Fluorodeoxyglucose Uptake in Human Cancer Cell Lines Is Increased by Hypoxia

Anaira C. Clavo, Raya S. Brown and Richard L. Wahl

Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan

Malignant neoplasms commonly have increased rates of glucose utilization, poor perfusion and areas of low oxygenation. Autoradiographic studies of excised tumors have shown increased FDG uptake in viable cells near necrotic portions of tumor. We evaluated in vitro whether tumor cell FDG uptake increased with hypoxia. **Methods:** The uptake of ^{3}H-FDG into two human tumor cell lines (HTB 63 melanoma and HTB 77 IP3 ovarian carcinoma) was determined after exposure to differing oxygen atmospheres ranging from 0% to 20% O_2 for varying time periods. Glucose transport was independently determined as well as estimates of the level of Glut-1 glucose transporter membrane protein. **Results:** FDG uptake in both the melanoma and the ovarian carcinoma cell lines increased significantly (39.6% ± 6.7% and 36.7% ± 9%, respectively) over basal (20% O_2) conditions when cells were exposed to a mild hypoxic environment (5% O_2) for 1.5 hr. With a 4-hr exposure to 1.5% O_2, the increase in FDG uptake was greater at 52.3% ± 8.9% and 43.5% ± 19%, respectively. With 4 hr of anoxia, the increase in FDG uptake over basal conditions was 42.7% ± 10% and 63.3% ± 13.7% for melanoma and ovarian carcinoma cells, respectively. Membrane transport of 3-O-methylglucose (3-OMG) was increased by hypoxia for melanoma and ovarian carcinoma. Immunochemical assays for Glut-1 showed an increase in the membrane expression of the Glut-1 transporter in cells exposed to hypoxia. **Conclusion:** Hypoxia increases cellular uptake of FDG in two different malignant human cell lines. Increased glucose transport, in part due to increased membrane expression of the Glut-1 glucose transporter, contributes to this phenomenon. Increased FDG uptake in tumors visualized during PET imaging may be partly reflective of tumor hypoxia.

Key Words: fluorodeoxyglucose; methylglucose; hypoxia; immunocytochemistry; cancer cell lines

J Nucl Med 1995; 36:1625–1632

Increased glycolysis is one of the most common biochemical characteristics of malignant tumors (*1–6*). Glucose transport in mammalian tissues is mediated by a family of structurally related but genetically distinct glucose-transporter proteins. The factors that regulate glucose-transporter gene expression in vitro include oncogenes (*7*), growth factors, insulin, oral hypoglycemic agents, vanadate, glucocorticoids, glucose levels and the state of cellular differentiation (*8*); however, in vivo, glucose-transporter gene expression in adipose cells, skeletal muscle and liver is markedly affected by nutritional and metabolic states (*9*).

Glucose can enter cells by two types of catalyzed processes: facilitated diffusion and sodium-dependent active transport. With facilitated diffusion, glucose transport occurs from a region of higher to lower concentration through specialized lipid-soluble transport proteins (gates) that form an integral part of the membrane. Active transport is also mediated by membrane transport proteins (pumps) but requires a primary source of energy, with glucose being carried into the cell against a concentration gradient (*10*). Increased glucose utilization is common in malignancies and can be imaged using PET by assessing the distribution of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG). Although we and others have shown that FDG accumulates into tumor cells in proportion to the number of viable cancer cells, such in vitro studies were performed under conditions of excellent oxygenation (*11–13*). Malignant neoplasms exhibit regions of low oxygenation due to poor perfusion and have increased rates of glucose utilization. Cells near the necrotic area of the tumor display an increased glucose (FDG) uptake as demonstrated by autoradiographic studies (*14,15*). Since hypoxia has clinical significance in the outcome of conventional radiotherapy and some forms of chemotherapy treatments (*16*), we have evaluated FDG uptake in cancer cells in the presence of several levels of oxygenation, including hypoxia. These in vitro studies may help explain the mechanism(s) responsible for increased FDG uptake in both PET tumor imaging and cells near necrotic regions of tumors.

MATERIALS AND METHODS

Cell Lines

The human melanoma HTB 63 and ovarian carcinoma HTB 77 cell lines were purchased and handled as described by the American Type Culture Collection (ATCC, Rockville, MD). The IP3 subline of the HTB 77 cell line, originally described by Wahl et al. (*17*), was used. Briefly, HTB 63 and HTB 77 IP3 cells were grown in either McCoy's 5a or RPMI 1640 medium (Sigma, St. Louis,

Received Sept. 19, 1994; revision accepted Mar. 6, 1995.

For correspondence or reprints contact: Richard L. Wahl, MD, Division of Nuclear Medicine, University of Michigan Medical Center, 1500 E Medical Center Dr., B1G 412, Ann Arbor, MI 48109-0028.

MO) and maintained in a 5% CO_2-humidified atmosphere at 37°C (Forma, Marietta, OH) until ready for use. All media were supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 50 IU/ml penicillin G and 50 μg/ml streptomycin sulfate. D-(+)-glucose (10% w/v) was added to glucose-free RPMI to obtain the desired final concentration. Generally, experiments were conducted at 100 mg/dl glucose (5.5 m*M*) unless otherwise indicated. All additives (antibiotics and supplements), enzymatic dispersing agents and buffers such as HBSS (Hanks' Balanced Salt Solution without Ca^{+2} or Mg^{+2}) and phosphate buffer saline (PBS) were obtained either from Sigma or Celox (Hopkins, MN). Feeding of the cultures was conducted on alternate days with a complete change of media. At confluency, cells were dissociated with either trypsin (0.25%) or trypsin-EDTA (0.05% trypsin; 0.02% EDTA), their concentration adjusted as needed and used as described in the specific protocol. Tumor cell viability was assessed by the Trypan Blue dye exclusion technique using an Olympus (Lake Success, NY) IMT-2 inverted microscope.

Oxygen Studies

Medical gas mixtures containing different oxygen concentrations (0, 1.5, 5, 10 or 20% O_2:5% CO_2:balance N_2) were obtained from Aga Gas (Cleveland, OH) and further tested in an ABL-30, CO Oximeter 282 (Radiometer, Copenhagen, Denmark) blood gas analyzer for accuracy of oxygen concentrations. The gasses were delivered into sterile, no additives, Vacutainer™ (Becton Dickinson, Rutherford NJ) tubes containing 0.5×10^6 cells in RPMI media (either 2 or 3 ml) using a N_2 CGA 580 regulator (Victor Equipment Co., Denton, TX) to provide a constant low pressure of 86.2 kPa (1 psi = 6.895 kPa) for up to 4 hr and of 43.1 kPa for the longer periods. Incubation times at the desired oxygen tension varied from 20 min to 4 and 24 hr before adding the radiolabeled compound and 1 hr longer for the uptake study, always maintaining the 37°C temperature. The 1-hr tracer uptake period was selected to mimic the in vivo uptake duration for FDG-PET studies in cancer patients.

Tritiated DG Tracer Uptake

FDG and 3-OMG were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Cells were seeded at an initial density of 1×10^6 cells/ml in 150 cm^2 tissue culture flasks, fed with a complete change in media on the second and fifth days and used on the sixth day. This procedure assured the use of cells in a near plateau phase as assessed by flow cytometric assays (data not shown). After dissociation, 0.5×10^6 cells in RPMI media (2 ml) were aliquoted into sterile Vacutainer™ tubes and exposed to the desired O_2 tension for 20 min, 1.5, 4 or 24 hr. Radioactive tracer was diluted in RPMI, added to cells (2 μCi in 100 μl of growth media) and incubation continued for approximately 60 min. Tracer incorporation was either halted by using ice-cold HBSS or cells were spun at 250× g for 10 min at 4°C and the supernates were saved in sterile Vacutainer™ tubes for lactate and glucose determinations. Cell pellets were resuspended, washed three times with 4.5 ml of ice-cold HBSS and immediately lysed with 0.3 *M* NaOH:1% SDS (incubated 30 min at room temperature). Whole-cell extracts were transferred to scintillation vials containing 10 ml Hionic Fluor (Packard, Meridien, CT) and the vials were stored overnight, in the dark, at 4°C. Bound radioactivity was measured by beta counting the following day in a 1600 TR Packard liquid scintillation analyzer (Meridien, CT). Tracer uptake was expressed as counts per minute per total number of cells in a tube (total tracer uptake) or the percent basal levels and represents the mean of four determinations ± s.e.m., unless stated otherwise.

Glucose and Lactate Determinations in the Growth Media

Lactic acid was measured by UV endpoint (Sigma, St. Louis, MO). The catalytic action of lactate dehydrogenase (LD) permits measurement of lactate by monitoring the generation of NADH spectrophotometrically at 340 nm (*18*). Glucose in the media was estimated with an Accu Check IIm and Chemstrips bG (Boehringer Mannheim, Indianapolis, IN). Results represent the mean of four determinations ± s.d.

3-OMG Transport

Transport assay of this nonmetabolizable sugar was performed as previously described by Reeves (*19*) with slight variations. Cells were grown until near confluence and dispersed into single cell suspensions. They were aliquotted in sterile Vacutainer™ tubes to obtain 1×10^6 cells in a final volume of 50 μl of RPMI. Tubes were incubated for 2 hr at 37°C in RPMI containing either 100 or 500 mg/dl glucose (5.5 and 28 m*M*, respectively). Radiotracer (1 μCi equal to 10 μ*M* of ^{3}H-3-OMG in 50 μl growth media per sample) was added to the tubes and the incubation proceeded for at least another 10 min at 37°C in a water bath/shaker. At the end of this period, cold HBSS buffer was added simultaneously to all tubes to stop tracer flux, and the cells were immediately harvested on a Whatman (Hillsboro, OR) GF/B filter using a Brandel apparatus (Gaithersburg, MD), and automated solid-liquid separation device. Filters (1.0 μm pore size) were presoaked for at least 2 hr in 50 m*M* glucose. After the diluted cells were aspirated, tubes were rapidly filled four more times with cold buffer to ensure complete collection of sample cells. Individual filter paper disks were placed into labeled scintillation vials and the cells were lysed as described above for FDG uptake. The results represent the average of four individual determinations ± s.d. When transport was measured in the presence of varying levels of oxygen (1.5% or 20%), the same number of cells were first incubated in 2 ml RPMI with 100 mg/dl glucose for 4 hr and then 2 μCi (in 100 μl of the same growth media) of tracer were added. Samples were rapidly collected and processed as before except a Reeves Angel (1.5 μm pore size) filter was used. The results, unless indicated otherwise, were expressed as the percent average of 18 individual determinations ± s.d. The rate of 3-OMG uptake was determined by curve fitting. Thus, transport was measured over several minutes after tracer introduction.

Immunocytochemistry

Cells were grown as described and seeded on sterile 11 × 22-mm coverslips at 4×10^4 cells per slide. The feeding schedule of the cells involved a complete change in growth media on the third day with experiments being performed on the fourth day. Each coverslip was placed into a sterile Vacutainer™ tube containing 3 ml RPMI growth media, and blood gas mixtures were delivered as previously described. After 4 hr of incubation at the desired oxygen atmosphere, the slides were removed one at a time from the tube, dip-washed three times in HBSS and fixed immediately in buffered formalin (4%, pH 7.2) for 10 min at room temperature. They were then rinsed three times (7 min each) in HBSS and stored overnight in a humidified chamber at 4°C. Glut-1 and Glut-2 expression were evaluated by immunostaining the cultures according to the ABC method (*20*) using rabbit anti-glucose transporter antibodies diluted 1:500 as previously described (*21*). The percentage of transport-positive cells from the total number of cells assessed under a given condition was calculated. Each experiment consisted of one to six cultures or slides

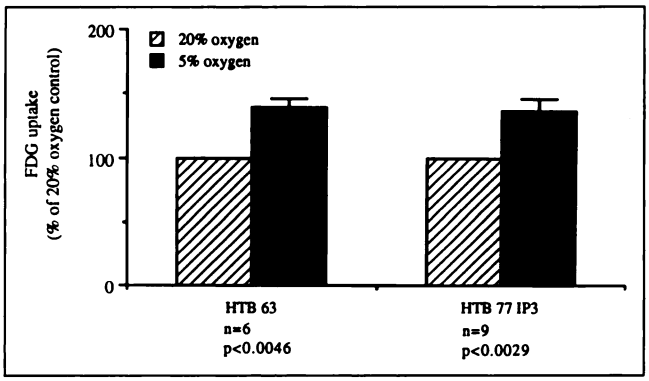

FIGURE 1. FDG uptake over 1 hr in human melanoma (HTB 63) and ovarian adenocarcinoma (HTB 77 IP3) cell lines after 1.5 hr of exposure at the given oxygen tension. Tracer uptake (counts per minute) was higher in the presence of a mildly hypoxic environment (5% O_2) compared to basal oxygenation levels (20% O_2). There was a 39.6% ± 6.7% increase in uptake for the melanoma cells and a 36.7% ± 9% increase for the ovarian cells. Data represent the percentage mean ± s.e.m. of two to three independent experiments (n = 4 per experiment).

and three to four fields per slide were counted. Each field measured 0.9 × 0.9 mm in size, and the average number of cells counted per field was 449 ± 97 cells for the normoxic state and 452 ± 147 cells for the hypoxic condition. A total of ten normoxic (38 fields) and nine hypoxic (34 fields) cultures were studied for Glut-1 and one normoxic (3 fields) and two hypoxic (6 fields) cultures for Glut-2.

RESULTS

Effect of Hypoxia on Tritiated-FDG Accumulation

Figure 1 shows an increase in FDG accumulation over 60 min for both cell lines (about 40% for melanoma cells and near 37% for ovarian carcinoma cells) following a 1.5-hr exposure to a 5% O_2 atmosphere versus a 1.5-hr exposure to a 20% O_2 atmosphere. Increased FDG accumulation was also observed in both melanoma and ovarian carcinoma cell lines (Fig. 2) when the cells were exposed for 4 hr to an anoxic (0% oxygen) or hypoxic (moderately 1.5% or mild 5% oxygen) environment, as compared to normoxic conditions (baseline 4 hr at 20% oxygen). The maximal increase in uptake seen over baseline for the melanoma cells was approximately 52% (Fig. 2A) and 44% for the ovarian carcinoma cells incubated at 1.5% oxygen (Fig. 2B). When cells were incubated at 0% oxygen, there was a 43% increase in uptake for the melanoma cells (Fig. 2A) and approximately a 63% increase for the ovarian cancer cells (Fig. 2B). For the other two time points tested (0.3 and 24 hr), FDG uptake varied and hypoxic effects were not as pronounced. After 24 hr of exposure under such conditions, cell viability declined, especially in the anoxic group, as did total FDG uptake. Lactate values in the final growth media increased over time (from about 9 to 100 mg/dl) but were not significantly different at each time point for the different oxygen concentrations tested (0%, 1.5%, 5% and 20%), except for IP3 ($p < 0.005$) cells incubated for 4 hr (Fig. 3). Glucose levels remained essentially unchanged over time and within groups except for a modest decline seen after 24 hr of incubation (Fig. 4).

Effect of Hypoxia on 3-OMG Uptake

When cells were exposed to a moderately hypoxic environment (1.5% O_2) for 4 hr, uptake was significantly increased over basal levels for the cell lines tested (Table 1 and Fig. 5B). Entry of the tracer in IP3 cells preincubated for 2 hr in RPMI containing either 100 or 500 mg/dl glucose was rapid and linear for the first 60 sec with good curve fits ($r > 0.96$) over a full 10-min uptake period, as shown in Figure 5A. Differences in transport were found, with greater transport membrane for cells incubated at the lower glucose concentration from 30 sec to 10 min following tracer exposure.

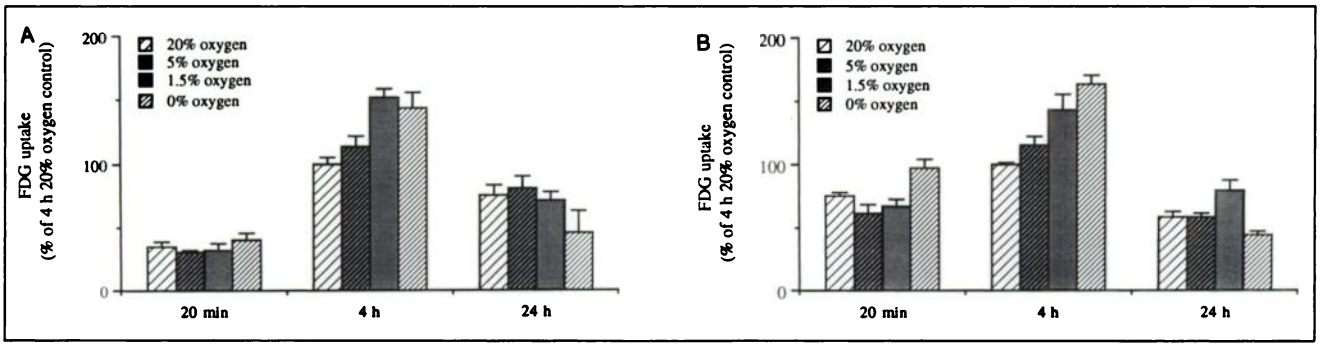

FIGURE 2. FDG uptake over 1 hr in HTB 63 (A) and HTB 77 IP3 cell lines (B) with different exposure times and varied oxygen tensions. One-hour tracer uptake (counts per minute) was higher after 4 hr of incubation than after 0.3 or 24 hr of incubation regardless of the oxygen tension. At 4 hr, there was a 52.3% ± 8.9% ($p < 0.006$) increase in FDG uptake for the melanoma cells (A) incubated at 1.5% oxygen and a 42.7% ± 10% ($p < 0.03$) increase in FDG uptake in the presence of 0% oxygen versus normoxic cells. Likewise, there was a 43.5% ± 19% ($p < 0.06$) increase in FDG uptake for the ovarian cancer cells (B) incubated at 1.5% oxygen and a 63.3% ± 13.7% ($p < 0.01$) increase in the presence of 0% oxygen (anoxia) versus normoxic cells. After 24 hr, cell viability was poor, which accounts for the low tracer uptake. Data represent the percentage mean ± s.e.m. of four individual samples per condition.

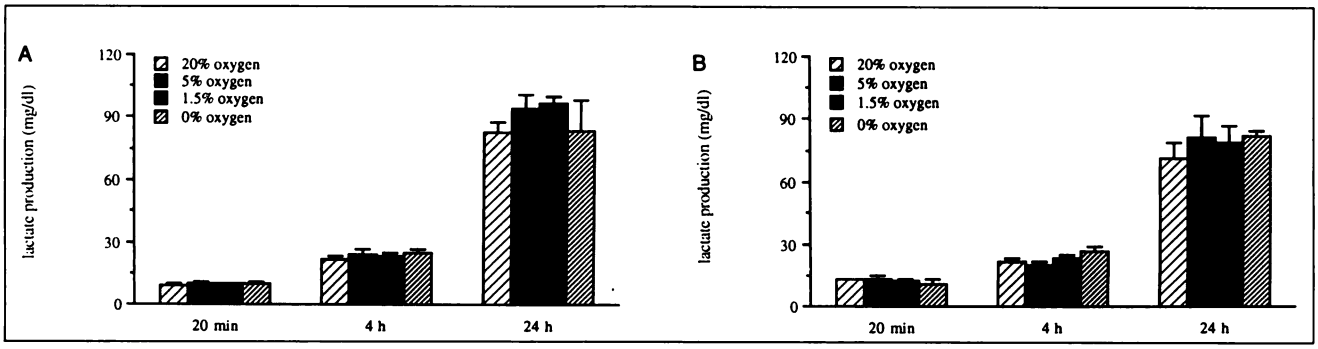

FIGURE 3. Lactate production by human melanoma (A) and ovarian adenocarcinoma (B) cells. There was no significant difference in lactate production over baseline 20% oxygen values among the varying oxygen concentrations at any time point for the melanoma cells. At the 4-hr time point, lactate production was modest but significantly greater for the anoxic IP3 cells at 4 hr than for the cells exposed to 20% oxygen for the same period of time ($p < 0.005$). Lactate levels were mainly related to the duration of incubation, not the oxygenation level. Data represent the mean ± s.d. of four individual samples per condition.

Effect of Hypoxia on the Cellular Expression of Glut-1 and Glut-2

Exposure of ovarian carcinoma cells to moderate hypoxia (1.5% O_2) for 4 hr resulted in the increased membrane expression of Glut-1 ($p < 0.05$) but not Glut-2 transporters, as indicated in Table 2 and Figure 6.

DISCUSSION

While tumors generally consume glucose excessively, the contribution of hypoxic cells [a common feature of human tumors (*22*)] to the increased utilization of glucose or FDG uptake is less clear (*23*). FDG is a glucose analog that competes for glucose transport sites on the membrane of cells and for phosphorylation by enzyme hexokinase. Unlike glucose, however, this analog cannot be significantly metabolized further (*24,25*) and accumulates within the cell because it cannot leak out nor enter most metabolic pathways (*26*). FDG-6-phosphate may be dephosphorylated, but the rate of conversion depends upon the tissue involved (*27*), with many tumors having low dephosphorylation rates. We observed a reasonably rapid enhancement in glucose (FDG) uptake in both melanoma and ovarian carcinoma cell lines in response to 1.5 hr of exposure to hypoxia. By contrast, 20 min of hypoxia were insufficient to induce such an effect. When the hypoxic incubations were prolonged to 4 hr, FDG accumulation increased even more over basal conditions. The increase in FDG accumulation, if any, in hypoxic cells maintained for the longest incubation period (24 hr), was modest, but as stated before, the number of viable cells also had somewhat declined by this time. These latter results are in agreement with our earlier findings of FDG uptake being correlated with the number of viable cancer cells (*12*).

A lower net FDG signal was seen with the melanoma and ovarian carcinoma cell lines after a 20-min incubation period. This is in contrast to the much higher tracer uptake obtained after 4 hr of incubation under the same conditions. The differences between 20-min and 4-hr incubation uptake values may be partly attributed to the effects of trypsin on cellular membranes and glucose transporters. Indeed, uptake at 20 min was lower than that of cells in normoxic conditions for 4 hr. It has been shown that treatment of Ehrlich cells with trypsin can produce a deep enzymatic cleavage which affects the structural integrity of the bilayered cell membrane (*28*) and reduces sodium-coupled amino acid transport, reduce ATP levels and abol-

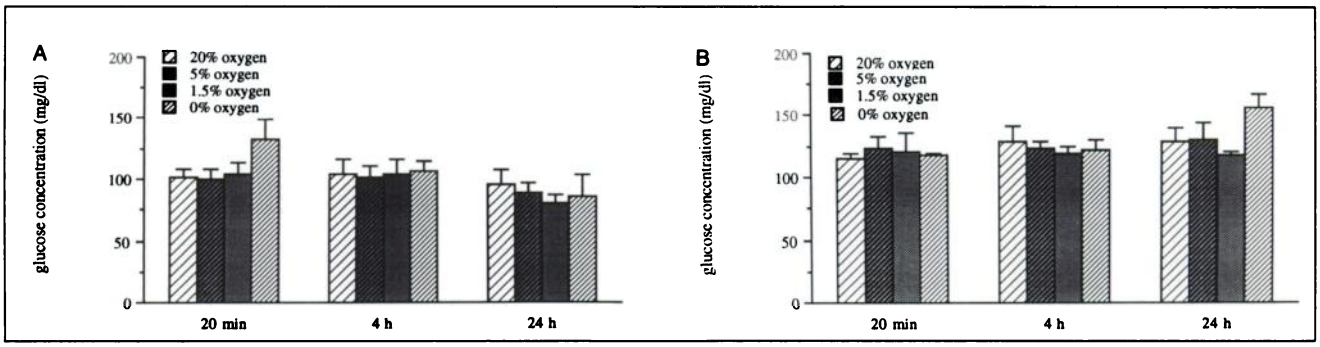

FIGURE 4. Glucose concentration in the growth media of the human melanoma (A) and ovarian adenocarcinoma (B) cell lines incubated for 0.3, 4 and 24 hr at varying oxygen concentrations. Approximate values (by glucometer) were stable throughout the 4-hr incubation, but the HTB 63 line showed a slight decline at 24-hr. Thus, altered media glucose levels were not responsible for the increased FDG uptake seen with 4 hr of hypoxia. Data represent the mean ± s.d. of four individual samples per condition.

TABLE 1
3-O-Methylglucose Transport and Accumulation in Melanoma and Ovarian Carcinoma Cancer Cells

Cell line	No.	20% oxygen Mean* ± s.d.	1.5% oxygen Mean* ± s.d.	p value
HTB 63	18	886 ± 91	1248 ± 57	0.0009
HTB 77 IP3	18	734 ± 42	1371 ± 210	0.0027

Prior to uptake studies, cells were exposed for 4 hr to either 20% or 1.5% oxygen atmosphere. Tracer was added and after 10 min of incubation, total tracer uptake (* expressed as cpm) was determined. Mean ± s.d. of 18 individual determinations.

TABLE 2
Immunoperoxidase Detection of Glut-1 and Glut-2 on Ovarian Carcinoma Cells Incubated for Four Hours Under Hypoxic (1.5% O_2) or Normoxic (20% O_2) Conditions

Transporter	20% oxygen Mean ± s.d.*	1.5% oxygen mean ± SD*	p value†
Glut 1	44.3 ± 13.3	53.3 ± 12.9	0.0331
Glut 2	35.7 ± 3.5	32.1 ± 3.9	0.4261

*Percentage of cells (mean ± s.d.) staining positively for either transporter. In each culture, 3-4-fields (0.9 × 0.9 mm each) were counted. For Glut-1, ten normoxic and nine hypoxic cultures were counted. For Glut-2, one normoxic and two hypoxic cultures were studied.
†One-tail t-test.

ish cation gradients (*29*). Moreover, tryptic digestion of erythrocyte ghosts has been shown to increase affinity of the residual transporter for D-glucose and inward-binding of sugar but to decrease the affinity for the outward-binding of sugar (*30*), as well as induce loss of the Glut-4 signal in a time- and concentration-dependent fashion (*31*). Thus, while we used as little trypsin as possible, this cell dissociation process may have induced the transient decrease of FDG uptake observed when tracer uptake was measured immediately after trypsin treatment. Nonetheless, under comparable conditions, hypoxic cells accumulated more FDG than well-aerated cells.

In general, glucose levels did not decrease over 4 hr, indicating that glucose deprivation was not responsible for the increased cellular FDG uptake seen with hypoxia. These results in tumor cells are in partial agreement with those reported by Bashan et al. (*32*), who studied rat muscle cells. The increase in FDG uptake seen in our studies with short-term and long-term hypoxia versus basal 20% oxygenation could partly be due to the well known Pasteur effect. This effect (decrease in glycolysis brought about by oxygen), recognized for more than 60 years and first described by Warburg (*3*), occurs when cells are made hypoxic. Under such circumstances, both glycolysis and lactic acid production increase. Considering that we observed an increase in FDG uptake without an increase in lactate (at a given incubation time versus normoxic conditions), the Pasteur effect does not seem to represent the sole mechanism for the phenomenon we describe here. Rather,

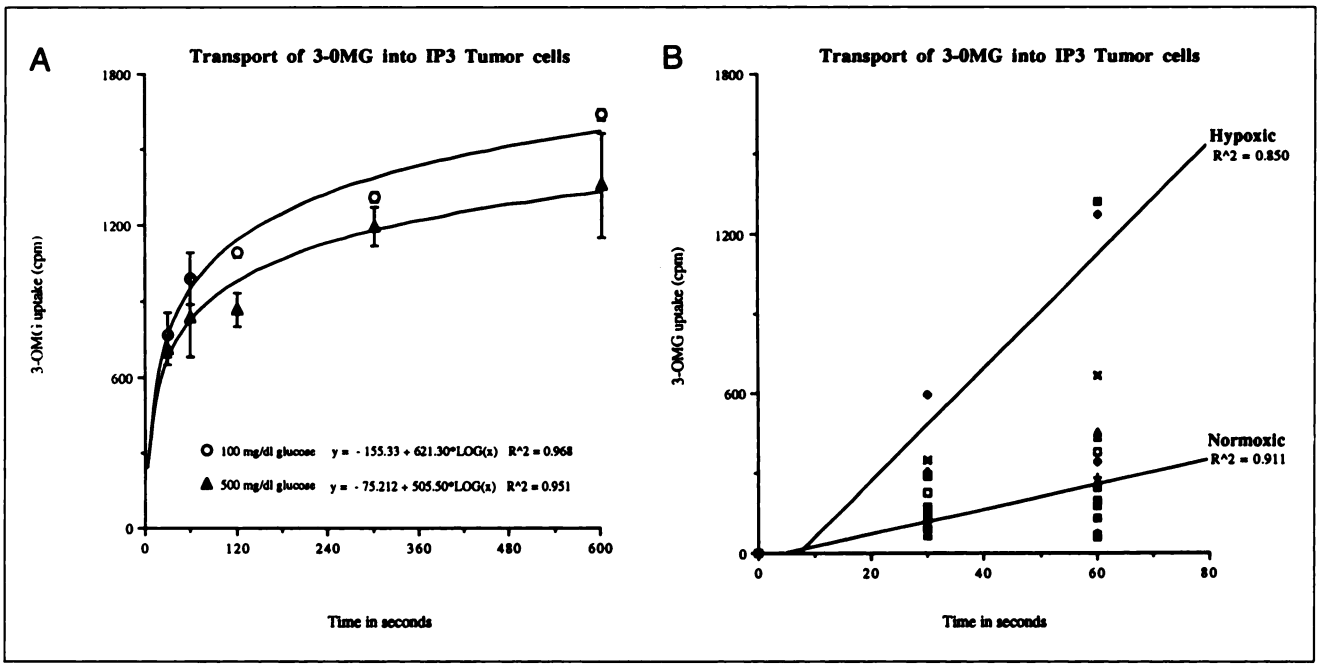

FIGURE 5. (A) Time course of 3-OMG entry into ovarian carcinoma cells. Transport (total counts per minute) in cells was rapid with a plateau. During the linear portion of transport (from 0 to 60 sec), 3-OMG transport was reduced by 15.1% in the 500-mg/dl glucose group. Cell volumes were comparable between groups (data not shown). Data represent the percentage mean ± s.d. of four individual samples per condition. (B) Time course of 3-OMG entry into normoxic (20% oxygen) and hypoxic (1.5% oxygen) ovarian carcinoma cells. Faster transport in the hypoxic group is apparent. The transport rate is increased approximately 4.5-fold by hypoxia ($p < 0.03$).

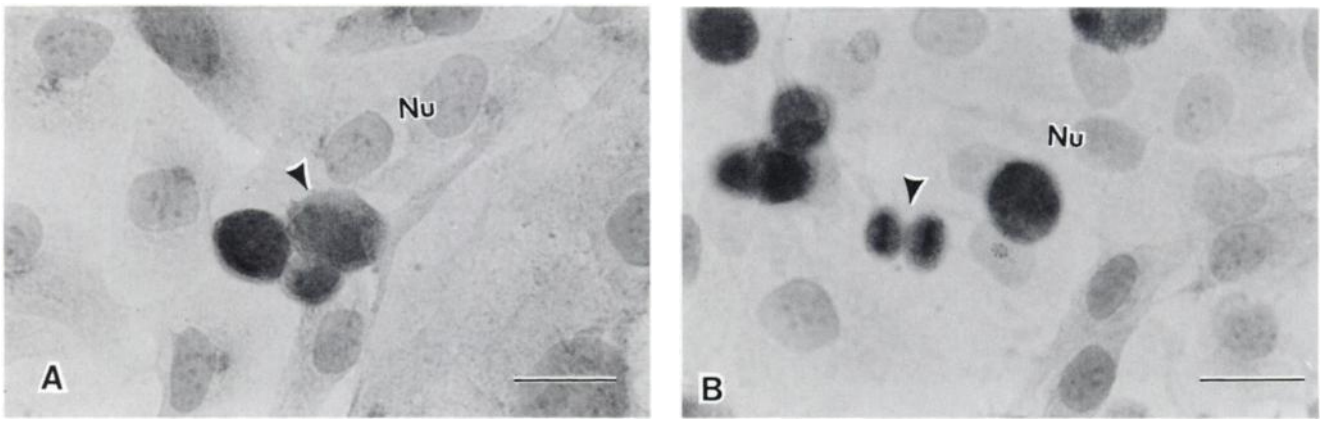

FIGURE 6. Glut-1 expression in human ovarian carcinoma cell cultures exposed to normoxic (A) or hypoxic (B) atmospheres. The number of Glut-1 positive cells (darkly stained cells) was 44.3% for the normoxic state and 53.3% for the hypoxic condition. Arrowheads indicate cells undergoing mitosis. Nu = nucleus; bar = 20 μm; mag = 670×.

our findings suggest that the increased transport is an important factor in the higher FDG uptake seen with hypoxia.

The in vivo relation between rates of transport and phosphorylation of deoxyglucose during long-term incubations can be complex (*33*). Complications in measuring transport can be avoided by performing measurements under conditions where no substrate metabolism occurs, such as when 3-OMG is used. This sugar analog is accepted by the D-glucose transport system (*34*) and taken into the cell, but it cannot be phosphorylated by hexokinase (*35*) nor further metabolized (*36*). Several researchers have demonstrated that 3-OMG uptake occurs by carrier-facilitated diffusion into transformed and primary cell lines (*37–43*). Our transport data indicate that cells grown in the high-glucose medium (28 m*M*) exhibit an 8%–20% reduction of 3-OMG transport activity compared to the reduction (67%) reported by Whitesell et al. (*43*) for 3T3-L1 fibroblasts. Their results may be accounted by the fact that the sugar concentration they chose (0.29 m*M*) was much higher than ours (10 μM). When transport was measured under normoxic or hypoxic conditions, our results (1.4 to 1.9-fold increase in 3-OMG uptake for melanoma and ovarian carcinoma cells, respectively) are consistent with those reported by Reeves (*19*) for normal rat thymocytes under hypoxic conditions. Our experiments assessed transport during anaerobic conditions, which more closely resembles physiological tumor conditions.

Expression of an increased number of the Glut-1 transporter protein molecules on the membrane of hypoxic ovarian carcinoma cells, as detected by immunocytochemistry, suggests the Glut-1 transporter has a role in the increased FDG uptake by these tumor cells. In fact, the glucose transporter content in the plasma membrane of perfused rat hearts increased about 50% in response to anoxia, as compared to a 150% increase in glucose transport, suggesting that transporter translocation accounted for at least a third of the effect (*44*). Therefore, an increase in transporter expression does not always exactly correspond to an increase in sugar uptake, since other intrinsic properties of the transporter (changes in affinity and/or Vmax; translocation, etc.) have to be considered as well. More recently, translocation of Glut-4 transporters in fat and muscle cells in response to hypoxia has been demonstrated (*45*). The higher uptake of FDG seen in melanoma cells as compared to the ovarian cancer cells in some assays (e.g., Fig. 2) may partly explain our inability to measure by immunochemistry changes in the level of Glut-1 transporter expression in the melanoma cell line (even a 1:1000 dilution of the antibody showed no differences between controls and experimental cultures). Thus, Glut-1 expression was high (essentially 100%) in the HTB 63 melanoma cells during both normoxia and hypoxia (data not shown).

Hypoxic cells are commonly seen within cancers, particularly in adenocarcinomas. It is therefore tempting to speculate, based on these in vitro data, that increased FDG uptake in tumors is at least partly indicative of tumor hypoxia. Some aspects of the distribution of FDG uptake in tumors on a microscopic level support this hypothesis. For instance, Brown et al. (*14*) observed increased FDG uptake in apparently viable Glut-1-positive cancer cells located near necrotic areas of tumors in studies of ovarian carcinoma xenografts. Glut-1, in particular, was regularly expressed in several cell layers surrounding necrotic areas within large basophilic tumors, while Glut-2 expression was reduced in the cells comprising those tumors (*46*). Since tumor necrosis is commonly due to outgrowth of the tumor's blood supply, viable cells near the necrotic area might logically be expected to be hypoxic. Kubota et al. have suggested that tumor cells near necrotic areas have high FDG uptake in vivo, despite their being dead or nearly dead, due to increased leakage of FDG into those cells (*15*). The mechanism for this proposed phenomenon is unclear, since continuous accumulation of FDG inside cells requires phosphorylation and therefore ATP. Moreover, Minn et al. (*11*) demonstrated that FDG uptake in nonhypoxic tumor cells in vitro has a positive correlation with intracellular ATP levels. Thus, it is feasible that tumor

hypoxia may be contributing to the increased FDG uptake seen in viable tumor cells located near necrotic areas of tumors.

Whether high FDG uptake in tumors is generally related to tumor hypoxia is not resolved by our study. Ultimately, in vivo studies comparing tumor oxygenation by direct measurement with the level of FDG uptake in tumors would be of considerable interest. Clearly, if high FDG uptake was indicative of tumor hypoxia, PET could be particularly useful for planning tumor treatments because hypoxic cells are more effectively treated with hyperfractionation, hypoxic sensitizers or particle beam treatments than with standard methods.

We cannot conclude that tumor hypoxia is the predominant reason for increased FDG uptake in tumors. Indeed, in preliminary studies, Zasadny et al. (*47*) have shown that tumor blood flow and FDG uptake are generally correlated in patients with untreated primary breast cancers, with areas of high flow having higher FDG uptake, which is consistent with the importance of radiotracer delivery. Since well-perfused regions are less likely to be hypoxic, hypoxia alone would not seem to be the sole explanation for these regions of increased FDG uptake in viable breast cancers. Rather, increased FDG uptake in viable cancer cells would seem the more likely rationale, as proposed by Higashi et al. (*12*). Therefore, it seems more probable that hypoxia is a modifying factor for FDG uptake in a given tumor, and that hypoxic cells would probably accumulate more FDG than normoxic cells for a given level of tracer delivery and viable cell number. It will also be of interest to determine if modifiers of tumor oxygenation can alter FDG uptake in tumors. While the role of tumor oxygenation on the FDG signal will need more study in vivo, our in vitro study clearly shows that FDG uptake is substantially enhanced by hypoxia.

CONCLUSION

FDG uptake is increased in both human melanoma and ovarian carcinoma cell lines exposed to varying degrees of hypoxia, including anoxia, as compared to uptake under normal conditions of oxygenation in vitro. The hypoxia-induced increase in FDG uptake was not generally associated with measurable increases in lactate production but was associated with an increase in the expression of the Glut-1 glucose transporter and with increased membrane transport of glucose. Hypoxia increases FDG uptake in tumor cell lines and may be an important contributing factor to the ^{18}F signal detected in PET imaging studies of cancer with FDG.

ACKNOWLEDGMENTS

The authors thank Dr. Kirk A. Frey, Dr. Sunil Mukhopadhyay and Michael Overbeck for helpful advice and Jeannette Roesner for her contribution to the early experimental stages of this study. This work was supported by National Cancer Institute grants CA 53172 and CA 52880.

REFERENCES

1. Warburg O, Posener K, Negelein E. The metabolism of the carcinoma cell. In: *The metabolism of tumors*. New York: Richard R. Smith Inc.; 1931: 129–169.
2. Warburg O. On the origin of cancer cells. *Science* 1956;123:309–314.
3. Weinhouse S. Glycolysis, respiration and anomalous gene expression in experimental hepatomas: G.H.A. Clowes memorial lecture. *Cancer Res* 1972;32:2007–2016.
4. Hatanaka M. Transport of sugars in tumor cell membranes. *Biochem Biophys Acta* 1974;355:77–104.
5. Weber G. Enzymology of cancer cells. *N Engl J Med* 1977b;296:541–551.
6. Weber G. Enzymology of cancer cells. *N Engl J Med* 1977a;296:486–492.
7. Flier JS, Mueckler MM, Usher P, Lodish HF. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. *Science* 1987;235:1492–1495.
8. Yamamoto T, Seino Y, Fukumoto H, et al. Overexpression of facilitative glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990;170:223–230.
9. Kahn BB, Flier JS. Regulation of glucose-transporter gene expression in vitro and in vivo. *Diabetes Care* 1990;13:548–564.
10. Kahn BB. Dietary regulation of glucose transporter gene expression: tissue specific effects in adipose cells and muscle. *J Nutr* 1994;124:1289S–1295S.
11. Minn H, Kangas L, Knuutila V, Paul R, Sipila H. Determination of 2-fluoro-2-deoxy-D-glucose uptake and ATP level for evaluation of drug effects in neoplastic cells. *Res Exp Med (Berlin)* 1991;191:27–35.
12. Higashi K, Clavo AC, Wahl RL. Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake. *J Nucl Med* 1993;34:414–419.
13. Minn H, Clavo AC, Grenman R, Wahl RL. In vitro comparison of cell proliferation kinetics and uptake of tritiated 2-fluoro-2-deoxy-D-glucose and L-methionine in squamous-cell carcinoma of the head and neck. *J Nucl Med* 1995;36:252–258.
14. Brown RS, Fisher SJ, Wahl RL. Autoradiographic evaluation of the intratumoral distribution of 2-deoxy-D-glucose and monoclonal antibodies in xenografts of human ovarian adenocarcinoma. *J Nucl Med* 1993;34:75–82.
15. Kubota R, Kubota K, Yamada S, Tada M, Ido T, Tamahashi N. Active and passive mechanisms of [fluorine-18]fluorodeoxyglucose uptake by proliferating and prenecrotic cancer cells in vivo: a microautoradiographic study. *J Nucl Med* 1994;35:1067–1075.
16. Tannock I, Guttman P. Response of Chinese hamster ovary cells to anticancer drugs under aerobic and hypoxic conditions. *Br J Cancer* 1981;43: 245–248.
17. Wahl RL, Barrett J, Geatti O, et al. The intraperitoneal delivery of radiolabeled monoclonal antibodies: studies on the regional delivery advantage. *Cancer Immunol Immunother* 1988;26:187–201.
18. Gloster JA, Harris P. Observations on an enzymic method for the estimation of pyruvate in blood. *Clin Chim Acta* 1962;7:206–211.
19. Reeves JP. Stimulation of 3-O-methylglucose transport by anaerobiosis in rat thymocytes. *J Biol Chem* 1975;250:9413–9420.
20. Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol* 1981;75:734–748.
21. Brown, RS, Wahl, RL. Overexpression of Glut-1 glucose transporter in human breast cancer: an immunohistochemical study. *Cancer* 1993;72: 2979–2985.
22. Mueller-Klieser W, Vaupel P, Manz R, Schmidseder R. Intracapillary oxyhemoglobin saturation of malignant tumors in humans. *Int J Radiat Oncol Biol Phys* 1981;7:1397–1404.
23. Brown JM, Giaccia AJ. Tumour hypoxia: the picture has changed in the 1990s. *Int J Radiat Biol* 1994;65:95–102.
24. Kipnis DM, Cori CF. Studies of tissue permeability. *J Biol Chem* 1959;234: 171–177.
25. Smith DE, Gorski J. Estrogen control of uterine glucose metabolism: an analysis based on the transport and phosphorylation of 2-deoxyglucose. *J Biol Chem* 1968;243:4169–4174.
26. Plagemann PG, Richey DP. Transport of nucleosides, nucleic acid bases, choline and glucose by animal cells in culture. *Biochim Biophys Acta* 1974; 344:263–305.
27. Gallagher BM, Fowler JS, Gutterson NI, MacGregor RR, Wan CN, Wolf AP. Metabolic trapping as a principle of radiopharmaceutical design: some factors resposible for the biodistribution of [^{18}F] 2-deoxy-2-fluoro-D-glucose. *J Nucl Med* 1978;19:1154–1161.
28. Anglhileri LJ, Dermietzel R. Cell coat in tumor cells—effects of trypsin and EDTA: a biochemical and morphological study. *Oncology* 1976;33:17–23.

29. Johnson PA, Johnstone RM. Alterations in membrane permeability with trypsin treatment. *Can J Biochem* 1981;59:668–675.
30. May JM, Qu ZC, Beechem JM. Tryptic digestion of the human erythrocyte glucose transporter: effects on ligand binding and tryptophan fluorescence. *Biochemistry* 1993;32:9524–9531.
31. Yano Y, May JM. Ligand-induced conformational changes modify proteolytic cleavage of the adipocyte insulin-sensitive glucose transporter. *Biochem J* 1993;295:183–188.
32. Bashan N, Burdett E, Hundal HS, Klip A. Regulation of glucose transport and GLUT1 glucose transporter expression by O_2 in muscle cells in culture. *Am J Physiol* 1992;262:C682–C690.
33. Graff JC, Wohlhueter RM, Plagemann PG. Deoxyglucose and 3-O-methylglucose transport in untreated and ATP-depleted Novikoff rat hepatoma cells: analysis by a rapid kinetic technique, relationship to phosphorylation and effects of inhibitors. *J Cell Physiol* 1978;96:171–188.
34. Renner ED, Plagemann PG, Bernlohr RW. Permeation of glucose by simple and facilitated diffusion by Novikoff rat hepatoma cells in suspension culture and its relationship to glucose metabolism. *J Biol Chem* 1972;247:5765–5776.
35. Narahara HT, Ozand P. Studies of tissue permeability. *J Biol Chem* 1963; 238:40–49.
36. Csaky TZ, Wilson JE. The fate of 3-O-$^{14}CH_3$-glucose in the rat. *Biochim Biophys Acta* 1956;22:185–186.
37. Peters JH, Hausen P. Effect of phytohemagglutinin on lymphocyte membrane transport. 2. Stimulation of "facilitated diffusion" of 3-O-methylglucose. *Eur J Biochem* 1971;19:509–513.
38. Venuta S, Rubin H. Sugar transport in normal and Rous sarcoma virus-transformed chick-embryo fibroblasts. *Proc Natl Acad Sci USA* 1973;70: 653–657.
39. Czech MP. Regulation of the D-glucose transport system in isolated fat cells. *Mol Cell Biochem* 1976;11:51–63.
40. Caygill CP, Pring JB. 3-O-Methylglucose transport into lymphocytes and the effect of insulin. *Biochem Soc Trans* 1977;5:1056–1058.
41. Aubery M, Bernard B, Bourrillon R. Age-dependent changes in 3-O-methylglucose transport in chick embryo fibroblasts. *Biochem Biophys Res Commun* 1979;91:508–514.
42. Whitesell RR, Gliemann J. Kinetic parameters of transport of 3-O-methylglucose and glucose in adipocytes. *J Biol Chem* 1979;254:5276–83.
43. Whitesell RR, Regen DM, Pelletier D, Abumrad NA. Evidence that down-regulation of hexose transport limits intracellular glucose in 3T3-L1 fibroblasts. *Diabetes* 1990;39:1228–1234.
44. Wheeler TJ. Translocation of glucose transporters in response to anoxia in heart. *J Biol Chem* 1988;263:19447–19454.
45. Sun D, Nguyen N, DeGrado TR, Schwaiger M, Brosius FI. Ischemia induces translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane of cardiac myocytes. *Circulation* 1994;89:793–798.
46. Ahn YS, Zerban H, Bannasch P. Expression of glucose transporter isoforms (Glut-1, Glut-2) and activities of hexokinase, pyruvate kinase, and malic enzyme in preneoplastic and neoplastic rat renal basophilic cell lesions. *Virchows Archiv B Cell Pathol* 1993;63:351–357.
47. Zasadny KR, Wahl RL. Regional FDG uptake versus flood flow in untreated primary breast cancers [Abstract]. *J Nucl Med* 1993;34:74P.