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Does 2-FDG-PET Accurately Reflect Quantitative *In vivo* Glucose Utilization?

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Abstract

2-Deoxy-2-[^{18}F]fluoro-D-glucose (2-FDG) with positron emission tomography (2-FDG-PET) is undeniably useful in the clinic, among other uses, to monitor change over time using the 2-FDG standardized uptake values (SUV) metric. This report suggests some potentially serious caveats for this and related roles for 2-FDG PET. Most critical is the assumption that there is an exact proportionality between glucose metabolism and 2-FDG metabolism, called the lumped constant, LC. This report describes that LC is not constant for a specific tissue and may be variable before and after disease treatment. The purpose of this work is not to deny the clinical value of 2-FDG PET; it is a reminder that when one extends the use of an appropriately qualified imaging method, new observations may arise and further validation would be necessary.

Current understanding of glucose-based energetics *in vivo* is based on the quantification of glucose metabolic rates with 2-FDG PET, a method that permits the *non-invasive* assessment in various human disorders. However, 2-FDG is only a good substrate for facilitated-glucose transporters (GLUTs) but not for sodium-dependent glucose co-transporters (SGLTs), which have recently been shown to be distributed in multiple human tissues. Thus, the GLUT-mediated *in vivo* glucose utilization measured by 2-FDG PET would be blinded to the potentially substantial role of functional SGLTs in glucose transport and utilization.

Therefore, in these circumstances the 2-FDG LC used to quantify *in vivo* glucose utilization should not be expected to remain constant. 2-FDG LC variations have been especially significant in tumors, particularly at different stages of cancer development, affecting the accuracy of

quantitative glucose measures and potentially limiting the prognostic value of 2-FDG, as well as its accuracy in monitoring treatments. SGLT-mediated glucose transport can be estimated using α-methyl-4-deoxy-4-[^{18}F]fluoro-D-glucopyranoside (Me-4FDG). Utilizing both 2-FDG and Me-4FDG should provide a more complete picture of glucose utilization *via* both GLUT and SGLT transporters in health and disease stages. Given the widespread use of 2-FDG PET to infer glucose metabolism, appreciating the potential limitations of 2-FDG as a surrogate for glucose metabolic rate and the potential reasons for variability in LC is relevant. Even when the readout for the 2-FDG PET study is only an SUV parameter, variability in LC is important, particularly if it changes over the course of disease progression (e.g., an evolving tumor).

Introduction

The introduction of positron emission tomography (PET) in medical research in the 1970s permitted for the first time the visualization of key biochemical process in living humans, providing a unique tool for the evaluation of the biological basis of disease (1,2). Since biochemical alterations precede anatomical abnormalities in most diseases, PET was quickly perceived as a unique opportunity for earlier diagnosis before the onset of clinical symptoms, a critical consideration for effective treatments.

The ultimate objective for the use of molecular imaging probes with PET is the quantification of specific biochemical processes. Because the need for energy is ubiquitous in cells, a generally applicable method for measuring energy production was sought. This is highlighted in the use of 2-FDG for the measurement of local glucose metabolism (MR_{Glc}), which is the cornerstone of PET imaging in humans. Glucose is the primary substrate for energy metabolism in tissues and a continuous supply of glucose is required for cells to function. Thus, glucose utilization measured using 2-FDG PET has become an established method for quantifying *in vivo* local functional activity in brain, heart and most cancers.

Due to the early PET scanner design and characteristics, the first human studies primarily used 2-FDG PET to investigate epilepsy, stroke, Alzheimer's disease, and for grading primary brain tumors (3). Mainly for these reasons, the vast majority of 2-FDG validation studies in tumors have been earlier applied to primary brain tumors.

The Sokoloff Method for in vivo Glucose Utilization

The methodology for using 2-FDG in human imaging was developed from the [^{14}C]2-deoxy-D-glucose (2-DG) autoradiography approach of Sokoloff *et al* (2) for the measurement of cerebral MR_{Glc} *in vivo* in the normal rat brain. The principle is as follows: the energy-independent facilitated transporters, GLUTs, whose isoform GLUT1 is expressed in the blood brain barrier and glial cells and GLUT3 in neurons (4) have long been considered the main transporters of glucose. Following transport, intracellular hexokinase (HK) catalyzes the phosphorylation of glucose to glucose-6-phosphate in the first step of glycolysis. This key phosphorylation step by HK was exploited in the 2-DG autoradiography approach (2) based on the fact that phosphorylated 2-DG is not metabolized further and is trapped in the cell. The approach has been quantitatively validated and inference of glucose metabolic rate from 2-FDG-PET relies on the use of a term called the lumped constant (LC), to account for differences in the kinetics of transport and phosphorylation of 2-DG or 2-FDG relative to glucose.

to a function of the~ LC= $LC = \frac{\lambda \cdot K_m \cdot V^*_{max}}{\emptyset \cdot V_{max} \cdot K^*_m}$3

where λ is the ratio of the distribution volume of 2-DG or 2-FDG to that of glucose and ϕ is the fraction of glucose that continues down the Embden-Meyerhof pathway after being phosphorylated, typically close to 1. K_m is the Michaelis-Menten constant for phosphorylation of glucose (* indicates 2-DG or 2-FDG), and V_{max} is the maximum velocity for phosphorylation of glucose (* indicates 2-DG or 2-FDG). The LC is used to convert the metabolic rate of 2-DG ($MR_{2\text{-}DG}$) to that for glucose (MR_{Glc}) by dividing $MR_{2\text{-}DG}$ by LC.

$$MR_{Glc} = \frac{MR_{2\text{-}DG}}{LC}$$

It is important to appreciate the assumptions of this model when using it to interpret a 2-FDG PET study. The 2-DG approach assumed that the GLUT pathway was the sole entry point for glucose utilization. Except for some regional variation (*i.e.,* Purkinje cells, glutamatergic neurons), this assumption is essentially true in the healthy brain as demonstrated by Sokoloff's initial validation studies (2) and by our own measurements presented in this manuscript. But there are at least two functional transporter systems in humans, GLUTs and SGLTs, that are open and variable in tissue, and this observation is most prevalent with evolving tumors. Since 2-FDG is blinded to SGLT-mediated glucose transporters (Table 1), the two-transporter mechanism has the potential to lead to misinterpretation of cancer diagnosis and staging, particularly at the earliest stages of disease, and also to inaccurate evaluation of treatment response with longitudinal studies. Thus, 2-FDG validation is not nearly as complete for disease conditions as it is for the healthy normal brain. If we are to have confidence in diagnostic or repeat 2-FDG PET studies (*e.g.* baseline and after treatment), it behooves the nuclear medicine community to invest in more thorough validation research for reasons presented in this report.

Variability of the Lumped Constant

The approach pioneered by Sokoloff (2) with 2-DG and autoradiography in normal rat brain was extended to the human brain, and first applied using 2-FDG and the Mark IV scanner at the University of Pennsylvania in August 1976 (5). It has since been incorporated worldwide as the 2-

FDG PET methodology (6). The 2-FDG PET method assumes that 2-FDG kinetics for transport and phosphorylation and glucose metabolism change in parallel, thereby justifying the LC as a proportionality constant. While that assumption is valid in healthy brain, global variability of the 2-FDG LC in a variety of pathophysiological conditions and even in many normal tissues of the body indicates that this assumption is not always correct. For example, using the relative $MR_{2\text{-}DG}$ and MR_{Glc} based on arteriovenous differences across the rat brain, Sokoloff *et al* (2) obtained a value for the 2-DG LC of 0.483 (SD 0.107). Reivich *et al* (7), by a similar approach in humans, estimated the LC for 2-FDG to be 0.52 (SD 0.028). Phelps *et al* (6), using dynamic PET imaging of 2-FDG, estimated the LC for 2-FDG to be 0.42 (SD 0.059). Whereas the direct method introduced by Sokoloff had produced rather stable 2-FDG LC values for the normal human brain, other indirect methods have established the 2-FDG LC to be higher. Graham *et al* (8) used kinetic analysis of sequential 2-FDG and 1-[^{11}C]-glucose PET studies to independently measure MR_{Glc} and $MR_{2FDG,}$ and obtain a uniform 2-FDG LC throughout the healthy brain of 0.89 (SD 0.08). Other groups have obtained similar values in the healthy human brain: 0.81 (SD 0.15) from one group (9) and 0.81 (SD 0.18) from another (10). Other methods to assess the stability of the 2-FDG LC under a variety of conditions have been proposed, including kinetic constant assessments to estimate glucose transport across the blood–brain barrier and derive glucose utilization in brain tissue (11 and references therein).

However, measurement of MR_{Glc} and MR_{2FDG} in patients with primary brain tumors resulted in LCs of 1.23±0.35 in 10 subjects with anaplastic astrocytoma, 1.47±0.48 in 30 subjects with glioblastoma and 0.86±0.14 in contralateral brain of these same subjects (12). The most striking aspect of these reports is that the between-subject variance was much larger for tumors than for

normal brain, suggesting that it is inappropriate to extend the assumptions from the Sokoloff model to tumors in the brain.

Extending the use of 2-FDG PET outside the normal brain and brain tumors to quantify glucose metabolism has been equally problematic because of limited validation. In traumatic brain injury, a significant reduction in the global and regional 2-FDG LC was observed and attributed to reduced hexokinase activity (13,14). Beyond the normal brain, no consistent 2-FDG LC could be demonstrated in normal rabbit myocardium (15), human skeletal muscle (16) or adipose tissue (17), all tissues where insulin dependency is important. The LC has also been measured in pig liver under fasting and hyperinsulinemia, with substantially different values (18).

Proposed Reasons for Variability of the Lumped Constant. The Sodium Glucose Transporter Effect

While the 2-FDG LC in the normal brain has been found to be reasonably stable, the publications from Krohn and coworkers (11,19-21) have consistently demonstrated that tumors are particularly sensitive to variations in 2-FDG LC. This was attributed to the specific differences between brain tumors and healthy brain, such as tissue heterogeneity in expression of hexokinases, lack of blood-brain barrier (BBB) integrity and regional differences in glucose concentration (19,21). This variability may not be surprising in view of the dysregulated enzymology of tumors.

The properties of hexokinases as well as glucose transporters contribute to the net values for K_m and V_{max} in the LC, with phosphorylation considered more rate-limiting than transport. HK isozymes often have different properties and subcellular localization (attached to mitochondrial

membrane rather than cytosolic) in pathological tissues. This has been suggested earlier as a possible explanation for LC variability (19,21). While it is likely that HK isoenzymes lead to some of the variability in LCs in human tissues, recent observations on the functional role of sodium-coupled glucose transporters (SGLTs) offer a compelling additional explanation for the variation in 2-FDG LC.

SGLT transporters derive from the SLC5A gene family. They harness the gradient of sodium ions across the plasma membrane to drive glucose into cells, using a co-transporter mechanism coupling the movement of one glucose and two sodium ions (22 -24). The most studied family members are SGLT1 and SGLT2, which are mainly involved in glucose transport in the intestine and kidneys to conserve this important substrate for energy metabolism. Even though there has been evidence for SGLT mRNA and protein in multiple human tissues (22-24), the full extent of functional SGLTs remained largely unknown until recently (25,26). Indeed, SGLT transporters are widely distributed throughout the body, including in cancer, which makes it tempting to speculate *a priori* that their participation in *in vivo* glucose utilization could be potentially significant (27). This adds a previously unrecognized factor that may critically influence the 2-FDG LC and the accuracy of 2-FDG for quantifying regional MR_{Glc}. As stated earlier, because 2-FDG is not transported by SGLTs (25), any component of glucose utilization *via* SGLT transporters (Figure 1) would be overlooked with 2-FDG, resulting in an underestimation of glucose utilization and an artificial variation in its LC. As a result, the regional and global MR_{Glc} values determined with 2-FDG PET will not be accurate in an unknown but potentially substantial number of cases (e.g., in tumors). Unfortunately, the literature does not provide measurements of LC in some of the tissues where SGLTs have been shown to date to be prevalent, like prostate,

pancreas and lung adenocarcinoma. However, it is safe to expect that human cancers and pathological states in general in many organs, involve significant variability in the 2-FDG LC (19). In spite of these limitations, the semiquantitative utilization of 2-FDG PET has remained useful clinically for diagnosis and to evaluate the prognosis for patients, especially when reported as SUVs.

Imaging Agents for SGLT- Mediated Glucose Transporters

SGLT-mediated glucose transport can be imaged with PET in animals and humans. SGLT-specific radiolabeled sugar analogs of 1-α-methyl-D-glucopyranoside have been developed as substrates for *in vivo* assessment of glucose transport using PET (Table 1). Mapping of the distribution of SGLT expression and function is possible with Me-4FDG, a SGLT-specific molecular imaging probe with desirable imaging characteristics for quantitative assessment of glucose metabolism *via* the SGLT pathway (*e.g.*, high affinity, selective specificity, no significant peripheral or tissue metabolism) (28). High affinity Me-4FDG trapping in the SGLT transporter is in contrast to the tissue accumulation of 2-FDG through conversion to 2-FDG-6-phosphate.

Using this approach, the expression of SGLT2, was demonstrated by immunohistochemistry (IHC) in human astrocytomas, pancreatic and prostate cancers. Their functional activity was also shown by transport assays on fresh surgical specimens with the SGLT-specific Me-4FDG. Imaging pancreatic and prostate adenocarcinomas using Me-4FDG PET was demonstrated in mouse xenograft models (27) and preliminarily in humans (Wright EM and Barrio JR, unpublished results). High grade astrocytomas were also imaged with Me-4FDG PET in humans (29). Furthermore, the importance of SGLT2 in tumor growth and survival in a mouse xenograft model

of pancreatic cancer treated with specific SGLT2 inhibitors that are used for treating diabetes has been reported (27). These tumors were highly heterogeneous by immunohistochemistry with cell aggregates accumulating Me-4FDG as well as 2-FDG (Figure 2).

For measurement of SGLT function in brain, the molecular imaging probe must also be a good substrate for GLUT1, the exclusive transporter of D-glucose and analogs across the BBB. SGLT-specific radiolabeled imaging probes without GLUT1 affinity, such as Me-4FDG, cannot be used for *in vivo* assessment of SGLT in the human brain since the SGLT transporters are primarily expressed on cortical and hippocampus pyramidal neurons, as well as Purkinje cells in the cerebellum (25) (Figure 4). However, Me-4FDG can be used when the BBB is compromised as in high grade astrocytomas, and its specific retention supported by SGLT2 expression in neoplastic glioblastoma cells and endothelial cells of the proliferating microvasculature (29). Based on earlier work on the *in vitro* transport of sugar-analog probes *via* SGLT1, 4-deoxy-4-[^{18}F]fluoro-D-glucose (4-FDG) was identified as a suitable biomarker for probing SGLTs in the intact brain (25,26). 4-FDG is qualified for *in vivo* imaging in that it is an excellent substrate for SGLT transporters with specific cell retention in proportion to functional activity, while maintaining its reversible transport *via* GLUT1 at the BBB (Table 1; Figure 1).

<u>The Shift to Oxidative Glycolysis with Cancer Progression: Effect on LC and SUV</u>

Tumor heterogeneity can change as cancers develop, further complicating interpretation of 2-FDG PET quantitative determinations, whether by MR_{Glc} or by SUV. The shift from SGLT- to GLUT-mediated glucose transport was recently demonstrated in human lung adenocarcinomas and in

murine genetically engineered models with a combination of IHC and Me-4FDG PET imaging (30). Pre-malignant and early-stage/low-grade lung adenocarcinoma (LADC) expressed high functional activity of SGLT2; GLUT-mediated uptake of 2-FDG was mainly observed in more advanced stages of LADC, suggesting a global shift from SGLT- to GLUT-mediated transport (Figure 3). This result is consistent with the clinical role of 2-FDG PET in LADC - high sensitivity and specificity in moderate and advanced stages but unreliable in earlier stages. Therefore, using 2-FDG-PET to evaluate its uptake in lung cancer would drastically change with the stage of cancer development, whether it was evaluated by MR_{Glc} or by SUV. This may be the best evidence to date that there is a discrepancy of glucose metabolic rate that is clinically significant and is not accounted for by a 2-FDG-PET determination. It is likely that the same shift will be observed in other cancers and emphasizes the importance of SGLT transporters in limiting our interpretation of SUVs for 2-FDG-PET.

If tissues have abundant access to circulating glucose *via* facilitated transporters (GLUTs), the critical question is why do they need energetically expensive glucose *via* sodium-mediated SGLTs? The well-recognized role of SGLTs in intestine and kidney is focused on recovery of glucose for metabolic utilization in the whole body, but locally in tissues this role does not apply. The expression of SGLTs is cellularly heterogeneous. The exquisite localization of SGLT-mediated glucose accumulation against an extracellular gradient (22-24) provides a higher level of glucose in cellular microenvironments that presumably is driven by mitochondrial demand for an accessible fuel. This mechanism may be an advantage in early cancer cell development and explain SGLTs in pre-malignant lesions and early tumorigenesis, as well as metastasis initiation. In sharp contrast, GLUT-mediated uptake of 2-FDG appears low at early stages of cancer (Figure 3), which

parenthetically limits its clinical role in early detection. In later stages of cancer, up-regulation of glycolysis results in increased consumption of 2-FDG, a nearly universal property of advanced primary and metastatic cancers. The persistent metabolism of glucose to lactate even under aerobic conditions (aerobic glycolysis) in cancer development (31) leads to micro-environmental acid-induced toxicity for surrounding normal cells, which may promote unconstrained proliferation and invasion (32).

Roadmap for Investigating the Impact of SGLTs on 2-FDG PET Imaging

Having argued that the expression and function of SGLTs in cancer deserves further attention by the nuclear medicine community and biological researchers, the obvious question is how this should be accomplished. While it might be tempting to invoke genetic approaches, including knockout models, this would likely lead to a necessary, but long road to answering the impact, which is the clinically relevant question. A compelling case could also be made for a bedside-to-bench translational approach. Measuring SUVs for 2-FDG-PET will always be the easiest and most commonly applied metric for estimating cellular energy metabolism. However, some investigations may find changes in SUV that are inconsistent with clinical assessment of the course of disease and this is the setting where the clinical observation should lead to more detailed assessment of the role of SGLTs, and other variables that might change LC. This approach starts by identifying situations where there is valid suspicion that energy metabolism is more involved than what 2-FDG PET results predict, thus incentivizing directed tissue analysis for even specific animal models to learn about differential regulation of GLUTs and SGLTs. Cancers with high levels of SGLT expression should be considered. In addition to early stage lung adenocarcinoma,

cancer in the prostate, kidney and pancreas are settings where 2-FDG has found a limited role but SGLTs are functionally expressed. Analysis of biopsy specimens of tumor and surrounded normal tissue will tell whether SGLTs are dysregulated as cancers develop, e.g., with selective upregulation in early disease and subsequent downregulation. If they are, that would be sufficient information to recommend caution in interpreting 2-FDG SUV and might foster imaging with Me-4FDG or 4-FDG, as appropriate. Also, these results might stimulate laboratory studies as to whether SGLTs have an early tumor promoting role or a later tumor suppression role. Answers to these mechanistic questions would have consequences in terms of tumor biology and development of new therapeutic targets.

Conclusions

An accurate value of the LC is essential for quantitative assessment of glucose metabolic rates from 2-FDG PET imaging. Calibration of 2-FDG PET results to reflect precisely glucose utilization rate has been always understood as an essential prerequisite to attributing any alteration in 2-FDG tissue kinetics to glycolytic activity. The overarching conclusion from this report is that 2-FDG PET images, even when analyzed semi-quantitatively using SUV approaches, are valuable in the clinic, but there is a need for a more complete understanding of factors that impact 2-FDG PET images. The present work summarizes evidence on the potential impact of previously unrecognized glucose utilization *via* sodium-coupled glucose transporters (SGLTs) on the GLUT-mediated glucose utilization measured with 2-FDG. SGLT transporters are widely distributed and functionally active under normal physiological conditions and respond to substantial up-regulation during early disease. Thus, SGLT-mediated glucose transporters may have a dynamic role in

glucose utilization and a crucial effect on the 2-FDG LC, directly affecting quantitative glucose metabolism measurements in humans using 2-FDG PET. Current practice recommends a minimum 30% change in SUV adjusted for lean body mass in order to report a response to tumor therapy (33). With a better understanding of factors that impact MR_{Glc} or SUV measurements, could this threshold be reduced, making the nuclear medicine study a more useful consult?

The relative effect of SGLT1 and SGLT2 functional expression in the normal human brain confirms that the 2-FDG LC is globally relatively stable in the brain where most of the glucose utilization is via GLUTs, with only about 5.4 % of the glucose being consumed via SGLTs, as measured with 4-FDG PET (See Appendix for relative glucose utilization estimates). However, locally the effects may be more significant: SGLTs are highly expressed in cortical hippocampal glutamatergic neurons and in Purkinje cells in cerebellum (25,26) (Figure 4), both of which are part of the neuronal circuit of cerebral vulnerability to brain hypoxia (34). Yet, only limited data exist as to the variations in cerebral glucose transport and utilization via GLUTs or SGLTs in related disease conditions. Figure 5 provides a hint as to their possible role in stroke.

In cancer tissues, the heterogenous cellular composition and the presence of quantitatively significant SGLT expression is consistent with the unreliable value of the 2-FDG LC. Cancer staging adds an imponderable variable at the earliest stages of its development, where SGLT-mediated transport may be especially high, blinding the 2-FDG PET signal until a more glycolytic stage of cancer metabolism develops. The same logic seems to apply to myocardial glucose utilization, which in early determinations appears high via SGLT in the young human heart and evolves into a more glycolytic form with age (Wright and Barrio, unpublished).

The observation on glucose transport and utilization *via* GLUTs and SGLTs in cancer adds an additional twist to the observation that cancer cells may use other substrates, in addition to glucose. Assumptions that some 'human cancers consume little glucose', based on low 2-FDG PET signal, needs to be reevaluated and only confirmed after glucose utilization via SGLTs is considered. Utilizing both 2-FDG and Me-4FDG (or 4-FDG in brain) in humans would help provide comprehensive insight into the very basic biochemical mechanism of glucose utilization *via* GLUT and SGLT transporters in health and disease stages.

More research is needed to comprehend the intricacies of the *in vivo* interplay between GLUT- and SGLT-mediated glucose transport in physiologic and pathophysiologic states throughout the human body. The initial observations reported here open a myriad of interesting basic questions about *in vivo* glucose utilization, well beyond the current use and limitations of the 2-FDG PET approach. The recent observation suggesting that the epidermal growth factor receptor (EGFR) regulates SGLT expression in cancer (35) adds a critical variable to the comprehensive understanding of *in vivo* glucose utilization for optimization of new therapies for cancer patients (36).

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Key Points

Question: Does the 2-FDG PET signal fully represent the total glucose utilization in tissue?

Pertinent Findings: The GLUT-mediated *in vivo* glucose utilization measured by 2-FDG PET would be blinded to the potentially significant role in glucose transport and utilization of functional sodium glucose transporters (SGLTs) present in normal human tissues (e.g., brain, heart, muscle) and cancer. The interplay between GLUT- vs SGLT-mediated glucose utilization would affect the 2-FDG PET signal, the lumped constant (LC) and SUV used for quantitative *in vivo* measurement of glucose utilization.

Implications for Patient Care: The apparent variations in 2-FDG PET signals have been especially significant in tumors, particularly at different stages of cancer development, potentially limiting in several instances the diagnostic value of the biomarker, as well as its relevance in monitoring treatments.

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Appendix

Estimation of the Relative SGLT-mediated vs. GLUT-mediated Glucose Utilization in Normal Human Brain

While specific retention of 2-FDG in human brain occurs *via* BBB GLUT1 transporters and hexokinase-mediated phosphorylation in neuronal cells, the specific trapping and retention of 4-FDG is through sodium-dependent glucose transporters on the neuronal cell membrane. Based on Michaelis-Menten constants of 4-FDG for SGLT and GLUT as compared to those of glucose, as shown in Table 1, the "Lumped Constant" of 4-FDG can be estimated to be about 5 times larger than that of 2-FDG.

The central question is, how is the brain uptake of 4-FDG related to actual glucose utilization rate? Dynamic scanning of normal healthy subject(s) after bolus injection of 4-FDG has been obtained together with dynamic blood 4-FDG levels during the 60 min scan time. For comparison, a similar dynamic 2-FDG PET and dynamic blood curve was also performed on the same subject(s). Patlak analyses were done for representative cortical regions (i.e., frontal cortex and cerebellum for both the 4-FDG and 2-FDG PET determinations) and the uptake constants (Ki's) so obtained were compared to estimate the glucose utilization rates via SGLTs and via GLUTs. The Ki of 4-FDG in the frontal cortex was found to be 0.0108 ml/min/g, while that of 2-FDG was 0.0430 ml/min/g. Based on the larger LC (5 times) of 4-FDG vs. that of 2-FDG as stated above, the glucose utilization rate through SGLTs in the frontal cortex is calculated to be about 5.4% of the rate assessed by 2-FDG. In other words, in the frontal cortex, the glucose utilization rate via SGLT is relatively small as compared to that measured using 2-FDG. However, since 4-FDG distribution is different from that of 2-FDG, as shown in Figure 4, it should be expected that regional variations would exist. For example, the high expression of SGLTs in the Purkinje cells in cerebellum, is shown by the high cerebellar 4-FDG uptake (Ki value for 4-FDG there was determined to be 0.017 ml/min/g in cerebellum vs. 0.0108 ml/min/g in the cortex). Thus, in normal cerebellum a larger fraction (~8.5%) of glucose utilization is through SGLTs.

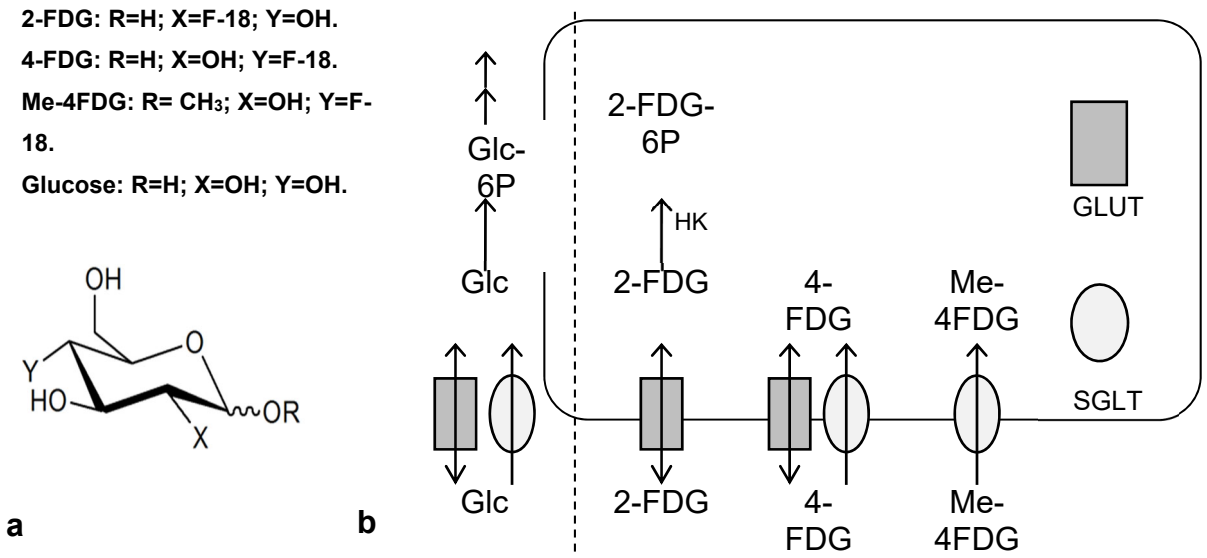

Figure 1. Glucose transporter imaging probes and their transport cascades into cells. (a) Structure of glucose analog probes. (b) Graphical interpretation of glucose utilization *via* GLUT and SGLT: Glucose (Glc) is transported into cells by both GLUT and SGLT. To discriminate between the various glucose transport pathways, probes were designed to be substrates for either GLUT (2-FDG) or SGLT (Me-4FDG), or both (4-FDG). 2-FDG is phosphorylated by hexokinase (HK), but cannot be further metabolized, resulting in metabolic trapping in cells. Both 4-FDG and Me-4FDG are not substrates for HK, consistent with their retention in cells by the sodium/glucose co-transport (SGLT) mechanism. Adapted from Yu et al, 2010 (Reference 25).

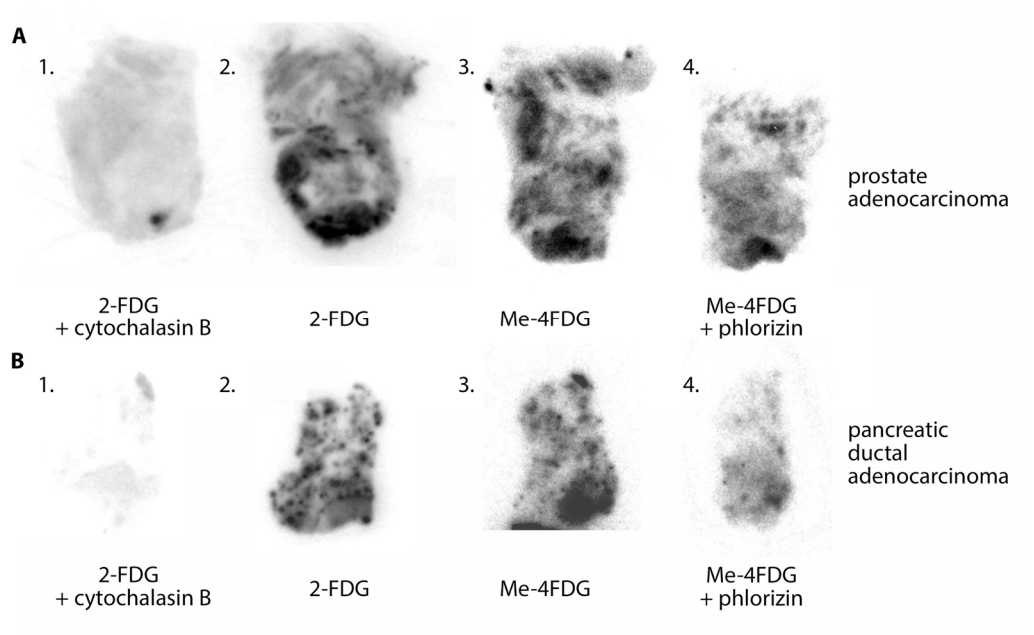

Figure 2. Spatial heterogeneity of glucose uptake in pancreatic and prostate cancers in humans. Spatial heterogeneity of glucose uptake in human pancreatic and prostate cancers. The regional distribution of glucose uptake via GLUTs and SGLTs was mapped in fresh samples of human prostate adenocarcinoma (A) and of pancreatic ductal adenocarcinoma (B) by *in vitro* uptake and autoradiography. Briefly, the specimens were cut into 300-μm-thick slices and consecutive slices were incubated with either 2-FDG (specific for GLUTs) or Me-4FDG (which detects SGLT activity), with or without coincubation with the corresponding inhibitors (cytochalasin A for GLUT and phlorizin for SGLT). The slices were subsequently exposed to autoradiographic plates to obtain images of regional tracer uptake. The numbers at the top left corner of each slice represent the order in which the slices were cut. As described in Scafoglio et al, 2015 (Reference 27)

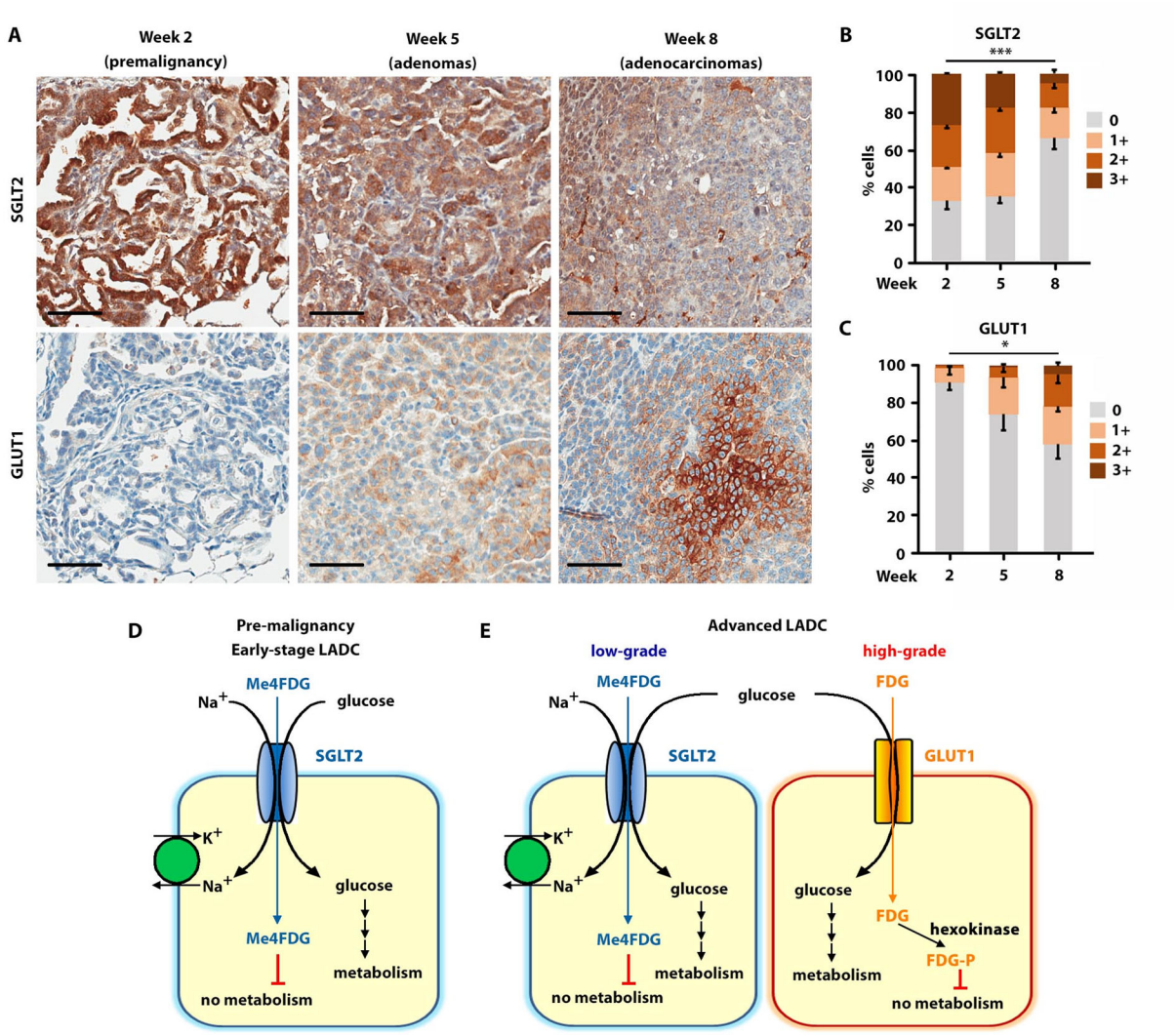

Figure 3. Temporal heterogeneity of glucose transporter expression in lung adenocarcinoma. SGLT2 and GLUT1 glucose transporter expression was investigated in the $Kras^{G12D}$; p53-/- (KP) model of lung adenocarcinoma at different time points after tumor induction. Scale bar, 50 µm. **Expression** of the SGLT2 (**B**) and GLUT1 (**C**) IHC signal at different time points after tumor induction; **D-E**) Schematic representation of glucose transporter expression in different stages of LADC: Premalignant lesions and early-stage LADC express only SGLT2 (**D**), whereas advanced tumors show spatial heterogeneity of glucose transport expression, with SGLT2 in well-differentiated and GLUT1 in poorly differentiated areas of the same tumor (**E**). Similar spatial heterogeneity of glucose transporter expression was also demonstrated in human lung adenocarcinoma. Adapted from Scafoglio et al, 2018 (Reference 30)

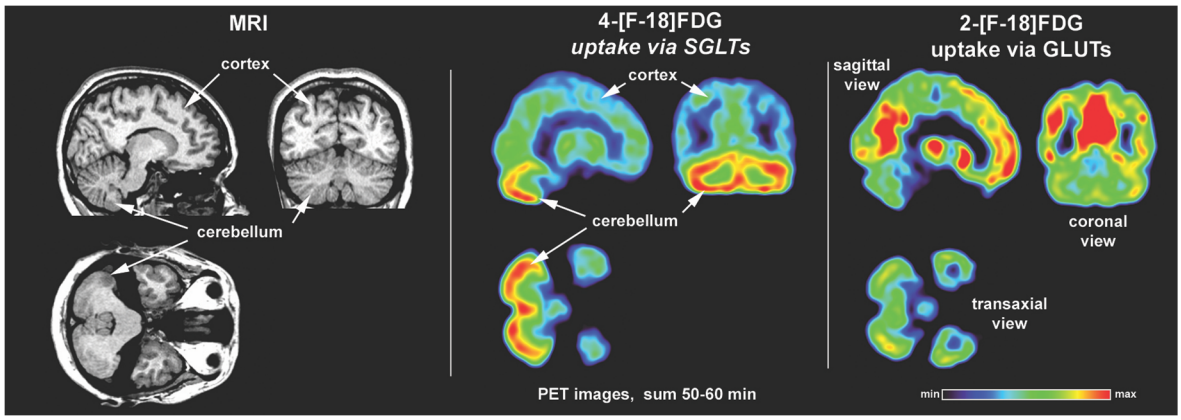

Figure 4. SGLT-mediated glucose utilization in the normal human brain. The two PET scan panels, display the typical 2-FDG PET scan in the normal human brain, reflecting GLUT-mediated glucose utilization (right panel); and a 4-FDG PET scan in the normal human brain reflecting functional SGLT-distribution (both sum images 30-60 min after IV injection). Structural MRI scans provide brain anatomical reference. Note the substantial relative regional differences between the two scans, most particularly the high functional SGLT localization in Purkinje cells in cerebellum, as independently verified by immunohistochemistry (Kepe V, Wright EM and Barrio JR, unpublished observations).

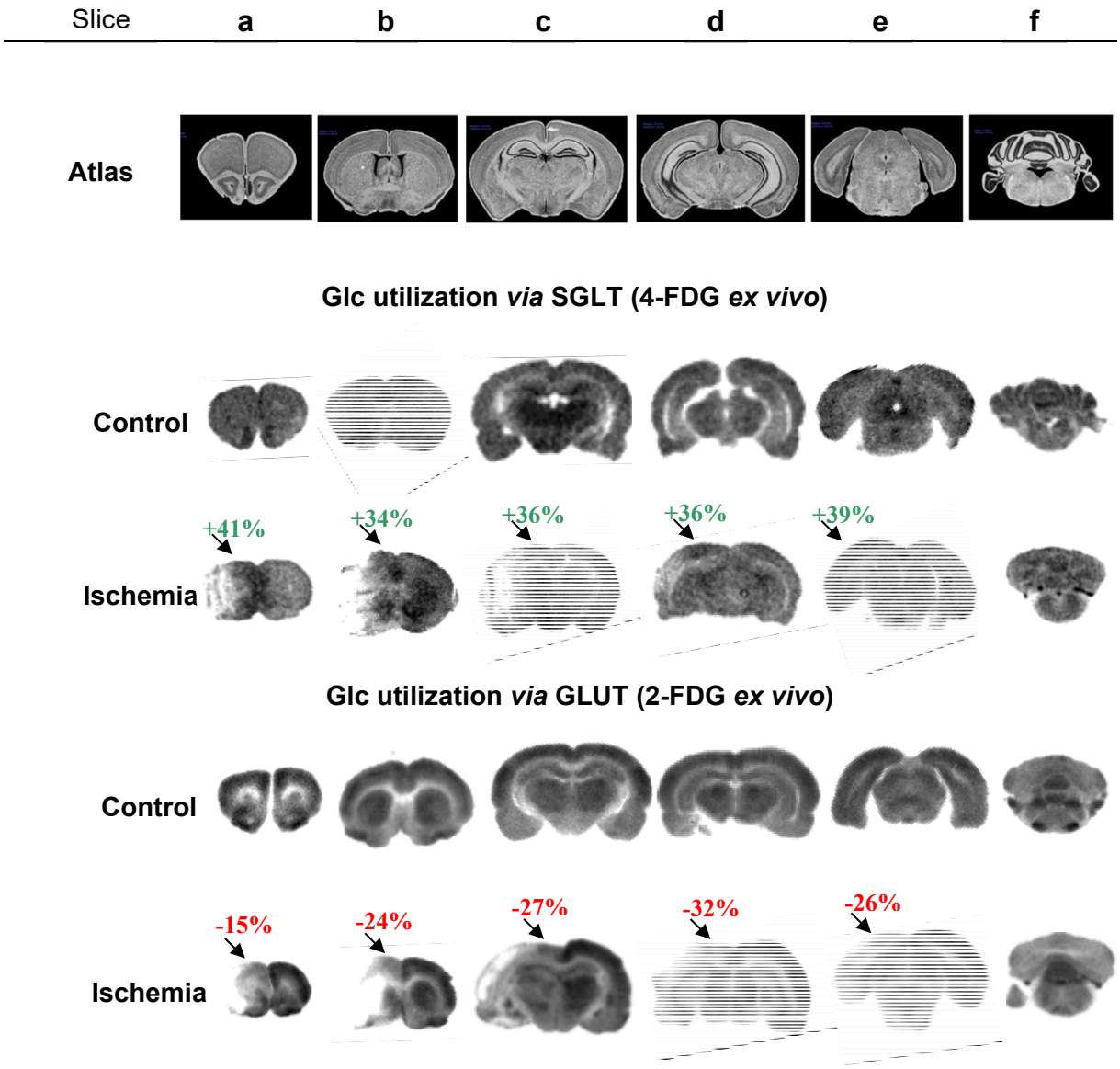

Figure 5. Upregulation of SGLT functional activity in the middle cerebral artery occlusion (MCAo) model. The regulation of SGLT activity in the disease state is exemplified by a stroke model in rat brain (43). One hr. after IV tail veil injection of the imaging probe (2-FDG or 4-FDG; 5-8 mCi), *ex vivo* autoradiography in the MCAo model was used to demonstrate the different responses between SGLTs and GLUTs under post-ischemic conditions. One day after reperfusion the accumulation of both 4-FDG and 2-FDG was reduced in the focal area but in the penumbra of focal lesions the accumulation of 4-FDG was increased by 34-41 %, in contrast to more than 15% reduction of 2-FDG uptake which is consistent with previous work (44). Regions of interest (ROI) for quantification of 4-FDG or 2-FDG were manually drawn on penumbral regions and the same regions on contralateral side using Multi Gauge V3.0 (Yu AS, Wright EM, Barrio JR, unpublished observations).

Table 1: **Kinetics of hexose membrane transport and phosphorylation**

	SGLT1/SGLT2			GLUT1			Hexokinase		
Hexose	K_m (mM)	Turnover (s^{-1}) [e]	Ref.	K_m (mM)	Turnover (s^{-1})	Ref.	K_m (mM)	V_{max}	Ref.
2-FDG	>100	u.d	[22]	3.2	1,000–13,000[a]	[39]	0.19	0.5	[41]
4-FDG	0.07	160[b]	[22]	n.a	1,000–13,000[a]		84	0.1	[41]
Me-4FDG	0.1[c]	160[b]		u.d[d]	~0		u.d[d]	u.d[d]	
Me-glucose	0.7 / 1.6	160	[22, 37, 38]	u.d	~0	[39]	u.d	u.d	[42]
D-Glucose	0.5 / 1.6	160	[16, 37, 38]	6.3	1,000–13,000	[39, 40]	0.17	1	[41]

Me-DG, 1-methyl-D-glucopyranoside; u.d, undetectable; n.a, not available; [a] Estimated to be identical to D-glucose at 38 °C; [b] Estimated to be identical to Me-glc (α-methyl-D-glucopyranosides) at 38 °C. [c] Mean of two experiments; [d] Ref 25 [e] Data available is only for SGLT1.
(Adapted from Yu A et al, 2010; reference 25)