

Role of oxygen vs. glucose in energy metabolism in a mammary carcinoma perfused *ex vivo*: Direct measurement by ^{31}P NMR

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ABSTRACT The role of glycolysis vs. respiration in tumor energy metabolism has been studied, to date, primarily *in vitro* by using single cells, multicellular spheroids, or tissue slices. With the advent of *in vivo* NMR spectroscopy, several investigators have shown that tumor energy status depends on its blood flow. Since manipulation of blood flow alters both oxygen and glucose delivery to a solid tumor, these studies have not been able to separate the relative contribution of oxygen vs. glucose in energy metabolism *in vivo*. In the present study, we have overcome this problem by combining two methods: the tissue-isolated R3230AC mammary adenocarcinoma perfused *ex vivo* and ^{31}P NMR spectroscopy. The isolated tumor permits one to control the perfusion pressure as well as the metabolite concentrations in the perfusate. NMR spectroscopy permits one to measure the ratio of nucleoside triphosphate to inorganic phosphate (NTP/ P_i) and pH. Our results show that (i) the NTP/ P_i ratio *ex vivo* is similar to that observed *in vivo* prior to surgery, (ii) the NTP/ P_i ratio is insensitive to flow changes at high flow rates but is proportional to flow rate at flows comparable to those found *in vivo*, (iii) the NTP/ P_i ratio of these tumors is resistant to hypoxia and is not maintained when glucose is removed or replaced with glutamine, and (iv) although both O_2 and glucose are consumed by these tumors, the effect of perfusate flow rate appears to be mediated largely through glucose delivery. The current approach not only provides information about the role of glycolysis vs. respiration in a rodent tumor but also is general and versatile enough to provide similar data in human tumors perfused *ex vivo*.

The production of ATP by mammalian cells occurs by glycolysis and oxidative phosphorylation. The current understanding of the relative contributions of each of these energy-producing pathways in tumors has been derived primarily from *in vitro* studies of single cells, multicellular spheroids, and tissue slices (1–3). However, the conclusions based on these *in vitro* systems may not be valid for solid tumors *in vivo* due to the temporal and spatial heterogeneity in tumor blood supply and the resulting mass transport limitations (4–6). Using NMR spectroscopy, several investigators have shown that changes in the NTP/ P_i ratio due to various manipulations (e.g., chemotherapy, radiation therapy, etc.) are correlated with alterations in blood flow (7–12). Since changes in blood flow modify both O_2 and glucose delivery to a solid tumor, these studies have not been able to separate the relative contributions of glycolysis and respiration. Although it is possible to change O_2 or glucose concentrations *in vivo* by altering O_2 level in inspired gases (13) or by exogenous administration of glucose or insulin (10, 14), the interpretation of results is complicated by alterations in other physiological parameters. As a result, the following central question remains unanswered: What is the relative

contribution of oxygen vs. glucose in the maintenance of ATP and pH in tumors *in vivo*?

The tissue-isolated tumor preparation perfused *ex vivo* makes it possible to study systematically the determinants of tumor energy metabolism. This tumor model, first developed by Gullino (15), is a transplantable tumor surgically prepared so that it has a single artery and a single vein as its vascular connections to its host. It is possible to completely remove the tumor from its host as an isolated perfused tumor (16). This *ex vivo* tumor has the vascular structure and potential mass transport limitations of a solid tumor *in vivo* but permits complete control over perfusate pressure and composition. The isolated perfused tumor model has been used to study O_2 and glucose consumption (17), the determinants of tumor blood flow (18, 19), capillary filtration (20), drug pharmacokinetics (21, 22), and most recently energy metabolism (23).

We report herein the use of a combination of the perfused tissue-isolated R3230AC mammary adenocarcinoma and ^{31}P NMR spectroscopy to isolate the factors that determine the energy status of the tumors. This tumor line has been characterized *in vitro* for its energy metabolism (24, 25). The *ex vivo* perfusion has been carried out using an erythrocyte-free medium with a relatively low O_2 content. The metabolic stability of the preparation is assessed both by comparing the *in situ* and *ex vivo* ^{31}P NMR spectra and by monitoring the stability of the energy status over time. The relationship between flow and energy status is examined by monitoring the ^{31}P NMR spectrum during progressive decreases in perfusate pressure and flow rate. Of course, altering the flow changes the delivery of both O_2 and glucose. These effects are separated in experiments where either O_2 or glucose is removed from the perfusing medium, thus evaluating whether the tumor has the ability to maintain its energy status in the absence of an exogenous carbon source (glucose) or in the absence of the usual electron acceptor for oxidative phosphorylation (O_2). Finally, by replacing the glucose with glutamine, a carbon source that does not enter glycolysis but produces ATP solely through oxidative phosphorylation, the ability of the tumor to maintain ATP levels by oxidative phosphorylation alone is evaluated.

METHODS

Tumor Preparation and Perfusion. Experiments were performed using the R3230AC mammary adenocarcinoma (Bio-measure, Hopkinton, MA), maintained in our laboratory by serial subcutaneous propagation in Fischer 344 weanlings. The tissue-isolated tumors were prepared surgically in adult Fischer 344 females by the procedure developed by Gullino (15) and modified in our laboratory (26). Briefly, tumor slurry is injected into the periovarian fat, which is then enveloped in a Parafilm bag (American National Can, Greenwich, CT) and placed subcutaneously. Tumor blood flow is supplied by the ovarian artery; all venous outflow returns via the ovarian vein. The tissue-isolated tumors were used for experiments 12–17 days after implantation [tumor weight, 0.7–2.5 g (av-

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erage, 1.4 g); diameter, 1.2–1.8 cm]. Surgery to isolate and perfuse the tumor *ex vivo* was performed by a procedure (27) based upon those used for tumors isolated in the ovarian fat pad (16, 18). The tumor was completely removed from its host and placed in a heated ($36.5 \pm 0.5^\circ\text{C}$) humidified chamber.

In all experiments, tumors were first perfused with a cell-free Krebs/Henseleit (KH) solution [118 mM NaCl/4.7 mM KCl/1.2 mM KH_2PO_4 /1.2 mM MgSO_4 /2.55 mM CaCl_2 /11.1 mM glucose/sodium heparin (7000 units/liter)/2.5% (wt/vol) bovine serum albumin (Sigma; A7030, essentially fatty acid free)]. By using NaHCO_3 (≈ 3 g/liter), the hydrogen ion concentration was adjusted so that the perfusate at 36.5°C , equilibrated with 95% O_2 /5% CO_2 , had a pH of 7.4. Osmolarity of the perfusate was measured with a cryoscopic osmometer (Osmomat 030; Gonotec, Berlin) and adjusted to 320 milliosmoles/liter by the addition of either water or NaCl. The perfusate was prefiltered, then filtered again through a sterile 0.22- μm (pore size) filter, and kept at 4°C until use. Two alternate perfusates were also prepared in the same manner. One of these buffers was identical to the original KH but contained no glucose; in the other, glucose was replaced with either 22.2 mM L-glutamine or 11.1 mM L-glutamine plus 11.1 mM pyruvate. Perfusate was kept in a heated bath (37°C) and bubbled with 95% O_2 /5% CO_2 .

The perfusate was pumped by a multichannel peristaltic pump (Ismatec 7618-30, Cole-Parmer) passing through a 100- μm nylon mesh filter, a gas exchanger consisting of 16 feet of silastic tubing (1.5 mm i.d./1.9 mm o.d.; Dow Corning) enclosed in a 250- cm^3 chamber through which humidified 95% O_2 /5% CO_2 was passed at ≈ 0.5 liter/min, and a bubble trap. Total flow was determined from the weight of perfusate collected over 1-min periods from the venous cannula. A second parallel perfusate line was added to allow a rapid switch between two perfusates of different composition; the two perfusate lines joined at the exit of the gas exchanger. An additional gas input was added to the gas exchanger to allow a rapid switch between two gases of different composition. The distance between the gas exchanger and the tumor was kept at 12 cm. With the 95% N_2 /5% CO_2 in the gas exchanger, the PO_2 of the perfusate at its entrance to the tumor was < 3 mmHg (1 mmHg = 133 Pa) for all of the flow rates used in the experiments.

NMR. ^{31}P NMR spectroscopy was performed with a 7.0-tesla (^1H resonance frequency, 300 MHz; ^{31}P resonance frequency, 121.5 MHz) 15-cm-bore Biospec II imaging spectrometer (Bruker, Billerica, MA). The radiofrequency probe consisted of a two-turn 1.4-cm-diameter solenoid tuned to the ^{31}P resonance frequency. The probe was placed over the surface of the tumor. Spectra consisted of 256 summed acquisitions obtained using 90° pulses (pulse length, ≈ 15 μsec) and a repetition time of 2.5 sec. Spectra were processed with manual phasing and a spline baseline correction. Peak assignments were made by comparison with spectra from perchloric acid extracts of subcutaneous tumors (28). Peak areas were determined by numerical integration. The NTP/P_i ratio was determined by taking the average of the γ - and βNTP peak areas and dividing this by the P_i area. The $\beta\text{NTP}/\gamma\text{NTP}$ ratio was not altered by any of the experimental manipulations (data not shown). No correction was made for partial saturation of the resonances. We assumed that there were no significant changes in the longitudinal relaxation time, T_1 , while calculating changes in peak areas. The NMR-measured pH (pH_{NMR}) was determined from the difference in the chemical shift between P_i and αATP , by using a calibration curve determined for liver (29).

Lactate and Oxygen. Lactate concentrations were measured with an enzymatic assay (Sigma). Oxygen content was measured using a blood-gas analyzer (Radiometer, Copenhagen). Lactate production and oxygen consumption were

calculated from the arterio-venous concentration difference and the flow rate.

Experiments. The ^{31}P NMR spectra of eight tumors were examined both *in situ* and *ex vivo*, i.e., immediately before and after the surgery necessary for *ex vivo* perfusion. *In vivo* spectra were obtained with the rat under the pentobarbital anesthesia used for the isolation surgery and the tumor within its Parafilm bag and with its vascular connections intact but freed from its subcutaneous pouch. The four tumors studied *in vivo* were made acutely ischemic by arresting the host rat's heart with a rapid infusion of 1 M KCl while the collection of ^{31}P spectra continued. Four sets of *ex vivo* experiments were performed. During each experiment, serial ^{31}P spectra were collected continuously and venous outflow was periodically sampled. Each of the experiments began with a 60- to 90-min collection of baseline spectra from the perfused tumor. Flow was adjusted to maintain a constant perfusion pressure of 100 mmHg. In the first set of experiments ($n = 4$), no manipulations of the perfusate composition or pressure were made throughout the perfusion. In the second ($n = 4$), the gas entering the exchanger was changed to 95% N_2 /5% CO_2 , removing $>99.5\%$ of the O_2 from the perfusate within several minutes. This hypoxia continued for 60–90 min. The gas composition was then returned to 95% O_2 /5% CO_2 . After an additional 30 min, perfusion pressure was progressively decreased at 50-min intervals to ≈ 50 , 25, 12, and 6 mmHg. Finally, the perfusate was returned to its original pressure and spectra were collected for another 60 min. In the third set of experiments ($n = 4$), the perfusate was changed to one that contained no glucose. Glucose-free perfusion continued for 60–90 min. The original glucose-containing medium was returned and spectra were collected for another 60 min. In the fourth set of experiments ($n = 3$), the perfusate was changed to one that contained 22.2 mM glutamine or 11.1 mM glutamine plus 11.1 mM pyruvate.

Of 24 attempted *ex vivo* perfusions, 15 proved suitable for the experiments. The tumors were not used if (i) no venous outflow existed, (ii) technical problems with the apparatus caused the loss of blood flow, or (iii) the energy status of the perfused tumor was outside the range observed for the same tumors measured *in situ* (i.e., $\text{NTP}/\text{P}_i < 0.25$). Most of the unsuitable tumors were excluded by one of the first two criteria; only two tumors had normal venous outflow but failed to meet the third criterion.

RESULTS AND DISCUSSION

^{31}P NMR monitoring shows that the energy status of the R3230AC mammary adenocarcinoma can be maintained during *ex vivo* perfusion with a cell-free medium. The ^{31}P NMR spectra of the tissue-isolated tumors assessed both *in vivo* and *ex vivo* showed no consistent changes after the extensive surgery required to perfuse the tumor (Fig. 1). The NTP/P_i of the tumors before surgery was 1.10 ± 0.67 (average \pm SD); after surgery the NTP/P_i was 0.79 ± 0.39 . For most of the tumors, there was a difference in the energy status before and after surgery; however, the direction of the change was not consistent. Four tumors showed a decrease in the NTP/P_i ratio; the other four showed no change or an increase. This variability may arise from heterogeneity in the biological behavior of the tumors but it more likely represents variable amounts of vascular and cellular damage from the extensive surgery necessary for *ex vivo* perfusion. These two groups showed no differences in their responses to later manipulations. Tumor pH_{NMR} averaged 7.2 before surgery and 6.99 after. Those tumors that exhibited a decrease in NTP/P_i also had a decreased pH_{NMR} , and those with increased or unchanged NTP/P_i showed no significant change in pH_{NMR} . In controls, the relative levels of high-energy phosphate intermediates were stable throughout the 6–8 h of *ex vivo* perfu-

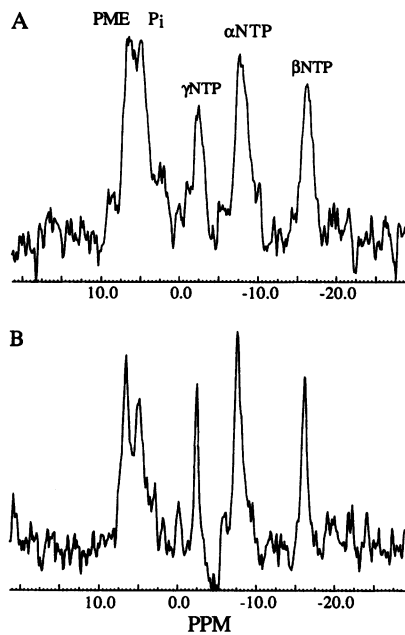


FIG. 1. ^{31}P NMR spectra obtained from a tissue-isolated R3230AC mammary tumor *in vivo* (A) and during the *ex vivo* perfusion (B). Peaks are phosphomonoesters (PME), inorganic phosphate (P_i), and the α , β , and γ phosphates of nucleoside triphosphates (α -, β -, and γ NTPs).

sion (data not shown). During the first 45 min of perfusion, NTP/ P_i of the tumors remained constant or increased slightly. In all the controls, the NTP/ P_i and pH remained constant after the first 45 min of the ^{31}P NMR spectroscopy. This stability during constant pressure perfusion occurred despite a gradual spontaneous 25–75% decrease in venous flow over the entire 6- to 8-h perfusion. Similarly, there was no indication of loss of total phosphates from the NMR spectra; the intensity of the NTP peaks was similar through all of the experimental manipulations.

The tumor energy status showed a marked response to changes in blood or perfusate flow. *In vivo*, the cessation of tumor blood flow results in a rapid drop in the NTP/ P_i ratio (Fig. 2). Similarly, when flow rate was decreased in the *ex vivo*-perfused tumors by lowering the perfusion pressure, the NTP/ P_i ratio dropped rapidly, reaching a new steady state within 25 min. Fig. 3 shows the steady-state responses of the *ex vivo*-perfused tumor energy status (NTP/ P_i) to all of the flow decrements. The initial venous flow rates were 0.6 ± 0.4 ml per min per g (average \pm SD). If one includes the data obtained during the period of constant pressure perfusion

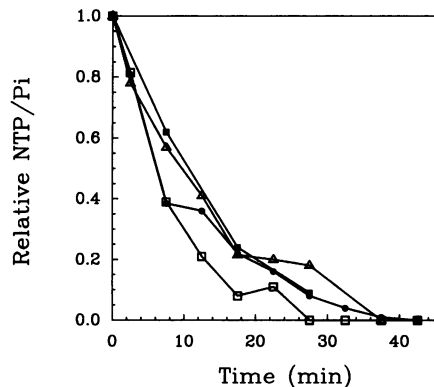


FIG. 2. Response of the *in vivo* tumor energy status to ischemia. The NTP/ P_i ratios have been normalized to 1.0 at 0 min, when blood flow stopped.

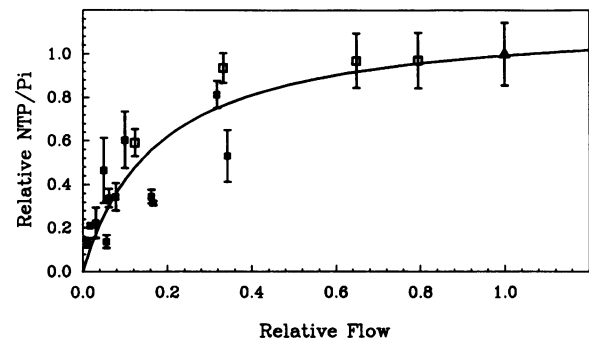


FIG. 3. Response of tumor energy status to acute changes in flow. Δ , The NTP/ P_i ratio vs. flow is given relative to its value during the first 60 min of ^{31}P monitoring; \square , points obtained just before the first pressure decrement (from 100 mmHg to 50 mmHg); \blacksquare , points obtained at the progressively decreased perfusion pressures. Error bars represent SD.

(Fig. 3), the relationship between NTP/ P_i and flow has an apparent K_m at roughly 10% of the initial flow. The absolute perfusate flow rate to which this relative flow corresponds averaged 0.05 ml per min per g. The changes in NTP/ P_i were paralleled by decreases in the pH_{NMR} by as much as 0.6 pH unit (data not shown).

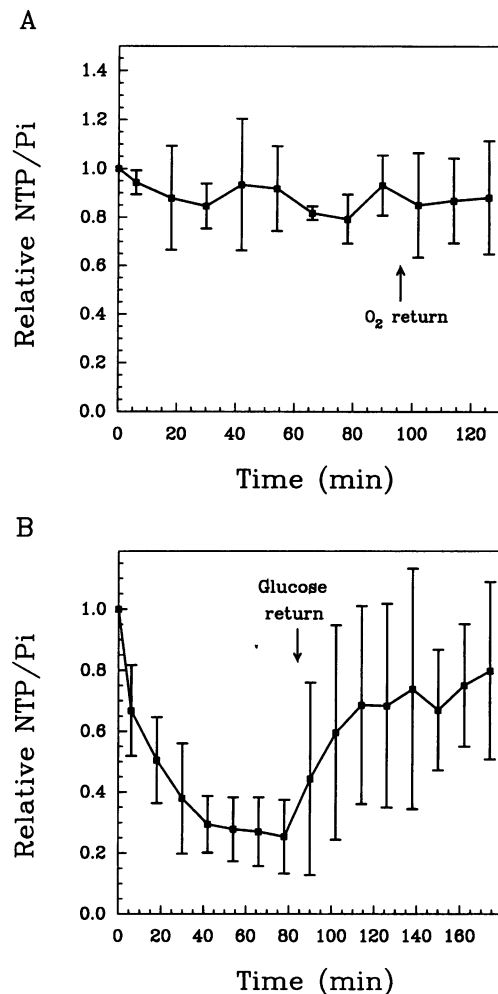


FIG. 4. Average response of NTP/ P_i ratio to acute hypoxia (A) and acute glucose deprivation (B). At 0 min in A, the gas in the gas exchanger was switched to 95% $\text{N}_2/5\%$ CO_2 . At 0 min in B, the glucose content of the medium was decreased to zero. The arrows mark the return of the 95% $\text{O}_2/5\%$ CO_2 or glucose, respectively. Error bars represent SD.

Table 1. Effect of hypoxia on tumor NTP/P_i, pH_{NMR}, and lactate production

Experiment	NTP/P _i		pH _{NMR}		Lactate production, μmol per min per g	
	+ O ₂	- O ₂	+ O ₂	- O ₂	+ O ₂	- O ₂
A	1.05 ± 0.16	1.06 ± 0.14	7.12 ± 0.20	7.14 ± 0.13	0.63 ± 0.26	0.65 ± 0.18
B	0.32 ± 0.04	0.26 ± 0.05	6.66 ± 0.09	6.68 ± 0.07	1.15 ± 0.26	1.84 ± 0.42*
C	0.63 ± 0.16	0.53 ± 0.09	6.91 ± 0.19	6.74 ± 0.07*	0.44 ± 0.26	0.30 ± 0.11
D	1.00 ± 0.13	0.94 ± 0.20	6.98 ± 0.23	6.94 ± 0.08	0.36 ± 0.11	0.38 ± 0.11

NTP/P_i, pH_{NMR}, and lactate production values labeled + O₂ are determined from the averages of measurements made both before hypoxia and after O₂ was returned; values labeled - O₂ are determined from the average of measurements made in 12–90 min after the onset of hypoxia. All values are given as mean ± SD. Each experiment represents a single tumor. *Values significantly changed ($P < 0.05$ by paired two-tailed t test) by hypoxia.

Despite the marked response of both the *in vivo*- and the *ex vivo*-perfused R3230AC mammary adenocarcinoma to ischemia, hypoxia alone had little effect on the energy status, the pH_{NMR}, or the lactate release of the perfused tumors (Fig. 4A and Table 1). The decrease in NTP/P_i was 10 ± 9% (mean ± SD). The return of O₂ to the perfusate resulted in no further change in any of these measured parameters. In contrast to the effect of hypoxia, the tumor NTP/P_i ratio dropped markedly during glucose deprivation (Fig. 4B and Table 2). The NTP/P_i ratio of the perfused tumors fell to a steady-state level 25 ± 10% of its baseline level with a half-life of 13 ± 5 min. This apparently stable residual NTP may reflect the ability of the neoplastic cells to utilize glycogen or to catabolize endogenous substrates (e.g., fatty acids) via oxidative phosphorylation. Upon the return of the glucose-containing medium, NTP/P_i slowly rose and, after 60 min, had reached 76 ± 23% of the baseline value. Switching the carbon substrate from glucose to glutamine or glutamine plus pyruvate in the presence of O₂ also resulted in a rapid loss of the high-energy phosphates. The rate of the NTP/P_i drop was similar to that observed for glucose deprivation (Table 2). On average, the steady-state NTP/P_i ratio was 15 ± 11% of the baseline value, not significantly different from that observed for the carbon-free medium.

The above results demonstrate a variety of the experimental manipulations allowed by the *ex vivo* tumor preparation. The direct manipulation of flow over a wide range shows the relationship between flow and energy status under the perfusion conditions used. It is important to note that the measurements are made during a gradually increasing vascular resistance, which by itself, however, had little effect on NTP/P_i in controls. Of course, this experiment addresses directly only the effect of acute changes in flow. The effect of chronic changes may be substantially different. However, the relationship between flow and NTP/P_i measured here strongly supports the conclusion of a number of studies that the response of tumor NTP/P_i to various manipulations results chiefly from the observed changes in the blood flow (7–12).

Changes in either O₂ or glucose delivery have the potential to be mechanisms by which changes in flow affect tumor

energy status. O₂ limitations are well-documented *in vivo* (6, 17, 30, 31), and there is much indirect evidence that they are related to tumor energy status (7–12). However, the results of studies that monitor directly the effect of acute hypoxia on neoplastic cells or tissues are complex. When cultured FSaII and MCalV cells are made acutely hypoxic, the energy charge of the former drops markedly over 2 h whereas that of the latter decreases significantly only after 4–6 h (32). When hybridoma cells grown at high density in hollow fiber reactors are acutely exposed to decreased O₂ concentrations, the relative levels of NTP drop markedly over 10 min (33). When tumor spheroids of EMT6 mouse mammary carcinoma are exposed to acute hypoxia, the energy status is markedly reduced and pH_{NMR} is decreased though the NTP/P_i ratio of the spheroids returns to baseline levels after 20 h despite continued hypoxia (34). When mice bearing subcutaneous FSaII fibrosarcoma or MCalV mammary carcinoma tumors are given 100% or 10% O₂ in their inspired gases, the NTP/P_i ratios rapidly show mild-to-moderate increases or decreases, respectively (13). Glucose (or another carbon source) is necessary for energy production and limitations to its delivery *in vivo* are also well-documented (30, 31, 35). Glucose deprivation results in rapid loss of high-energy phosphates (14, 36).

The results of the current experiments show that the removal of oxygen caused only a small drop in NTP levels in the tissue-isolated R3230AC mammary tumors. The insensitivity of tumor energy status to hypoxia is first seen in the transition from *in vivo* to *ex vivo* conditions and again in the further removal of O₂ from the perfusion medium. The cell-free perfusate, brought into equilibrium with 95% O₂/5% CO₂, contains only 10% of the O₂ of whole blood; the removal of erythrocytes also decreases the resistance to flow through the perfused tumors by roughly a factor of 2 (19). Thus, O₂ delivery to the *ex vivo* tumor is decreased by 80%. Despite the markedly decreased O₂ delivery and consumption imposed *ex vivo*, the NTP/P_i ratio of the *ex vivo* perfused tumors is comparable to their *in vivo* levels. Further reduction of O₂ delivery to 1% of the baseline *ex vivo* consumption had little effect on the NTP/P_i ratio. This result is at odds with the apparent contribution of oxidative phosphorylation calcu-

Table 2. Effect of glucose deprivation and glutamine perfusion on tumor NTP/P_i and pH_{NMR}

Experiment	NTP/P _i			pH _{NMR}		
	+ glucose	- glucose	+ glutamine	+ glucose	- glucose	+ glutamine
E	1.55 ± 0.28	0.30 ± 0.06*		6.89 ± 0.09	7.09 ± 0.00*	
F	0.61 ± 0.07	0.11 ± 0.02*		6.79 ± 0.05	7.09 ± 0.01*	
G	0.36 ± 0.05	0.14 ± 0.01*		6.81 ± 0.07	7.09 ± 0.01*	
H	1.15 ± 0.20	0.27 ± 0.01*		7.14 ± 0.06	7.14 ± 0.06	
I	0.73 ± 0.05		0.08 ± 0.03*	6.89 ± 0.11		6.91 ± 0.14
J	1.53 ± 0.30		0.42 ± 0.03*	7.22 ± 0.18		7.14 ± 0.25
K	0.48 ± 0.04		0.03 ± 0.02*	6.87 ± 0.08		7.09 ± 0.28

See Table 1 for explanation.

*Values significantly changed ($P < 0.05$ by paired two-tailed t test) by glucose deprivation and glutamine perfusion.

lated from oxygen consumption and lactate production rates. When measured in independent experiments, the O₂ consumption of the tissue-isolated R3230AC mammary tumors was 46 $\mu\text{mol per h per g}$ *in vivo* (R.K.J. and B. Bartel, unpublished results) and 13 $\mu\text{mol per h per g}$ *ex vivo*. The *ex vivo* glucose consumption was 41 $\mu\text{mol per h per g}$ (27) though only 50% of the glucose consumed by these tumors is accounted for by lactate production, similar to other tumors *in vivo* (37). If two ATPs are produced per glucose utilized and six ATPs are produced per O₂ consumed, this would mean that 50% of the ATP is generated through oxidative phosphorylation.

Many phenomena might explain the observed insensitivity of these tumors' energy status to hypoxia. These include the inhibition of O₂ consumption from the elevated rates of glucose delivery *ex vivo* (secondary to increased flow rates with the acellular medium) via a Crabtree effect, the diffusion of O₂ across the exposed tumor surface, a parallel reduction in both ATP consumption and production, or a rapid switch from one energy-producing pathway to another during the transition to *ex vivo* perfusion. It also is possible that oxygen consumption does not reflect ATP generation through oxidative phosphorylation. There may be significant mitochondrial-uncoupling or other oxygen-utilizing processes. The inability of the tumors to use glutamine or pyruvate to maintain relative NTP levels must be interpreted with caution. The decreased O₂ delivery with the cell-free perfusate makes it uncertain whether these results can be applied to *in vivo* metabolism; i.e., it is possible that greater O₂ delivery might enable the cells to use such substrates as glutamine to maintain energy status.

In conclusion, the energy status of the isolated *ex vivo*-perfused mammary adenocarcinoma is similar to that *in vivo*. The perfused tumor is dependent on glucose for maintenance of NTP levels. Severe hypoxia has little effect on tumor NTP levels. Thus, the effects of changes in blood flow on energy status are mediated largely through glucose delivery in this tumor. It is possible that this behavior represents a difference in the metabolic properties of this mammary adenocarcinoma *in vitro* and *in vivo*. Of course, many questions remain unanswered. There is apparent consumption of O₂ when it is available, despite the insensitivity of relative NTP levels to hypoxia. Roughly half of the glucose consumed is not converted to lactate. The ability of the tumor to utilize such substrates as glutamine and pyruvate with higher rates of O₂ delivery is not known. Further experiments using ¹³C-labeled substrates and perfusates with higher O₂-carrying capacity may prove useful in addressing such questions. Finally, with the availability of a similar *ex vivo* preparation for human tumors (38), it should now be possible to discern the role of glycolysis vs. respiration in human tumors.

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