J Cancer Res Clin Oncol (2000) 126:549–559

REVIEW

E. K. J. Pauwels · E. J. C. Sturm · E. Bombardieri
F. J. Cleton · M. P. M. Stokkel

Positron-emission tomography with [^{18}F]fluorodeoxyglucose

Part I. Biochemical uptake mechanism and its implication for clinical studies

Received: 19 November 1999 / Accepted: 1 February 2000

Abstract Over the past decades, Positron Emission Tomography has opened a new field of imaging. Nowadays, this technique is being used for diagnosing, staging disease as well as for prognostic stratification and monitoring therapy. In this respect, [^{18}F]fluorodeoxyglucose (FdGlc) is by far the most commonly used PET agent. Many factors have been identified being responsible for a high uptake of this agent in malignancy. However, additional factors such as tumour treatment may interfere with the uptake mechanism. Knowledge of all these factors is a prerequisite for an optimal interpretation of PET studies and, consequently, for a reliable judgement of tumour status. In this article, a review is given of the factors influencing FdGlc uptake and the implications for clinical studies.

Keywords PET · FDG · Uptake mechanism · Malignancy

Introduction

Over the past decades, tumour imaging has evolved from basic science to clinical practice. The introduction of ultrasonography, magnetic resonance imaging and computed tomography has improved on clinical examination for tumour staging. However, these anatomy-related techniques assess primary tumours and metastases by size and structural changes. Furthermore, superficially growing tumours and small lymph node metastases may be missed, whereas enlarged lymph nodes may contain inflammation instead of tumour, causing false positive and false negative results respectively.

In nuclear medicine, the development of new radionuclide tracers and the improvement of equipment for imaging during the past decades has enabled a more functional approach based on metabolic imaging rather than anatomy-related techniques. Traditionally the contribution of nuclear medicine to oncology has been predominantly diagnostic imaging for tumour localisation. Compounds labelled with iodine-131, gallium-67, thallium-201 chloride and technetium-99m have an established role in clinical oncology, all using a specific aspect of tumour pathophysiology and biochemistry. The introduction of positron-emission tomography (PET) has opened a new field of imaging. Currently, this technique is being used for initial diagnosis, assessing disease extension and prognosis, planning and monitoring treatment and, finally, detecting recurrent disease. In this respect, [^{18}F]fluorodeoxyglucose (FdGlc) is by far the most commonly used PET agent. The uptake mechanism of FdGlc, a glucose analogue, in malignant cells is well known, and the higher uptake and metabolism than in normal cells make it possible to detect malignant tumours. However, several factors, such as serum glucose levels, tumour hypoxia and malignancy grade, may influence the uptake at these sites. In addition, iatrogenic and therapeutic changes may interfere with one's ability to make a reliable judgement of the primary or recurrent tumour status. Therefore, knowledge of the basic aspects of FdGlc uptake is a prerequisite for an optimal interpretation of FdGlc PET studies.

In the present article, an overview is given of the uptake mechanism and biochemical processing of glucose and FdGlc in normal and malignant cells. Furthermore, the factors that influence uptake of FdGlc and the implications for clinical studies are discussed.

E. K. J. Pauwels (✉) · E. J. C. Sturm · M. P. M. Stokkel
Leiden University Medical Centre,
Division of Nuclear Medicine, C4-Q,
P.O. Box 9600, 2300 RC, Leiden, The Netherlands
e-mail: pauwels@mail.medfac.leidenuniv.nl
Tel.: +31-71-526-3475; Fax: +31-71-526-6751

F. J. Cleton
Leiden University Medical Centre,
Division of Clinical Oncology,
Leiden, The Netherlands

E. Bombardieri
Instituto Nazionale per lo Studio e la Cura dei Tumori,
Milano, Italy

Uptake of glucose by normal cells

Glucose is an essential compound for living organisms. It is the most important carbon supplier for the synthesis of tissue, and energy-producing metabolic processes depend largely on glucose availability.

The uptake of glucose and other hexoses by human cells can take place via three mechanisms of transport. The first, passive diffusion, is a relatively slow process and is made possible by the hydrophilic nature of glucose. It is of minor importance for human tissue. The second mechanism is active transport by a Na^+-dependent glucose transporter, occurring across the apical brush border of intestinal and kidney epithelial cells. The third method of transport involves the facilitative glucose transporter (GLUT), which is presented on membranes of almost all cells and is the main pathway for glucose to enter the cell body.

Na^+-dependent glucose transporter

Na^+ is required as a co-factor for the active transport of glucose across intestinal and kidney epithelial cells by sodium/glucose transporters. This means that Na^+ enters the cell down its electrochemical gradient, accompanying glucose. The accumulated Na^+ inside the cell is cleared by the Na^+/K^+-ATPase, which exchanges 3 Na^+ against 2K^+ ions in an energy-dependent way. So far, two different isoforms have been discovered: SGLT (sodium/glucose transporter)-1 and SGLT-2. SGLT-1 has a high affinity for glucose and transports Na^+ and glucose in the ratio 2:1, whereas SGLT-2 has a low affinity and the Na^+/glucose ratio is 1:1 (Kanai et al. 1994). Another difference is that SGLT-1 can transport glucose as well as galactose (Ikeda et al. 1989). It is remarkable that expression of SGLT-1 transport in the intestine can be induced by a variety of hexoses, even by the non-metabolisable sugar 3-*O*-methylglucose (Miyamoto et al. 1993). Apparently, expression of this transporter does not require transport or metabolism. Two genetic syndromes are associated with mutations in DNA encoding these transporters: hereditary glucose/galactose malabsorption and benign glycosuria (Turk et al. 1994).

Facilitated transport

Six isoforms of facilitative-diffusion glucose transporters have been identified so far: GLUT-1 to GLUT-5 and GLUT-7. The *GLUT-6* gene is not expressed and is called a pseudogene (Kayano et al. 1990), which is a gene inactivated by an accumulation of mutations or the absence of regulating sequences. These isoforms share the same transmembrane topology, but they differ in kinetic properties, tissue localisation, sugar specificities and regulation in states of imbalanced glucose homeostasis. GLUT-1, GLUT-3 and GLUT-4 have a relatively high affinity for glucose, so they are important in tissues highly dependent on glucose as their energy source. The amount of glucose transported across the membrane not only depends on the number of transporters expressed on the cell membrane but also on the kinetic capacity of the transporter expressed. The sequence of GLUT-7, cloned in rat liver, resembles that of GLUT-2 and it possibly has a role in the dephosphorylation process of glucose 6-phosphate inside the endoplasmatic reticulum (Waddell et al. 1992). GLUT-5 is a fructose transporter (Inukai et al. 1995).

GLUT-1

This transporter is widely expressed at membranes of many different cells in the human body; the highest concentrations are found in fetal tissue (Tadokoro et al. 1995) and placenta (Barros et al. 1995). Thus, it is not very surprising that growth stimuli, like insulin (Todaka et al. 1994), insulin-like growth factor I (Wilson et al. 1995), growth hormone (Tai et al. 1990) and thyroid hormone (Weinstein and Haber 1993), induce a higher expression of GLUT-1.

In adults, high expression is seen in erythrocytes (Mueckler 1994) and in epithelial cells of blood/tissue barriers, especially the blood/brain barrier (Maher 1995). Beside glucose, GLUT-1 can also transport galactose and mannose. This takes place in an asymmetrical way, i.e. the affinity for sugar influx is about ten times as high as that for glucose efflux (Carruthers 1990).

It has been suggested that GLUT-1 belongs to the family of glucose-regulated proteins, of which the genes are more expressed in situations of cellular stress (Sviderskaya et al. 1996).

GLUT-2

This low-affinity transporter can be found in the intestine, kidney, liver and the β cells of the pancreas (Thorens et al. 1990). It is also present in different regions of the brain (Leloup et al. 1994) (Jetton et al. 1994). Together with the glycolytic enzyme glucokinase, it constitutes a glucose-sensing system, which signals the differences in glycaemia to the liver and the β cells of the pancreas (Thorens et al. 1988). Glucose, galactose, mannose and fructose can all be carried by the GLUT-2 transporter.

GLUT-3

Its high affinity for glucose means that GLUT-3 can ensure a constant glucose supply to neurons in the brain, even at low extracellular glucose concentrations (Maher et al. 1995 and 1996). In the brain, glucose is transported across the blood/brain barrier by GLUT-1 and, once it reaches the neurons, GLUT-3 is the most efficient

transporter in the hypoglycaemic conditions of the cerebral interstitial space. GLUT-3 has a high expression in the testis as well, especially in spermatozoa (Haber et al. 1993).

GLUT-4

Expression of this transporter is high in brown and white adipose tissue and in skeletal and cardiac muscle. In hyperglycaemic circumstances, like the period after dinner, glucose transport can be increased up to 30 times in response to insulin. Insulin stimulates intracellular vesicles, which contain the GLUT-4 transporter, to translocate to and fuse with the plasma membrane. The thus increased GLUT-4 expression results in an increased glucose uptake of the cell (Rea and James 1997; Holman and Kasuga 1997).

GLUT-5

This transporter is only 39%–40% identical to the other isoforms (Kayano et al. 1990). It is the main transporter of fructose and is expressed at high levels in the jejunal region of the small intestine, although its mRNA can also be found in small concentrations in kidney, brain, insulin-sensitive tissues, testicles and spermatozoa (Mantych et al. 1993). There is some evidence that glucose is a competitive inhibitor of fructose uptake at GLUT-5. This differential sensitivity of fructose to glucose may be due to proteins interacting with GLUT-5, as suggested by Miyamoto et al. (1994).

Molecular structure and function of GLUT protein

The GLUT protein has a structure of 12 membrane domains, connected with hydrophilic loops. A distinction can be made between the cytoplasmic side and the extracellular side. Both the N terminus and the C terminus are on the cytoplasmic side. A large intracellular loop connects the 6th and the 7th transmembrane domains, thus holding the two parts of 6 transmembrane domains together (Fig. 1).

An alternating conformer model is used to describe the transport of glucose by the GLUT protein across the cell membrane (Fig. 2). The transporter has one sugar-binding site, which can be exposed both at the cytoplasmatic surface and the extracellular surface but not simultaneously at both. Sugar binds to the extracellular surface and a carrier-sugar complex is formed. This carrier-sugar complex can reorientate to the opposite side of the membrane by virtue of a conformational change and, finally, sugar is released into the cell (Baldwin 1993). The cytoplasmatic side of the divergent amino acid sequence seems to be important in the regulation of GLUT. Jung (1998) hypothesised a role for a specific cellular protein, binding via a certain pathway to the cytoplasmtic side. This, as yet unidentified, protein seems to be of major importance in the insulin-induced GLUT recruitment.

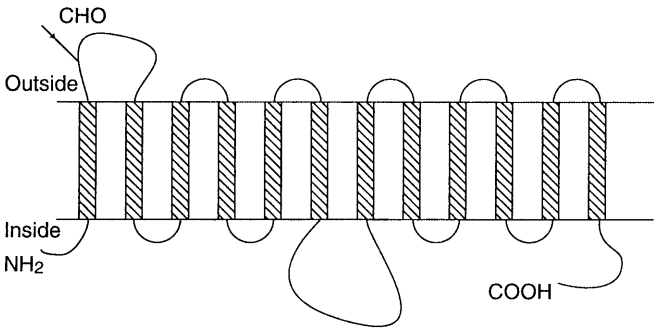

Fig. 1 Orientation of glucose transporter protein in the plasma membrane after Kasanicki and Pilch (1990)

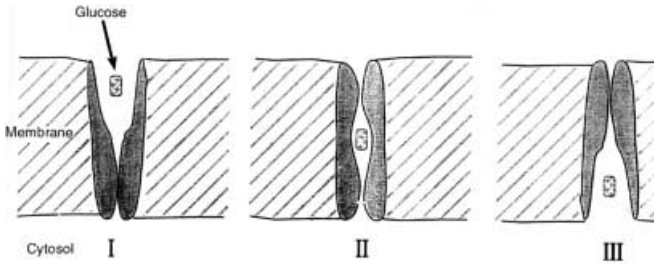

Fig. 2 Transport of glucose molecule across the cell membrane facilated by the glucose transporter protein (see text)

Fates of glucose in normal cells

Once glucose has entered the cell, it is the subject of an intricate network of several pathways, which all have the goal of producing building blocks for synthesis of tissue or generating energy. The first step in the metabolism of glucose is called glycolysis, the conversion of glucose to pyruvate and lactate.

Glycolysis

This is a set of multiple reactions, occurring in the cytosol, eventually converting one glucose molecule into two molecules of pyruvate. Glycolysis has three control elements represented by irreversible reactions, having regulatory as well as catalytic roles. The first reaction is a control element, the conversion of glucose to glucose 6-phosphate (Fig. 3). This reaction is catalysed by the enzyme hexokinase, which binds to the outer membrane of the mitochondrion near the porin molecule. These porins represent voltage-dependent anion-channel isoforms (Golshani Hebroni and Bessman 1997) and they are protein channels through which ATP moves outside the mitochondrion to enter the cytosol. Hexokinase re-

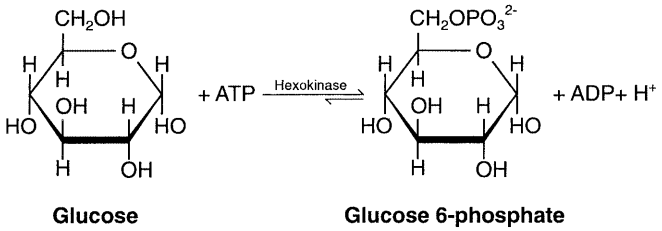

Fig. 3 Conversion of glucose to glucose 6-phosphate, catalysed by the enzyme hexokinase

duces the ATP to ADP and produces glucose 6-phosphate. Meanwhile, ADP enters the mitochondrion again and becomes an acceptor for phosphoryl groups. The porin-hexokinase complex is stimulated by insulin.

So far four different types of hexokinase are known, distinguished on the basis of their electrophoretic mobility. Type I is most abundant in brain and erythrocytes, type II is predominant in insulin-sensitive tissue, such as skeletal muscle, heart, diaphragm and adipose tissue, type III is not predominant in any tissue (Rempel et al. 1994) and type IV: also called glucokinase, is predominant in liver cells. The enzymatic activity of all hexokinases except glucokinases is inhibited by glucose 6-phosphate. When glucose is abundant, glucokinase becomes activated in the liver to produce glucose 6-phosphate for the synthesis of glycogen.

Another important step is the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate (Fig. 4). This reaction is catalysed by phosphofructokinase, which is the most important control element in glycolysis. Unlike the other control elements hexokinase and pyruvate kinase, this is the only unique irreversible step in glycolysis (i.e. it catalyses the reaction of molecules that are strictly glycolytic intermediates). Phosphofructokinase is allosterically inhibited by ATP, inhibiting glycolysis when there is enough energy, and by H^+ (preventing excessive formation of lactate). Another inhibitor is citrate, which is an intermediate of the citric acid cycle (see below) and abundant when there are high levels of biosynthetic precursors. A remarkable activator of phosphofructokinase is fructose 2,6-bisphosphate, which is formed out of fructose 6-phosphate by an enzyme called phosphofructokinase 2. When fructose 6-phosphate is abundant, this system will lead to a rise in fructose 2,6-bisphosphate levels and hence to stimulation of phosphofructokinase.

The third irreversible step in glycolysis is the reaction phospho*enol*pyruvate–pyruvate, catalysed by pyruvate kinase (Fig. 5). Three types of pyruvate kinase have

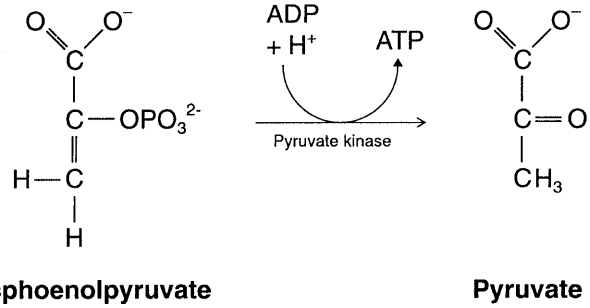

Fig. 5 Conversion of phospho*enol*pyruvate to pyruvate, catalysed by the enzyme pyruvate kinase

been found: the L type predominates in the liver, the M type predominates in muscle and brain and the A type is abundant in other tissues. ATP allosterically inhibits the isoenzyme (so glycolysis is slow when there is enough energy). Alanine, synthesised from pyruvate, is also an inhibitor, thus signalling that there are enough building blocks. The enzyme is stimulated by fructose 1,6-bisphosphate.

At the end of glycolysis, pyruvate is subject to four different metabolic fates. Under anaerobic conditions, for example in muscle during intense activity, pyruvate is reversibly converted into lactate. This reaction serves to regenerate NAD^+, which is necessary for glycolysis to proceed. The overall reaction in the conversion of glucose in lactate delivers two ATP molecules, the maximum amount of energy under anaerobic conditions. A second fate of pyruvate is transamination into the amino acids alanine, valine and leucine, from which the second and third are essential. This occurs in the cytosol. These reactions are also reversible, making it possible for the amino acids to enter the central metabolic pathway and be oxidised in the citric acid cycle to generate energy. Further, pyruvate can be carboxylated inside mitochondria into oxaloacetate, an intermediate of the citric acid cycle (Fig. 6). This carboxylation serves two purposes: one to replenish intermediates of the citric acid cycle and the other to make possible gluconeogenesis (the synthesis of glucose from non-carbohydrate sources, such as lactate, amino acids and glycerol). It shares a number of reactions with glycolysis but also includes a number of other reactions to bypass the essential irreversibility of the corresponding reactions in glycolysis. The conversion of pyruvate to oxaloacetate and the following conversion of oxaloacetate into phospho*enol*pyruvate is such a bypass (Fig. 6). A fourth, very important, fate of pyruvate is its conversion into acetyl-

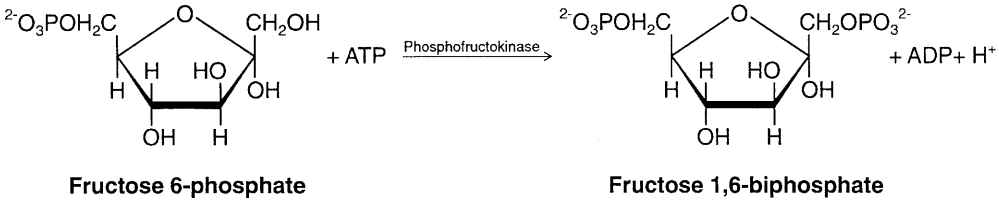

Fig. 4 Conversion of fructose 6-phosphate to fructose 1,6-bisphosphate, catalysed by the enzyme phosphofructokinase

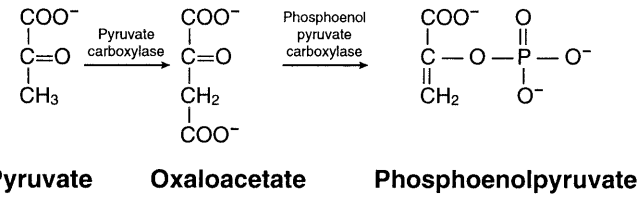

Fig. 6 Carboxylase conversion of pyruvate into oxaloacetate, followed by the conversion of oxaloacetate into phospho*enol*pyruvate

CoA inside mitochondria. This molecule can be the supplier of two-carbon fragments needed for the synthesis of lipids or it can enter the citric acid cycle if there is a need for ATP.

The citric acid cycle

The citric acid cycle (also called the tricarboxylic acid cycle or Krebs cycle), depicted in Fig. 7, occurring in the mitochondria, is the final common pathway for the oxidation of glucose and other fuel molecules. Some intermediates can also be transaminated into amino acids.

The first step in the cycle is the condensing of acetyl-CoA with oxaloacetate, thus generating a six-carbon molecule: citrate. In the following steps citrate is again converted into oxaloacetate. Thus, two carbon atoms from acetyl-CoA enter the cycle, leaving the cycle as two CO_2 molecules. The energy yield from the several steps is 3 NADH, 1 FADH and 1 GTP per molecule of pyruvate. The NADH and FADH molecules transfer their electrons to oxygen in the electron-transport chain to generate 3 ATP per NADH and 2 ATP per FADH. This is necessary for the continuity of the cycle, since a constant supply of the electron acceptors NAD^+ and FAD^+ is required for the cycle to proceed. Hence, the citric acid cycle is obligatorily aerobic.

The rate of the cycle depends on the need for ATP. There are several control elements in it, which are dependent on the amount of ATP: citrate synthetase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase. Another important control element for the cycle is the irreversible conversion of pyruvate to acetyl-CoA. As mentioned above, some intermediates can be converted into amino acids. Oxaloacetate can be transaminated into aspartate, a precursor for several other amino acids, most of them being essential. Glutamate, again a precursor for amino acids, is the product of 2-oxoglutarate.

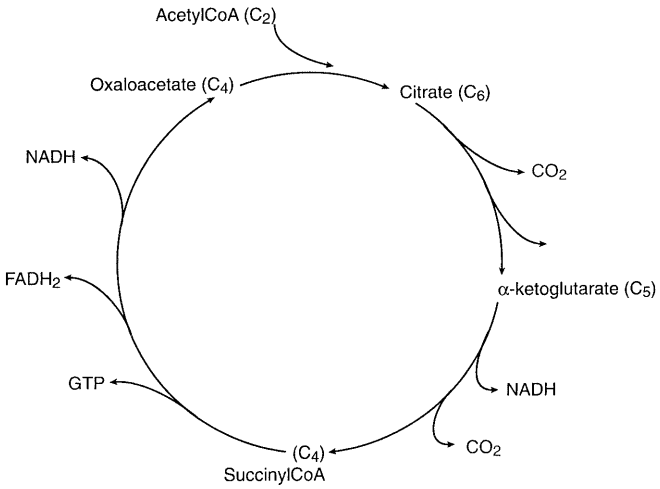

Fig. 7 Citric acid cycle (see text)

Pentose phosphate pathway

A pathway that starts with some intermediates of glycolysis is the pentose phosphate pathway, taking place in the cytosol. It is largely involved in the de novo synthesis of nucleic acids and therefore cell proliferation in vitro and in vivo. The pathway is also able to produce NADPH, which serves as an electron donor (hybride ion donor) in reductive biosynthesis. There are two pathways in the synthesis of nucleic acids. The oxidative pathway, starting with glucose 6-phosphate, is catalysed by the enzyme glucose 6-phosphate dehydrogenase and the non-oxidative pathway, starting with fructose 6-phosphate, is catalysed by transketolase, with thiamine (vitamin B_1) as its co-factor. The ribose 5-phosphate is converted into 5-phosphoribosyl-1-pyrophosphate, a building block for the synthesis of nucleotides. It is likely that the non-oxidative pathway is the predominant one in nucleic acid ribose synthesis, as found by Boros et al. (1997). It can also be assumed that glucose influences the regulation of the enzymes pyruvate kinase and hexokinase through this non-oxidative pathway: glucose signals to the glucose-responsive element, the expression of which is regulated by xylulose 5-phosphate, an intermediate of the non-oxidative pathway.

Glucose uptake in tumour cells

Besides an overproduction of glycolytic enzymes (see below), an increased uptake of glucose is seen in tumour cells (Warburg, 1930).The facilitative glucose transporters described above, the expression and activity of which are regulated by oncogenes and growth factors, are generally held responsible for this and it is becoming more and more likely that overexpression of GLUT-1, GLUT-3 and/or GLUT-5 has a role in this enhanced transport (Merrall et al. 1993). Revealing the distribution and function of these transporters in malignant cells could lead to the development of new diagnostic and prognostic markers as well as the development of specific monoclonal antibodies that could be used in tumour localisation and treatment.

Research performed over the past decade has revealed the following: an increase in the GLUT-1 and GLUT-3 mRNA in cancers of the oesophagus, colon [together with GLUT-5 (Mesonero et al. 1995)] and pancreas (Yamamoto et al. 1990); overexpression of

GLUT-1 and GLUT-3 mRNA in the brain (Nishioka et al. 1992) together with GLUT-1 protein expression in head and neck tumours (Mellanen et al. 1994); GLUT-1 overexpression in renal cell carcinoma (Nagase et al. 1995), insulinomas (Boden et al. 1994), haemangioblastomas (Cornford et al. 1995) and thyroid cancers (Haber et al. 1997); GLUT-1 and GLUT-3 overexpression in lung carcinomas (Younes et al. 1997); GLUT-1 and GLUT-5 overexpression in breast tumours (Brown and Wahl 1993, Zamora Leon et al. (1996). The overexpression of the fructose transporter GLUT-5 in breast cancer cells is especially remarkable since fructose uptake in normal breast tissue is mediated by GLUT-2, whereas GLUT-5 is not expressed. This indicates that neoplastic transformation of breast tissue leads to expression of a high-affinity fructose transporter, permitting enhanced uptake of fructose, a substrate apparently used by few human tissues. Zamora-Leon et al. 1996) speculated that this could be a metabolic advantage over normal breast tissue, since the regulatory steps that control glucolysis, cannot affect fructolysis.

Younes et al. (1996) showed that, with some rare exceptions, GLUT-1 has limited expression in normal human tissue and benign lesions. They confirmed the work of Nelson et al. (1996) by showing that GLUT-1 overexpression in cancer cells is not characteristic of all tumours. Furthermore, GLUT-1 overexpression varies in positive tumours being manifest in a few cells or in the majority of tumour cells. The overexpression of GLUT-1 and GLUT-3 is only localised in the perinecrotic zones of the tumour, where the environment of the tumour cells is hypoxic. An abundance of glucose transporters could be an adaptation that helps the tumour cell to survive and gives it an advantage over normal cells under such circumstances (Mellanen et al. 1994).

Differences in GLUT expression between tumour cells and normal tissue and the heterogeneity of GLUT-1 expression in the different tumours suggest that such expression may be of biological significance. Correlation was seen between expression of GLUT-1 and the aggressiveness of the tumour (Younes et al. 1996). For example, GLUT-1 expression in colon cancer and the frequency of lymph node metastases were found to be correlated (Younes et al. 1996).

Of clinical importance is the question whether these changes in expression of facilitative transporters are strictly related to the malignant phenotype or occur at an early stage in carcinogenesis. Reisser et al. (1999) found that preneoplastic lesions already showed an increased expression of GLUT-1, so increases in GLUT-1 expression might be used as a diagnostic tool for detection of preneoplastic lesions. In the same work they found a relationship between reduced glycogen storage and the degree of dysplasia, associated with increased GLUT expression.

New therapeutic strategies in the future, based on GLUT overexpression, could be developed. Inhibition of GLUT-1 with chemicals such as phloretin, a known inhibitor of glucose membrane transport could be useful (Miller et al. 1992). Other therapeutic approaches could be the competitive inhibition of glucose uptake by glucose analogues such as 3-*O*-methylglucose (Hwang et al. 1992) or intracellular introduction of glucose-linked cytotoxic molecules, using the transport mechanism (Pohl et al. 1995).

Hexokinase

For about seven decades, malignant cells have been known to use their excessive glucose uptake to maintain a high glycolytic rate, even under aerobic conditions (Warburg 1930). A normal cell derives most of its energy from oxidative phosphorylation, so the fact that, in a rapidly growing tumour cell, the citric acid cycle shows minor activity and the cell depends on glycolysis for more than half of its energy needs indicates a very important metabolic difference between a normal and a malignant cell (Nakashima et al. 1984). Besides producing of energy, glycolytic intermediates are also involved in two other features of growing tumours: enhanced DNA/RNA synthesis by means of the pentose phosphate pathway and the synthesis of lipids (out of fructose 1,6-bisphosphate), essential for membrane synthesis. The glycolytic capacity of a given tumour is now generally assumed to be characteristic of its state of differentiation (Flier et al. 1987; Birnbaum et al. 1987).

Singh et al. (1974) suggested that the promoting force for this increased glycolytic flux is the phosphorylation of glucose, the first reaction of the glycolytic pathway. This step is catalysed by hexokinase and it is this enzyme that shows an increased activity in tumour cells (Arora and Pedersen 1988; Rempel et al. 1994). At least two factors contribute to this increased activity of hexokinase. One is the difference in binding to the outer membrane of the mitochondrion, the other is the overproduction of this enzyme in malignant cells compared to normal cells.

Hexokinase binding to mitochondria

Rempel et al. (1994) studied hexokinase in liver cells and found that the percentage of membrane-bound enzyme activity increases with the grade of malignancy, varying from about 30% of the total activity in normal liver cells to about 70% in dedifferentiated tumour cells.

The hexokinase binds to the porin molecule on the outer mitochondrial membrane and thus has easy access to the ATP produced in the mitochondrion (Arora and Pedersen 1988). The amino-terminal half of hexokinase is thought to be involved in this connection with the mitochondrial membrane, while the C-terminal half has both catalytic and regulatory roles (Arora et al. 1993). The bound hexokinase is less sensitive to the feedback inhibition of glucose 6-phosphate, which is an important mechanism in normal cells (Bustamante and Pedersen

1977). Also proteolytic degradation is of less influence on bound hexokinase.

It is intriguing to know that the soluble and the bound enzyme are chemically identical. Hexokinases can be resolved into two subtypes (IIa and IIb) by hydrophobic interaction chromatography. Owing to its greater hydrophobicity and lower negative charge, type IIb, binds more readily to mitochondria than does type IIa. There is evidence that regression in differentiation is accompanied by alterations in the proportion and subcellular distribution of these subtypes (Rempel et al. 1994).

Hexokinase overproduction in cancer cells

As seen above, four different isoenzymes of hexokinase are known and there is a pronounced tissue-specific distribution. In malignant cells, type II hexokinase and to a lesser extent type I hexokinase are overexpressed, regardless whether the tissue of origin expresses these enzymes (Rempel et al. 1994; Mathupala et al. 1995). These two isoenzymes are exactly the same as in normal tissue and in their experiments no evidence was found for a specific tumour hexokinase. This does not exclude a post-translational modification, regulation by phosphorylation for example, which has been seen in vitro but not yet in vivo (Adams et al. 1991; Arora and Pedersen 1993).

Increased transcription of the hexokinase gene seems to be, at least in part, the cause for the overproduction of the enzyme in cancer, as a marked elevation of hexokinase mRNA levels has been demonstrated in tumour cells (Johansson et al. 1985). This was demonstrated by Mathupala et al. (1995) who found a tenfold transcriptional rate of hexokinase in tumour cells.

Research has been done on the promotor region of type II hexokinase to uncover the transcriptional regulation of the overexpression in tumour cells. The promotor-binding site for RNA polymerase on a gene in the process of RNA synthesis contains various response elements. These are sequences via which several transcription factors can exert their regulatory influence on the expression of the enzyme. On the promotor of hexokinase there appear to be several response elements for the transcription factor AP-1, the complex formed by the oncoproteins Fos and Jun and Pea-3, a factor inducible by several other oncoproteins (Gutman and Wasylyk 1990). This indicates that oncoproteins exert their influence on cancer cells partly by increasing glycolysis. Mathupala et al. (1995) found that, in rat ascites AS-30D hepatomas (which are rapidly growing, highly glycolytic tumours, preferentially expressing the type II enzyme), the promotor was up-regulated by glucose, insulin, glucagon and by pathways for tyrosine kinase, protein kinase A and protein kinase C. None of these factors activated the promotor in normal hepatocytes, indicating the involvement of a different set or level of transcription factors in the regulation of hexokinase expression and therefore the rate of glycolysis.

Fed/fasted state

In normal cells, insulin is active in the fed state and glucagon in the fasted state. Therefore opposing effects on gene expression were found: insulin activates the transcription of hexokinase and glucagon inhibits this transcription. This is certainly the case for type IV hexokinase (glucokinase), which is the predominant isoform expressed in normal liver, where the type II isoform is silent (Printz et al. 1993). cAMP, the major mediator of glucagon signalling, is also an inhibitor of glucokinase (Granner and Pilkis 1990). Both insulin and glucagon (cAMP) activate the promotor of the hexokinase II gene in cancer cells. This can be seen as a strategy of the cancer cell to keep its glycolysis high, even with a limited supply of glucose. If the glucose level in the environment rises, the cancer cell can utilise the available glucose immediately, without a lag period. This means that transcription of hexokinase in tumour cells is independent of the metabolic state of neighbouring healthy cells. Thus, cancer cells have an advantage over their cell of origin.

Hypoxia

Wang and Semenza (1995) determined the presence of a specific region inducing hypoxia in the promotor of hexokinase. The hypoxic state of a solid tumour may cause up-regulation of hexokinase, which enhances the rate of glycolysis, serving as a mechanism for the tumour cell to survive. Interestingly, the response element for glucose was found to overlap the response element for hypoxia. It is not yet known whether these two elements act synergistically in the presence of both glucose and hypoxia.

Gene amplification

The overexpression of hexokinase II in malignant cells results from another mechanism besides the differential transcriptional regulation of hexokinase expression described above. Rempel et al. (1996) found that, in the rapidly growing rat AS-30D hepatoma cell lines, increased hexokinase activity was partly the result of an at least fivefold amplification of the hexokinase II gene, compared to normal hepatocytes. This is not very surprising as instability of the genome is often seen in transformed cells and overexpression of oncogenes is often caused by amplification.

p53

In normal cells, p53 acts as a cell-cycle checkpoint protein, thus halting the cell in G1phase in the event of DNA damage. Mathupala et al. (1997) discovered the presence of functional p53 response elements on the type

II hexokinase promotor in another experiment with AS-30D hepatomas.

In this experiment there appeared to be a positive regulatory effect of mutated p53 (two point mutations in its cDNA were demonstrated) on the hexokinase promotor. In the AS-30D hepatoma cells, p53 was highly abundant in its mutated form, with a longer half-life than wild-type p53 (Mukhopadhyay et al. 1995). This could be the key to a possible link between loss of cell-cycle control in rapidly growing tumour cells and their propensity to catabolise glucose at high rates, one of the commonest biochemical signatures of cancer cells.

Fluorodeoxyglucose

The glucose analogue deoxyglucose, which has one oxygen atom less than the glucose molecule (Fig. 8), is transported into the cell in the same way as glucose. Once it has reached the cytosol, it is phosphorylated to deoxyglucose 6-phosphate by the enzyme hexokinase, just like normal glucose. The next reaction in glycolysis, producing deoxyfructose 6-phosphate, in effect a rearrangement of the carbonyl group from the C-1 to the C-2 position in the ring structure, is not possible because this would require an oxygen atom at the C-2 position. Thus, deoxyglucose 6-phosphate is trapped in the cell, as the reverse reaction to deoxyglucose does not take place because of the low concentration of glucose 6-phosphatase in tumour cells.

When labelled with ^{18}F this molecule can be revealed by PET. Hence, a high uptake of [^{18}F]fluorodeoxyglucose reflects an increased glucose metabolism in, for example, tumour cells.

Besides tumours, there are several other tissues that exhibit a high glucose metabolism. Among them are the brain and the heart, which have very low concentrations of glucose 6-phosphatase (Brock et al. 1997), and FdGlc uptake in muscles could also be considerably increased. Hence complete rest is prescribed for patients before the scintigraphic examination (Rigo et al. 1996). In addition, the uptake of FdGlc can be further reduced by fasting, thus causing a hypoinsulinaemia, which is responsible for the decrease in glucose transporters of the muscle cell. Tissues with a relatively low FdGlc uptake are the liver and gut, which show high glucose 6-phosphatase levels (Brock et al. 1997).

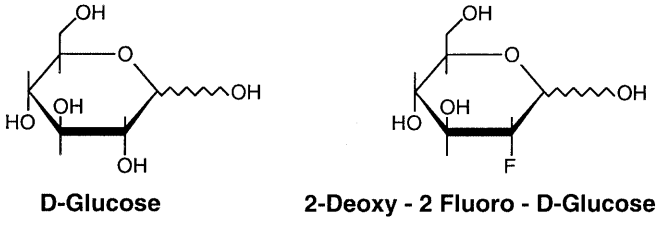

Fig. 8 Structural formula of 2-deoxy-2-fluoro-D-glucose as compared to D-glucose

Uptake of FdGlc in tumour cells is influenced by a certain number of factors, of which we presumably only know a few. Besides biological factors, such as increased expression of glucose transporters and the presence of a highly active hexokinase isoform (described above), other influences are, for example, hypoxia and hyperglycaemia.

Hypoxia

In experiments with melanoma and ovarian carcinoma cell lines, Clavo et al. (1995) showed that FdGlc uptake was increased when cells were exposed to a hypoxic environment. The hypoxia was associated with an increased expression of the GLUT-1 glucose transporter and an increased membrane transport of glucose and not with increased lactate production.

Hyperglycaemia

FdGlc uptake into human cancer cells is inhibited by high glucose levels in the medium, because of the competition between FdGlc and D-glucose for uptake into the malignant cell (Wahl et al. 1991, 1992). Simmons et al. (1993) showed that GLUT-1 protein and mRNA levels were increased when fetal lung and muscle cells were cultured in low-glucose medium, while high glucose concentrations were associated with increased GLUT-1 protein and mRNA levels. This could be an adaptive response of the cell: a reduction in GLUT-1 transporters could serve as a method to keep glucose at a normal level in a hyperglycaemic environment. On the other hand, an increase in GLUT-1 levels can allow the tumour cell to survive in a hypoglycaemic environment (Torizuka et al. 1997).

Despite the gaps in our knowledge of FdGlc, FdGlc PET is recognized to be an accurate, non-invasive tool for imaging a wide range of different tumours. For a decade it has been suggested for the specific diagnosis and staging of cancer (Strauss and Conti 1991). More recently, it is thought to be useful in the evaluation of cancer prognosis and efficacy of anticancer therapy (Okazumi et al. 1992), which is usually measured in terms of inhibition of proliferation, whereas FdGlc PET measures the metabolic activity (Slosman et al. 1993). For example, Crippa et al. (1998) demonstrated that FdGlc uptake, represented by a standardised uptake value, was correlated with prognostic factors such as histopathological grading and p53 expression.

Measuring metabolic activity is especially important since clinical studies on gliomas and lymphomas have shown that hypermetabolic tumours usually have a poorer prognosis than hypometabolic ones (Brock et al. 1997). Minn et al. (1988) demonstrated that the proliferative activity of tumours could be measured by FdGlc PET. However, more recent research in vitro by Higashi et al. (1993) showed that FdGlc accumulation represented the number of viable tumour cells, not their

proliferative grade. However, a number of aspects of the behaviour and properties of the tumour cell should be taken into account.

First, there is the fact that a tumour is not homogeneous, which means that malignant cells, premalignant cells and benign cells are all seen in the tumour, distributed in a scattered way, e.g. clusters of malignant cells alternate with clusters of premalignant or even normal cells. Because scintigraphy can not detect these clusters separately, the average energy demand of the cells within a tumour is depicted, which hampers the assessment of the real metabolic status of the tumour.

Second, tumours often induce an inflammatory response, resulting in the invasion of macrophages and formation of granulation tissue. Kubota et al. (1992, 1994) reported that both macrophages and granulation tissue showed a higher uptake of FdGlc than did viable tumour cells. In their editorial, they summarised the components of a tumour: neoplastic tissue consisting of viable cells (high FdGlc uptake) and necrosis (no FdGlc uptake) and non-neoplastic tissue could be divided into macrophages, young granulation tissue (high FdGlc uptake) and a scar (no FdGlc uptake). This phenomenon directly created the false positive result in the work of Haberkorn (1991). He studied patients with colorectal tumours: a higher FdGlc uptake after radiotherapy was seen at the tumour site, despite the success of the therapy.

Third, it is not clear how long after the administration of FdGlc one has to wait before the start of the scintigraphic examination. FdGlc uptake with time is most likely dependent on local parameters, such as mitotic activity and growth rate. There is hardly any literature on this subject. Optimal post-therapeutic timing is also very important, thus reducing false-positive scans as a consequence of the uptake of the above-mentioned inflammatory cells.

Fourth, Sokoloff et al. (1977) and Phelps et al. (1979) were involved in the design of a three-compartment model for FdGlc to quantity its kinetic behaviour. This method assumes that radioactivity is spread homogeneously over the tumour. Since this is not the case, some doubts have arisen about the validity of this method (Schmidt et al. 1996). Also, the lumped constant, the ratio of FdGlc phosphorylation rate to the glucose phosphorylation rate under steady-state conditions, was developed in an attempt to relate the quantitative behaviour of FdGlc to that of glucose (Schmidt et al. 1996). The numerical value of this ratio is influenced by tumour treatment because of the physiological and biochemical response, such as the action of lysosomal acid hydrolases, necrosis (Fischman and Alpert 1993) and the changing activity of different isoforms of hexokinases.

Concluding remarks

This article elaborates on the resemblances and differences between tumour cells and normal cells in the uptake and biochemical processing of glucose. As described, up-regulation of the membrane glucose transporters and enhanced production of glycolytic enzymes (hexokinase) are considered to be the main causes for the increased uptake and metabolism of glucose by tumour cells. Furthermore, the differences in the behaviour of glucose and FdGlc within tumour cells were reviewed, as well as the factors that influence uptake of FdGlc and the limitations of FdGlc scintigraphy in relation to pathophysiology.

Despite the increasing clinical use of FdGlc PET, which has proved to be of great value in solving individual clinical problems, the issue of quantification remains. Three major questions are still unanswered. First, what level of accumulation determines whether a cell is malignant or not? Second, how great a decrease in FdGlc uptake by a tumour must be achieved to assume effective treatment? Third, does FdGlc uptake measure the degree of aggressiveness of a tumour? One could speculate whether a single measurement of glucose metabolism could give an answer to these clinical questions. Future research may at least partly unravel these deficiencies in our knowledge.

References

Adams V, Griffin LD, Gelb BD, McCabe ER (1991) Protein kinase activity of rat brain hexokinase. Biochem Biophys Res Commun 177: 1101–1106

Arora KK, Pedersen PL (1988) Functional significance of mitochondrial bound hexokinase in tumor cell metabolism. Evidence for preferential phosphorylation of glucose by intramitochondrially generated ATP. J Biol Chem 263: 17 422–17 428

Arora KK, Pedersen PL (1993) Glucose utilization by tumor cells: the enzyme hexokinase autophosphorylates both its N- and C-terminal halves [published erratum appears in Arch Biochem Biophys 307: 224 (1993)]. Arch Biochem Biophys 304: 515–518

Arora KK, Filburn CR, Pedersen PL (1993) Structure/function relationships in hexokinase. Site-directed mutational analyses and characterization of overexpressed fragments implicate different functions for the N- and C-terminal halves of the enzyme. J Biol Chem 268: 18 259–18 266

Baldwin SA (1993) Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochim Biophys Acta 1154: 17–49

Barros LF, Yudilevich DL, Jarvis SM, Beaumont N, Baldwin SA (1995) Quantitation and immunolocalization of glucose transporters in the human placenta. Placenta 16: 623–633

Birnbaum MJ, Haspel HC, Rosen OM (1987) Transformation of rat fibroblasts by FSV rapidly increases glucose transporter gene transcription. Science 235: 1495–1498

Boden G, Murer E, Mozzoli M (1994) Glucose transporter proteins in human insulinoma [published erratum appears in Ann Intern Med 121: 470 (1994)]. Ann Intern Med 121: 109–112

Boros LG, Puigjaner J, Cascante M, Lee WN, Brandes JL, Bassilian S, Yusuf FI, Williams RD, Muscarella P, Melvin WS, Schirmer WJ (1997) Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. Cancer Res 57: 4242–4248

Brock CS, Meikle SR, Price P (1997) Does fluorine-18 fluorodeoxyglucose metabolic imaging of tumours benefit oncology? (See comments). Eur J Nucl Med 24: 691–705

Brown RS, Wahl RL (1993) Overexpression of Glut-1 glucose transporter in human breast cancer. An immunohistochemical study. Cancer 72: 2979–2985

Bustamante E, Pedersen PL (1977) High aerobic glycolysis of rat hepatoma cells in culture: role of mitochondrial hexokinase. Proc Natl Acad Sci USA 74: 3735–3739

Carruthers A (1990) Facilitated diffusion of glucose. Physiol Rev 70: 1135–1176

Clavo AC, Brown RS, Wahl RL (1995) Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. J Nucl Med 36: 1625–1632

Cornford EM, Hyman S, Black KL, Cornford ME, Vinters HV, Pardridge WM (1995) High expression of the Glut1 glucose transporter in human brain hemangioblastoma endothelium. J Neuropathol Exp Neurol 54: 842–851

Crippa F, Seregni E, Agresti R, Chiesa C, Pascali C, Bogni A, Decise D, De Sanctis V, Greco M, Daidone MG, Bombardieri E (1998) Association between [18F]fluorodeoxyglucose uptake and postoperative histopathology, hormone receptor status, thymidine labelling index and p53 in primary breast cancer: a preliminary observation. Eur J Nucl Med 25: 1429–1434

Fischman AJ, Alpert NM (1993) FDG-PET in oncology: there's more to it than looking at pictures [editorial; comment]. J Nucl Med 34: 6–11

Flier JS, Mueckler MM, Usher P, Lodish HF (1987) Elevated levels of glucose transport and transporter messenger RNA are induced by *ras* or *src* oncogenes. Science 235: 1492–1495

Golshani Hebroni SG, Bessman SP (1997) Hexokinase binding to mitochondria: a basis for proliferative energy metabolism. J Bioenerg Biomembr 29: 331–338

Granner D, Pilkis S (1990) The genes of hepatic glucose metabolism. J Biol Chem 265: 10 173–10 176

Gutman A, Wasylyk B (1990) The collagenase gene promoter contains a TPA and oncogene-responsive unit encompassing the PEA3 and AP-1 binding sites. EMBO J 9: 2241–2246

Haber RS, Weinstein SP, E OB, Morgello S (1993) Tissue distribution of the human GLUT3 glucose transporter. Endocrinology 132: 2538–2543

Haber RS, Weiser KR, Pritsker A, Reder I, Burstein DE (1997) GLUT1 glucose transporter expression in benign and malignant thyroid nodules. Thyroid 7: 363–367

Haberkorn U, Strauss LG, Dimitrakopoulou A, Engenhart R, Oberdorfer F, Ostertag H, Romahn J, van Kaick G (1991) PET studies of fluorodeoxyglucose metabolism in patients with recurrent colorectal tumors receiving radiotherapy. J Nucl Med 32: 1485–1490

Higashi K, Clavo AC, Wahl RL (1993) Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake (see comments). J Nucl Med 34: 414–419

Holman GD, Kasuga M (1997) From receptor to transporter: insulin signalling to glucose transport. Diabetologia 40: 991–1003

Hwang YY, Kim SG, Evelhoch JL, Ackerman JJ (1992) Non-glycolytic acidification of murine radiation-induced fibrosarcoma 1 tumor via 3-*O*-methyl-D-glucose monitored by 1H, 2H, 13C, and 31P nuclear magnetic resonance spectroscopy. Cancer Res 52: 1259–1266

Ikeda TS, Hwang ES, Coady MJ, Hirayama BA, Hediger MA, Wright EM (1989) Characterization of a Na^+/glucose cotransporter cloned from rabbit small intestine. J Membr Biol 110: 87–95

Inukai K, Katagiri H, Takata K, Asano T, Anai M, Ishihara H, Nakazaki M, Kikuchi M, Yazaki Y, Oka Y (1995) Characterization of rat GLUT5 and functional analysis of chimeric proteins of GLUT1 glucose transporter and GLUT5 fructose transporter. Endocrinology 136: 4850–4857

Jetton TL, Liang Y, Pettepher CC, Zimmerman EC, Cox FG, Horvath K, Matschinsky FM, Magnuson MA (1994) Analysis of upstream glucokinase promoter activity in transgenic mice and identification of glucokinase in rare neuroendocrine cells in the brain and gut. J Biol Chem 269: 3641–3654

Johansson T, Berrez JM, Nelson BD (1985) Evidence that transcription of the hexokinase gene is increased in a rapidly growing rat hepatoma. Biochem Biophys Res Commun 133: 608–613

Jung CY (1998) Proteins that interact with facilitative glucose transporters: implication for function. Exp Physiol 83: 267–273

Kanai Y, Lee WS, You G, Brown D, Hediger MA (1994) The human kidney low affinity Na^+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. J Clin Inves 93: 397–404

Kasanicki MA, Pilch PF (1990) Regulation of glucose-transporter function (review, 85 references). Diabetes Care 13: 219–227

Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS, Eddy RL, Byers MG, Shows TB, Seino S, Bell GI (1990) Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J Biol Chem 265: 13 276–13 282

Kubota R, Yamada S, Kubota K, Ishiwata K, Tamahashi N, Ido T (1992) Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. J Nucl Med 33: 1972–1980

Kubota R, Kubota K, Yamada S, Tada M, Ido T, Tamahashi N (1994) Microautoradiographic study for the differentiation of intratumoral macrophages, granulation tissues and cancer cells by the dynamics of fluorine-18-fluorodeoxyglucose uptake. J Nucl Med 35: 104–112

Leloup C, Arluison M, Lepetit N, Cartier N, Marfaing Jallat P, Ferre P, Penicaud L (1994) Glucose transporter 2 (GLUT 2): expression in specific brain nuclei. Brain Res 638: 221–226

Maher F (1995) Immunolocalization of GLUT1 and GLUT3 glucose transporters in primary cultured neurons and glia. J Neurosci Res 42: 459–469

Maher F, Davies Hill TM, Simpson IA (1996) Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons. Biochem J 315: 827–831

Mantych GJ, James DE, Devaskar SU (1993) Jejunal/kidney glucose transporter isoform (Glut-5) is expressed in the human blood-brain barrier. Endocrinology 132: 35–40

Mathupala SP, Rempel A, Pedersen PL (1995) Glucose catabolism in cancer cells. Isolation, sequence, and activity of the promoter for type II hexokinase. J Biol Chem 270: 16 918–16 925

Mathupala SP, Heese C, Pedersen PL (1997) Glucose catabolism in cancer cells. The type II hexokinase promoter contains functionally active response elements for the tumor suppressor p53. J Biol Chem 272: 22 776–22 780

Mellanen P, Minn H, Grenman R, Harkonen P (1994) Expression of glucose transporters in head-and-neck tumors. Int J Cancer 56: 622–629

Merrall NW, Plevin R, Gould GW (1993) Growth factors, mitogens, oncogenes and the regulation of glucose transport. Cell Signal 5: 667–675

Mesonero J, Matosin M, Cambier D, Rodriguez Yoldi MJ, Brot Laroche E (1995) Sugar-dependent expression of the fructose transporter GLUT5 in Caco-2 cells. Biochem J 312: 757–762

Miller JH, Mullin JM, McAvoy E, Kleinzeller A (1992) Polarity of transport of 2-deoxy-D-glucose and D-glucose by cultured renal epithelia (LLC-PK1). Biochim Biophys Acta 1110: 209–217

Minn H, Joensuu H, Ahonen A, Klemi P (1988) Fluorodeoxyglucose imaging: a method to assess the proliferative activity of human cancer in vivo. Comparison with DNA flow cytometry in head and neck tumors. Cancer 61: 1776–1781

Miyamoto K, Hase K, Takagi T, Fujii T, Taketani Y, Minami H, Oka T, Nakabou Y (1993) Differential responses of intestinal glucose transporter mRNA transcripts to levels of dietary sugars. Biochem J 295: 211–215

Miyamoto K, Tatsumi S, Morimoto A, Minami H, Yamamoto H, Sone K, Taketani Y, Nakabou Y, Oka T, Takeda E (1994) Characterization of the rabbit intestinal fructose transporter (GLUT5). Biochem J 303: 877–883

Mueckler M (1994) Facilitative glucose transporters. Eur J Biochem 219: 713–725

Mukhopadhyay T, Maxwell AS, Roth JA (1995) p53 supppressor gene, Landes, Austin, Tex

Nagase Y, Takata K, Moriyama N, Aso Y, Murakami T, Hirano H (1995) Immunohistochemical localization of glucose transporters in human renal cell carcinoma. J Urol 153: 798–801

Nakashima RA, Paggi MG, Pedersen PL (1984) Contributions of glycolysis and oxidative phosphorylation to adenosine 5'-triphosphate production in AS-30D hepatoma cells. Cancer Res 44: 5702–5706

Nelson CA, Wang JQ, Leav I, Crane PD (1996) The interaction among glucose transport, hexokinase, and glucose-6-phosphatase with respect to 3H-2-deoxyglucose retention in murine tumor models. Nucl Med Biol 23: 533–541

Nishioka T, Oda Y, Seino Y, Yamamoto T, Inagaki N, Yano H, Imura H, Shigemoto R, Kikuchi H (1992) Distribution of the glucose transporters in human brain tumors. Cancer Res 52: 3972–3979

Okazumi S, Isono K, Enomoto K, Kikuchi T, Ozaki M, Yamamoto H, Hayashi H, Asano T, Ryu M (1992) Evaluation of liver tumors using fluorine-18-fluorodeoxyglucose PET: characterization of tumor and assessment of effect of treatment (see comments). J Nucl Med 33: 333–339

Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)2-fluoro-2-deoxy-D-glucose: validation of method. Ann Neurol 6: 371–388

Pohl J, Bertram B, Hilgard P, Nowrousian MR, Stuben J, Wiessler M (1995) D-19575 – a sugar-linked isophosphoramide mustard derivative exploiting transmembrane glucose transport. Cancer Chemother Pharmacol 35: 364–370

Printz RL, Magnuson MA, Granner DK (1993) Mammalian glucokinase. Annu Rev Nutr 13: 463–496

Rea S, James DE (1997) Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles. Diabetes 46: 1667–1677

Reisser C, Eichhorn K, Herold Mende C, Born AI, Bannasch P (1999) Expression of facilitative glucose transport proteins during development of squamous cell carcinomas of the head and neck. Int J Cancer 80: 194–198

Rempel A, Bannasch P, Mayer D (1994) Differences in expression and intracellular distribution of hexokinase isoenzymes in rat liver cells of different transformation stages. Biochim Biophys Acta 1219: 660–668

Rempel A, Mathupala SP, Perdersen PL (1996) Glucose catabolism in cancer cells: regulation of the Type II hexokinase promoter by glucose and cyclic AMP. FEBS Lett 385: 233–237

Rigo P, Paulus P, Kaschten BJ, Hustinx R, Bury T, Jerusalem G, Benoit T, Foidart Willems J (1996) Oncological applications of positron emission tomography with fluorine-18 fluorodeoxyglucose. Eur J Nucl Med 23: 1641–1674

Schmidt KC, Lucignani G, Sokoloff L (1996) Fluorine-18-fluorodeoxyglucose PET to determine regional cerebral glucose utilization: a re-examination. J Nucl Med 37: 394–349

Simmons RA, Flozak AS, Ogata ES (1993) Glucose regulates glut 1 function and expression in fetal rat lung and muscle in vitro. Endocrinology 132: 2312–2318

Singh M, Singh VN, August JT, Horecker BL (1974) Alterations in glucose metabolism in chick embryo cells transformed by Rous sarcoma virus. Transformation-specific changes in the activities of key enzymes of the glycolytic and hexose monophosphate shunt pathways. Arch Biochem Biophys 165: 240–246

Slosman DO, Pittet N, Donath A, Polla BS (1993) Fluorodeoxyglucose cell incorporation as an index of cell proliferation: evaluation of accuracy in cell culture. Eur J Nucl Med 20: 1084–1088

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: 897–916

Strauss LG, Conti PS (1991) The applications of PET in clinical oncology. J Nucl Med 32: 623–648

Sviderskaya EV, Jazrawi E, Baldwin SA, Widnell CC, Pasternak CA (1996) Cellular stress causes accumulation of the glucose transporter at the surface of cells independently of their insulin sensitivity. J Membr Biol 149: 133–140

Tadokoro C, Yoshimoto Y, Sakata M, Imai T, Yamaguchi M, Kurachi H, Oka Y, Maeda T, Miyake A (1995) Expression and localization of glucose transporter 1 (GLUT1) in the rat oviduct: a possible supplier of glucose to embryo during early embryonic development. Biochem Biophys Res Commun 214: 1211–1218

Tai PK, Liao JF, Chen EH, Dietz J, Schwartz J, Carter Su C (1990) Differential regulation of two glucose transporters by chronic growth hormone treatment of cultured 3T3-F442A adipose cells. J Biol Chem 265: 21 828–21 834

Thorens B, Sarkar HK, Kaback HR, Lodish HF (1988) Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. Cell 55: 281–290

Thorens B, Cheng ZQ, Brown D, Lodish HF (1990) Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells [corrected and republished with original paging, article originally printed in J Am Physiol 259: C279–285 (1990)]. Am J Physiol 259: C279–285

Todaka M, Nishiyama T, Murakami T, Saito S, Ito K, Kanai F, Kan M, Ishii K, Hayashi H, Shichiri M, et al (1994) The role of insulin in activation of two enhancers in the mouse GLUT1 gene. J Biol Chem 269: 29 265–29 270

Torizuka T, Clavo AC, Wahl RL (1997) Effect of hyperglycemia on in vitro tumor uptake of tritiated FDG, thymidine, L-methionine and L-leucine. J Nucl Med 38: 382–386

Turk E, Martin MG, Wright EM (1994) Structure of the human Na^+/glucose cotransporter gene SGLT1. J Biol Chem 269: 15 204–15 209

Waddell ID, Zomerschoe AG, Voice MW, Burchell A (1992) Cloning and expression of a hepatic microsomal glucose transport protein. Comparison with liver plasma-membrane glucose-transport protein GLUT 2. Biochem J 286: 173–177

Wahl RL, Cody RL, Hutchins GD, Mudgett EE (1991) Primary and metastatic breast carcinoma: initial clinical evaluation with PET with the radiolabeled glucose analogue 2-[F-18]-fluoro-2-deoxy-D-glucose. Radiology 179: 765–770

Wahl RL, Henry CA, Ethier SP (1992) Serum glucose: effects on tumor and normal tissue accumulation of 2-[F-18]-fluoro-2-deoxy-D-glucose in rodents with mammary carcinoma. Radiology 183: 643–647

Wang GL, Semenza GL (1995) Purification and characterization of hypoxia-inducible factor 1. J Biol Chem 270: 1230–1237

Warburg (1930) The metabolism of tumors. Arnold Constable, London

Weinstein SP, Haber RS (1993) Glucose transport stimulation by thyroid hormone in ARL 15 cells: partial role of increased GLUT1 glucose transporter gene transcription. Thyroid 3: 135–142

Wilson CM, Mitsumoto Y, Maher F, Klip A (1995) Regulation of cell surface GLUT1, GLUT3, and GLUT4 by insulin and IGF-I in L6 myotubes. FEBS Lett 368: 19–22

Yamamoto T, Seino Y, Fukumoto H, Koh G, Yano H, Inagaki N, Yamada Y, Inoue K, Manabe T, Imura H (1990) Over-expression of facilitative glucose transporter genes in human cancer. Biochem Biophys Res Commun 170: 223–230

Younes M, Lechago LV, Somoano JR, Mosharaf M, Lechago J (1996) Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers. Cancer Res 56: 1164–1167

Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT (1997) Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer 80: 1046–1051

Zamora Leon SP, Golde DW, Concha II, Rivas CI, Delgado Lopez F, Baselga J, Nualart F, Vera JC (1996) Expression of the fructose transporter GLUT5 in human breast cancer [published erratum appears in Proc Natl Acad Sci USA 93: 15 522 (1996)]. Proc Natl Acad Sci USA 93: 1847–1852