

# Cellular Origin and Molecular Mechanisms of $^{18}\text{F}$ -FDG Uptake: Is There a Contribution of the Endothelium?

**T**he glucose analog  $^{18}\text{F}$ -FDG is a surrogate marker for glucose metabolism and the most commonly used radiopharmaceutical for PET. The number of clinical applications for  $^{18}\text{F}$ -FDG and PET continues to increase, especially in the field of oncology. It has been known for many years that increased glycolysis is a distinctive feature of malignant tumors compared with normal tissues (1). Di Chiro et al. first demonstrated a more intense accumulation of  $^{18}\text{F}$ -FDG in human brain tumors than in surrounding brain (2). The concept of preferential  $^{18}\text{F}$ -FDG uptake in malignant tumors was rapidly extended to many other cancers. However, the underlying mechanism of  $^{18}\text{F}$ -FDG uptake in various tissues is not completely understood. Further evidence of the cellular origin and molecular mechanisms of  $^{18}\text{F}$ -FDG uptake is reported by Maschauer et al. in this issue of *The Journal of Nuclear Medicine* (3).

These authors (3) evaluated the uptake of  $^{18}\text{F}$ -FDG in human umbilical vein endothelial cells (HUVECs) serving as a model of vascular endothelium. In cell culture, they report similar uptake values as compared with tumor cells and a higher  $^{18}\text{F}$ -FDG uptake as compared with human macrophages. Therefore, they suggest a contribution of the endothelium to  $^{18}\text{F}$ -FDG uptake patterns observed in neoplastic and vascularized lesions. They further demonstrate a modulation of  $^{18}\text{F}$ -FDG

uptake under certain experimental conditions. Though increased glucose and concomitantly  $^{18}\text{F}$ -FDG consumption after glucose deprivation has been shown before (4), an interesting finding is the increased  $^{18}\text{F}$ -FDG uptake after incubation of HUVECs with vascular endothelial growth factor (VEGF). VEGF is an endothelial cytokine that stimulates proliferation and migration of vascularly derived endothelial cells and is overexpressed in a variety of tumors, including renal, breast, ovary, and colon cancer. Consecutively, an upregulation of respective receptors VEGF-R1 and VEGF-R2 can be found in tumor-derived endothelium. After adding VEGF to the medium, the authors describe an 82% increase of  $^{18}\text{F}$ -FDG uptake in HUVECs compared with untreated controls. According to these findings, VEGF-stimulated endothelial cells may contribute to total  $^{18}\text{F}$ -FDG uptake.

However, caution is necessary when applying the results to physiologic conditions. The main obstacle of the study is the cell type examined. HUVECs can hardly be used to estimate the contribution of the endothelium to  $^{18}\text{F}$ -FDG uptake patterns in vivo. Furthermore, the necessity to perform uptake studies at  $4^{\circ}\text{C}$  makes it difficult to relate the findings to human disease. Therefore, the metabolism of  $^{18}\text{F}$ -FDG in vascular endothelium must be examined in further studies. So far, only a limited number of models are available to study the metabolism of vascular endothelium or metabolic alterations during angiogenesis. A suitable model for this application might be the rabbit eye model in which tumors are implanted in the anterior eye chamber (5). Subsequently, tumor

growth and neoangiogenesis can be evaluated in the rabbit cornea. Another approach is the use of tumor xenotransplant models using immunodeficient mice (6).

The idea of endothelial cells contributing to  $^{18}\text{F}$ -FDG uptake patterns in tumors as well as vascularized lesions is innovative. Previous in vitro studies indicated that  $^{18}\text{F}$ -FDG uptake is determined mainly by the number of viable tumor cells (7). Whereas necrotic or fibrotic tissue may reduce tracer uptake, the presence of inflammatory cells potentially results in an increased  $^{18}\text{F}$ -FDG accumulation (8,9). Animal studies have shown that inflammatory cells significantly contribute to  $^{18}\text{F}$ -FDG uptake in tumors. Using a tumor mouse model, Kubota et al. reported that 29% of  $^{18}\text{F}$ -FDG uptake was related to nontumoral tissue (10). Recent clinical investigations indicate that  $^{18}\text{F}$ -FDG uptake in tumors does not precisely reflect the number of viable tumor cells (11–13).

The detailed molecular mechanism of  $^{18}\text{F}$ -FDG accumulation within cells has been investigated in vitro and in vivo.  $^{18}\text{F}$ -FDG enters the cell by the same membrane transport mechanism as glucose. After penetration of the cellular membrane via glucose transporters, both  $^{18}\text{F}$ -FDG and glucose are phosphorylated by hexokinase. Unlike glucose-6-phosphate,  $^{18}\text{F}$ -FDG-6-phosphate is not a substrate of glucose-6-phosphate isomerase and does not undergo further metabolism in the glucose pathway.  $^{18}\text{F}$ -FDG is therefore trapped within cells. Three phenomena have been recognized to cause increased glucose utilization in tumor cells: overexpression of membrane glucose transporters, especially Glut-1

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For correspondence or reprints contact: Andreas K. Buck, MD, University of Ulm, Robert-Koch-Strasse 8, D-89081 Ulm, Germany.

E-mail: andreas.buck@medizin.uni-

and Glut-3 (14,15); increased hexokinase activity (16); and decreased levels of glucose-6-phosphatase (17). In tumors with higher activity of glucose-6-phosphatase, a lower  $^{18}\text{F}$ -FDG uptake may occur—for example, in hepatocellular carcinoma (18). It remains to be determined which of those steps is rate limiting in the process of  $^{18}\text{F}$ -FDG uptake in cancer cells. The overexpression of Glut-1 in nearly all human cancers has lead to the speculation that glucose metabolism is predominantly regulated by glucose transporters. However, it was demonstrated recently that a tumor cell line with low expression of Glut-1 accumulated  $^{18}\text{F}$ -FDG more rapidly than a cell line with high expression (19). In a clinical study, Torizuka et al. have shown by kinetic modeling that the phosphorylation step appears to be rate determining in the uptake of  $^{18}\text{F}$ -FDG in breast but not in lung cancer (20). Therefore, mechanisms regulating  $^{18}\text{F}$ -FDG uptake may vary among cancers derived from different tissues.

Glut-1 expression has also been found in vascular endothelial cells. Exposure of endothelial cells to hypoxia results in a 9-fold upregulation of Glut-1 messenger RNA (mRNA) expression and a 2- to 3-fold increase of 2-deoxyglucose influx, suggesting an analogous uptake mechanism of  $^{18}\text{F}$ -FDG (21). There are only limited studies on the effects of cytokines on glucose transport in endothelial cells. However, a VEGF-mediated increase of glucose transport was also demonstrated by Pan et al., who exposed bovine aortic endothelial cells to VEGF and detected an increased glucose transport and elevated Glut-1 mRNA levels (22).

Overexpression of VEGF and other stress-related proteins or signaling molecules, such as interleukin-1, transforming growth factor- $\alpha$  (TNF- $\alpha$ ), or transforming growth factor- $\beta$  (TGF- $\beta$ ), have also been observed in hypoxic tissue derived from tumors. Recently, it was suggested that hypoxia increases the uptake of  $^{18}\text{F}$ -FDG through activation of the glycolytic pathway. After exposing breast cancer cells to a hypoxic atmosphere, Clavo et al. detected

a significant increase of  $^{18}\text{F}$ -FDG uptake (23). Burgman et al. reported that Glut-1 receptors are upregulated 2-fold but not overexpressed in hypoxic tumor cells (24). Furthermore, a concomitant overexpression of VEGF and Glut-1 was reported in hypoxic lung cancer xenografts (25). Therefore, a connection of hypoxia and induction of glycolysis via VEGF-induced Glut-1 expression seems feasible.

Under physiologic conditions, cell turnover in the endothelium is a rare event and the energy demand is low. At a specific time point, only 1 of 100,000 endothelial cells passes through the cell cycle (26). Solitary tumors can achieve a size of >1–2 mm only if sufficient oxygen and blood supply are provided by newly formed blood vessels. This angiogenic switch is also the basis for invasive growth of carcinomas and metastasis (26). Besides VEGF, neoangiogenesis and concomitant proliferation of endothelial cells are mediated by several growth factors, such as fibroblast growth factors, platelet-derived growth factor (PDGF), TGF- $\alpha$ , and vascular cell adhesion molecules (e.g., VCAM-1). A correlation between  $^{18}\text{F}$ -FDG uptake and these parameters may exist but has not been investigated so far.

The hypothesis of endothelial cell  $^{18}\text{F}$ -FDG accumulation is further supported by 2 clinical studies (12,13) that report a significant correlation of microvessel density and tumor  $^{18}\text{F}$ -FDG uptake in 70 and 55 patients with breast cancer, respectively. Microvessel density turned out to be an indicator of higher malignancy (27) and an independent predictor of prognosis—for example, in breast cancer and melanoma (28,29). Vascular endothelium may promote  $^{18}\text{F}$ -FDG uptake not exclusively by enhanced delivery of  $^{18}\text{F}$ -FDG to the tumor cells. A significant  $^{18}\text{F}$ -FDG uptake in vascular endothelial cells may also contribute to overall  $^{18}\text{F}$ -FDG uptake.

In summary, the definite mechanism for  $^{18}\text{F}$ -FDG uptake patterns in tumors remains to be determined. Various cellular components and a variety of molecular mechanisms contribute to re-

spective  $^{18}\text{F}$ -FDG uptake. The most important factors comprise expression of Glut-1 and hexokinase, the number of viable tumor cells, microvessel density, tumor cell proliferation, and the presence of inflammatory cells. The study of Maschauer et al. (3) gives further evidence that VEGF-stimulated endothelium may contribute to  $^{18}\text{F}$ -FDG uptake in a lesion. Additional studies using models especially designed for the evaluation of angiogenesis are necessary to further elucidate the relevance of the endothelium to  $^{18}\text{F}$ -FDG uptake patterns.

**Andreas K. Buck, MD**  
**Sven N. Reske, MD**  
*University of Ulm*  
*Ulm, Germany*

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