UCSF

UC San Francisco Previously Published Works

Title

Methodological considerations for studies of brain glycogen.

Permalink

<https://escholarship.org/uc/item/9488650x>

Journal

Journal of neuroscience research, 97(8)

ISSN

0360-4012

Authors

Wu, Long
Wong, Candance P
Swanson, Raymond A

Publication Date

2019-08-01

DOI

10.1002/jnr.24412

Peer reviewed

Technical and Comparative Aspects of Brain Glycogen Metabolism

Long Wu, Nicholas J. M. Butler, and Raymond A. Swanson

Abstract It has been known for over 50 years that brain has significant glycogen stores, but the physiological function of this energy reserve remains uncertain. This uncertainty stems in part from several technical challenges inherent in the study of brain glycogen metabolism, and may also stem from some conceptual limitations. Factors presenting technical challenges include low glycogen content in brain, non-homogenous labeling of glycogen by radiotracers, rapid glycogenolysis during postmortem tissue handling, and effects of the stress response on brain glycogen turnover. Here, we briefly review aspects of glycogen structure and metabolism that bear on these technical challenges, and discuss ways these can be overcome. We also highlight physiological aspects of glycogen metabolism that limit the conditions under which glycogen metabolism can be useful or advantageous over glucose metabolism. Comparisons with glycogen metabolism in skeletal muscle provide an additional perspective on potential functions of glycogen in brain.

Keywords 2-deoxyglucose · Brain · Carbon-13 · Glycogen · Immunohistochemistry · Microwave fixation · Radioisotope

1 Introduction

It has been known for over 50 years that brain has significant glycogen stores, but the physiological function of this energy reserve remains uncertain. This uncertainty stems in part from several technical difficulties inherent in the study of brain glycogen metabolism, and may also stem from some conceptual limitations. This chapter will briefly outline basic structural and bioenergetic aspects of glycogen metabolism, discuss how these lead to certain technical challenges for the study of brain glycogen, and compare what is known about glycogen metabolism in brain with skeletal muscle.

L. Wu · N. J. M. Butler · R. A. Swanson (✉)
Department of Neurology, University of California, San Francisco,
and San Francisco Veterans Affairs Health Care System, San Francisco, CA, USA
e-mail: Raymond.swanson@ucsf.edu

2 Glycogen Structure and Distribution

Glycogen is a large, branched polymer of glucose. The polymer form eliminates the high osmolarity that would result from an equimolar concentration of free glucose. It also protects the otherwise free “reducing” ends of glucose from auto-oxidation (Fig. 1), and provides a means of intracellular localization of the energy store. Glycogen polymers are roughly spherical and organized into concentric tiers, with the inner tiers composed by chains that normally contain two branches, and the

Fig. 1 Schematic two-dimensional cross-sectional view of glycogen. A core protein of **glycogenin** is surrounded by branches of **glucose** units. The entire globular granule may contain around 30,000 glucose units. The individual glucose moieties of glycogen are linked by α -1, 4-glycosidic bonds, with branch points at approximately every 12 glucose residues linked by α -1, 6-glycosidic bonds. The exposed ends of all glycogen chains are non-reducing. Image from Haggstrom (2014)

outer tiers composed of unbranched chains (Gunja-Smith et al. 1970). The linear chains of glucose residues are joined by α -1,4-glycosidic bonds, with branch points at approximately every 10–14 glucose residues linked by α -1,6-glycosidic bonds (Calder 1991). The exposed ends of all glycogen chains are non-reducing. Mathematical modeling suggests that the size of a glycogen molecule is limited to 12 tiers, which means a total of approximately 53,000 glucose residues and a radius of 21 nm. In accordance with this modeling, the glycogen particles found in the mouse brain and resting human skeletal muscle ranged from 10 to 44 nm in diameter (Cataldo and Broadwell 1986; Wender et al. 2000; Marchand et al. 2002). The average diameter of these glycogen particles is 20–30 nm, which is estimated to be 7–8 tiers (Goldsmith et al. 1982; Melendez-Hevia et al. 1993).

Glycogen polymers (“granules”) are present in the cytosol, endoplasmic reticulum (ER)/ sarcoplasmic reticulum (SR) and lysosomes (Cardell Jr. 1977; Stapleton et al. 2010; Geddes et al. 1992). In liver cells, approximately 10% of all glycogen particles are found in lysosomes (Jiang et al. 2010), where they undergo slow degradation by acid maltase. Almost all tissues contain some detectable glycogen, but the relative amounts of glycogen vary enormously. Glycogen levels in the adult rat under physiological conditions are as follows: liver >> skeletal muscle > cardiac muscle > brain > kidney (Table 1). Glycogen in mammalian brain is localized primarily to astrocytes (Cali et al. 2016; Cataldo and Broadwell 1986; Wender et al. 2000; Koizumi 1974), but much smaller amounts are also found in meningeal cells,

Table 1 Glycogen content in different tissue types

Tissue	Glycogen (mg/g tissue)	Citation
Liver	30.23 $\pm$ 2.5	Vissing et al. (1989)
Liver	43.12 $\pm$ 5.5 *	Kusunoki et al. (2002)
Liver	32.3 $\pm$ 2.0	Khandelwal et al. (1979)
Heart	4.51 $\pm$ 0.33	Vissing et al. (1989)
Heart	4.16 $\pm$ 0.23	Conlee et al. (1989)
Kidney	0.06 $\pm$ 0.01	Khandelwal et al. (1979)
Kidney	0.03 $\pm$ 0.01 *	Nannipieri et al. (2001)
Muscle (GPS) ^c	5.94 $\pm$ 0.25 ^a	Baker et al. (2005)
Muscle (white gastrocnemius)	5.79 $\pm$ 0.72	Garetto et al. (1984)
Muscle (white gastrocnemius)	7.01 $\pm$ 0.17	Vissing et al. (1989)
Brain (cortex)	0.68 $\pm$ 0.03 ^b	Sagar et al. (1987)
Brain (cortex)	0.60 $\pm$ 0.03	Kong et al. (2002)
Brain (cortex)	2.09 $\pm$ 0.27 *	Cruz and Dienel (2002)
Brain (whole brain)	2.06 $\pm$ 0.21	Oe et al. (2016)

Glycogen content in adult rat or mouse tissues under physiological conditions were shown in the table. Data are reported as means $\pm$ SD (*) or means $\pm$ SEM

^aReported as mmol/kg dry weight; converted here using values of 160.3 mg /mmol as molecular weight of glycogen and 76% as the water content of muscle (Pivarnik and Palmer 1994)

^bReported as mmol/mg protein; converted here using values of 122 mg protein/gram wet weight in brain (Banay-Schwartz et al. 1992)

^cGPS gastrocnemius-plantaris-soleus muscle complex

endothelial cells, and other cell types. Neurons contain appreciable amounts of glycogen during development, but this falls to very low levels in the mature brain except in certain brainstem neurons (Koizumi 1974; Ibrahim 1975; Cavalcante et al. 1996; Saez et al. 2014; Cataldo and Broadwell 1986; Borke and Nau 1984; Oe et al. 2016).

3 Glycogen Metabolism

The synthesis of a *de novo* glycogen granule is thought to be initiated by glycogenin, although recent studies with glycogenin deficient mice indicate other mechanisms of glycogen initiation are likely possible (Testoni et al. 2017). Glycogenin glycosylates itself at a tyrosine residue and catalyzes the extension of glucan chains (Smythe and Cohen 1991; Cao et al. 1993). The glucan chains serve as primer for glycogen synthetase, which catalyzes the formation of α -1, 4-glycosidic linkages of glycogen. Glycogen synthesis requires uridine diphosphate glucose (UDP-glucose) as a substrate, which is formed from uridine triphosphate and glucose 1-phosphate by UDP-glucose pyrophosphorylase (Fig. 2). α -1, 6-glycosidic branch points are subsequently produced by glycogen branching enzyme (1,4- α -glucan-branching enzyme) at approximately every 12 glucose residues.

Glycogenolysis is mediated by glycogen phosphorylase, which hydrolyzes glucose residues at α -1, 4 linkage points to generate glucose 1-phosphate. Glycogen debranching enzyme linearizes glycogen chains near the α -1, 6 branch points to provide linear substrate for glycogen phosphorylase (Nakayama et al. 2001). Glycogen phosphorylase is thought to be the rate limiting enzyme in glycogen breakdown. The activity of glycogen phosphorylase is regulated by changes in energy state through allosteric actions of AMP, which accelerates activity; and by ATP and glucose-6-phosphate, which slow enzymatic activity. Glycogen phosphorylase is also regulated by its phosphorylation state, through the action of glycogen phosphorylase kinase. Glycogen phosphorylase kinase is in turn regulated by a variety of signaling pathways through phosphorylation and allosteric interactions. These regulatory actions provide a mechanism for “anticipatory” glycogen mobilization to prevent any actual decline of cellular energy state. For example, glycogen phosphorylase kinase is activated by epinephrine through epinephrine-induced elevations in cAMP and activation of protein kinase A. Glycogen phosphorylase kinase can also be partly activated by elevated levels of Ca^{2+} , via binding to its calmodulin subunit. This mechanism is particularly important in skeletal muscle where muscle contraction triggers the release of Ca^{2+} from the sarcoplasmic reticulum.

Glycogen phosphorylase liberates glucose residues from glycogen as glucose-1-phosphate, which is freely converted to glucose-6 phosphate (Fig. 2). The shuttling of each glucose moiety on and off glycogen requires the net consumption of one ATP equivalent. This bioenergetic expense has implications for the potential physiological functions of brain glycogen, as discussed later in this chapter. It may also be significant that formation of glucose-6 phosphate from glycogen does not consume ATP, unlike the initial hexokinase step of glycolysis.

Fig. 2 Bioenergetics and regulation of glycogen metabolism. Glycogen synthase extends an existing glucosan chain of α -1, 4-glycosidic linkages using UDP glucose as substrate. Glycogen branching enzyme subsequently forms α -1, 6-glycosidic bonds to create branch points. Glycogen degradation is mediated by debranching enzyme and glycogen phosphorylase. Glycogen phosphorylase is regulated allosterically in response to hormones, e.g. norepinephrine and vasoactive intestinal peptide (VIP); by changes in energy state (AMP, glucose-6-phosphate (G6P), and others), and by second messengers such as cAMP. The immediate product of glycogen phosphorylase is glucose 1-phosphate, which is freely converted to glucose-6-phosphate. Hepatocytes (but not other cell types) can rapidly dephosphorylate glucose-6-phosphate to generate free glucose for export. There is a net cost of one ATP per molecule of glucose-6-phosphate that is cycled onto and off of the glycogen polymer, as 2ATP equivalents are consumed in forming UDP glucose from glucose-1-phosphate and 1 ATP equivalent is gained back at the formation of glucose-1-phosphate from a glucose residue and inorganic phosphate (Pi) at the glycogen phosphorylase step

4 Brain-Specific Aspects of Glycogen Metabolism

Although neurons are thought to be the primary energy consuming cells in brain, astrocytes contain the vast majority of brain glycogen. Electron microscopy identifies glycogen granules throughout astrocyte cell bodies and processes, particularly near axonal boutons and dendritic spines (Cali et al. 2016). Glutamate uptake is an energy-intensive astrocyte function, and interestingly glycogen phosphorylase has been found to be associated with the astrocyte glutamate transporter, GLT-1 (Genda et al. 2011). Astrocyte glycogen is quickly degraded under conditions of energy failure (Swanson et al. 1989; Suh et al. 2007a; Lowry et al. 1964), as would be expected. Glycogen metabolism in astrocytes is also induced by a several neurotransmitters and other signaling molecules, including vasoactive intestinal peptide (VIP), noradrenaline, arachidonic acid, glutamate, cAMP, and K⁺ (Magistretti

1988; Sorg et al. 1995; Cummins et al. 1983; Cambray-Deakin et al. 1988a, b; Subbarao et al. 1995; Subbarao and Hertz 1990; Walls et al. 2009). These signaling molecules serve to couple astrocyte glycogen metabolism to neuronal activity. For VIP and noradrenaline in particular, the anatomical organization of these inputs provides a framework for coordinated signaling. The narrow radial pattern of arborization of intracortical VIP neuron and the tangential intracortical trajectory of the noradrenergic fibers suggests that these two systems may function in a complementary fashion: VIP regulating energy metabolism locally, within individual columnar modules, and norepinephrine exerting a more global effect (Magistretti et al. 1981).

Direct autoradiographic and biochemical measures of glycogen turnover show it to be increased by sensory neuron stimulation in the awake rat (Swanson et al. 1992; Dienel et al. 2007). Conversely, conditions causing focally or globally reduced neuronal activity lead to a corresponding local or global increases in glycogen content, suggesting reduced glycogen utilization (Swanson 1992b). These conditions include focal brain injury, anesthetics, slow wave sleep, and hibernation (Pudenz et al. 1975; Nelson et al. 1968; Phelps 1972; Watanabe and Passonneau 1973; Swanson 1992b).

5 Experimental and Technical Considerations in Study of Brain Glycogen

Low glycogen content in brain. Several aspects of glycogen structure and regulation in brain pose unique challenges for experimental observation. First among these is the relatively low concentration of glycogen in brain. Many of the classical histochemical methods for detecting glycogen were developed using liver or muscle tissues, in which glycogen content is far higher than in brain. For example, the periodic acid schiff (PAS) method of staining polysaccharides works well in liver and muscle, but in brain the low glycogen content and relatively higher content of glycoproteins and glycolipids, which also react with PAS, makes this approach less useful, even when coupled with dimedone blocking of aldehydes (Cammermeyer and Fenton 1981). Immunohistochemical methods using glycogen antibodies are far more sensitive (Oe et al. 2016). Nonspecific glycogen labeling by either of these methods can be identified as label that persists in tissues after digestion with amylase or rendered ischemic for a few minutes prior to fixation. Low glycogen levels similarly pose a challenge for magnetic resonance studies of glycogen, as recently reviewed (Soares et al. 2017).

Use of radiotracers to assess glycogen turnover. A challenge common to many studies of metabolism is that there is no fixed relationship between the metabolite flux and measured metabolite concentrations. Glycogen turnover in particular—i.e. the breakdown and resynthesis of individual glycogen molecules—may accelerate, decelerate, or stop altogether with little or no change in net glycogen content (Fig. 3). Radiolabeled or isotope enriched substrates are therefore widely used for assessments of metabolic rates. However, accurate quantification by these

Fig. 3 Schematic relationship between neuronal activity and astrocyte glycogen metabolism. Short bursts of neuronal activity induce mobilization of astrocyte glycogen. The glycogen is resynthesized between bursts, resulting in glycogen turnover with no net change in glycogen content over time. It has been proposed that glycogenolysis is triggered by neuronal activity to provide substrate for increased glycolytic demand over intervals too short to be met by changes in local blood flow

approaches requires that the labeled molecules be homogeneously distributed among the unlabeled molecules. This is difficult to achieve in glycogen because individual glucose moieties are sequentially added to and removed from the outer glycogen tiers, and have widely variable dwell times in the glycogen polymer (Youn and Bergman 1987; Elsner et al. 2002). The relationships between rates of glycogenolysis and label release therefore depend upon the patterns of glycogen synthesis and breakdown at the level of individual glycogen granules (as diagrammed in Fig. 4). These limitations do not render assessments of glycogen turnover impossible, but they do limit the precision of these measures and often require certain assumptions. For example, very prolonged administration of tracers can be used to improve homogeneity of glycogen labeling, and this can be further refined by modeling patterns of glycogen turnover (DiNuzzo 2013; Oz et al. 2015; Soares et al. 2017).

The use of radiolabeled glucose to monitor glycogen metabolism is also complicated by the fact that normal glucose metabolism rapidly distributes the label to amino acids, lipids and many other cell constituents other than glycogen. This problem can be surmounted in part by physical isolation of glycogen prior to isotope analysis, or by the use of 2-deoxyglucose, which is incorporated into and released from glycogen but is much more slowly metabolized to other molecules (Nelson et al. 1984; Kai Kai and Pentreath 1981). Glucose labeled at the 3 or 4 carbon positions also mitigates this problem (Swanson et al. 1992), because glucose entering the tricarboxylic acid cycle (from which most other metabolites are formed) loses the 3 and 4 carbons in the pyruvate dehydrogenase reaction. In vivo magnetic resonance spectroscopy studies can in some cases use native ^{13}C abundance to assess brain glycogen concentrations, but the low abundance of ^{13}C coupled with low concentrations of glycogen in brain currently limit the temporal and spatial resolution of this approach (Soares et al. 2017).

Simultaneous polymer growth

Sequential polymer growth

Fig. 4 Results of radiolabeling tracer experiments are influenced by the mode of glycogen polymer growth and breakdown. Schematic diagram showing distribution in glycogen of labeled glucose (filled circles) injected early during glycogen synthesis. Each column of circles represents an individual glycogen polymer. In one scenario, all polymers simultaneously add glucose moieties. In the opposite scenario, each polymer is synthesized to its maximum size before a second one begins to expand. Many other intermediates or more complex patterns are also possible, as are differing patterns of glycogen polymer breakdown. These differing patterns produce a different relationship between rates of glycogen turnover and rates of label release. Redrawn from Youn and Bergman (1987)

Rapid post-mortem glycogenolysis. Glycogenolysis is triggered by very small elevations in AMP, befitting its role as an emergency energy store. This process begins almost instantly with cessation of blood flow, and continues until the brain is frozen, acidified, or otherwise treated to inactivate glycogen phosphorylase. (For frozen brains, the process resumes upon thawing unless other measures are also taken.) This presents a major challenge to histochemical detection of glycogen, particularly with the low concentration of glycogen in brain. Post-mortem glycogenolysis can be prevented by “funnel freezing” with direct application of liquid nitrogen to the animal skull (Ponten et al. 1973; Dienel et al. 2002), or more conveniently by high-energy microwave fixation of brain *in situ*, in which brain temperature is elevated to levels that denature glycogen metabolizing enzymes within 1 s or less (Medina et al. 1975; Sagar et al. 1987). The microwave approach has limitations, however. The high heat induced by and required for microwave fixation can reduce the water content of the brain, thus artificially elevating the glycogen content expressed per gram wet weight. This can be avoided by normalizing to mg protein rather than to wet weight, but special care must be taken to ensure that the fixed (denatured) protein is fully solubilized for protein determination. Failure to correct for brain dehydration or fully solubilize proteins leads to erroneously elevated glycogen measurements in microwave fixed brain (Swanson and Sagar, unpublished observations). A second limitation is that the heating and water vapor formation often distort normal cell architecture and destroy immunoreactivity of many anti-

gens; however, these problems can be overcome by careful titration of microwave power (Oe et al. 2016; Oe et al., this volume).

It is often useful to express glycogen measurements in terms of glucose molecule equivalents. The molecular weight of glucose is 180.16 g/mol; however, the molecular weight of each glucose moiety in glycogen is somewhat less, because the glycosidic linkages between the glucose moieties in glycogen each subtract the equivalent of one H₂O from their molecular weight. There is one α -1,4 glycosidic bond between each glucose moiety in the linear chains, and there is in addition one α -1,6 glycosidic bond at each branch point, which occurs approximately every tenth glucose residue. The calculated molecular weight for glycogen can thus be estimated to be 160.3 g/mol glucose equivalent. The true value will vary slightly depending upon the degree of polymer branching.

Hormonal and neurotransmitter effects on glycogenolysis. This aspect of glycogen metabolism may be uniquely problematic for studies of glycogen in brain because it involves sensory experience of the subject. There is a generally inverse relationship between neuronal activity and glycogen levels, as evidenced by elevated brain glycogen levels during anesthesia, hibernation, and slow wave sleep. Conversely, as outlined above, glycogen phosphorylase is activated by a number of signaling molecules to initiate glycogenolysis in anticipation of actual energy demand. For example, epinephrine and norepinephrine released during the stress response are potent glycogenolytic signaling molecules. It follows that animals that are stressed during the interval before brain harvest may have regionally lower brain glycogen levels than non-stressed animals. Experimental evidence supports this concern (Cruz and Dienel 2002). This observation suggests that, like glucose utilization, glycogen levels and turnover rates are likely influenced in a regionally discrete manner by the subject experience and response near the time of the observation.

6 Physiological Functions of Glycogen in Brain

Function under energy failure conditions. There is no question that glycogen can serve as an energy reserve in brain. Electrophysiology experiments using optic nerve and corpus callosum have shown that astrocyte glycogen can sustain axon function during glucose deprivation or during high frequency stimulation (Wender et al. 2000; Brown et al. 2005, 2012). These and other studies also suggest that lactate or pyruvate derived from astrocyte glycogen can be used by neurons for oxidative metabolism (Dringen et al. 1993; Poitry-Yamate et al. 1995; Pellerin et al. 1998; Tekkok et al. 2005). Other studies have demonstrated that increased glycogen in astrocytes improves survival of neurons under conditions of oxygen deprivation or glucose deprivation, both in culture and *in vivo*.

The total glycogen store in brain is approximately 3 mM (as expressed in glucose equivalents). Given estimates of basal brain glucose utilization rate (Sokoloff et al.

1977), this amount of glycogen would be expected to fuel brain metabolism for less than 15 min if it were the only energy supply available. However, there is a compensatory reduction in the rate of glucose utilization and synaptic activity during energy compromise (Suda et al. 1990). In addition, it is rare to have a complete cessation of glucose delivery to brain. During severe insulin-induced hypoglycemia, for example, the flux of glucose from blood to brain is reduced but not zero, and brain glycogen is consumed very gradually (Ratcheson et al. 1981; Choi et al. 2003). A quantitative analysis suggests that normal levels of astrocyte glycogen should be able to support brain metabolism for about 100 min during hypoglycemia (Gruetter 2003). In agreement with this assessment, an experimental study demonstrated that an 80% increase in rat brain glycogen at the onset of severe hypoglycemia extended the period of brain electrical activity by 90 min (Suh et al. 2007b). The rats with elevated initial brain glycogen concentrations also had improved neuronal survival.

Glycogen is far less useful as an energy store in brain under ischemic conditions, in which only anaerobic (glycolytic) energy metabolism is possible. Under these conditions each glucose molecule stored in glycogen can generate only 3 ATP, as opposed to the 34–36 ATP generated under aerobic conditions. Accordingly, glycogen stores are very rapidly depleted (within 3 min) during complete ischemia (Lowry et al. 1964). Moreover, there is no possibility of transferring usable energy substrates from astrocytes to neurons under anoxic conditions because substrates that can exit from astrocytes, such as lactate and pyruvate, can only be metabolized aerobically. However in focal ischemia (as results from occlusion of a cerebral artery), there is often a border zone area with partially preserved blood supply in which energy supply is barely adequate to maintain cell viability. Spreading depression from the ischemic core can strikingly increase energy demands in this “penumbra” area and exacerbate ischemic injury (von Bornstadt et al. 2015). Astrocyte glycogen stores are rapidly consumed during spreading depression (Feuerstein et al. 2016), and increased glycogen stores delay its advancing wave front (Seidel and Shuttleworth 2011). A study of focal brain ischemia showed reduced infarct size in rats that had elevated brain glycogen content (Swanson et al. 1990).

While the aggregate evidence that glycogen can serve as an emergency energy store in brain is convincing, it nevertheless does not establish this as the physiological, evolutionarily directed function of brain glycogen. (As an analogy, the catabolism of skeletal muscle to support nutrition during extreme starvation does not demonstrate this as the primary function of muscle.) In fact, several aspects of brain glycogen are poorly explained by this interpretation. These include the continuous turnover of brain glycogen under normal conditions; the complex neuromodulatory influences that promote astrocyte glycogenolysis in the absence of energy failure; and perhaps most fundamentally, the location of glycogen in astrocytes rather than in neurons.

Given the energetic cost of shuttling glucose into and out of glycogen polymers, it is useful to delineate the conditions under which this might carry advantages over direct glucose utilization. One obvious condition would be to provide buffering for transient, local insufficiencies in glucose supply, as might happen for example in the

brief interval between a burst of neuronal activity and subsequent increase in local blood flow (Fig. 3). This idea was proposed by Swanson (Swanson 1992a) and more formally developed by Shulman and colleagues as the “glycogen shunt” hypothesis (Shulman et al. 2001; Shulman and Rothman 2001). A related idea proposes that astrocyte glycogenolysis occurs preemptively in activated tissues as a glucose sparing mechanism, to prevent reduction in available glucose that would otherwise occur (Dienel and Cruz 2015; DiNuzzo et al. 2010). Although these concepts have gained wide support, there is uncertainty as to when or if such a transient glucose insufficiency occurs under physiological conditions. Glucose concentration will not become rate-limiting for energy metabolism unless it falls near the glucose K_m of hexokinase, which is less than 0.1 mM (Thompson and Bachelard 1977). Glucose concentration in normal rodent brain is approximately 2–2.5 mM, which can also be taken as the approximate concentration of glucose in the intracellular space. (Values for humans are about 30% lower). Can neuronal activity outstrip supply to the extent that local glucose concentrations fall near the hexokinase K_m value? The classic study by Silver and Erecińska (Silver and Erecinska 1994) showed that spreading depression, which causes nearly simultaneous bursting of neurons at its wave front, reduced extracellular glucose concentrations from 2.4 mM to 2 mM, a value well above the hexokinase K_m . Similarly, studies of brain energy metabolites during pentylenetetrazole-induced status epilepticus showed reductions in brain glucose concentration to no lower than 0.9 mM (McCandless et al. 1987; Folbergrova et al. 1985; Ingvar et al. 1984), excluding settings in which cerebral blood flow was impaired. Though not definitive, these results obtained using spreading depression and seizure activity, both of which massively increase brain energy demands, cast some doubt on the concept that changes in brain glucose utilization resulting from normal activity could require buffering by local mobilization of glycogen stores.

7 Comparisons to Liver and Skeletal Muscle

Assessment of glycogen functions in other tissues may suggest alternative possible roles for glycogen utilization in brain. Liver has a specialized role as a glycogen storage depot for systemic use. Hepatocytes express high concentrations of glucose-6-phosphatase for liberating free glucose into the blood circulation for use by other organs. However, astrocytes do not have high levels of glucose-6-phosphatase, and it follows that astrocytes do not have a role analogous to hepatocytes as a store of free glucose for use by other cells. Skeletal muscle contains glycogen levels intermediate between liver and brain, and like brain the stored glycogen in muscle undergoes continuous turnover and is not released to the systemic circulation. Skeletal muscle fiber types can be classified as Type 1 “red” or Type 2 “white”, with the red fibers containing far more mitochondria and white fibers containing far more glycogen (Nielsen et al. 2011). The Type 2 white fibers contract more quickly but fatigue more rapidly than Type 1 red fibers. Muscle glycogen turnover increases during exercise (Nielsen et al. 2011; Shulman and Rothman 2001), similar to the glycogen

turnover increase induced by neuronal activity in brain. However, contracting muscle has repetitive intervals of ischemia corresponding to muscle contractions, during which glycolytic metabolism is essential for ATP production, and this does not have a correlate in brain. Moreover, glycogen in muscle is localized to the contacting myocytes themselves, and there is no evidence that glycogen is metabolized in one cell to fuel activity in another cell as has been proposed to occur in brain.

Muscle fatigue correlates with glycogen depletion (Allen et al. 2008; Matsui et al. this volume), but, surprisingly, the mechanistic link between these events has not been established (Allen et al. 2008; Ortenblad et al. 2013). This point is exemplified by observations in McCardle's disease, an inherited condition caused by a genetic deficiency in muscle glycogen phosphorylase. Individuals with McCardle's disease are generally well, but develop early fatigue with exercise. This occurs even though myocyte glucose levels *increase* with exercise (MacLean et al. 1999; Sahlin 1990). Moreover, muscle performance in McCardle's disease is not significantly improved by hyperglycemia, as should occur if glycogen serves only to buffer intervals of reduced glucose availability. The biochemical defect in these muscles appears not to be a simple lack of glycolytic substrate, but rather a reduced capacity to sequester inorganic phosphate along with other changes that reduce the free energy ($\Delta G'$) provided by ATP hydrolysis (Malucelli et al. 2011).

8 Summary

Studies of brain glycogen metabolism are complicated by factors that stem directly from specific structural and regulatory features of glycogen. These factors must be addressed and considered in interpreting study results. Bioenergetic aspects of glycogen metabolism place certain constraints on the settings under which glycogen utilization may be advantageous to brain. These aspects, in conjunction with some aspects of muscle glycogen metabolism, suggest that current concepts of brain glycogen physiology may need to be expanded.

References

- Allen DG, Lamb GD, Westerblad H (2008) Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88(1):287–332. <https://doi.org/10.1152/physrev.00015.2007>
- Baker DJ, Timmons JA, Greenhaff PL (2005) Glycogen phosphorylase inhibition in type 2 diabetes therapy: a systematic evaluation of metabolic and functional effects in rat skeletal muscle. *Diabetes* 54(8):2453–2459
- Banay-Schwartz M, Kenessey A, DeGuzman T, Lajtha A, Palkovits MJA (1992) Protein content of various regions of rat brain and adult and aging human brain. 15(2):51–54. <https://doi.org/10.1007/bf02435024>
- Borke RC, Nau ME (1984) Glycogen, its transient occurrence in neurons of the rat CNS during normal postnatal development. *Brain Res* 318(2):277–284

- von Bornstadt D, Houben T, Seidel JL, Zheng Y, Dilekoz E, Qin T, Sandow N, Kura S, Eikermann-Haerter K, Endres M, Boas DA, Moskowitz MA, Lo EH, Dreier JP, Woitzik J, Sakadzic S, Ayata C (2015) Supply-demand mismatch transients in susceptible peri-infarct hot zones explain the origins of spreading injury depolarizations. *Neuron* 85(5):1117–1131. <https://doi.org/10.1016/j.neuron.2015.02.007>
- Brown AM, Sickmann HM, Fosgerau K, Lund TM, Schousboe A, Waagepetersen HS, Ransom BR (2005) Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter. *J Neurosci Res* 79(1–2):74–80
- Brown AM, Evans RD, Black J, Ransom BR (2012) Schwann cell glycogen selectively supports myelinated axon function. *Ann Neurol* 72(3):406–418. <https://doi.org/10.1002/ana.23607>
- Calder PC (1991) Glycogen structure and biogenesis. *Int J Biochem* 23(12):1335–1352
- Cali C, Baghabra J, Boges DJ, Holst GR, Kreshuk A, Hamprecht FA, Srinivasan M, Lehtvaslainen H, Magistretti PJ (2016) Three-dimensional immersive virtual reality for studying cellular compartments in 3D models from EM preparations of neural tissues. *J Comp Neurol* 524(1):23–38. <https://doi.org/10.1002/cne.23852>
- Cambray-Deakin M, Pearce B, Morrow C, Murphy S (1988a) Effects of extracellular potassium on glycogen stores of astrocytes in vitro. *J Neurochem* 51(6):1846–1851
- Cambray-Deakin M, Pearce B, Morrow C, Murphy S (1988b) Effects of neurotransmitters on astrocyte glycogen stores in vitro. *J Neurochem* 51(6):1852–1857
- Cammermeyer J, Fenton IM (1981) Improved preservation of neuronal glycogen by fixation with iodoacetic acid-containing solutions. *Exp Neurol* 72(2):429–445
- Cao Y, Mahrenholz AM, DePaoli-Roach AA, Roach PJ (1993) Characterization of rabbit skeletal muscle glycogenin. Tyrosine 194 is essential for function. *J Biol Chem* 268(20):14687–14693
- Cardell RR Jr (1977) Smooth endoplasmic reticulum in rat hepatocytes during glycogen deposition and depletion. *Int Rev Cytol* 48:221–279
- Cataldo AM, Broadwell RD (1986) Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. II. Choroid plexus and ependymal epithelia, endothelia and pericytes. *J Neurocytol* 15(4):511–524
- Cavalcante LA, Barradas PC, Vieira AM (1996) The regional distribution of neuronal glycogen in the opossum brain, with special reference to hypothalamic systems. *J Neurocytol* 25(8):455–463
- Choi IY, Seaquist ER, Gruetter R (2003) Effect of hypoglycemia on brain glycogen metabolism in vivo. *J Neurosci Res* 72(1):25–32
- Conlee RK, Berg TL, Han DH, Kelly KP, Barnett DW (1989) Cocaine does not alter cardiac glycogen content at rest or during exercise. *Metab Clin Exp* 38(11):1039–1041
- Cruz NF, Dienel GA (2002) High glycogen levels in brains of rats with minimal environmental stimuli: implications for metabolic contributions of working astrocytes. *J Cereb Blood Flow Metab* 22(12):1476–1489. <https://doi.org/10.1097/01.WCB.0000034362.37277.C0>
- Cummins CJ, Lust WD, Passonneau JV (1983) Regulation of glycogen metabolism in primary and transformed astrocytes in vitro. *J Neurochem* 40(1):128–136
- Dienel GA, Cruz NF (2015) Contributions of glycogen to astrocytic energetics during brain activation. *Metab Brain Dis* 30(1):281–298. <https://doi.org/10.1007/s11011-014-9493-8>
- Dienel GA, Wang RY, Cruz NF (2002) Generalized sensory stimulation of conscious rats increases labeling of oxidative pathways of glucose metabolism when the brain glucose-oxygen uptake ratio rises. *J Cereb Blood Flow Metab* 22(12):1490–1502
- Dienel GA, Ball KK, Cruz NF (2007) A glycogen phosphorylase inhibitor selectively enhances local rates of glucose utilization in brain during sensory stimulation of conscious rats: implications for glycogen turnover. *J Neurochem* 102(2):466–478. <https://doi.org/10.1111/j.1471-4159.2007.04595.x>
- DiNuzzo M (2013) Kinetic analysis of glycogen turnover: relevance to human brain ¹³C-NMR spectroscopy. *J Cereb Blood Flow Metab* 33(10):1540–1548. <https://doi.org/10.1038/jcbfm.2013.98>
- DiNuzzo M, Mangia S, Maraviglia B, Giove F (2010) Glycogenolysis in astrocytes supports blood-borne glucose channeling not glycogen-derived lactate shuttling to neurons: evidence

- from mathematical modeling. *J Cereb Blood Flow Metab* 30(12):1895–1904. <https://doi.org/10.1038/jcbfm.2010.151>
- Dringen R, Gebhardt R, Hamprecht B (1993) Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Res* 623(2):208–214
- Elsner P, Quistorff B, Hansen GH, Grunnet N (2002) Partly ordered synthesis and degradation of glycogen in cultured rat myotubes. *J Biol Chem* 277(7):4831–4838. <https://doi.org/10.1074/jbc.M108226200>
- Feuerstein D, Backes H, Gramer M, Takagaki M, Gabel P, Kumagai T, Graf R (2016) Regulation of cerebral metabolism during cortical spreading depression. *J Cereb Blood Flow Metab* 36(11):1965–1977. <https://doi.org/10.1177/0271678x15612779>
- Folbergrova J, Ingvar M, Nevander G, Siesjo BK (1985) Cerebral metabolic changes during and following fluoroethyl-induced seizures in ventilated rats. *J Neurochem* 44(5):1419–1426
- Garetto LP, Richter EA, Goodman MN, Ruderman NB (1984) Enhanced muscle glucose metabolism after exercise in the rat: the two phases. *Am J Phys* 246(6 Pt 1):E471–E475. <https://doi.org/10.1152/ajpendo.1984.246.6.E471>
- Geddes R, Jeyarathan P, Taylor JA (1992) Molecular and metabolic aspects of lysosomal glycogen. *Carbohydr Res* 227:339–349
- Genda EN, Jackson JG, Sheldon AL, Locke SF, Greco TM, O'Donnell JC, Spruce LA, Xiao R, Guo W, Putt M, Seeholzer S, Ischiropoulos H, Robinson MB (2011) Co-compartmentalization of the astroglial glutamate transporter, GLT-1, with glycolytic enzymes and mitochondria. *J Neurosci* 31(50):18275–18288. <https://doi.org/10.1523/JNEUROSCI.3305-11.2011>
- Goldsmith E, Sprang S, Fletterick R (1982) Structure of maltoheptaose by difference Fourier methods and a model for glycogen. *J Mol Biol* 156(2):411–427
- Gruetter R (2003) Glycogen: the forgotten cerebral energy store. *J Neurosci Res* 74(2):179–183
- Gunja-Smith Z, Marshall JJ, Mercier C, Smith EE, Whelan WJ (1970) A revision of the Meyer-Bernfeld model of glycogen and amylopectin. *FEBS Lett* 12(2):101–104
- Hagström M (2014) Glycogen structure. *Wiki J Med* 1(2):8. <https://doi.org/10.15347/wjm/2014.008>
- Ibrahim MZ (1975) Glycogen and its related enzymes of metabolism in the central nervous system. *Adv Anat Embryol Cell Biol* 52(1):3–89
- Ingvar M, Soderfeldt B, Folbergrova J, Kalimo H, Olsson Y, Siesjo BK (1984) Metabolic, circulatory, and structural alterations in the rat brain induced by sustained pentylenetetrazole seizures. *Epilepsia* 25(2):191–204
- Jiang S, Heller B, Tagliabracchi VS, Zhai L, Irimia JM, DePaoli-Roach AA, Wells CD, Skurat AV, Roach PJ (2010) Starch binding domain-containing protein 1/genethonin 1 is a novel participant in glycogen metabolism. *J Biol Chem* 285(45):34960–34971. <https://doi.org/10.1074/jbc.M110.150839>
- Kai Kai MA, Pentreath VW (1981) High resolution analysis of [³H]2-deoxyglucose incorporation into neurons and glial cells in invertebrate ganglia: histological processing of nervous tissue for selective marking of glycogen. *J Neurocytol* 10(4):693–708
- Khandelwal RL, Zinman SM, Knull HR (1979) The effect of streptozotocin-induced diabetes on glycogen metabolism in rat kidney and its relationship to the liver system. *Arch Biochem Biophys* 197(1):310–316
- Koizumi J (1974) Glycogen in the central nervous system. *Prog Histochem Cytochem* 6(4):1–37
- Kong J, Shepel PN, Holden CP, Mackiewicz M, Pack AI, Geiger JD (2002) Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. *J Neurosci* 22(13):5581–5587
- Kusunoki M, Tsutsumi K, Hara T, Ogawa H, Nakamura T, Miyata T, Sakakibara F, Fukuzawa Y, Suga T, Kakumu S, Nakaya Y (2002) Correlation between lipid and glycogen contents in liver and insulin resistance in high-fat-fed rats treated with the lipoprotein lipase activator NO-1886. *Metab Clin Exp* 51(6):792–795
- Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW (1964) Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J Biol Chem* 239:18–30

- MacLean DA, Bangsbo J, Saltin B (1999) Muscle interstitial glucose and lactate levels during dynamic exercise in humans determined by microdialysis. *J Appl Physiol* (1985) 87(4):1483–1490. <https://doi.org/10.1152/jappl.1999.87.4.1483>
- Magistretti PJ (1988) Regulation of glycogenolysis by neurotransmitters in the central nervous system. *Diabetes Metab* 14(3):237–246
- Magistretti PJ, Morrison JH, Shoemaker WJ, Sapin V, Bloom FE (1981) Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proc Natl Acad Sci U S A* 78(10):6535–6539
- Malucelli E, Iotti S, Manners DN, Testa C, Martinuzzi A, Barbiroli B, Lodi R (2011) The role of pH on the thermodynamics and kinetics of muscle biochemistry: an in vivo study by (31) P-MRS in patients with myo-phosphorylase deficiency. *Biochim Biophys Acta* 1807(9):1244–1249. <https://doi.org/10.1016/j.bbabbio.2011.06.013>
- Marchand I, Chorneyko K, Tamopolsky M, Hamilton S, Shearer J, Potvin J, Graham TE (2002) Quantification of subcellular glycogen in resting human muscle: granule size, number, and location. *J Appl Physiol* (1985) 93(5):1598–1607. <https://doi.org/10.1152/japplphysiol.00585.2001>
- McCandless DW, Dworsky S, Modak AT, Stavinoha WB (1987) Pentylenetetrazole-induced changes in cerebral energy metabolism in Tupaia glis. *Epilepsia* 28(2):184–189
- Medina MA, Jones DJ, Stavinoha WB, Ross DH (1975) The levels of labile intermediary metabolites in mouse brain following rapid tissue fixation with microwave irradiation. *J Neurochem* 24(2):223–227
- Melendez-Hevia E, Waddell TG, Shelton ED (1993) Optimization of molecular design in the evolution of metabolism: the glycogen molecule. *Biochem J* 295(Pt 2):477–483
- Nakayama A, Yamamoto K, Tabata S (2001) Identification of the catalytic residues of bifunctional glycogen debranching enzyme. *J Biol Chem* 276(31):28824–28828. <https://doi.org/10.1074/jbc.M102192200>
- Nannipieri M, Lanfranchi A, Santerini D, Catalano C, Van de Werve G, Ferrannini E (2001) Influence of long-term diabetes on renal glycogen metabolism in the rat. *Nephron* 87(1):50–57. <https://doi.org/10.1159/000045884>
- Nelson SR, Schulz DW, Passonneau JV, Lowry OH (1968) Control of glycogen levels in brain. *J Neurochem* 15(11):1271–1279
- Nelson T, Kaufman EE, Sokoloff L (1984) 2-Deoxyglucose incorporation into rat brain glycogen during measurement of local cerebral glucose utilization by the 2-deoxyglucose method. *J Neurochem* 43(4):949–956
- Nielsen J, Holmberg HC, Schroder HD, Saltin B, Ortenblad N (2011) Human skeletal muscle glycogen utilization in exhaustive exercise: role of subcellular localization and fibre type. *J Physiol* 589(Pt 11):2871–2885. <https://doi.org/10.1113/jphysiol.2010.204487>
- Oe Y, Baba O, Ashida H, Nakamura KC, Hirase H (2016) Glycogen distribution in the microwave-fixed mouse brain reveals heterogeneous astrocytic patterns. *Glia* 64(9):1532–1545. <https://doi.org/10.1002/glia.23020>
- Ortenblad N, Westerblad H, Nielsen J (2013) Muscle glycogen stores and fatigue. *J Physiol* 591(18):4405–4413. <https://doi.org/10.1113/jphysiol.2013.251629>
- Oz G, DiNuzzo M, Kumar A, Moheet A, Seaquist ER (2015) Revisiting glycogen content in the human brain. *Neurochem Res* 40(12):2473–2481. <https://doi.org/10.1007/s11064-015-1664-4>
- Pellerin L, Pellegrini G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ (1998) Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev Neurosci* 20(4–5):291–299
- Phelps CH (1972) Barbiturate-induced glycogen accumulation in brain. An electron microscopic study. *Brain Res* 39(1):225–234
- Pivarnik JM, Palmer RA (1994) In: Wolinsky I, Hickson JF (eds) Water and electrolyte balance during rest and exercise. Nutrition in exercise and sport. CRC Press, Boca Raton, FL
- Poitry-Yamate CL, Poitry S, Tscapopoulos M (1995) Lactate released by Muller glial cells is metabolized by photoreceptors from mammalian retina. *J Neurosci* 15(7 Pt 2):5179–5191

- Ponten U, Ratcheson RA, Siesjö BK (1973) Metabolic changes in the brains of mice frozen in liquid nitrogen. *J Neurochem* 21(5):1211–1216
- Pudenz RH, Bullara LA, Jacques S, Hambrecht FT (1975) Electrical stimulation of the brain. III The neural damage model. *Surg Neurol* 4(4):389–400
- Ratcheson RA, Blank AC, Ferrendelli JA (1981) Regionally selective metabolic effects of hypoglycemia in brain. *J Neurochem* 36(6):1952–1958
- Saez I, Duran J, Sinadinos C, Beltran A, Yanes O, Tevy MF, Martinez-Pons C, Milan M, Guinovart JJ (2014) Neurons have an active glycogen metabolism that contributes to tolerance to hypoxia. *J Cereb Blood Flow Metab* 34(6):945–955. <https://doi.org/10.1038/jcbfm.2014.33>
- Sagar SM, Sharp FR, Swanson RA (1987) The regional distribution of glycogen in rat brain fixed by microwave irradiation. *Brain Res* 417(1):172–174
- Sahlin K (1990) Muscle glucose metabolism during exercise. *Ann Med* 22(3):85–89
- Seidel JL, Shuttlesworth CW (2011) Contribution of astrocyte glycogen stores to progression of spreading depression and related events in hippocampal slices. *Neuroscience* 192:295–303. <https://doi.org/10.1016/j.neuroscience.2011.05.006>
- Shulman RG, Rothman DL (2001) The "glycogen shunt" in exercising muscle: a role for glycogen in muscle energetics and fatigue. *Proc Natl Acad Sci U S A* 98(2):457–461. <https://doi.org/10.1073/pnas.98.2.457>
- Shulman RG, Hyder F, Rothman DL (2001) Cerebral energetics and the glycogen shunt: neurochemical basis of functional imaging. *Proc Natl Acad Sci U S A* 98(11):6417–6422
- Silver IA, Erecinska M (1994) Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *J Neurosci* 14(8):5068–5076
- Smythe C, Cohen P (1991) The discovery of glycogenin and the priming mechanism for glycogen biogenesis. *Eur J Biochem* 200(3):625–631
- Soares AF, Gruetter R, Lei H (2017) Technical and experimental features of magnetic resonance spectroscopy of brain glycogen metabolism. *Anal Biochem* 529:117–126. <https://doi.org/10.1016/j.ab.2016.12.023>
- Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [¹⁴C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28(5):897–916
- Sorg O, Pellerin L, Stolz M, Beggah S, Magistretti PJ (1995) Adenosine triphosphate and arachidonic acid stimulate glycogenolysis in primary cultures of mouse cerebral cortical astrocytes. *Neurosci Lett* 188(2):109–112
- Stapleton D, Nelson C, Parsawar K, McClain D, Gilbert-Wilson R, Barker E, Rudd B, Brown K, Hendrix W, O'Donnell P, Parker G (2010) Analysis of hepatic glycogen-associated proteins. *Proteomics* 10(12):2320–2329. <https://doi.org/10.1002/pmic.200900628>
- Subbarao KV, Hertz L (1990) Effect of adrenergic agonists on glycogenolysis in primary cultures of astrocytes. *Brain Res* 536(1–2):220–226
- Subbarao KV, Stolzenburg JU, Hertz L (1995) Pharmacological characteristics of potassium-induced, glycogenolysis in astrocytes. *Neurosci Lett* 196(1–2):45–48
- Suda S, Shinohara M, Miyaoka M, Lucignani G, Kennedy C, Sokoloff L (1990) The lumped constant of the deoxyglucose method in hypoglycemia: effects of moderate hypoglycemia on local cerebral glucose utilization in the rat. *J Cereb Blood Flow Metab* 10(4):499–509
- Suh SW, Bergher JP, Anderson CM, Treadway JL, Fosgerau K, Swanson RA (2007a) Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*,S*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1H-indole-2-carboxamide). *J Pharmacol Exp Ther* 321(1):45–50. <https://doi.org/10.1124/jpet.106.115550>
- Suh SW, Bergher JP, Anderson CM, Treadway JL, Fosgerau K, Swanson RA (2007b) Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*,S*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-

- 1-(phenylmethyl)pro pyl]-1H-indole-2-carboxamide). *J Pharmacol Exp Ther* 321(1):45–50. <https://doi.org/10.1124/jpet.106.115550>
- Swanson RA (1992a) Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can J Physiol Pharmacol* 70(Suppl):S138–S144
- Swanson RA (1992b) Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can J Physiol Pharmacol* 70(Suppl):S138–S144
- Swanson RA, Sagar SM, Sharp FR (1989) Regional brain glycogen stores and metabolism during complete global ischaemia. *Neurol Res* 11(1):24–28
- Swanson RA, Shiraishi K, Morton MT, Sharp FR (1990) Methionine sulfoximine reduces cortical infarct size in rats after middle cerebral artery occlusion. *Stroke* 21(2):322–327
- Swanson RA, Morton MM, Sagar SM, Sharp FR (1992) Sensory stimulation induces local cerebral glycogenolysis: demonstration by autoradiography. *Neuroscience* 51(2):451–461
- Tekkok SB, Brown AM, Westenbroek R, Pellerin L, Ransom BR (2005) Transfer of glycogen-derived lactate from astrocytes to axons via specific monocarboxylate transporters supports mouse optic nerve activity. *J Neurosci Res* 81(5):644–652
- Testoni G, Duran J, Garcia-Rocha M, Vilaplana F, Serrano AL, Sebastian D, Lopez-Soldado I, Sullivan MA, Slebe F, Vilaseca M, Munoz-Canoves P, Guinovart JJ (2017) Lack of glycogenin causes glycogen accumulation and muscle function impairment. *Cell Metab* 26(1):256–266. e254. <https://doi.org/10.1016/j.cmet.2017.06.008>
- Thompson MF, Bachelard HS (1977) Differences in catalytic properties between cerebral cytoplasmic and mitochondrial hexokinases. *Biochem J* 161(3):593–598
- Vissing J, Wallace JL, Galbo H (1989) Effect of liver glycogen content on glucose production in running rats. *J Appl Physiol* (1985) 66(1):318–322. <https://doi.org/10.1152/jappl.1989.66.1.318>
- Walls AB, Heimburger CM, Bouman SD, Schousboe A, Waagepetersen HS (2009) Robust glycogen shunt activity in astrocytes: effects of glutamatergic and adrenergic agents. *Neuroscience* 158(1):284–292. <https://doi.org/10.1016/j.neuroscience.2008.09.058>
- Watanabe H, Passonneau JV (1973) Factors affecting the turnover of cerebral glycogen and limit dextrin in vivo. *J Neurochem* 20(6):1543–1554
- Wender R, Brown AM, Fern R, Swanson RA, Farrell K, Ransom BR (2000) Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. *J Neurosci* 20(18):6804–6810
- Youn JH, Bergman RN (1987) Patterns of glycogen turnover in liver characterized by computer modeling. *Am J Phys* 253(4 Pt 1):E360–E369