RADIATION RESEARCH 172, 220–225 (2009)
0033-7587/09 $15.00

DOI: 10.1667/RR1448.1

A New EPR Oximetry Protocol to Estimate the Tissue Oxygen Consumption *In Vivo*

Caroline Diepart, Bénédicte F. Jordan and Bernard Gallez[1]

Laboratory of Biomedical Magnetic Resonance, Université catholique de Louvain, B-1200 Brussels, Belgium

Diepart, C., Jordan, B. F. and Gallez, B. A New EPR Oximetry Protocol to Estimate the Tissue Oxygen Consumption *In Vivo*. *Radiat. Res.* 172, 220–225 (2009).

The oxygen consumption rate in tumors affects tumor oxygenation and response to therapies. A new EPR method was developed to measure tissue oxygen consumption noninvasively. The protocol was based on the measurement of pO_2 during a carbogen challenge. The following sequence was used: (1) basal value during air breathing; (2) saturation of tissue with oxygen by carbogen breathing; (3) switch back to air breathing. The assumption was that the kinetics of the return to the basal value after oxygen saturation would be governed mainly by tissue oxygen consumption. This challenge was applied in hyperthyroid mice (generated by chronic treatment with L-thyroxine) and control mice because hyperthyroidism is known to dramatically affect the oxygen consumption rate of tumor and muscle cells. Muscle and tumor cells from the hyperthyroid mice consumed oxygen faster than muscle and tumor cells from the control mice, which is consistent with the results of *in vitro* studies. Tumor perfusion was not affected by the treatment with L-thyroxine. This method gives an index that may reasonably be ascribed to the local oxygen consumption and has the unique advantage of being adaptable to *in vivo* studies.

INTRODUCTION

The partial pressure of oxygen (pO_2) is a crucial factor that affects the response of tumors to radiation and other cytotoxic treatments. Over the past decade, clinical studies using oxygen microelectrodes have demonstrated the potential value of measuring pO_2 in tumors to determine the probability of response to conventional radiation therapy. Several studies have shown improved outcomes for cancer patients whose tumors have smaller hypoxic fractions. Tumor hypoxia also promotes metastasis, selects cells with malignant phenotypes, and promotes angiogenesis (*1*).

[1] Address for correspondence: CMFA/REMA, Avenue Mounier 73.40, B-1200 Brussels, Belgium; e-mail: bernard.gallez@uclouvain.be.

Tumor oxygenation depends on a balance between oxygen supply and consumption, and both should be considered in developing strategies to reduce tumor hypoxia. A number of strategies have been considered in an effort to improve tumor oxygenation during radiation treatment, with the goal of increasing tumor sensitivity to radiation. Theoretical simulation of oxygen transport to tumors (*2*) suggests that decreasing the oxygen consumption rate of tumor tissue could be a particularly effective strategy to reduce the fraction of hypoxic tissue in solid tumors. Several drugs that inhibit cellular oxygen consumption have been characterized for their potential to increase tumor oxygenation and thereby enhance radiosensitivity. Meta-iodobenzylguanidine (*3*), insulin (*4*), anti-inflammatory drugs (*5*), corticoids (*6*), some antagonists of vascular endothelial growth factor (VEGF, SU5416) (*7*), and anti-thyroid hormones (*8*) all modify the rate of oxygen consumption by tumor cells. Other means to reduce oxygen consumption have also been proposed, including using the Crabtree effect through administration of glucose (*9*) and decreasing local tumor temperature to less than 25°C (*10*).

There are a number of useful methods for measuring pO_2 or $[O_2]$ *in vivo* (*11, 12*), but methods to measure the tissue oxygen consumption rate are limited. Electron paramagnetic resonance (EPR) can measure oxygen consumption *in vitro* and *ex vivo*. The method is based on the variation of the line width of a paramagnetic material in the presence of consuming cells in a closed system. Because there is a relationship between the line width and the pO_2, the effects of treatments on the tumor cell oxygen consumption rate can be measured (*4*). Positron emission tomography (PET) allows *in vivo* measurements and quantification of physiological processes using short-lived positron-emitting radiopharmaceuticals. The PET technique enables measurement of cerebral oxygen consumption using a short inhalation of $[^{15}O]$-O_2 (*13*); however, this method cannot be applied in tissues with disorganized vascular architecture, such as tumors. Only one method can measure the oxygen consumption rate of tumor cells *in vivo* (*14*). Dewhirst *et al.* reported determinations of the oxygen consumption

rate in microscopic tumor regions bound by microvessels. Profiles of tissue pO_2 were measured along lines crossing the regions using oxygen microelectrodes. Theoretical simulations of the oxygen diffusion in the regions were made, and consumption rates were estimated for the best fit between measured and simulated values (*14*). However, such electrodes are difficult to use, involve a significant degree of invasiveness, and cannot be used for repeated measurements.

In vivo EPR oximetry presents some advantages that make the technique a tool of choice to monitor tissue oxygenation: non-invasiveness, repeatability, sensitivity at low pO_2, localized measurements, little or no toxicity, and ability to make several measurements simultaneously (*15*). Here we developed a new *in vivo* EPR method that can estimate tissue oxygen consumption non-invasively. For this purpose, we designed a protocol where pO_2 was monitored in the tissue of interest during a breathing challenge. During carbogen breathing, local pO_2 increases progressively. When breathing is switched back to air, pO_2 returns to its basal values. Our hypothesis was that the return kinetics could be influenced by the local oxygen consumption. As proof of concept, we applied this protocol in muscle and tumor tissues in hyperthyroid mice and control mice. Hyperthyroidism was induced by chronic treatment with L-thyroxine; this treatment is known to dramatically affect the oxygen consumption rate of muscle and tumor cells (*8, 16*).

MATERIALS AND METHODS

Tumor Model

A transplantable mouse liver tumor (TLT) model was implanted in the legs of NMRI mice. The measurements were performed when the tumor reached 8.0 ± 1.0 mm. All animal experiments were conducted in accordance with national animal care regulations.

Treatments

Mice were treated with L-thyroxine (0.003%) in drinking water for 3 weeks. Animals were anesthetized by inhalation of isoflurane mixed with 21% oxygen in a continuous flow, delivered by a nose cone. Induction of anesthesia was performed using 3% isoflurane; isoflurane was then stabilized at 1.8% for a minimum of 15 min before any measurement.

In Vivo Evaluation of Tissue Oxygenation and Oxygen Consumption

EPR oximetry using charcoal (CX 0670-1; EM Sciences, Gibbstown, NJ) as the oxygen-sensitive probe was used to evaluate changes in tumor oxygenation, using a protocol described previously (*17*). EPR spectra were recorded using an EPR spectrometer (Magnettech, Berlin, Germany) with a low-frequency microwave bridge operating at 1.2 GHz and an extended loop resonator. A suspension of charcoal was injected in the center of the tumor and in the muscle (100 mg/ml; 70 µl injected, particle size of 1–25 µm). Charcoal particles were used because of their metabolic stability and their high sensitivity to subtle changes in tissue oxygenation. The localized EPR measurements correspond to an average of the pO_2 values in a volume of ~10 mm^3 (*17*). The protocol to measure oxygen consumption was performed as follows: When three similar measurements had been obtained under air-breathing conditions, the breathing gas was switched to carbogen. Carbogen breathing was maintained until the local pO_2 reached a maximum (plateau of at least three similar measurements). The gas was then switched back to air and measurements were made until the pO_2 returned to its basal values. Measurements were performed at 5-min intervals during the first part of the experiment (before switching back to air) and at 2- to 4-min intervals during the second part of the experiment. The return kinetics (Δ pO_2 as a function of time, after switching back to air breathing) wass measured by a monoexponential fitting. We also performed an additional experiment in which blood flow to the hind limb was stopped by ligation of the leg after carbogen breathing. The ligation was performed when we obtained the maximal pO_2 after carbogen breathing. Immediately after the ligation, the pO_2 was measured over time. A blue hind limb indicated the success of the clamping. The technique of clamping has been used and validated in experiments with radiation in our laboratory (*8*). In the present experiment, the mean pO_2 measured in tumors before carbogen breathing was 2.9 ± 0.2 mmHg, and the pO_2 measured after clamping was 0.5 ± 0.1 mmHg.

Patent Blue Staining

Patent Blue (Sigma-Aldrich) was used to obtain a rough estimate of the TLT tumor perfusion after treatment with L-thyroxine. This technique involves the injection of 200 µl of Patent Blue solution (1.25%) into the tail vein of the mice. After 1 min, a uniform distribution of the staining through the body was obtained, and mice were killed humanely. Tumors were carefully excised and cut in two size-matched halves. Pictures of each tumor cross section were taken with a digital camera. To compare the stained and unstained areas, an in-house program running on IDL (Interactive Data Language, RSI, Boulder, CO) was used. For each tumor, a region of interest (stained area) was defined on the two pictures, and the percentage of stained area in the whole cross section was determined. The mean of the percentage of the two pictures was then calculated and used as an indicator of tumor perfusion.

RESULTS

Thyroid status influences the oxygen consumption rate of tumor cells (*8*). Tumor cells extracted from control mice consumed oxygen more slowly than tumor cells extracted from hyperthyroid mice. The mean slopes were -1.41 ± 0.11 µ*M*/min ($n = 11$) and -1.91 ± 0.15 µ*M*/min ($n = 9$), respectively (*8*), and were significantly different from each other (Student's t test, $P < 0.05$). Measurements in isolated muscle cells using this protocol were not possible due to difficulties in dissociating the muscle structure rapidly.

Typical evolutions of pO_2 during the breathing challenge are shown in Fig. 1. The first arrow corresponds to the start of the carbogen challenge. The second arrow corresponds to the time when carbogen was switched back to air. Measurements of the evolution of pO_2 in the tumors are shown in the top panels (Fig. 1A and B, for control and hyperthyroid mice, respectively), while experiments in muscles are shown in the bottom panels (Fig. 1C and D, for control and hyperthyroid mice, respectively). In this figure, it can be seen that (1) the kinetics of the return to basal values was faster in hyperthyroid mice than in control mice and (2) the kinetics of the return to basal values was faster in

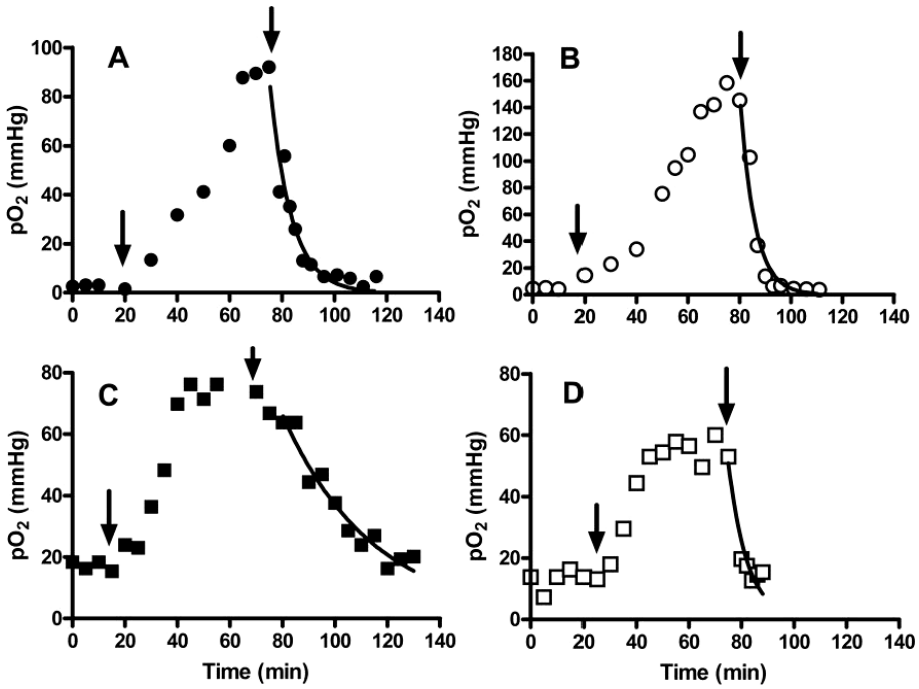


FIG. 1. Evolution of the pO_2 during breathing challenge in tumor and muscle of hyperthyroid and control mice. The measurements were carried out using an EPR spectrometer operating at 1.2 GHz, using charcoal particles as oxygen sensors. First arrow: air switched to carbogen; Second arrow: carbogen switched to air. Upper panels: Typical evolution of pO_2 during the breathing challenge in tumors of a control (panel A) and a hyperthyroid (panel B) mouse. Bottom panels: Typical evolution of pO_2 during the breathing challenge in muscle of a control (panel C) and a hyperthyroid (panel D) mouse. The measurements were done using charcoal in mice at L-band.

tumors than in muscle. Only a few data points were measurable when the return kinetics was rapid, as observed in hyperthyroid mice (Fig. 1B and D). The maximal pO_2 reached after carbogen breathing was lower in hyperthyroid mice than in the control group (84 $\pm$ 48.6 mmHg and 123 $\pm$ 38 mmHg, respectively). This is consistent with the rapid oxygen consumption by the tissues in hyperthyroid mice. It should be noted that the extent of increase in pO_2 was variable from one mouse to another, especially in tumors. However, the return kinetics was reproducible within groups when the results were expressed as a percentage of the maximal pO_2 reached after carbogen breathing. This is illustrated in Fig. 2 for tumor experiments carried out in hyperthyroid and control mice. The difference between return kinetics observed for tumors in control mice and in hyperthyroid mice is illustrated in Fig. 2A and B (log plot), and individual evolutions of pO_2 are presented in Fig. 2C (control mice) and D (hyperthyroid mice). These graphs illustrate that the measurements were reasonably reproducible within a group and that the return kinetics was systematically faster for hyperthyroid mice (most data points are below 25% of the maximal pO_2 value after 10 min, in contrast to control mice; Fig. 2C and D). The return kinetics measured in muscles of control and hyperthyroid mice is presented in Fig. 3A (normal plot) and B (log plot).

Quantitative estimation of the return kinetics was carried out using a monoexponential curve, because this estimation fitted the experimental data well. The constants of the kinetics (k expressed as min^{-1}) are presented in Fig. 4. Thyroid status significantly modified the constants of the kinetics. The constants for hyperthyroid mice were higher than those for control mice for both tumors and muscles. The k values in tumors were 0.13 $\pm$ 0.01 min^{-1} (mean $\pm$ standard error, $n = 7$) and 0.18 $\pm$ 0.01 min^{-1} (mean $\pm$ standard error, $n = 8$) in control and hyperthyroid mice, respectively; this difference was significant (Student's t test, $P < 0.05$). The k values in muscles were 0.09 $\pm$ 0.01 min^{-1} (mean $\pm$ standard error, $n = 11$) and 0.18 $\pm$ 0.02 min^{-1} (mean $\pm$ standard error, $n = 13$) in control and hyperthyroid mice, respectively; this difference was highly significant ($P < 0.001$). The constants of the kinetics were greater ($P < 0.05$) in tumors from euthyroid mice than in muscles. However, there were no statistically significant differences between the constants of the kinetics for tumors and muscles in the hyperthyroid mice. To measure oxygen consumption without the potential confounding factors of variability in perfusion and vascular pO_2, we performed an additional experiment consisting of clamping the blood flow to the hind limb after carbogen breathing. The constants after clamping were not significantly different from those in the

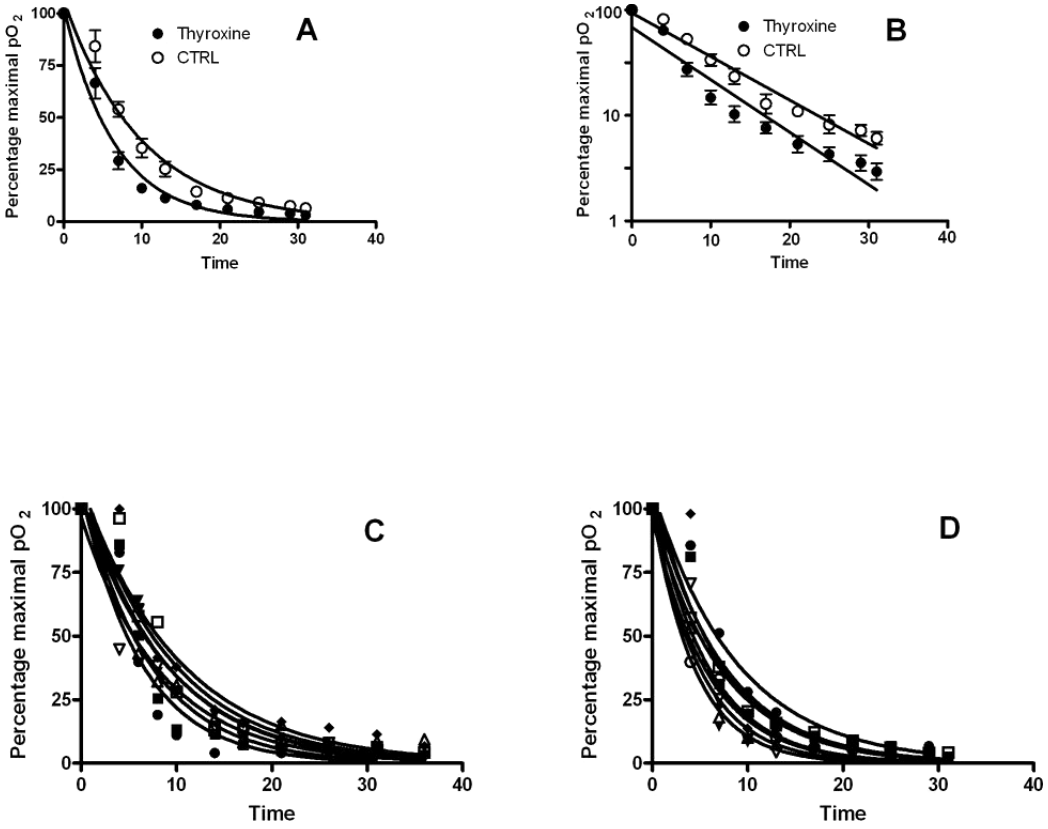


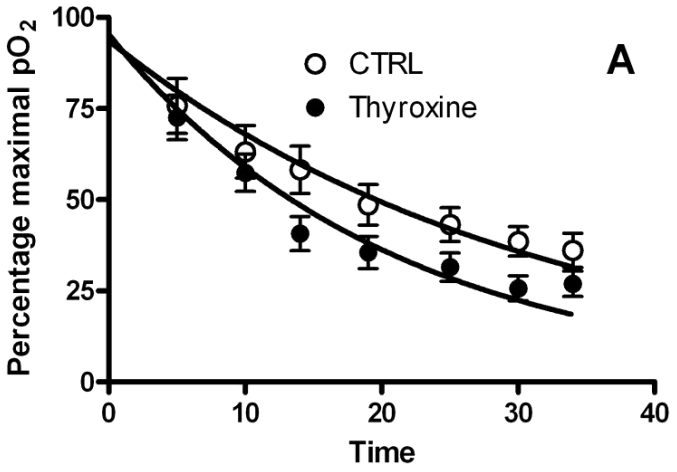
FIG. 2. Return kinetics after carbogen breathing measured in tumors of control (CTRL) and hyperthyroid mice. The results are expressed as a percentage of the maximal pO_2 reached after carbogen breathing (mean ± standard error). Panel A: Normal plot; panel B: log plot; panel C: individual measurements obtained in tumors from control mice; panel D: individual measurements obtained in tumors from hyperthyroid mice.

previous experiment (without clamping). The k values were 0.14 ± 0.02 min^{-1} (mean ± standard error, $n = 5$) and 0.22 ± 0.02 min^{-1} (mean ± standard error, $n = 8$) in control and hyperthyroid mice, respectively.

The effect of L-thyroxine treatment on the tumor blood perfusion was estimated using the colored area observed in tumors 1 min after i.v. injection of Patent Blue (Fig. 5). This method has been validated previously by our group and compared with DCE-MRI data (*6, 7, 20*). No difference was observed between control mice and hyperthyroid mice [71.5 ± 2.7% ($n = 10$) and 70.0 ± 3.5% ($n = 12$), respectively, Student's t test].

DISCUSSION

Tissue oxygen consumption is a key factor in physiology and physiopathology. Modulating tissue oxygen consumption (for example, by hibernation) is an essential part of therapeutic strategy in intensive care medicine and in organ transplantation. In oncology, it has been predicted (*3*) and verified experimentally (*5, 6, 8*) that modification of oxygen consumption is more efficient at correcting tumor hypoxia than modification of oxygen delivery. Although oxygen consumption is thus very important, limited non-invasive methods are available to measure it. In this study, we attempted to develop a new method that can highlight differences in oxygen consumption by different tissues. The use of normal and hyperthyroid mice provided ideal models of tissues with different oxygen consumption rates. First it was confirmed *ex vivo* that treatment with L-thyroxine did change tumor oxygen consumption. The oxygen consumption rate was shown to be significantly enhanced in hyperthyroid mice compared to control mice (*8*). Remarkably, this difference in the oxygen consumption rate was correlated with higher constants of the kinetics, reflecting the rapidity of the return of oxygenation to basal values after presaturation with oxygen (Fig. 4). It is also important to note that the perfusion of the tissues was not affected by the L-thyroxin treatment (Fig. 5). It has also been shown that perfusion of tumors remains unchanged during carbogen breathing (*18, 19*). Thus the perfusion is not responsible for the change in return kinetics, and local oxygen consumption is likely the main factor responsible for this return to initial oxygenation levels. The clamping experiment definitely confirms that our technique is measuring the oxygen consumption of tumor cells without any major influence of confounding factors such as perfusion and vascular pO_2. Interestingly, the constants of kinetics for euthyroid mice were higher in tumors than in muscles (Fig. 4). It is well



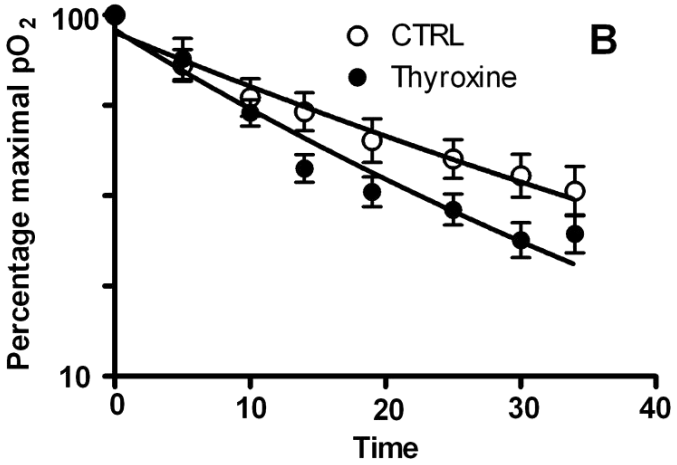


FIG. 3. Return kinetics after carbogen breathing measured in muscles of control (CTRL) and hyperthyroid mice. The results are expressed as a percentage of the maximal pO_2 reached after carbogen breathing (mean ± SEM). Panel A: Normal plot; panel B: log plot.

known that the high rate of tumor cell proliferation and metabolism present in tumors increases oxygen consumption by tumor tissues. However, careful interpretation of the results is important in this case since we are comparing two different tissues with different perfusion rates (i.e., oxygen will be refreshed faster in muscle than in tumor). Perfusion can be a confounding factor when comparing results from poorly and highly perfused tissues. This problem is not present when we study the effect of a treatment on a single tissue. Also, we showed by our clamping experiments that measurements on tumors are not influenced by such a factor, probably because tumors already have very low perfusion rates. Although the experiments were carried out in two tissues that are physiologically and morphologically very

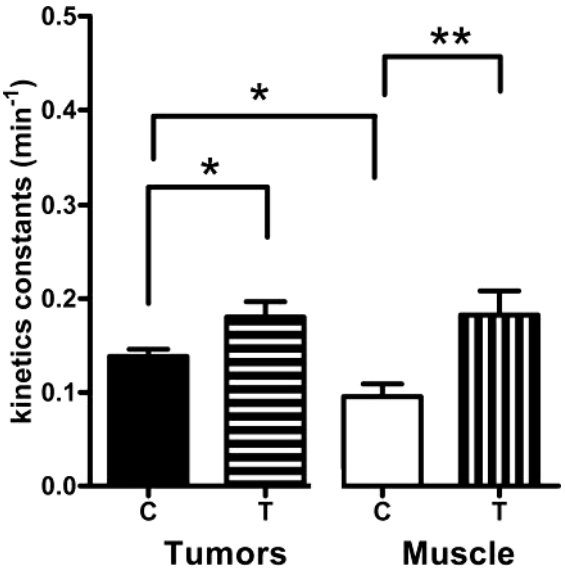


FIG. 4. Constants of kinetics measured in tumor and muscle *in vivo*. $*P < 0.05$; $**P < 0.01$. C: Control, T: thyroxine. The constants measured in tumors and muscles from hyperthyroid mice were higher than in control mice. In euthyroid mice, the constants were higher in tumors than in muscles. In hyperthyroid mice, there was no significant difference between the constants for tumors and muscles.

different, the results obtained are in agreement with the known oxygen consumption rates for hyper- and euthyroid mice. The variability of response after carbogen breathing can be explained by the fact that EPR oximetry is measuring a single point (mean value of ~10 mm^3) in the tumor. Some highly responding regions (such as in the periphery of the tumor) can dramatically influence the mean value. This could be addressed by developing a method to obtain parametric maps of oxygen consumption to be able to probe the heterogeneity of response (see below).

It should be emphasized that our method does not provide a quantitative absolute value of oxygen con-

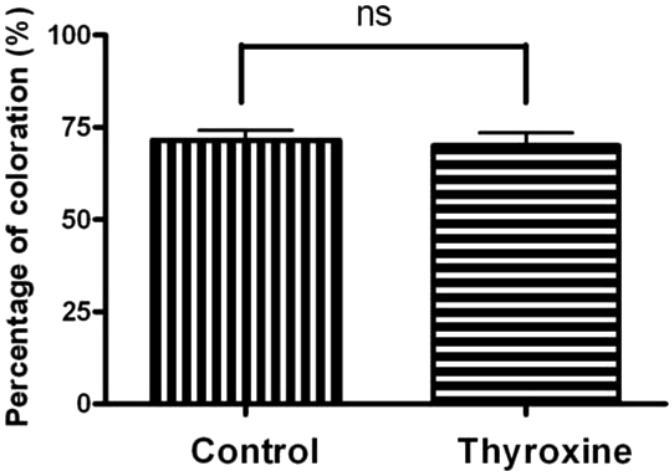


FIG. 5. Perfusion assessed in TLT tumors by the Patent Blue staining technique. No significant difference was observed between control and hyperthyroid mice.

sumption (for example, in terms of μmol of oxygen consumed by millions of cells and per unit of time). Rather, it gives an index that may reasonably be ascribed to the local oxygen consumption. Such a qualitative parameter is also used in *in vivo* EPR to estimate the redox status of a tissue by measuring the kinetics of reduction of nitroxide in tissues (*21, 22*). There are several potential applications of the present work. First, this method should be considered for evaluating *in vivo* the efficacy of different modifiers of oxygen consumption or inhibitors of respiration. Second, the protocol could be transferred to other imaging modalities that are able to visualize oxygen in tissues, such as EPR imaging and ^{19}F NMR imaging, to provide parametric maps reflecting oxygen consumption. Finally, the possibility that EPR may be used in humans in the near future is another reason to pay more attention to this technique (*23*). It should be noted that the carbogen challenge is already being used in the first clinical EPR studies, and our technique to measure oxygen consumption *in vivo* could be an important tool for use in human subjects.

ACKNOWLEDGMENTS

This work is supported by grants from the Belgian National Fund for Scientific Research (FNRS), the Televie, the Fonds Joseph Maisin, the Fondation Belge contre le Cancer, the Saint-Luc Foundation, the ''Actions de Recherches Concertées-Communauté Française de Belgique-ARC 04/09-317'', and the ''Pôle d'attraction Interuniversitaire PAI VI (P6/38)''. BJ is a research associate of the FNRS.

Received: April 28, 2008; accepted: April 30, 2009

REFERENCES

1. P. Vaupel and L. Harrison, Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncologist* **9** (Suppl. 5), 4–9 (2004).
2. T. W. Secomb, R. Hsu, E. T. Ong, J. F. Gross and M. W. Dewhirst, Analysis of the effects of oxygen supply and demand on hypoxic fraction in tumors. *Acta Oncol.* **34**, 313–316 (1995).
3. J. E. Biaglow, Y. Manevich, D. Leeper, B. Chance, M. W. Dewhirst, W. T. Jenkins, S. W. Tuttle, K. Wroblewski, J. D. Glickson and S. M. Evans, MIBG inhibits respiration: potential for radio- and hyperthermic sensitization. *Int. J. Radiat. Oncol. Biol. Phys.* **42**, 871–876 (1998).
4. B. F. Jordan, V. Gregoire, R. J. Demeure, P. Sonveaux, O. Feron, J. O'Hara, V. P. Vanhulle, N. Delzenne and B. Gallez, Insulin increases the sensitivity of tumors to irradiation: involvement of an increase in tumor oxygenation mediated by a nitric oxide-dependent decrease of the tumor cells oxygen consumption. *Cancer Res.* **62**, 3555–3561 (2002).
5. N. Crokart, K. Radermacher, B. F. Jordan, C. Baudelet, G. O. Cron, V. Gregoire, N. Beghein, C. Bouzin, O. Feron and B. Gallez, Tumor radiosensitization by antiinflammatory drugs: evidence for a new mechanism involving the oxygen effect. *Cancer Res.* **65**, 7911–7916 (2005).
6. N. Crokart, B. F. Jordan, C. Baudelet, G. O. Cron, J. Hotton, K. Radermacher, V. Gregoire, N. Beghein, P. Martinive and B. Gallez, Glucocorticoids modulate tumor radiation response through a decrease in tumor oxygen consumption. *Clin. Cancer Res.* **13**, 630–635 (2007).
7. R. Ansiaux, C. Baudelet, B. F. Jordan, N. Crokart, P. Martinive, J. Dewever, V. Gregoire, O. Feron and B. Gallez, Mechanism of reoxygenation after antiangiogenic therapy using su5416 and its importance for guiding combined antitumor therapy. *Cancer Res.* **66**, 9698–9704 (2006).
8. B. F. Jordan, N. Christian, V. Gregoire, O. Feron and B. Gallez, Thyroid status is a key modulator of tumor oxygenation: Implication for radiation therapy. *Radiat. Res.* **168**, 428–432 (2007).
9. S. A. Snyder, J. L. Lanzen, R. D. Braun, G. Rosner, T. W. Secomb, J. Biaglow, D. M. Brizel and M. W. Dewhirst, Simultaneous administration of glucose and hyperoxic gas achieves greater improvement in tumor oxygenation than hyperoxic gas alone. *Int. J. Radiat. Oncol. Biol. Phys.* **51**, 494–506 (2001).
10. D. K. Kelleher, C. Nauth, O. Thews, W. Krueger and P. Vaupel, Localized hypothermia: impact on oxygenation, microregional perfusion, metabolic and bioenergetic status of subcutaneous rat tumours. *Br. J. Cancer* **78**, 56–61 (1998).
11. B. Gallez, C. Baudelet and B. F. Jordan, Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications. *NMR Biomed.* **17**, 240–262 (2004).
12. D. S. Vikram, J. L. Zweier and P. Kuppusamy, Methods for noninvasive imaging of tissue hypoxia. *Antioxid. Redox Signal.* **9**, 1745–1756 (2007).
13. P. H. Poulsen, D. F. Smith, L. Ostergaard, E. H. Danielsen, A. Gee, S. B. Hansen, J. Astrup and A. Gjedde, *In vivo* estimation of cerebral blood flow, oxygen consumption and glucose metabolism in the pig by [^{15}O]water injection, [^{15}O]oxygen inhalation and dual injections of [^{18}F]fluorodeoxyglucose. *J. Neurosci. Methods* **77**, 199–209 (1997).
14. M. W. Dewhirst, T. W. Secomb, E. T. Ong, R. Hsu and J. F. Gross, Determination of local oxygen consumption rates in tumors. *Cancer Res.* **54**, 3333–3336 (1994).
15. H. M. Swartz, Using EPR to measure a critical but often unmeasured component of oxidative damage: oxygen. *Antioxid. Redox Signal.* **6**, 677–686 (2004).
16. R. Gredilla, T. M. Lopez, M. Portero-Otin, R. Pamplona and G. Barja, Influence of hyper- and hypothyroidism on lipid peroxidation, unsaturation of phospholipids, glutathione system and oxidative damage to nuclear and mitochondrial DNA in mice skeletal muscle. *Mol. Cell. Biochem.* **221**, 41–48 (2001).
17. B. Gallez, B. F. Jordan, C. Baudelet and P. D. Misson, Pharmacological modifications of the partial pressure of oxygen in murine tumors: evaluation using *in vivo* EPR oximetry. *Magn. Reson. Med.* **42**, 627–630 (1999).
18. M. W. Dewhirst, E. T. Ong, G. L. Rosner, S. W. Rehmus, S. Shan, R. D. Braun, D. M. Brizel and T. W. Secomb, Arteriolar oxygenation in tumour and subcutaneous arterioles: effects of inspired air oxygen content. *Br. J. Cancer Suppl.* **27**, S241–S246 (1996).
19. M. E. Powell, S. A. Hill, M. I. Saunders, P. J. Hoskin and D. J. Chaplin, Effect of carbogen breathing on tumour microregional blood flow in humans. *Radiother. Oncol.* **41**, 225–231 (1996).
20. R. Ansiaux, C. Baudelet, B. F. Jordan, N. Beghein, P. Sonveaux, W. J. De, P. Martinive, V. Gregoire, O. Feron and B. Gallez, Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. *Clin. Cancer Res.* **11**, 743–750 (2005).
21. P. Kuppusamy, H. Li, G. Ilangovan, A. J. Cardounel, J. L. Zweier, K. Yamada, M. C. Krishna and J. B. Mitchell, Noninvasive imaging of tumor redox status and its modification by tissue glutathione levels. *Cancer Res.* **62**, 307–312 (2002).
22. G. Ilangovan, H. Li, J. L. Zweier, M. C. Krishna, J. B. Mitchell and P. Kuppusamy, *In vivo* measurement of regional oxygenation and imaging of redox status in RIF-1 murine tumor: effect of carbogen-breathing. *Magn. Reson. Med.* **48**, 723–730 (2002).
23. N. Khan, B. B. Williams, H. Hou, H. Li and H. M. Swartz, Repetitive tissue pO_2 measurements by electron paramagnetic resonance oximetry: current status and future potential for experimental and clinical studies. *Antioxid. Redox Signal.* **9**, 1169–1182 (2007).