Increased Glucose Metabolism in Untreated Non-Hodgkin’s Lymphoma: A Study With Positron Emission Tomography and Fluorine-18-Fluorodeoxyglucose

By Maria Lapela, Sirkku Leskinen, Heikki R.I. Minn, Paula Lindholm, Pekka J. Kierni, Karl-Ove Soderstrom, Jorgen Bergman, Merja Haaparanta, Ulla Ruotsalainen, Olof Solin, and Heikki Joensuu

Glucose metabolism has been shown to be increased in neoplastic tissue. It has been suggested that high activity of glucose metabolism is associated with a high grade of malignancy of human cancer. We studied in vivo glucose metabolism in 22 patients with untreated non-Hodgkin’s lymphoma with fluorine-18-fluorodeoxyglucose (FDG) and positron emission tomography (PET). FDG uptake in lymphoma deposits was measured blinded to clinical data, and compared with histologic classification and proliferative activity. Tracer uptake was measured by using two indices of FDG accumulation: the standardized uptake value (SUV) and the regional metabolic rate (rMR) for the tracer. The median SUV of the lymphomas was 8.5 (range, 3.5 to 31.0), and the median rMR 22.7 μmol/100 g/min (range, 9.0 to 124.3 μmol/100 g/min). A high FDG uptake in tumors was associated with high histologic degree of malignancy as defined by the Working Formulation (P = .005 for the SUV, and P = .04 for the rMR) or by the Kiel classification (P = .003 for the SUV, and P = .02 for the rMR). A high FDG accumulation was also associated with a high S-phase fraction (r = .786 for the SUV, P = .0002; and r = .774 for the rMR, P = .02). We conclude that in untreated non-Hodgkin’s lymphomas high FDG uptake is associated with high histologic grade of malignancy and a high proliferation rate. This minimally invasive method may find application in assessing lymphoma lesions in patients who are poor candidates for surgery, and it may provide further information in cases where the grade of aggressiveness of lymphoma is not settled based on clinical or histologic data.

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Increased METABOLIC demand of cancer tissue for glucose was shown over half a century ago.1 It was later recognized that activity of glucose utilization tended to parallel differences in growth rate and differentiation of experimental cancer.2,3 During the last 2 decades it has become possible to evaluate this putative association in vivo by external imaging with positron emission tomography (PET) and 2-[18F]fluoro-2-deoxy-D-glucose (FDG), a glucose analog widely used to study glucose metabolism of normal and pathologic tissues.

PET is a quantitative imaging technique that measures externally radiation from a positron-emitting radiopharmaceutical in tissue as a function of time and space. The distribution of the radioactivity is reconstructed to tomographic slices from serial measurements taken from different angles.4 By using different isotopes, many biochemical processes can be studied repetitively and noninvasively in vivo.5 FDG is an analog of D-glucose that competes with glucose for facilitated intracellular transport and phosphorylation by hexokinase.6 The phosphorylated FDG is unable to enter the subsequent glycolytic steps and accumulates in cells with low phosphatase activity.7 Based on the simple uptake kinetics of FDG, models for quantification of glucose utilization in tissue have been developed.8,9

With FDG PET, significant correlation between the degree of malignancy and rate of glucose utilization has been found in primary intracranial neoplasms,10,12 although results have not been consistent.13 An association between tumor FDG uptake and the histologic grading has also been found in musculoskeletal tumors14,15 and breast cancer,16 but not in lung carcinoma or head and neck cancer.17,18

In lymphoma, elevated uptake of FDG was first shown with a specially collimated gamma camera.19 FDG PET has been successfully used for imaging of lymphoma.20-22 Complementing findings in computed tomography (CT),23 Initial experience suggested a difference in FDG uptake between high-grade and low-grade non-Hodgkin’s lymphomas (NHLs),24,26 but this difference was not confirmed in a recent study.25 However, presence of an association between FDG uptake and prognosis25 and proliferative activity22,26 in lymphomas has been suggested.

The present study was designed to evaluate glucose metabolism in untreated NHL as measured with FDG PET. FDG uptake in tumor was compared with histologic classification and with proliferative activity, as measured by DNA flow cytometry.

MATERIALS AND METHODS

Patients. Twenty-two patients with NHL seen at the Department of Oncology and Radiotherapy, Turku University Central Hospital between February 1989 and June 1994 participated in the study. The criteria for eligibility were untreated, histologically verified lymphatic malignancy with at least one tumor larger than 2 cm in diameter in a non-diabetic, co-operative patient with the World Health Organization performance status better than three. Each patient gave a written informed consent, and the study protocol was approved by the Ethical Committee of the Turku University Central Hospital.

Fifteen (68%) of the patients were women, and the median age was 58 years (range, 47 to 78 years). The body mass index, calculated as weight in kilograms divided by the square of height in meters, varied from 21.3 to 43.3 kg/m² (median, 25.1 kg/m²). Clinical staging was done according to the Ann Arbor classification system.27

From the Departments of Oncology and Radiotherapy, and Pathology, and Turku University Cyclotron-PET Center, University of Turku; Accelerator Laboratory, Åbo Academy University, Turku; and the Department of Oncology, University of Helsinki, Helsinki, Finland.

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Supported by the Finnish Cancer Society and the Turku University Foundation.

Address reprint requests to Maria Lapela, MD, Department of Oncology and Radiotherapy, Turku University Central Hospital, FIN—20 520 Turku, Finland.

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FDG PET study by the glucose oxidase method (Analox activity in plasma over total PET acquisition time. Plasma glucose corrected for deadtime, decay, and photon attenuation, and reconstructed in a 256 × 256 matrix. The reconstructed images were 30 × 30 cm in size. The final in-plane resolution in reconstructed and Hann-filtered (cut-off frequency 0.5) images was 8 mm in full width at half maximum.

Serial venous blood samples were taken for measurement of radioactivity in plasma over total PET acquisition time. Plasma glucose was determined in duplicate before, midway, and after the dynamic study by the glucose oxidase method (Analox GM7; Analox Instruments LTD, London, UK). The reference range for plasma glucose concentration was 3.3 to 6.4 mmol/L. In three cases no plasma glucose determinations and in one case no radioactivity measurements in blood were performed.

PET study. All subjects fasted at least for 6 hours before the study. The FDG synthesis was a modification of the method reported by Hamacher et al. The radiochemical purity of FDG was better than 99% and the specific activity at the end of synthesis was about 74 GBq/mmol (2 Ci/pmol). Two venous lines were inserted, one antecubitally for injection of FDG, and another in the contralateral preheated arm for blood sampling. Patients were positioned supine in an eight-ring ECAT 931/08 PET scanner (Siemens/CTI Corp, Knoxville, TN), which has an axial resolution of 6.7 mm and an in-plane spatial resolution of 6.5 mm in full width at half maximum. To correct for photon attenuation, a transmission scan was performed for 10 to 20 minutes before injection. Tracer uptake was studied by bolus injection of FDG. In three cases no plasma glucose determinations and in one case no radioactivity measurements in blood were performed.

PET analysis. The last frame of dynamic imaging (ie, 55 to 60 minutes postinjection) was used to define the regions of interest (ROIs) for quantitative analysis. Elliptical ROIs (about 60 mm in size) were drawn on the maximum FDG uptake area of the tumors. The average counts per pixel in each ROI were used for further calculations.

Tracer accumulation in the ROIs at the end of the dynamic study was reported as the standardized uptake value (SUV), which is the radioactivity concentration in an ROI divided by the injected dose normalized to the patient’s weight at a fixed time point. In addition, a graphical analysis of tracer uptake was performed by plotting tissue radioactivity concentration divided by plasma activity against cumulative radioactivity in plasma divided by plasma activity. The slope of the linear plot is equal to the utilization constant of FDG (influx constant, K_i), which represents the fractional rate of tracer transport and phosphorylation per unit time in tissues with negligible reverse metabolism. In the current study, the last nine timepoints representing the time from 15 to 60 minutes after injection were used to determine the slope by linear regression. The FDG influx constant was multiplied by the average plasma glucose level during imaging to obtain a metabolic index for FDG utilization (the regional metabolic rate [rMR], μmol/100 mg/min). All SUV and rMR values were calculated blinded without any knowledge of the clinical or other data.

Histologic classification. The tumor tissue was fixed in 10% neutral buffered formalin. The original tumor slides were reviewed, and new slides were sectioned from paraffin blocks. All cases were stained with hematoxylin and eosin and with the Van Gieson method.

### Table 1. Results

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age</th>
<th>Histology</th>
<th>WF</th>
<th>Kiel</th>
<th>Stage</th>
<th>SUV</th>
<th>rMR</th>
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<td>IV A</td>
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<td>9.0</td>
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<td>Low</td>
<td>II A</td>
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<td>NA</td>
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<td>Low</td>
<td>Low</td>
<td>IV A</td>
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<td>14.7</td>
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<td>Low</td>
<td>IV B</td>
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<tr>
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<td>Low</td>
<td>IV A</td>
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<td>III B</td>
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<td>I A</td>
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<td>IV B</td>
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Twenty-two patients with NHL studied with FDG and PET. Staging according to Ann Arbor staging system. Abbreviations: CC, centrocytic; CB, centroblastic; LC, lymphocytic; 16, immunoblastic; Class, Immunophenotype; SPF, S-phase fraction; SUV, standardized uptake value of FDG in tumor; rMR, regional metabolic rate of FDG in tumor, μmol/100 g/min; NA, not available.
All lymphomas were immunohistochemically stained for CD45, CD20, and CD3 antigens (DAKO, Copenhagen, Denmark). Histology of lymphomas was classified according to the Working Formulation scheme and the updated Kiel classification. Seven patients had low-grade, 11 intermediate-grade, and 4 had high-grade lymphoma according to Working Formulation (Table 1).

Flow cytometry and S-phase fraction (SPF). Flow cytometry from propidium-iodide–stained cell samples was performed with a FACSscan Flow Cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA) from deparaffinized tissue as described in more detail elsewhere. The peak width-area analysis to gate out doublets was used when analyzing the series. The S-phase fraction was calculated with a commercial version of the Multicycle software (Phoenix Flow Systems, San Diego, CA; Autofit version 2.50) without correction for the background debris. In DNA aneuploid cases, the SPF was calculated from the aneuploid stemlines. The SPF values were assessed without any knowledge of the clinical data (Table 1).

Statistical analysis. Statistical analyses were performed with the BMDP computer program (BMDP Statistical Software, Department of Biomathematics, University of California, Los Angeles). Comparison of non-normal distributions was done using Kruskal-Wallis analysis of variance or Mann-Whitney’s U-test. Scatter plots were analyzed by linear regression analysis. All P values are two-tailed.

RESULTS

The SUVs were calculated in all of the 22 lesions and the rMRs in 18 patients (Table 1). The median SUV of tumor FDG uptake was 8.5 (range, 3.5 to 31.0) and the median rMR was 22.7 μmol/100 g/min (range, 9.0 to 124.3 μmol/100 g/min). The SUV and the rMR measured for the same ROI correlated well with each other (r = .947, P < .0001).

A significant correlation was found between the histologic degree of malignancy as determined by the Working Formulation and the FDG uptake in tumors (P = .005 for the SUV, and P = .04 for the rMR, Figs 1 and 2). The difference between high-grade (n = 10) and low-grade (n = 12) lymphoma was significant also when the lymphomas were graded by the Kiel classification (P = .003 for the SUV, and P = .02 for the rMR, Fig 3).

The S-phase fraction was evaluable in 17 patients. A significant association was shown between high FDG accumulation and a high S-phase fraction (r = .786 for the SUV, P = .0002, n = 17, Fig 4; and r = .774 for the rMR, P = .02, n = 13). DNA aneuploid lymphomas (n = 5, median, 66.6 μmol/100 g/min, range, 17.8 to 124 μmol/100 g/min) had larger rMRs than the diploid ones (n = 9, median, 20.4 μmol/100 g/min, range, 9.0 to 52.4 μmol/100 g/min; P = .03), and they tended to have larger SUVs as well (n = 6, median, 19.0, range, 6.8 to 31 v n = 12, median, 8.3, range 3.5 to 19, respectively; P = .07).

No significant association was found between FDG uptake and the Ann Arbor stage or the IPI (P > .10 for both).

DISCUSSION

It is generally accepted that increased glycolysis is one of the most important metabolic characteristics of cancer cells. This concept was originally based on the classical fermentation studies by Warburg and enzymological studies on a series of rat hepatomas with increasing malignancy. Modern biochemical techniques have confirmed these initial findings.

Fig 1. FDG PET images of NHL: Patient 246 with high-grade lymphoma in a cervical lymph node (left panel, the SUV 16.8), and patient 221 with low-grade lymphoma in a supraclavicular lymph node (right panel, the SUV 6.2). The images are presented with the contours of the body and the color scale is calibrated to the maximum counts per pixel of the image in the left panel.

Fig 2. Standardized uptake values (SUV, n = 22, star) and metabolic rates (rMR, n = 18, circle) for FDG in tumors as measured with PET in patients with high-grade, intermediate-grade, and low-grade lymphoma according to the Working Formulation.
FDG PET IN NHL

Both at the level of increased glucose transport and phosphorylation. Clearly, glycolysis may be a major pathway for energy metabolism in neoplastic cells. The application of PET with FDG as a tracer to study glucose metabolism provides now a noninvasive method that can give quantitative information about the regional metabolic processes. FDG PET makes it possible to investigate glucose utilization of human tumors in vivo.

Our results suggest that Warburg's initial suggestion of an association between glucose utilization and the grade of differentiation of malignancy may be true for untreated, human NHLs. Warburg and other investigators observed that the transformation from slow-growing, well-differentiated tumors to rapidly growing, poorly differentiated lesions was accompanied by a progressive increase in glycolysis. In vitro, FDG uptake may be related to tumor cell doubling time. Yoshioka et al. showed that uptake of FDG in human cancers heterotransplanted in nude mice increased with loss of differentiation. In humans such an association has been suggested in malignant brain, musculoskeletal, and breast tumors when studied by PET and FDG. Newman et al. did not find a correlation between the grade of NHL and tumor uptake of FDG. However, all of the 11 NHL patients in their study had received chemotherapy (three patients also radiotherapy), which may have influenced the comparison of quantitative FDG uptake values between the subjects. In our series, all patients were studied before any therapy, and significant correlation between FDG uptake and the grade of differentiation was found.

In the present study, an association between tracer uptake and the S-phase fraction was also found supporting the results by Okada et al. They showed that proliferative activity as measured by Ki-67 staining had some correlation with the degree of FDG uptake in 23 patients with lymphoma of the head and neck region. There is a strong association between the S-phase fraction size determined from paraffin-embedded tissue and the histologic grade of malignancy in NHL.

The metabolism of FDG and glucose is known to be equivalent in tumors. The rMR depicts FDG utilization normalized to plasma concentration of the competing substrate, glucose, and does not directly reflect the glucose metabolic rate because of uncertainties in ratios of affinities of FDG and glucose for transport mechanisms and phosphorylation in heterogenous tissues. The parameter used to correct for the difference in substrate affinity, termed as lumped constant in the original Sokoloff equation, may well be larger than unity in tumors. Hence, rMR as used in the current study may overestimate the true glucose metabolic rate of neoplastic tissue. This uncertainty about the correction factor may explain why SUV was found to correlate better with the histologic grade than rMR in this study. Interestingly, the correlation was better also for influx constant (Ki), in comparison to rMR (Working Formulation, P = .009; Kiel classification, P = .023). This discrepancy could simply be explained by the small number of the patients in this series. However, a question is raised as to whether rMR with the linear plasma glucose correction in euglycemic patients is a more misleading parameter in tumors than SUV or Ki, which depict solely metabolism of FDG. Therefore, quantitation with SUV seems to be an adequate method for clinical purposes.

The present results support the hypothesis that high activity of FDG metabolism is associated with high-grade malignancy of human cancer. High-grade malignant NHLs have a higher affinity for transport and phosphorylation of FDG than the low-grade ones, which may be associated with increased glucose metabolism in accordance with the genuine hypothesis by Warburg. Also, a high S-phase fraction or DNA aneuploidy is associated with a high uptake of FDG in NHL, speaking in favor of relationship between cell proliferation and FDG uptake. Because the method is only minimally invasive, FDG PET could be used to study the metabolic activity of lymphoma deposits located in sites that are difficult to biopsy, and, therefore, the technique could be of value in some patients who are poor candidates for surgery because of their poor general condition or concurrent dis-
ases. FDG uptake might provide further information in cases where the degree of aggressiveness of lymphoma is not settled based on histology alone. The method may also be used in the whole-body imaging mode and might be of value in staging and selecting the metabolically most active tumor site for biopsy, and in the evaluation of a residual tumor mass for the presence of persisting disease. An interesting field for further research might be to study transformation of low-grade lymphomas to more malignant types. Further studies are also needed to discover the potential of FDG PET in predicting the outcome of patients with NHL, and in monitoring the efficacy of therapy.

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Increased glucose metabolism in untreated non-Hodgkin's lymphoma: a study with positron emission tomography and fluorine-18-fluorodeoxyglucose

M Lapela, S Leskinen, HR Minn, P Lindholm, PJ Klemi, KO Soderstrom, J Bergman, M Haaparanta, U Ruotsalainen and O Solin