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In vivo Magnetic Resonance Spectroscopy of cerebral glycogen metabolism in animals and humans

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Abstract

Glycogen serves as an important energy reservoir in the human body. Despite the abundance of glycogen in the liver and skeletal muscles, its concentration in the brain is relatively low, hence its significance has been questioned. A major challenge in studying brain glycogen metabolism has been the lack of availability of non-invasive techniques for quantification of brain glycogen in vivo. Invasive methods for brain glycogen quantification such as post mortem extraction following high energy microwave irradiation are not applicable in the human brain. With the advent of ^{13}C Magnetic Resonance Spectroscopy (MRS), it has been possible to measure brain glycogen concentrations and turnover in physiological conditions, as well as under the influence of stressors such as hypoglycemia and visual stimulation. This review presents an overview of the principles of the ^{13}C MRS methodology and its applications in both animals and humans to further our understanding of glycogen metabolism under normal physiological and pathophysiological conditions such as hypoglycemia unawareness.

Keywords

Brain glycogen; Magnetic Resonance Spectroscopy (MRS); Hypoglycemia; Metabolism; Stress response

Introduction

Glycogen is the most abundant storage form of glucose in the human body. As such, it serves a critical role in supporting cellular function at times of increased energy demand. The relationship between glycogen content in skeletal muscle and its utilization during muscular activity has been studied extensively (Bergström et al. 1967; Cori and Cori 1940), with muscle fatigue being the functional sequel of contraction-associated glycogen depletion. Glycogen metabolism in the liver supports glucose homeostasis of the entire body. In addition, enzymatic pathways involved in glycogen metabolism in the liver participate in intra-hepatic lipid metabolism. Dysregulation of these enzymes has been implicated in liver diseases such as non-alcoholic fatty liver disease (Cao et al. 2013). In contrast to what is known about glycogen metabolism in the liver and skeletal muscle, the role of brain glycogen in cerebral energy metabolism is not well understood. Brain glycogen content is significantly lower than the content in other organs (Roden et al. 2001), which has made it challenging to elucidate its physiological role. However, with the advent of ^{13}C Magnetic Resonance Spectroscopy (MRS), it has become possible to measure glycogen content and turnover during different physiological and pathophysiological states in the living brain non-invasively (Choi et al. 2003; Öz et al. 2009). In this review, we will discuss the structure, metabolism and physiological role of glycogen in general as well as in the central nervous system (CNS). We will also discuss brain glycogen metabolism under different physiological states including exercise, sleep, sleep deprivation and hypoglycemia. This will be followed by a discussion of the principles and limitations of the ^{13}C MRS method and how it has been used to quantify brain glycogen content and assess overall rates of brain glycogen metabolism in different physiological conditions.

Glycogen biochemistry

Glycogen is formed by the polymerization of individual glucose molecules through α 1–4 and α 1–6 glycosidic bonds. The α 1–4 glycosidic bonds constitute linear linkages, and the α 1–6 glycosidic bonds occurring after every 8–10 residues give glycogen its branched structure. Each glycogen molecule contains an estimated 53,000–60,000 glycosyl units (Benarroch 2010; Smythe and Cohen 1991). Glycogen synthase (branching enzyme) and phosphorylase (debranching enzyme) are the two rate limiting enzymes involved in the synthetic and catabolic pathways of glycogen metabolism, respectively. Both enzymes are regulated by phosphorylation but in reciprocal manners. Phosphorylation increases the activity of phosphorylase and decreases that of synthase. Protein phosphatase-1 (PP-1) dephosphorylates both enzymes, leading to increased synthase activity and decreased phosphorylase activity (Brown and Ransom 2007). Glycogen concentration varies markedly in different organs, from 100–500 $\mu\text{mol/g}$ in the liver to 2–12 $\mu\text{mol/g}$ in the CNS (Obel et al. 2012). Studies have demonstrated that glycogen content in the liver and skeletal muscles is very dynamic and changes significantly with physical activity, nutritional status and non-hepatic diseases like diabetes mellitus (Philip et al. 2012; Radziuk and Pye 2001; Magnusson et al. 1992).

CNS glycogen distribution and pathways of utilization

Our understanding of brain glycogen metabolism and how it supports CNS function has been limited until recently by the lack of non-invasive methods to study the brain glycogen content in vivo. Most studies in the past have used either post mortem (Gertz et al. 1985) or samples obtained by biopsy (Castejon et al. 2002; Lowry et al. 1983) to quantify brain glycogen concentration. Both methods have their own effects on brain glycogen content that can confound the measurements made. Brain glycogen is known to undergo rapid degradation post mortem (Hutchins and Rogers 1970), and investigations that relied on this approach are believed to have underestimated brain glycogen content. In addition, the stress induced by handling of the animals prior to sacrifice was shown to affect brain glycogen concentration (Cruz and Dienel 2002). The measurements of samples collected by biopsy are believed to have overestimated the content because the anesthesia required for the procedure frequently promoted glycogen accumulation (Nelson et al. 1968), presumably due to decreased neuronal activity. Despite these limitations, it has been well established that glycogen is found in all regions of the brain and is stored in the astrocytes (Wiesinger et al. 1997; Brown 2004; Swanson 1992; Swanson et al. 1992). Interestingly, the expression of enzymes that support glycogen synthesis has also been demonstrated in rat neurons, but these enzymes are suppressed by phosphorylation under physiological conditions (Vilchez et al. 2007). Failure to suppress the enzymatic machinery required for neuronal glycogen synthesis leads to glycogen accumulation and apoptosis (Vilchez et al. 2007).

On the cellular level, brain glycogen utilization involves complex interactions between astrocytes and neurons (Fig. 1). As glucose crosses the blood brain barrier (BBB) and enters into the astrocyte, it is phosphorylated into glucose-6-phosphate (G6P) which then enters either the glycolytic pathway or the glycogen synthetic pathway via activity of glycogen synthase (Wiesinger et al. 1997). Stored glycogen can be catabolized under the direction of several mediators, including adenosine (Magistretti et al. 1986), adenosine triphosphate (Sorg et al. 1995), cytosolic Ca^{+2} (Hamprecht et al. 1993) and extracellular K^{+} (Subbarao et al. 1995). The end product of glycogen degradation is G6P, but the fate of this molecule remains uncertain. According to one hypothesis, G6P is further metabolized to glucose via glucose-6-phosphatase, which can then leave the astrocyte to provide substrate elsewhere or enter glycolysis (Ghosh et al. 2005; Walls et al. 2009). Another hypothesis suggests that the G6P derived from glycogen is converted to pyruvate and subsequently to lactate (Brown et al. 2004; Tekkök et al. 2005; Sickmann et al. 2009; Suzuki et al. 2011), which is then transported to adjacent neurons by the so-called astrocyte-neuron lactate shuttle for oxidative phosphorylation in neurons (Pellerin and Magistretti 1994; Belanger et al. 2011).

Brain glycogen metabolism under different physiological states

Brain glycogen metabolism is affected by a variety of physiological and pathological stressors. For example, changes in brain glycogen metabolism were reported in response to brain injury (Shimizu and Hamuro 1958), hypoxia (Brucklacher et al. 2002) and ischemia (Folbergrova et al. 1996). In addition, brain glycogen metabolism responds to normal physiological stressors, such as exercise, sleep deprivation and hypoglycemia, as discussed below. Together with the known induction of glycogen breakdown by neurotransmitters that

regulate stress response, such as norepinephrine and vasoactive intestinal peptide (VIP) (Magistretti and Sorg 1993), these data support a role for glycogen in the stress response of the brain.

Exercise

Increased glucose utilization in the brain has been demonstrated in rodents after exhaustive exercise (Vissing et al. 1996), and many studies have shown increased glucose uptake and expression of c-Fos protein, which is a marker of cell activation, in different brain regions with different exercise regimens (Vissing et al. 1996; Soya et al. 2007; Ohiwa et al. 2006). Interestingly, some studies suggest this may be supported by glycogen metabolism. Matsui found a reduction in glycogen and an elevation in lactate in the cortex, hippocampus, hypothalamus, cerebellum and brainstem in response to intense exercise in rodents (Matsui et al. 2011). The reduction in brain glycogen content during exercise is thought to be due to exercise-induced increases in brain catecholamine and serotonin (Pagliari and Peyrin 1995, Gomez-Merino et al. 2001, Matsui et al. 2011).

Sleep/sleep deprivation

Sleep deprivation has been shown to increase glycogen turnover in the brain (Morgenthaler et al. 2009). Brain glycogen levels during slow wave sleep have been shown to increase up to 70 % above waking concentrations in rats (Karnovsky et al. 1983). Conversely, brain glycogen levels are reduced in the caudate nucleus, hippocampus, brainstem (Karadzic and Mrsulja 1969a) and cerebellum (Gip et al. 2002) in sleep deprived rats and in all brain structures in sleep deprived cats (Karadzic and Mrsulja 1969b). This is further supported by the observation that the release of norepinephrine and other neurotransmitters known to increase glycogenolysis is decreased during both rapid eye movement (REM) and non-REM sleep, which would result in unopposed activity of glycogen synthase (Benington and Heller 1995). In addition to changes in CNS neurotransmitters, adrenal glucocorticoid levels are also increased with sleep deprivation. Brain glycogen level has been found to be lower in sleep deprived rats with normal adrenal function when compared to adrenalectomized rats. This points towards a potential role of glucocorticoid in brain glycogenolysis (Gip et al. 2004). Kong et al. demonstrated that sleep deprivation of 12 to 24 h in rats reduces brain glycogen levels by approximately 40 %. This decrease was reversed by recovery sleep of 15 h (Kong et al. 2002). In contrast, in one study, glycogen synthase activity was increased 2.5 fold during sleep deprivation in mice (Petit et al. 2005). Another study has shown that brain glycogen metabolism in mice with sleep deprivation may vary according to genotype (Franken et al. 2003). Data regarding variations in glycogen metabolism during sleep and sleep deprivation in humans is lacking.

Hypoglycemia

Effect of hypoglycemia on brain glycogen has long been the subject of interest (Olsen and Klein 1947) but one of the major limitations in quantification of brain glycogen had been the widespread unavailability of non-invasive techniques. Hypoglycemia was found to increase brain glycogen utilization in cultured astrocytes (Suh et al. 2007) and in rodent models where brain glycogen content was measured following use of high energy microwave irradiation (Herzog et al. 2008) or decapitation in liquid nitrogen (Canada et al. 2011) to

prevent post mortem reductions in content. Similar methods have been applied to determine whether brain glycogen content “supercompensates”, i.e. overshoots above baseline levels, after hypoglycemia, with inconsistent results. Brain glycogen supercompensation was shown to occur in mice recovering from acute as well as recurrent hypoglycemia (Canada et al. 2011), in rats following hypoglycemic coma (Folbergrova et al. 1996) and in rats after repetitive neuroglucopenia (Alquier et al. 2007) using biochemical analyses, but in another study supercompensation in brain glycogen content in rats exposed to recurrent hypoglycemia was not observed (Herzog et al. 2008).

MRS principles, technique and limitations

Nuclear magnetic resonance (NMR) is based on the magnetic properties of nuclei in interaction with magnetic fields. Two main techniques of NMR, magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS), provide complimentary views of living intact organs. MRI readily provides anatomical and functional information whereas MRS supplies insights into tissue metabolism, including assessments of energy balance and enzyme kinetics. In vivo MRS can be performed using several nuclei such as ^1H , ^{13}C , ^{15}N , ^{17}O , ^{19}F , ^{23}Na , and ^{31}P . Each NMR active nucleus provides unique MR spectral patterns informing different aspects of physiology and metabolism, for example, ^{31}P MRS provides information about pH and high energy phosphates (Madden et al. 1991; Petroff et al. 1985; Roberts et al. 1981; Howe 1993). The most abundant nuclei in living systems, hydrogen and carbon, are the basis of ^1H and ^{13}C MRS, respectively. Early applications of in vivo ^1H MRS at clinical magnetic fields (1.5T) were limited to measurements of a few neurochemicals such as *N*-acetylaspartate (NAA), lactate and creatine due to technical challenges, which include suppression of intense signals from tissue water and lipid that are several orders of magnitude higher than those from metabolites, and low spectral resolution with significant spectral overlap. Advances in ^1H MRS techniques in water suppression, localization and quantification, as well as the sensitivity and resolution advantages provided by high and ultra-high fields, have resulted in simultaneous quantification of over 20 metabolites in the living animal brain (Tkáč et al. 1999; Pfeuffer et al. 1999). However, in vivo detection of brain glycogen using MRS has been limited due to very short T1 and T2 values. Only few reports demonstrated quantitative measurements of brain glycogen using ^{13}C MRS with administration of ^{13}C -enriched glucose (Choi et al. 1999; Choi and Gruetter 2003; Öz et al. 2002). The technical aspects of MRS to examine brain glycogen both in animal models and humans are described below.

Rodent brain glycogen using ^{13}C MRS

Traditionally rodent brain glycogen has been quantified using acid extraction based biochemical analysis methods, such as a phenol-sulfuric acid method (DuBois et al. 1956; Chee et al. 1983) or with phosphorylase and other glycogen-metabolizing enzymes (Passonneau et al. 1967; Nelson et al. 1968). However, the difficulties in brain extraction procedures hampered accurate measurements of glycogen concentrations including unaltered action of glycogen-metabolizing enzymes during the extraction process and/or artifacts from high acid levels that interfere with the biochemical analysis processes using HPLC. The use of high power microwave fixation methods facilitates inactivation of glycogen-metabolizing

enzymes, thus yielding overall higher brain glycogen concentrations (Kong et al. 2002). However, the biochemical analyses of brain glycogen content in rodents resulted in a wide range of brain glycogen concentrations of 2–12 $\mu\text{mol/g}$ and up to over 20 $\mu\text{mol/g}$ depending on the brain region (Nelson et al. 1968; Kong et al. 2002; Strang and Bachelard 1971; Sagar et al. 1987; Garriga and Cusso 1992; Dienel et al. 2007; Dienel and Cruz 2003). Recent advances in in vivo MRS methodology allowed quantification of glycogen and its metabolism in living organs including the liver, muscle and the brain. While the efforts to detect brain glycogen with ^1H MRS have been unsuccessful, even at the ultra-short echo time of 1–2 ms used in order to minimize transverse relaxation (T_2) of glycogen (Tkáč et al. 1999), direct ^{13}C MRS has emerged as the method of choice to study brain glycogen concentrations and metabolism in the living animal brains. The estimated glycogen concentrations using the ^{13}C MRS method in anesthetized, hyperglycemic rat brains are about 5–6 $\mu\text{mol/g}$ (Choi et al. 1999; Lei and Gruetter 2006; Morgenthaler et al. 2008; van Heeswijk et al. 2010; van Heeswijk et al. 2012) and about 3–4 $\mu\text{mol/g}$ in awake, normoglycemic rat brains (Choi and Gruetter 2003; Morgenthaler et al. 2009). In one study, metabolic turnover rates of glycogen in the normal living rat brains were approximately 0.5 $\mu\text{mol/g/h}$ (Choi et al. 1999). During insulin-induced hypoglycemia, the glycogenolytic rate in the rat brain was about 0.04 $\mu\text{mol/g/min}$ (Choi et al. 2003), or about 7–20 % of the basal cerebral metabolic rate of glucose (CMR_{glc}), depending on the activity level and/or anesthesia (Hyder et al. 2006 and references therein). The rapid glycogen degradation during hypoglycemia where average plasma glucose was <2 mM coincided with sharp increases of cerebral blood flow in order to increase the availability of brain glucose. This finding is consistent with utilization of the endogenous energy reservoir, brain glycogen, when plasma glucose concentrations are lower than the level required for brain energy metabolism, consistent with a deoxyglucose study (Horinaka et al. 1997). Interestingly, a sudden surge of cerebral blood flow occurs immediately as the brain glucose concentration reaches ~ 0 $\mu\text{mol/g}$, at which point the glucose influx from the blood to the brain is in equilibrium with glucose utilized by the brain (Choi et al. 2001). When the glucose levels recovered after hypoglycemia, the depletion of glycogen was followed by repletion to levels beyond those under normoglycemic conditions, leading to super-compensation of brain glycogen (Choi et al. 2003). Thus, consumption and over-accumulation of glycogen may represent compensatory metabolic responses to insulin-induced hypoglycemic stress.

Human brain glycogen using ^{13}C MRS

The earliest report of in vivo detection of human brain glycogen was in a patient with suspected McArdle disease, which elevates cerebral glycogen, and utilized ^1H MRS (Salvan et al. 1997). After that, ^{13}C MRS methodology was developed to detect glycogen and its metabolism in the human brain (Öz et al. 2002). This approach was shown to specifically measure brain glycogen and have negligible contamination from subcutaneous muscle glycogen (Öz et al. 2005). Using this method, brain glycogen content in healthy humans was shown to be ~ 3.5 $\mu\text{mol/g}$ and turnover of bulk brain glycogen was shown to occur at a rate of approximately 0.16 $\mu\text{mol/g/h}$; an observation that implies complete turnover takes 3–5 days (Öz et al. 2007). Next, the effect of hypoglycemia on brain glycogen turnover and recovery was assessed using the methodology (Öz et al. 2009 and 2012). Utilization of brain glycogen was demonstrated during hypoglycemia in healthy humans (Öz et al. 2009). In

addition, supercompensation appeared to occur in subjects after exposure to two hours of experimental hypoglycemia as compared to two hours of euglycemia at the same concentration of insulin (Öz et al. 2009). Similar evidence of glycogen supercompensation was not observed in subjects with type 1 diabetes mellitus with reported history of hypoglycemia unawareness and recurrent hypoglycemia, but the depth, duration, and proximity of the hypoglycemic episodes to the brain glycogen measurements were not controlled (Öz et al. 2012).

Conclusion

In vivo MRS allows investigation of glycogen in the living brain in real time. The noninvasive methods of brain glycogen measurements have been advanced to date to provide consistent, reliable quantification and metabolic rates, and new findings of its active role in the metabolic dynamics between neurons and astrocytes under various conditions including hypoglycemia.

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Fig. 1.

Brain glycogen metabolism at cellular level involving interactions between neurons and astrocytes. GLUT: Glucose transporter, TCA cycle: Tricarboxylic acid cycle, MCT: Monocarboxylate transporter, Glycogen synthase – P (phosphorylated). The hypothesized astrocyte-neuron lactate shuttle is shown with dashed arrow