspension was passed three times through a 20-mL syringe with a 13G cannula. The cell suspension was aliquoted in 25 cm^2 tissue culture flasks at the density of 0.35 cortices/flask for incubation studies. Cortical astrocytes for glutamate and glucose uptake studies were seeded in 24-well plates (1.5 cortices/plate). When the CO_2 production assay was to be performed, the astrocytes were prepared in six-well plates (1.3 cortices/plate). The culture medium was changed twice a week with weekly 5% reduction in FCS content of the culture medium starting at 20%. Cells were cultured for ∼3 weeks. All cultures were kept in a humidified incubator at 37°C in a mixture of atmospheric air and CO_2 (95/5%). To induce stellation of the astrocytes, dBcAMP (0.25 mM) was added during the last week of culturing (Hertz et al., 1989). The day before experiments, the cell culture medium was changed. The cultures were essentially pure astrocytes with only negligible presence of other cell types. Cultures prepared from 7-day-old mice have been thoroughly characterized regarding astrocytic function and markers and found to be essentially indistinguishable from those classically prepared from newborn mice (Skytt et al., 2010).

Western blot. To verify the presence of hGDH2 in transgenic mice-derived astrocytic cultures, cells were washed once with phosphate-buffered saline (PBS) and collected in a homogenization buffer containing 10 mM Tris HCl, pH 7.4, 0.5 mM EDTA, 1% Triton, 0.5 M NaCl (with protease inhibitors added). Cells were homogenized using a prechilled glass homogenizer at 200 strokes/3 min, on ice. After incubating for 10 min on ice, the whole homogenate was centrifuged for 10 min, at 11,000*g*, 4°C. The supernatant was transferred to a new tube and used for Western blotting. The cell lysate was separated in an 8.5% or 10% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and blotted with an anti-hGDH2 antibody (Zaganas et al., 2012), as well as a commercially available nonspecific anti-GDH antibody raised against full-length bovine GDH (Biodesign International, Saco, ME). The latter antibody is able to identify GDH1 from various sources, including mouse and human GDH1. Also, a commercially available anti-glial fibrillary acidic protein (GFAP) antibody was used as loading control (60 μg/lane) and to confirm the phenotype of the astrocytes. Human testis extract (Zaganas et al., 2012), a tissue that expresses both hGDH1 and hGDH2, was used as a positive control for hGDH2 expression.

Glutamate uptake and metabolism. Before the start of the experiment the astrocytes were deprived of glucose for 45 min in basic DMEM (i.e., DMEM + phenol red and bicarbonate, but without FCS, antibiotics, glucose, and glutamine), followed by 30 min exposure to L-[3,4-^{3}H]glutamate, both at 37°C. Glutamate uptake and utilization was monitored by incubating the astrocytes for 30 min in basic DMEM ± 2.5 mM glucose and L-glutamate ranging in concentration from 100 to 500 μM with a tracer amount of L-[3,4-^{3}H]glutamate (4 μCi mL^{-1} or 51.1 Ci $mmol^{-1}$). The assay was stopped by rapidly washing the astrocytes twice with ice-cold PBS (137 mM NaCl, 2.7 mM KCl, 7.3 mM Na_2HPO_4, 1.5 mM, KH_2PO_4, pH 7.4). Subsequently, the astrocytes were re-suspended in KOH (250 μl, 0.4 M), sealed in the plates, and left overnight at 4°C. The next day, an aliquot (100 μL) of the cell suspension was neutralized with 10% formic acid, mixed with 3.6 mL Ecoscint A, and analyzed on a liquid scintillation counter (Tri-Carb 2900TR, Perkin Elmer, Waltham, MA). The amounts of intracellular glutamate and metabolites thereof were normalized to the protein content in each sample. Protein content was determined in the remaining cell suspension using the BCA method with bovine serum albumin (BSA) as standard.

Determination of $^{14}CO_2$ production. Production of CO_2 from L-glutamate was measured using a method modified from Frigerio et al. (2010). Cells were incubated for 1 h (37°C) in basic DMEM ± glucose (2.5 mM) supplemented with L-glutamate (100 μM) with a tracer amount of L-[U-^{14}C]glutamate (0.1 μCi mL^{-1} or 278.0 mCi $mmol^{-1}$). $^{14}CO_2$ released from the individual wells during the incubation process was absorbed into six pieces of chromatography paper soaked in NaOH (2M) and attached to the lid. Metabolic activity was stopped after 1 h by placing the plates at −20°C. Subsequently, medium from each well was transferred to separate glass vials to which a 1.5 mL Eppendorf tube containing a new piece of chromatography paper soaked in 2 M NaOH was added. The glass vials were sealed individually with a rubber stopper. Using a cannula, 5 M HCl was injected directly into the medium, liberating any trapped $^{14}CO_2$ during 2 h of shaking at 37°C. The corresponding chromatography papers from incubation and shaking were combined for the determination of radioactivity of the trapped $^{14}CO_2$, using a liquid scintillation counter. Following removal of the incubation medium, cell extracts were made by rinsing the astrocytes twice with ice-cold PBS, extraction in 70% ethanol and centrifugation (20,000*g*, 20 min, 4°C) to separate the soluble extract (supernatant) from insoluble components (pellet). The pellet was re-suspended in 0.4 M KOH. The amount of $^{14}CO_2$ produced was

corrected for total protein content determined in the 0.4 M KOH cell suspension by the BCA method using BSA as a standard.

Incubation of astrocytes with [U-^{13}C]glucose and [U-^{13}C]-glutamate. The medium was discarded and astrocytes were rinsed twice with PBS supplemented with 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$. The astrocytes were incubated for 2 h at 37°C in 3.5 mL basic DMEM supplemented with 100 μM L-[U-^{13}C]glutamate ± 2.5 mM unlabeled glucose. Because of the efficient uptake of glutamate into astrocytes it is necessary to add additional L-[U-^{13}C]glutamate in order to maintain the extracellular concentration close to 100 μM throughout the two hours of incubation. The cultures of astrocytes were spiked with 17.5 μL L-[U-^{13}C]glutamate (20 mM stock) one hour into the incubation. The amount of L-[U-^{13}C]glutamate used to spike the medium is equivalent to a final concentration of 100 μM L-[U-^{13}C]glutamate in addition to the remainder of the 100 μM present from the start of the incubation. Glucose metabolism was studied by incubating the astrocytes in basic DMEM containing 2.5 mM D-[U-^{13}C]glucose without any additional addition of substrate after the first hour. After 2 h of incubation the medium was collected and the astrocytes were rinsed twice with ice-cold PBS. Subsequently, the astrocytes were extracted in 70% ethanol and centrifuged at 20,000*g* for 20 min at 4°C. The supernatant containing the cell extract was kept for further analysis and the pellet was used for protein determination by the Lowry method (Lowry et al., 1951) using BSA as a standard. Media and cell extracts were freeze dried and reconstituted in water for analysis by HPLC and GC-MS.

Determination of amino acid content. The amino acid content of selected amino acids (glutamate, glutamine, aspartate, valine, isoleucine, and leucine) in the cellular extracts was determined by reversed-phase HPLC using LC-10ADVP liquid chromatograph coupled to an RF-10AXL fluorescence detector (Shimadzu). Samples were derivatized online with o-phthaldialdehyde (OPA) before injection to the column and amino acids were detected by fluorescence (excitation $\lambda = 350$ nm, emission $\lambda = 450$ nm). An Agilent Eclipse AAA column (4.6 mm × 150 mm, pore size 5 μm) was used to separate the amino acids employing a mobile phase gradient based on mobile phase A (0.04 M citrate, 0.2 M phosphate, 4.8% acetonitrile, pH 5.9) and mobile phase B (90% acetonitrile). From 4.5 to 16.5 min the contribution of mobile phase B increased from 0 to 7% and from 7 to 50% from 16.5 to 35 min, all linearly. Contribution of mobile phase B was reduced to 0% from 36 min to 38 min. All of the amino acids of interest eluted within 35 min.

Metabolic mapping by GC-MS. The samples for GC-MS analysis were prepared essentially as described by Walls et al. (2014). In short, the aliquoted samples and standards were adjusted to pH 1–2 with HCl and dried under nitrogen flow. Analytes were then extracted into an organic phase of ethanol/benzene and again dried under nitrogen flow. Derivatization of the analytes of interest (amino acids, keto acids, and lactate) was done using MTBSTFA in the presence of 15% DMF (modified after Mawhinney et al. 1986). The metabolites of interest in samples and standards were analyzed

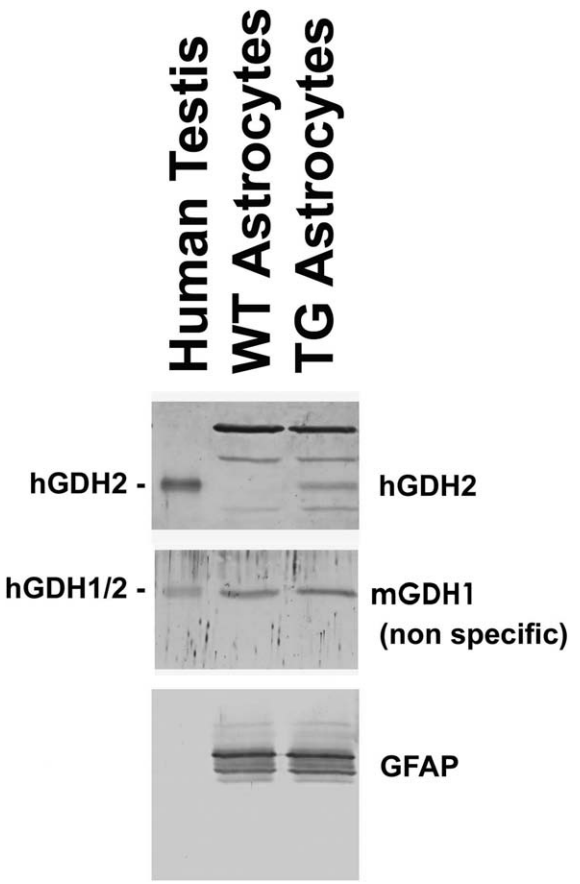

FIGURE 1: Western blots of the glutamate dehydrogenase isoenzymes (mGDH1 and hGDH2) in astrocytes from *GLUD2* transgenic mice (TG) and their wild type littermates (WT). Human testis was used as control for hGDH2. A non-specific antibody recognizing mGDH1 as well as hGDH2 was used to detect GDH in both TG and WT cultures and a hGDH2-specific antibody was used to determine the existence of hGDH2 in the *GLUD2* transgenic mice. Note that under these running conditions, the mGDH1 (wild-type and transgenic astrocytes), hGDH1 (human testis) and hGDH2 (human testis, transgenic astrocytes) proteins run together and the 2 KDa difference in molecular weight between hGDH1 and hGDH2 (Zaganas et al., 2012) is not observed. GFAP staining confirmed the phenotype of the cells and served as a loading control (60 μg/lane). The extra bands in the hGDH2 blots are nonspecific binding as they show also in WT mice, and thus are not hGDH2 but cross-reacting effects.

in a Shimadzu GC-2010 chromatograph (Zebron ZB-5MS column, parts no. 7CD-G010-08) coupled to Shimadzu GC-MS-Q2010plus mass spectrometer. Isotopic enrichment was corrected for natural abundance of ^{13}C in the standards and calculated according to Biemann (1962) and Walls et al. (2014). Isotopic enrichment is presented as percentage distribution of all possible isotopomers of a particular metabolite. Total molecular carbon labeling (MCL) is calculated by multiplying the labeling (%) of the different isotopomers of a compound with the number of labeled atoms in the isotopomer, summing these products, and dividing them by the total number of carbon atoms in the relevant compound as described by Walls et al. (2014). The data from the different isotopomers will be presented as

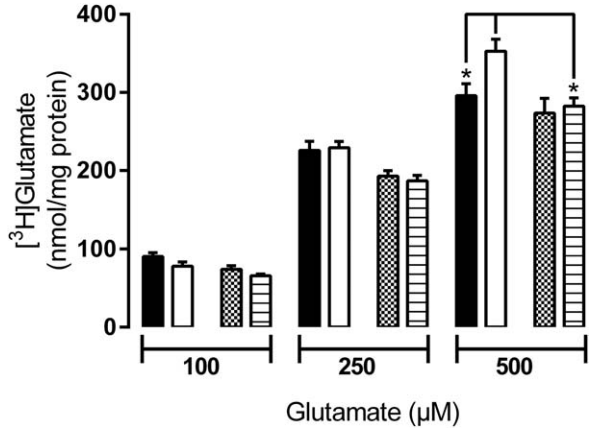

FIGURE 2: hGDH2-expressing astrocytes exhibit an elevated [^{3}H]glutamate uptake and metabolism compared to controls under normoglycemic conditions during exposure to high extracellular glutamate concentration. The [^{3}H]glutamate uptake was calculated by relating the Ci count of the cell suspensions from each condition to the ratio of labeled to unlabeled glutamate present in the medium using the specific activity of the isotope to convert the Ci count to nmol of [^{3}H]glutamate. Black bars: controls in presence of 2.5 mM glucose; white bars: hGDH2 in presence of 2.5 mM glucose; chequered bars: controls in absence of glucose; striped bars: hGDH2 in absence of glucose. *N* = 6–18 from three to six individual cell batches. Two-way ANOVA with Holm–Sidak post-test. * denotes $P \leq 0.05$ for indicated comparisons.

M + X, where X is the number of labeled atoms in the respective isotopomer and M is the molecular mass of the unlabeled molecule.

Statistical Analysis

All statistical analyses were performed in GraphPad Prism 6.0 software. Unpaired Student's *t* test was used when comparing two sets of data. If three or more groups of data were analyzed, one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was performed. For analysis of the effect of multiple variables, two-way ANOVA with Holm–Sidak post-test was performed. The established cut-off value for statistically significant difference was $P \leq 0.05$ and indicated by an asterisk (*). Results are presented as mean value ± standard error of the mean (SEM).

Results

Western Blots

To verify expression of mouse GDH1 (mGDH1) and human GDH2 (hGDH2) in the astrocytes cultured from the transgenic mice, Western blots were made on cell lysates. Astrocytes cultured from wild type (WT) littermates (controls) only express mGDH1, while the astrocytes cultured from cerebral cortex from the transgenic (TG) animals express both mGDH1 and hGDH2 (Fig. 1).

Glutamate Uptake and Metabolism

To obtain a direct and total measure of the importance of hGDH2 expression for astrocytic glutamate uptake and utilization a series of experiments were performed using astrocyte cultures expressing hGDH2 in addition to mGDH1 and corresponding controls (expressing only mGDH1). The astrocytes were incubated in media containing increasing concentrations of [^{3}H]glutamate (range 100–500 μM) either in the absence or presence of glucose (2.5 mM). Glutamate uptake and metabolism increased as a function of the glutamate concentration in the incubation media in all experimental groups (Fig. 2). However, in the hGDH2-expressing astrocytes a significant increase in glutamate uptake and metabolism was observed compared to controls at an extracellular glutamate concentration of 500 μM in the presence of glucose. This difference was not seen when glucose was removed (Fig. 2).

At the high concentration (500 μM) of glutamate the energy requiring glutamate uptake presumably causes the ADP level to increase, a condition activating hGDH2, thereby increasing glutamate metabolism and promoting uptake. The simultaneous presence of glucose likely results in a GTP level sufficient to inhibit GDH1 whereas hGDH2 is resistant to GTP mediated inhibition. The augmentation of glutamate

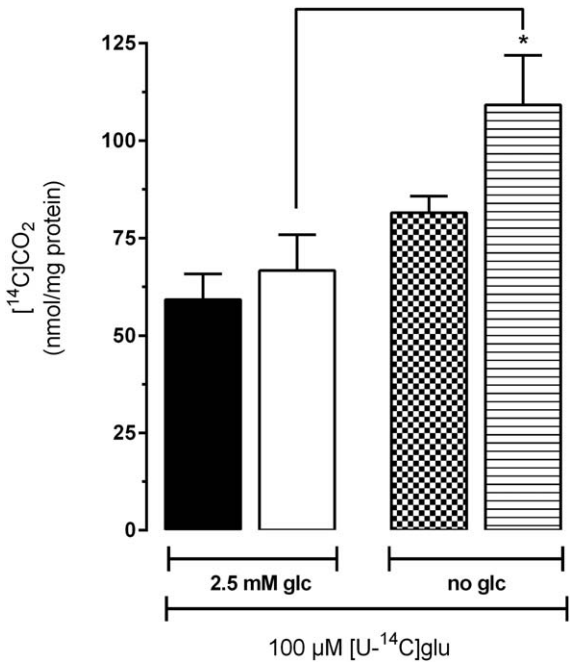

FIGURE 3: hGDH2-expressing astrocytes increase [^{14}C]CO_2 production in aglycemic conditions during exposure to 100 μM extracellular [U-^{14}C]glutamate. All incubation media contained 0.8 mM of each BCAA in addition to the indicated substrates (glutamate ± glucose). Black bars: controls in presence of 100 μM [U-^{14}C]glutamate and 2.5 mM glucose; white bars: hGDH2 in presence of 100 μM [U-^{14}C]glutamate and 2.5 mM glucose; chequered bars: controls in presence of 100 μM [U-^{14}C]glutamate and absence of glucose; striped bars: hGDH2 in presence of 100 μM [U-^{14}C]glutamate and absence of glucose. *N* = 4–16 from three to eight individual cell batches. Glc: glucose; Glu: glutamate. Two-way ANOVA with Holm–Sidak post-test. * denotes $P \leq 0.05$ for indicated comparisons.

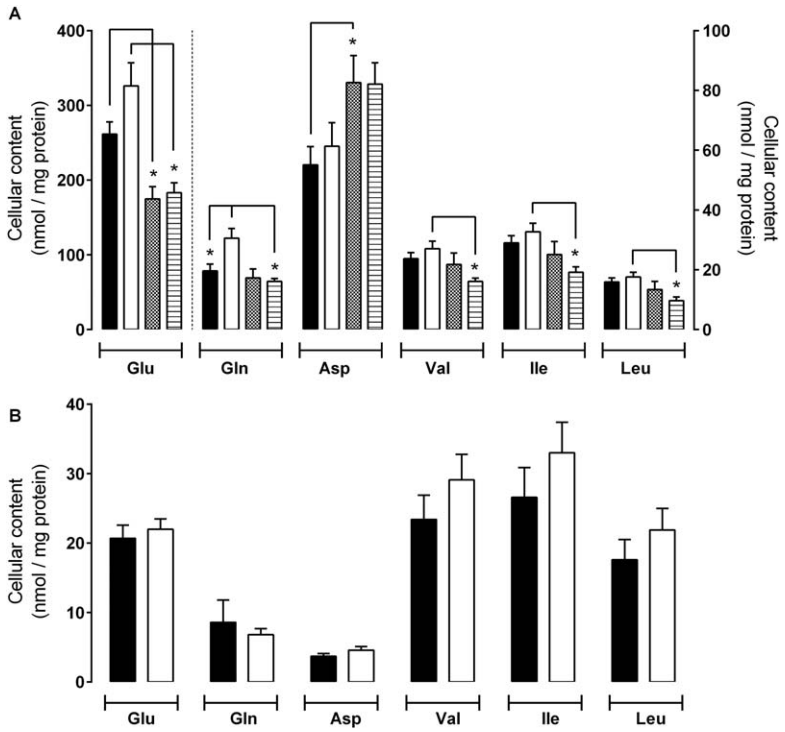

FIGURE 4: hGDH2-expressing astrocytes reduce intracellular BCAA content during aglycemic conditions compared to corresponding controls. A: Amino acid amounts in control and hGDH2-expressing astrocytes in the presence of glutamate with or without the addition of glucose. All incubation media contained 0.8 mM of each BCAA in addition to the indicated substrates (glutamate and/or glucose). Left axis: Glu; Right axis: Gln, Asp, Val, Ile, and Leu; black bars: controls in presence of 100 μM glutamate and 2.5 mM glucose; white bars: hGDH2 in presence of 100 μM glutamate and 2.5 mM glucose; chequered bars: controls in presence of 100 μM glutamate and absence of glucose; striped bars: hGDH2 in presence of 100 μM glutamate and absence of glucose. B: Amino acid amounts in control and hGDH2-expressing astrocytes maintained in the presence of glucose. All incubation media contained 0.8 mM of each BCAA in addition to glucose. Black bars: controls in presence of 2.5 mM glucose; white bars: hGDH2 in presence of 2.5 mM glucose. $N = 6–12$ from three to five individual cell batches. Glu: glutamate; Gln; glutamine; Asp: aspartate; Val: valine; Ile: isoleucine; Leu: leucine. A: Two-way ANOVA with Holm–Sidak post-test. * denotes $P \leq 0.05$ for indicated comparisons. B: Student's *t* test.

uptake in the astrocytes expressing hGDH2 was abolished in the absence of glucose where both isozymes are activated by ADP. It should be noted, that the level of glutamate uptake was sustained in the absence of glucose in control astrocytes underlining that glutamate is able to fuel its own uptake.

Tritiated glutamate, once taken up by the astrocytes, is to a certain extent metabolized and thus tritium will appear in TCA cycle intermediates as well as aspartate, glutamine and NADH/$FADH_2$. The tritium labeling of metabolites can only occur upon uptake of tritiated glutamate and therefore does not represent any error in the calculation of uptake capacity. However, tritium may be lost from the astrocytes via loss of metabolites, e.g., lactate, glutamine, citrate, and H_2O as they are exported from the astrocytes or osmotically equilibrates with the medium (Waagepetersen et al., 2001). Such loss of radioactivity may cause an underestimate of the glutamate uptake.

To obtain information about the metabolic fate of glutamate subsequent to its uptake, astrocyte cultures were incubated with ^{14}C-radiolabeled glutamate (100 μM) to assess the extent to which it was oxidized to carbon dioxide. hGDH2-expressing astrocytes exhibited an increased $^{14}CO_2$ production upon glucose removal not seen in control astrocytes (Fig. 3). These observations are compatible with a relatively more extensive activation of hGDH2 by an increase in ADP resulting from the lack of glucose as an energy substrate and a very low basal activity of hGDH2. Our finding may seem in conflict with the increased glutamate uptake observed in the presence of glucose. However, this increase was only significant during extreme workloads (500 μM glutamate) which can be assumed to cause elevated ADP levels (Fig. 2).

The Cellular Contents of Amino Acids

The intracellular levels of glutamate and aspartate are linked through the action of aspartate aminotransferase (AAT). This enzyme facilitates the formation of aspartate by transamination of oxaloacetate formed via TCA cycle metabolism of α-ketoglutarate originating from deamination of glutamate. The BCAAs are available in the incubation medium (0.8 mM each) and we have previously shown that GDH expression

affects metabolism of these amino acids (Nissen et al., 2015). No significant differences were seen between hGDH2-expressing astrocytes and corresponding controls regarding cellular contents of glutamate, aspartate, or the BCAAs when incubated in the presence of glutamate with or without the addition of glucose (Fig. 4A), or in the presence of glucose only, where glutamine, in addition to the already mentioned amino acids, was not affected either (Fig. 4B). The cellular content of glutamine was, however, increased in the hGDH2-expressing astrocytes compared to controls when incubated in the presence of glutamate and glucose (Fig. 4A). When glucose was removed, glutamine levels decreased to similar levels in hGDH2-expressing astrocytes and corresponding controls indicating a specific activation of hGDH2 during energy deprivation.

Upon glucose withdrawal, inter-group differences were observed in hGDH2-expressing astrocytes as well as in their respective controls (Fig. 4A). This effect of glucose withdrawal was seen as an increase in the intracellular level of aspartate and a decrease in the intracellular glutamate level. The increase in the aspartate level upon glucose removal in hGDH2 (not significant) and their respective controls is indicative of increased truncated TCA cycle activity [the conversion of glutamate to α-ketoglutarate by AAT catalyzed transamination of oxaloacetate, formed via TCA cycle metabolism of α-ketoglutarate, to aspartate (Westergaard et al., 1996)] due to low glucose availability. Interestingly, glucose removal resulted in a decreased cellular content of BCAAs in hGDH2-expressing astrocytes indicating an increased utilization of these amino acids. In contrast, no difference was seen in the corresponding controls (Fig. 4A) indicating that hGDH2 is involved in an increased utilization of BCAAs.

Metabolism of [U-^{13}C]Glutamate

In the presence of glucose. The measurements of uptake of tritiated glutamate and $^{14}CO_2$ production from L-[U-^{14}C]glutamate provide no information about the particular pathways involved in metabolism of glutamate. To specifically obtain information about metabolic pathways for glutamate, astrocytes were incubated with L-[U-^{13}C]glutamate (100 μM) in the presence of glucose for 2 h and cell extracts were analyzed for ^{13}C labeling of metabolites using GC-MS. The labeling patterns of TCA cycle metabolism of L-[U-^{13}C]glutamate are illustrated in Figure 5.

No significant difference in amount (%) of M + 5 labeled glutamate was found between hGDH2-expressing astrocytes and controls (Fig. 6A). Thus, any differences in amount (%) of labeling seen in the TCA cycle intermediates and other metabolites do not originate from lower labeling of the intracellular substrate pool of L-[U-^{13}C]glutamate obtained by uptake from the incubation medium. No change was seen in the amount (%) of M + 5 labeled glutamine (Fig. 6B). However, the amount of glutamine was higher in the hGDH2-expressing astrocytes suggesting that expression of hGDH2 facilitates synthesis of glutamine since the unchanged labeling (%) combined with an increased intracellular glutamine amount translates to an absolute increase in the amount of the isotopomer.

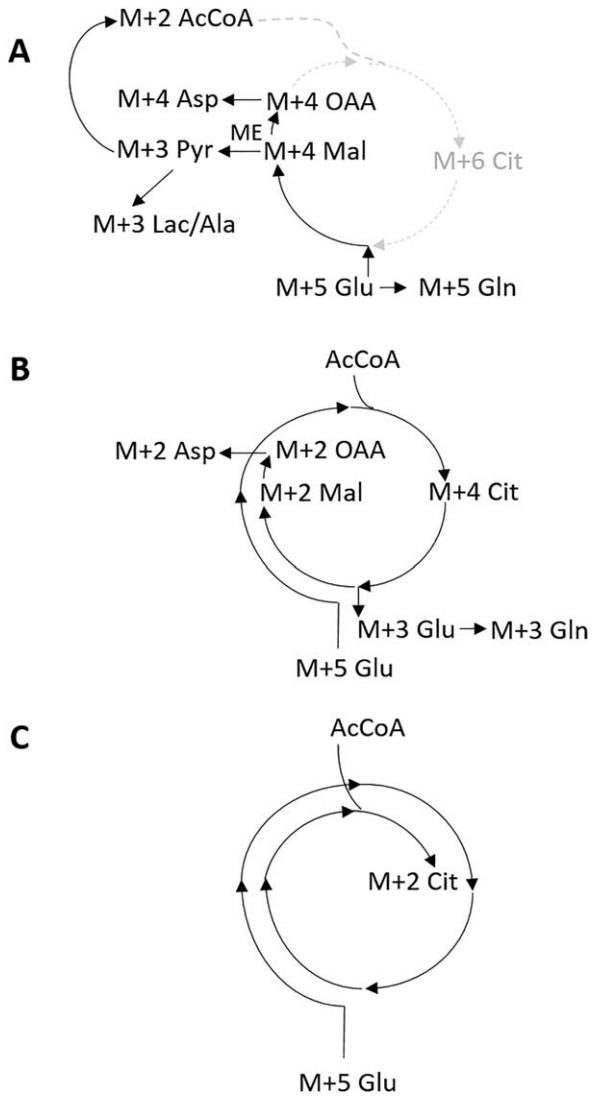

FIGURE 5: A: Illustration of pathway for direct metabolism and partial and full pyruvate recycling giving rise to M + 3 pyruvate/lactate/alanine or M + 6 citrate, respectively. B: Illustration of the pathway for 1st-turn metabolism. C: Illustration of the pathway for 2nd-turn metabolism. Details are described in the text.

The carbon skeleton of glutamate entering the TCA cycle as α-ketoglutarate results in production of M + 4 oxaloacetate (Fig. 5A). The amino group of glutamate can be transferred to oxaloacetate resulting in M + 4 aspartate by the activity of AAT. In hGDH2-expressing astrocytes, such metabolism of glutamate leading to production of M + 4

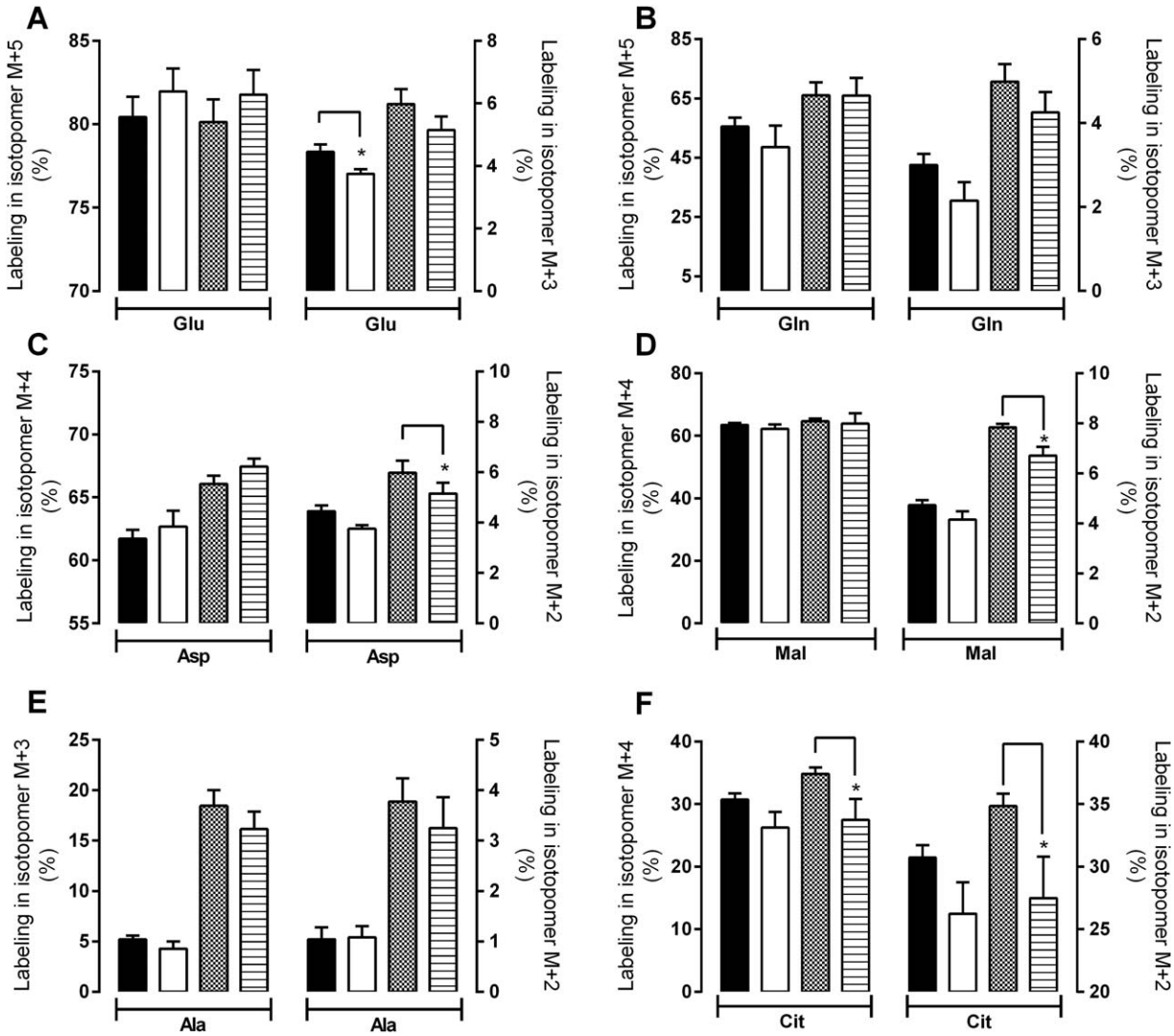

FIGURE 6: hGDH2-expressing astrocytes decrease amount (%) of labeling in intracellular metabolites from oxidative metabolism of [U-^{13}C]glutamate in the absence of glucose. Labeling pattern in selected amino acids and TCA cycle intermediates following incubation with 100 μM glutamate ± 2.5 mM glucose in hGDH2 and corresponding controls. All incubation media contained 0.8 mM of each BCAA in addition to the indicated substrates (glutamate ± glucose). A: Amount (%) of M + 5 and M + 3 labeled glutamate. B: Amount (%) of M + 5 and M + 3 labeled glutamine. C: Amount (%) of M + 4 and M + 2 labeled aspartate. D: Amount (%) of M + 4 and M + 2 labeled malate. E: Amount (%) of M + 3 and M + 2 labeled alanine. F: Amount (%) of M + 4 and M + 2 labeled citrate. The origin and meaning of the labeling patterns are explained in the text. Black bars: controls in presence of 100 μM glutamate and 2.5 mM glucose; white bars: hGDH2 in presence of 100 μM glutamate and 2.5 mM glucose; chequered bars: controls in presence of 100 μM glutamate and absence of glucose; striped bars: hGDH2 in presence of 100 μM glutamate and absence of glucose. Glu: glutamate; Gln; glutamine; Mal: malate; Asp: aspartate; Ala: alanine; Cit: citrate. $N = 6$–11 from three to four individual cell batches. Two-way ANOVA with Holm–Sidak post-test. * denotes $P \leq 0.05$ for indicated comparisons.

malate and aspartate showed no significant differences from those of their corresponding controls (Fig. 6C and D). Alanine M + 3 results from alanine aminotransferase (ALAT) catalyzed conversion of M + 3 pyruvate generated via conversion of M + 4 malate through partial pyruvate recycling via malic enzyme (ME) (Fig. 5A). hGDH2-expressing astrocytes showed no difference in M + 3 alanine (Fig. 6E) or M + 3 lactate, the latter derived from lactate dehydrogenase (LDH) catalyzed reduction of M + 3 pyruvate (Fig. 5A) (results not shown). The direct metabolism results in M + 4 labeled metabolites among which M + 4 labeled oxaloacetate initiates the 1st-turn of TCA cycle metabolism of L-[U-^{13}C]glutamate by condensation with unlabeled acetyl-CoA (Fig. 5B). In hGDH2-expressing astrocytes reduced amount (%) of labeling in 1st-turn metabolites was only significant for M + 3 labeled glutamate (Fig. 6A) although a tendency was seen in the remaining 1st-turn metabolites (M + 3 glutamine, M + 2 aspartate, M + 2 malate and M + 4 citrate) (Fig. 6B–D and F). Alanine M + 2 formed from M + 2 pyruvate derived from M + 2 malate was unchanged in hGDH2 astrocytes

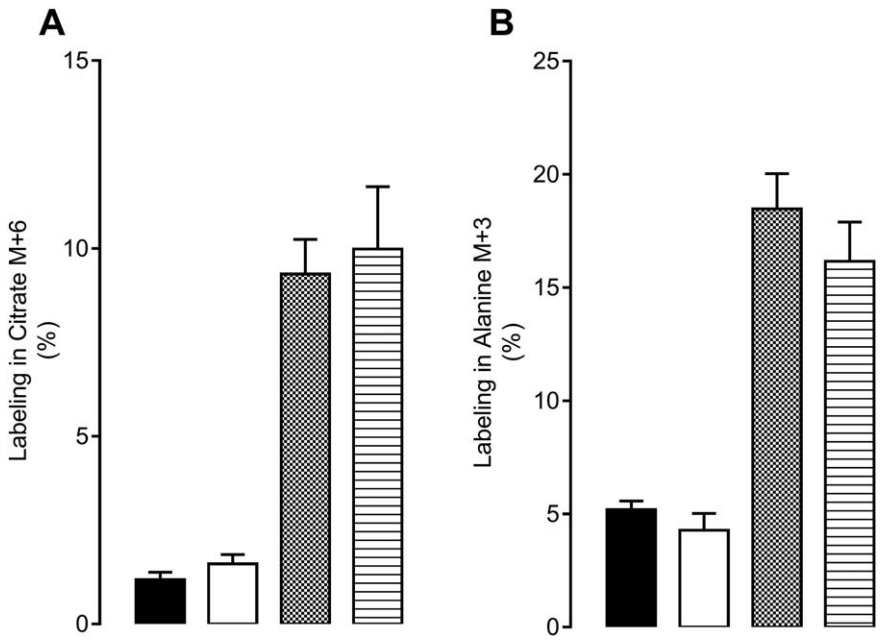

FIGURE 7: Pyruvate recycling is not affected by hGDH2 expression in astrocytes compared to controls when incubated with 100 μM glutamate regardless of the presence or absence of glucose. Amount (%) of labeling in different metabolites from oxidative metabolism of [U-^{13}C]glutamate. All incubation media contained 0.8 mM of each BCAA in addition to the indicated substrates (glutamate ± glucose). A: Amount (%) of M + 6 labeled citrate. B: Amount (%) of M + 3 labeled alanine. The origin and meaning of the labeling patterns are explained in the text. Black bars: controls in presence of 100 μM glutamate and 2.5 mM glucose; white bars: hGDH2 in presence of 100 μM glutamate and 2.5 mM glucose; chequered bars: controls in presence of 100 μM glutamate and absence of glucose; striped bars: hGDH2 in presence of 100 μM glutamate and absence of glucose. Ala: alanine; Cit: citrate. *N* = 6–11 from three to five individual cell batches. Two-way ANOVA with Holm-Sidak post-test.

compared to controls (Fig. 6E). In hGDH2 astrocytes the tendency toward a decline in 1st-turn metabolites was reflected in the 2nd-turn metabolite citrate M + 2 (Fig. 5C) compared to the corresponding control (Fig. 6F). The alteration of glutamate oxidation in hGDH2-expressing astrocytes may be due to a lower glucose oxidation and entry of acetyl-CoA in the TCA cycle.

In the absence of glucose. Because of the activation of GDH1 and hGDH2 by ADP the importance of GDH during glucose deprivation was also assessed as this condition is believed to increase ADP levels. The incubations described above were repeated in the absence of glucose. In hGDH2-expressing astrocytes, glucose removal resulted in a significantly decreased amount (%) of labeling compared to controls in 1st-turn TCA cycle metabolites (M + 2 aspartate, M + 2 malate and M + 4 citrate) as well as the 2nd-turn metabolite M + 2 citrate (Fig. 6C,D and F). Both control and hGDH2-expressing astrocytes seem to rely on the truncated TCA cycle to the same degree during glucose deprivation as seen from the unchanged intracellular amounts of glutamate and aspartate (Fig. 4) and unchanged amount (%) of labeling in metabolites resulting from metabolism of M + 5 α-ketoglutarate to M + 4 aspartate and malate, and M + 3 alanine (Fig. 6C–E). The apparent decrease in oxidative glutamate metabolism during glucose deprivation may be due to a more effective utilization of alternative substrates such as the BCAAs explaining the decrease seen in the cellular content of the BCAAs in hGDH2-expressing astrocytes upon glucose removal (Fig. 4A). An elevated level of ADP during glucose deprivation activates particularly hGDH2 thereby potentially increasing the catabolism of the BCAAs which enter the TCA cycle via succinyl-CoA and/or acetyl-CoA.

Pyruvate recycling. Formation of M + 6 citrate and M + 3 alanine results from full and partial pyruvate recycling, respectively (Fig. 5A). In hGDH2-expressing astrocytes the unchanged amount (%) of labeling in these isotopomers of citrate and alanine compared to controls indicates similar levels of pyruvate recycling (Fig. 7A and B). The increase in amount (%) of M + 3 labeled alanine and M + 6 labeled citrate following glucose removal is due to less dilution from the production of unlabeled pyruvate when glucose is not available as a cosubstrate.

Metabolism of [U-^{13}C]glucose

As demonstrated by Li et al. (2016), hGDH2 expression mainly seems to affect carbon metabolism via effects on the TCA cycle which is pivotal in the oxidative metabolism of glucose as well as glutamate. To assess the effect of hGDH2 expression on glucose metabolism, astrocytes were incubated

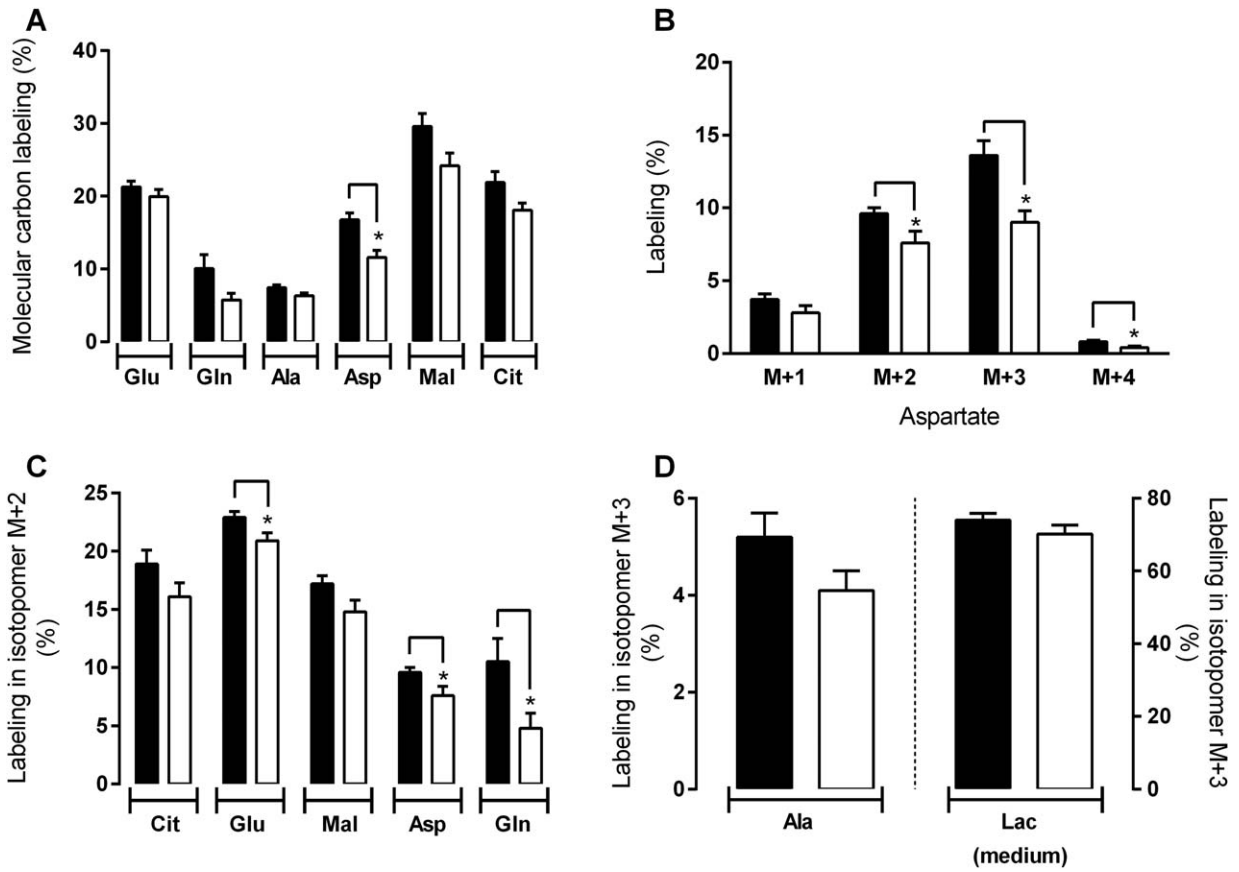

FIGURE 8: hGDH2-expressing astrocytes reduce oxidative but preserve glycolytic metabolism of glucose during incubation with 2.5 mM [U-^{13}C]glucose. All metabolites were measured in cell extracts except for lactate which was measured in the incubation medium. All incubation media contained 0.8 mM of each BCAA in addition to glucose. A: Molecular carbon labeling in metabolites of [U-^{13}C]glucose. B: Amount (%) of labeling of isotopomers of aspartate. C: Amount (%) of labeling in 1st-turn TCA cycle metabolites from incubation with [U-^{13}C]glucose. D: Amount (%) of labeling in glycolytically derived metabolites of [U-^{13}C]glucose. The origin and meaning of the labeling patterns are explained in the text. Black bars: controls in presence of 2.5 mM [U-^{13}C]glucose; white bars: hGDH2 in presence of 2.5 mM [U-^{13}C]glucose. Glu; glutamate; Gln: glutamine; Ala: alanine; Asp: aspartate; Mal: Malate; Cit: citrate. $N = 6$–12 from three to four individual cell batches. Student's t test. * denotes $P \leq 0.05$ for indicated comparisons.

in a medium containing [U-^{13}C]glucose with no exogenous glutamate. Labeling patterns in metabolites were subsequently analyzed by GC-MS. In hGDH2-expressing astrocytes a tendency towards a decrease in MCL (%) of metabolites was seen though only significant for aspartate (Fig. 8A) suggesting a reduced oxidative metabolism of glucose. The hGDH2-expressing astrocytes displayed a decreased amount (%) of labeling in aspartate resulting mainly from reduced amounts (%) of M + 2 and M + 3 labeling (Fig. 8B). Labeling intensities (%) of 1st-turn TCA cycle derived metabolites (M + 2) were significantly reduced in hGDH2-expressing astrocytes compared to controls (Fig. 8C). These 1st-turn TCA cycle derived metabolites (M + 2) are derived from condensation of M + 2 acetyl-CoA with unlabeled oxaloacetate and subsequent metabolism in the TCA cycle. Further, unchanged amount (%) of M + 3 labeled lactate and alanine (Fig. 8D) indicates that hGDH2 expression preferentially affects TCA cycle metabolism of glucose.

These findings point to a decreased total oxidative metabolism of glucose in hGDH2-expressing astrocytes compared to controls. The ratio of M + 3 and M + 2 aspartate is indicative of the level of pyruvate carboxylation versus oxidative decarboxylation. This ratio, calculated from Figure 8B, was significantly lower ($P = 0.024$) for hGDH2-expressing astrocytes (1.187 ± 0.058) compared to controls (1.410 ± 0.061) indicating that the need for anaplerosis via pyruvate carboxylation is reduced due to an augmented entrance of carbon skeleton into the TCA cycle from other sources, e.g. the BCAAs.

Summary of Results

Our findings are summarized in Figure 9 showing the metabolic pathways affected in hGDH2 expressing astrocytes. In short, we find that hGDH2 expression affects glucose metabolism by decreasing the flux through PDH and entry of glucose derived pyruvate into the TCA cycle for oxidative

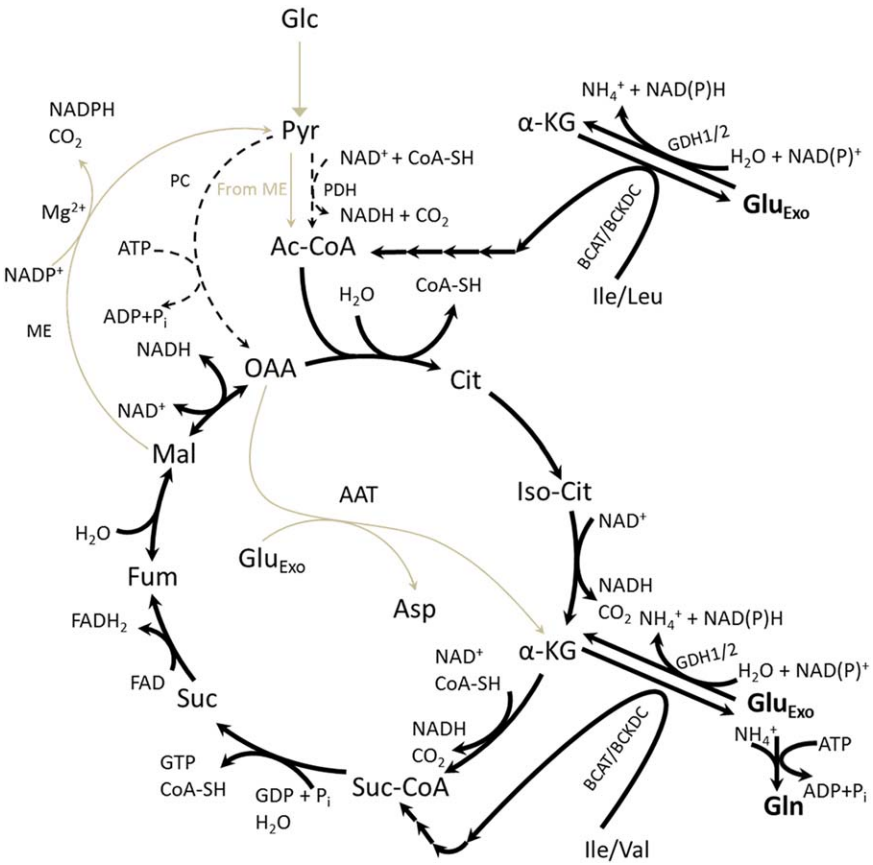

FIGURE 9: Illustration of metabolic pathways in mouse astrocytes affected by hGDH2 expression. Thick lines indicate increased flux through the pathway/enzyme and dashed lines indicate decreased flux. Grey lines indicate unaffected reactions. hGDH2 expression affects glucose metabolism by decreasing the flux through PDH and entry of glucose derived pyruvate into the TCA cycle for oxidative metabolism. Likewise, the flux through PC is decreased in hGDH2-expressing astrocytes. Glutamate metabolism is affected by an increased influx of glutamate into the TCA cycle via mGDH1 + hGDH2 activity and unchanged AAT activity and pyruvate recycling. Additionally, an increase in utilization of BCAAs (Ile, Leu, and Val) was seen in hGDH2-expressing astrocytes in the absence of glucose as a substrate. Lastly, hGDH2 expression increased amidation of glutamate to glutamine in the presence of glucose. PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; GDH, glutamate dehydrogenase; AAT, aspartate aminotransferase; ME, malic enzyme; BCAT, branched-chain amino acid aminotransferase; BCKDC, branched-chain α-ketoacid dehydrogenase complex. Glc: glucose, Pyr: pyruvate, Ac-CoA: acetyl-CoA, Cit: citrate, Iso-Cit: isocitrate, α-KG: α-ketoglutarate, Suc-CoA: succinyl-CoA, Suc: succinate, Fum: fumarate, Mal: malate, OAA: oxaloacetate, Glu: glutamate, Gln: glutamine, Ile: isoleucine; Leu: leucine; Val: valine. Exo denotes exogenously derived compounds. [Color figure can be viewed at wileyonlinelibrary.com]

metabolism. Likewise, the flux through PC is decreased in hGDH2-expressing astrocytes. Glutamate metabolism is affected by an increased influx of glutamate into the TCA cycle via mGDH1 + hGDH2 activity and unchanged AAT activity and pyruvate recycling. Additionally, an increase in utilization of BCAAs (Ile, Leu, and Val) was seen in hGDH2-expressing astrocytes in the absence of glucose as a substrate. Lastly, hGDH2 expression increased amidation of glutamate to glutamine in the presence of glucose.

It should be noted that the transgenic model is based on random insertion of the transgene and we cannot exclude the possibility that the observed metabolic alterations are due to deleterious effects of the insertion on other genes. However, Li et al. (2016) have shown consistent effects of hGDH2 insertion in two different transgenic lines and the metabolic alterations observed in the present study are in line with the expected effects of hGDH2 expression.

Discussion

Western blot analysis demonstrated that astrocytes cultured from cerebral cortex of the transgenic mouse strain expressed both isoforms of GDH (mGDH1 and hGDH2) in contrast to the astrocytes from wild type animals which as expected only expressed the mGDH1 isoform, which is highly homologous to hGDH1. This clearly demonstrates that the astrocyte preparations can be utilized to study how expression of hGDH2 alters the handling of glutamate and TCA cycle metabolism/capacity in astrocytes. The presence of hGDH2 in the transgenic mice was also confirmed by the characterization study by Li et al. (2016). Hence, this experimental

model provides a means to obtain information about glutamate metabolism and homeostasis in the human brain in which both isoforms of hGDH are expressed (Plaitakis et al., 1984; Spanaki et al., 2010).

hGDH2 Expression Increases Astrocytic Capacity for Glutamate Uptake and Metabolism

In agreement with multiple previous studies on astrocyte cultures we found high capacity for glutamate uptake and metabolism (for references, see Danbolt, 2001; McKenna et al., 1996; Sonnewald et al., 1997). The expression of hGDH2 augmented the capacity of the astrocytes to take up and utilize glutamate at high extracellular glutamate concentration (500 μM), a condition likely elevating the ADP level due to the workload on the glutamate transporters and the Na^+/K^+ ATPase (Fig. 9). It may be suggested that an increased workload in combination with availability of glucose increases the activity of only hGDH2 and not mGDH1. The availability of glucose sustains TCA cycle metabolism and GTP production which inactivates mGDH1 independent of the presence of ADP (Mastorodemos et al., 2005; Plaitakis et al., 2000).

An augmented capability to metabolize glutamate in hGDH2 expressing astrocytes increases the potential of glutamate to fuel its own uptake and it may reduce the sensitivity of the astrocytes to glucose deprivation. This is supported by the observation that only astrocytes expressing hGDH2 were able to increase the production of CO_2 from glutamate during glucose deprivation. The increased astrocytic glutamate uptake and metabolism caused by expression of hGDH2 is in line with the effects on carbon metabolism observed in the hGDH2 transgenic mice (Li et al., 2016). The role of glutamate as an energy substrate supplying ATP to maintain the capacity for glutamate uptake has recently been discussed by McKenna (2013) and Nissen et al. (2015), and confirmed experimentally by Pajęcka et al. (2015). Genda et al. (2011) showed that GLT-1 is colocalized with both hexokinase-1 and mitochondria suggesting that glutamate uptake may be energetically supported by glycolysis in combination with oxidative phosphorylation and thus, glutamate may be able to support its entire uptake via metabolism in the TCA cycle during periods of low glucose availability (McKenna, 2013; Pajęcka et al., 2015). The increase in glutamate oxidation following glucose withdrawal underscores the potential importance of hGDH2 during energy deprivation and is supported by the specific modes of regulation of hGDH1 and hGDH2 described in multiple studies (Erecinska and Nelson, 1990; Plaitakis et al., 2000; Spanaki et al., 2012; Takagaki et al., 1957).

hGDH2-Expressing Astrocytes Do Not Oxidize Glutamate at the Expense of Glutamine

A significant part of neurotransmitter glutamate taken up by astrocytes is amidated to glutamine in order for the astrocytes to support surrounding neurons with neurotransmitter precursor as part of the glutamate-glutamine cycle (McKenna, 2007). An alteration in the capacity of astrocytes to take up and oxidize glutamate by expression of hGDH2 may potentially affect the availability of glutamate for amidation to glutamine. However, we observed an elevated synthesis of glutamine in the hGDH2-expressing astrocytes. Thus, the increased capacity for oxidation is not at the expense of glutamine synthesis. Instead, the increased uptake supports glutamine synthesis in addition to the increased oxidative glutamate metabolism (Fig. 9). During glucose deprivation hGDH2 is further activated and promotes glutamate oxidation which reduces the availability of glutamate for amidation by the action of glutamine synthetase evident from a decline in the intracellular content of glutamine (Fig. 4A). Thus, in hGDH2-expressing astrocytes glutamate oxidation as well as glutamine synthesis are enhanced, however, during glucose deprivation the increased glutamate oxidation is at the expense of the glutamine pool.

Increased Utilization of the BCAAs in hGDH2-Expressing Astrocytes

We have recently suggested a link between the expression of GDH and utilization of BCAAs where reduction in GDH1 expression resulted in an increased utilization of isoleucine, one of the BCAAs, through a compensatory increased AAT activity and truncated TCA cycle activity (Nissen et al., 2015). In the present study we observe an increased utilization of BCAAs in hGDH2-expressing astrocytes upon glucose removal. However, as expected the utilization of BCAAs is not linked to an elevated truncated TCA cycle in hGDH2-expressing astrocytes. We speculate that the augmented capacity to oxidize glutamate to α-ketoglurate in hGDH2-expressing astrocytes particularly during glucose deprivation increases the transamination of the BCAAs to their corresponding ketoacids while α-ketoglurate is transaminated to glutamate (Fig. 9). The amount (%) of labeling obtained in TCA cycle intermediates and related amino acids from [U-^{13}C]glutamate was lower in hGDH2-expressing astrocytes during glucose deprivation compared to control. This may seem in contrast to the increased CO_2 production, however, it is likely caused by the usage of alternative energy substrates, such as the BCAAs, in line with the reduced cellular content of these amino acids. An increased utilization of BCAAs in the hGDH2-expressing astrocytes could possibly be explained by a close interaction of GDH and enzymes involved in BCAA metabolism. Previous studies have suggested that

glutamate and BCAA metabolism might be closely interconnected by the identification of a metabolon comprised of branched-chain amino acid aminotransferase (BCAT), branched-chain α-ketoacid dehydrogenase complex (BCKDC), and GDH1 in rat tissue (Hutson et al., 2011; Islam et al., 2010). Moreover, the fact that leucine activates both hGDH1 and hGDH2 (Plaitakis et al., 2000; Yielding and Tomkins, 1961) further contributes to the notion of a close relationship between metabolism of glutamate and BCAAs. Lastly, it has previously been shown by Johansen et al. (2007) and Bak et al. (2009) that astrocytes do in fact metabolize BCAAs and BCAAs are transaminated to a considerable extent in astrocytes (Yudkoff, 1997). The increased utilization of BCAAs in the hGDH2-expressing astrocytes seems to be in line with the above mentioned studies describing a metabolon formation of GDH1 with BCAT and BCKDC (Hutson et al., 2011; Islam et al., 2010). However, these studies did not demonstrate the same interaction of hGDH2 with BCAA catabolic enzymes. If such an interaction did in fact exist, glucose deprivation which greatly increases the functional activity of hGDH2 would provide the possibility for an increased oxidation of BCAAs in the TCA cycle. It should be noted though, that the studies of Hutson et al. (2011) were performed by combination of isolated enzymes and relevant substrates. Thus, metabolon formation was never investigated in the simultaneous presence of GDH1, GDH2, BCAT, and BCKDC. Further, the fact that we used intact astrocytes in the present study may also have affected our results compared to those of Hutson et al. (2011). In the present study the hGDH2-expressing astrocytes were exposed to a 2-h incubation with glutamate in the absence of glucose leading to a possible ADP build up in the astrocytes which strongly activates both GDH isozymes. Only partial activation by ADP of hGDH2 was employed in the studies of Hutson et al. (2011) leaving the possibility that full activation might be needed for metabolon formation. It might also be speculated that co-expression of the two GDH isoenzymes enables a synergistic effect on metabolon assembly and activity. Theoretically, both GDH1 and hGDH2 are able to form a metabolon with BCAT and BCKDC, and it could be speculated that the effect of the hGDH2-containing metabolon is only significant in the simultaneous presence of GDH1. Alternatively, only GDH1 actually forms the metabolon but the simultaneous presence of hGDH2 allows for additional formation of α-ketoglutarate by transamination via BCAT thereby increasing the total activity of the GDH1-BCAT-BCKDC metabolon.

Reduced Glucose Oxidation in hGDH2-Expressing Astrocytes

We find a decreased total oxidative metabolism of glucose in hGDH2-expressing astrocytes. Moreover, aspartate M + 3 was specifically reduced indicating attenuated activity of PC (Fig. 9). This may be due to an augmented entrance of carbon skeleton into the TCA cycle from other sources, e.g. amino acids. GDH activity in concert with aminotransferases is pivotal for oxidative catabolism of amino acids providing the TCA cycle with additional intermediates and acetyl-CoA for oxidation. Thus, the expression of hGDH2 may facilitate oxidation of amino acids even in the presence of glucose due to its lack of sensitivity to GTP inhibition (Plaitakis et al., 2011; Spanaki et al., 2012; Zaganas et al., 2012). Interestingly, astrocytes with reduced GDH1 activity displays an opposite metabolic phenotype, i.e. increased glucose metabolism via both oxidative and anaplerotic pathways (Nissen et al., 2015). This confirms the importance of GDH in general for energy homeostasis, particularly anaplerosis.

The above findings of affected carbon metabolism in the hGDH2-expressing astrocytes are in agreement with the study of Li et al. (2016) demonstrating altered concentration profile of glycolytic as well as TCA cycle metabolites in the hGDH2 transgenic mice. These alterations are linked to differential expression of metabolically associated proteins but no functional characterization of the metabolic pathways affected was performed (Li et al., 2016).

In the present study, we conclude that murine astrocytes expressing hGDH2 exhibit an increased capacity for uptake and oxidative metabolism of glutamate, particularly during increased workload and glucose deprivation. In addition, astrocytes expressing hGDH2 exhibit an increased utilization of BCAAs in the absence of glucose as well as a general decrease in oxidative glucose metabolism. We speculate, that the expression of hGDH2 allows the astrocytes to spare glucose and utilize the BCAAs during substrate shortages. These findings support the proposed role of hGDH2 in astrocytes as an important fail-safe during situations of intense glutamatergic activity where it supports glutamate removal as well as energy homeostasis and anaplerosis of TCA cycle intermediates.

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