# Conversion of Hyperpolarized [1-$^{13}$C]Pyruvate in Breast Cancer Cells Depends on their Malignancy, Metabolic Program and Nutrient Microenvironment

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## Supplementary Material

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**Table S1**: **Intracellular concentrations of lactate and pyruvate in MCF-7 and MDA-MB231 cells**.*

**A. MCF-7**

| **combinations** | | ratio | | | | calculated |
|---|---|---|---|---|---|---|
| glucose mM | glutamine mM | **lactate mM** | *dev.* | **pyr/lac** | **lac/pyr** | **pyruvate µM** |
| 1 | 0.1 | **1.50** | *0.69* | 0.021 | 46.5 | 32.2 |
| 2.5 | 0.1 | **2.90** | *1.25* | 0.015 | 65.8 | 44.0 |
| 1 | 1 | **1.33** | *0.69* | 0.015 | 68.3 | 19.4 |
| 2.5 | 1 | **1.24** | *0.58* | 0.023 | 43.3 | 28.7 |
| 25 | 4 | **9.92** | *7.13* | 0.020 | 48.8 | **203.2** |

**B. MDA-231**

| **combinations** | | ratio | | | | calculated |
|---|---|---|---|---|---|---|
| glucose mM | glutamine mM | **lactate mM** | *dev.* | **pyr/lac** | **lac/pyr** | **pyruvate µM** |
| 1 | 0.1 | 8.36 | 0.93 | 0.0043 | 234.0 | 35.7 |
| 2.5 | 0.1 | 13.06 | 1.07 | 0.0056 | 179.6 | 72.7 |
| 1 | 1 | 5.67 | 0.8 | 0.0045 | 222.1 | 25.5 |
| 2.5 | 1 | 4.29 | 0.46 | 0.0071 | 140.9 | 30.5 |
| 25 | 4 | 12.46 | 1.72 | 0.0027 | 376.3 | 33.1 |

* Lactate levels per sample were determined using the LDH-based assay (Material and Methods). These were used for calculating intracellular concentrations based on a cellular volume of 2 pL /cell and the cell number in the sample [$lac_{LDH}$]. For determining the pyruvate concentrations, the ratio of the relative levels of pyruvate/lactate, as determined by GC-MS relative to norvaline, were multiplied with the respective $lac_{LDH}$ concentrations.

**Table S2**: **Calculations of effective LDH activities ($LDH_{eff}$)***

**A. MCF-7 cells**

| **culture conditions** | | **NAD+** (n=2) | | **NADH** (n=2) | | **lactate** (n=3) | | **pyruvate** (Table S1) | **LDH activity** ($V_{max}$) | ***effective* LDH activity** ($LDH_{eff}$ ) |
|---|---|---|---|---|---|---|---|---|---|---|
| glucose mM | glutamine mM | **µM** | *dev.* | **µM** | *dev.* | **mM** | *dev.* | **µM** | µmol/min/ 10e6 cells* | µmol/min/ 10e6 cells* |
| 1 | 0.1 | **607** | *73* | **95** | *29* | **1.50** | *0.69* | **32.2** | 0.175 | **0.052** |
| 2.5 | 0.1 | **771** | *142* | **143** | *30* | **2.90** | *1.25* | **44.0** | 0.167 | **0.054** |
| 1 | 1 | **596** | *73* | **105** | *15* | **1.33** | *0.69* | **19.4** | 0.135 | **0.030** |
| 2.5 | 1 | **624** | *13* | **94** | *8* | **1.24** | *0.58* | **28.7** | 0.144 | **0.052** |
| 25 | 4 | 650** | *71* | *110*** | *17* | **9.92** | *7.13* | **203.2** | **0.199** | **0.114** |

B. **MDA-MB231 cells**

| **culture conditions** | | **NAD+** (n=2) | | **NADH** (n=2) | | **lactate** (n=3) | | **pyruvate** (Table S1) | **LDH activity** ($V_{max}$) | ***effective* LDH activity** ($LDH_{eff}$ ) |
|---|---|---|---|---|---|---|---|---|---|---|
| glucose mM | glutamine mM | **µM** | *dev.* | **µM** | *dev.* | **mM** | *dev.* | **µM** | µmol/min/ 10e6 cells* | µmol/min/ 10e6 cells* |
| 1 | 0.1 | **452** | *164* | **2.1** | *1* | **8.36** | 0.93 | **32.1** | 0.187 | **0.043** |
| 2.5 | 0.1 | **601** | *68* | **97.2** | *32* | **13.06** | 1.07 | **65.7** | 0.119 | **0.045** |
| 1 | 1 | **519** | *22* | **23.8** | *7* | **5.67** | 0.80 | **21.2** | 0.197 | **0.035** |
| 2.5 | 1 | **625** | *78* | **65.4** | *5* | **4.29** | 0.46 | **27.0** | *nd* | *nd* |
| 25 | 4 | **549**** | *42* | ***81**** | *16* | **12.46** | 1.72 | **26.6** | 0.155 | **0.034** |

*Values of intracellular substrate concentrations and potential LDH activities ($V_{max}$) were used to calculate the effective LDH activities ($LDH_{eff}$) of cells from MRS measurements. For simplification and calculations using the Michaelis-Mention equation, it was assumed that the $K_M$ for pyruvate is 100 µM (see Results, Fig. 3 (Goto et al., 2016) and that NADH was already enzyme-bound, pyruvate thus being the rate-limiting component.

** Values of $NAD^+$ and NADH averaged from respective values which are apparently similar in the different glucose/glutamine conditions

Reference for Table 2:

Goto, T., Sugawara, K., Nakamura, S., Kidokoro, S. I., Wakui, H., & Nunomura, W. (2016). Enzymatic and thermodynamic profiles of a heterotetramer lactate dehydrogenase isozyme in swine. Biochemical and Biophysical Research Communications, 479(4), 860-867. doi:10.1016/j.bbrc.2016.09.118

MCF-7 MDA-MB231

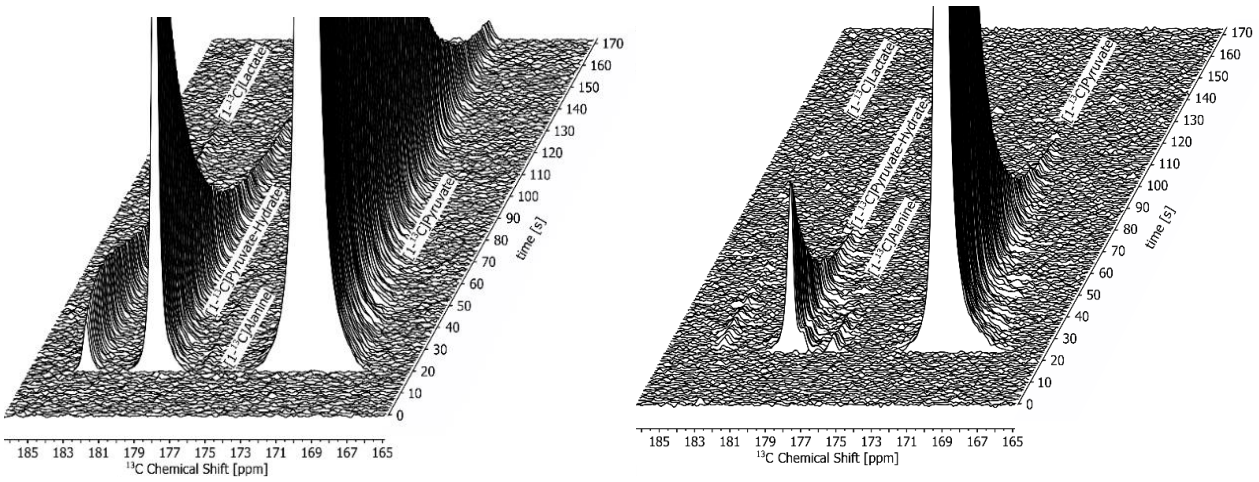


**Figure S1:** Example of water fall plots of dynamic hyperpolarized $^{13}$C-MRS spectra of a 2 min-acquisition upon injection of 1 mM [1-$^{13}$C] into MCF-7 and MDA-MB231 cell suspensions. Cells had been incubated 72 h in medium containing 2.5 mM glucose and 0.1 mM glutamine.

MCF-7

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| glucose mM | 1 | 1 | 2.5 | 2.5 | 1 | 1 | 2.5 | 2.5 | |
| glutamine mM | 0.1 | 0.1 | 0.1 | 0.1 | 1 | 1 | 1 | 1 | |
| marker | | | | | | | | | marker |


MDA-MB231

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| glucose mM | 1 | 1 | 2.5 | 2.5 | 1 | 1 | 25 | 25 | |
| glutamine mM | 0.1 | 0.1 | 0.1 | 0.1 | 1 | 1 | 4 | 4 | |
| marker | | | | | | | | | marker |

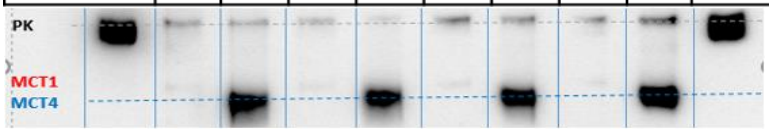


**Figure S2.** Western blots of protein expression of MCT1 in MCF-7 and MCT4 in MDA-MB231, relative to pyruvate kinase (as marker) in variable glucose / glutamine conditions.
Shown are representative blots of 2-3 similar experiments for each cell line. The unusual high PK signal in MCF-7 cells may be a technical deviation, as it is not observed in other blots for the same glucose/glutamine conditions.

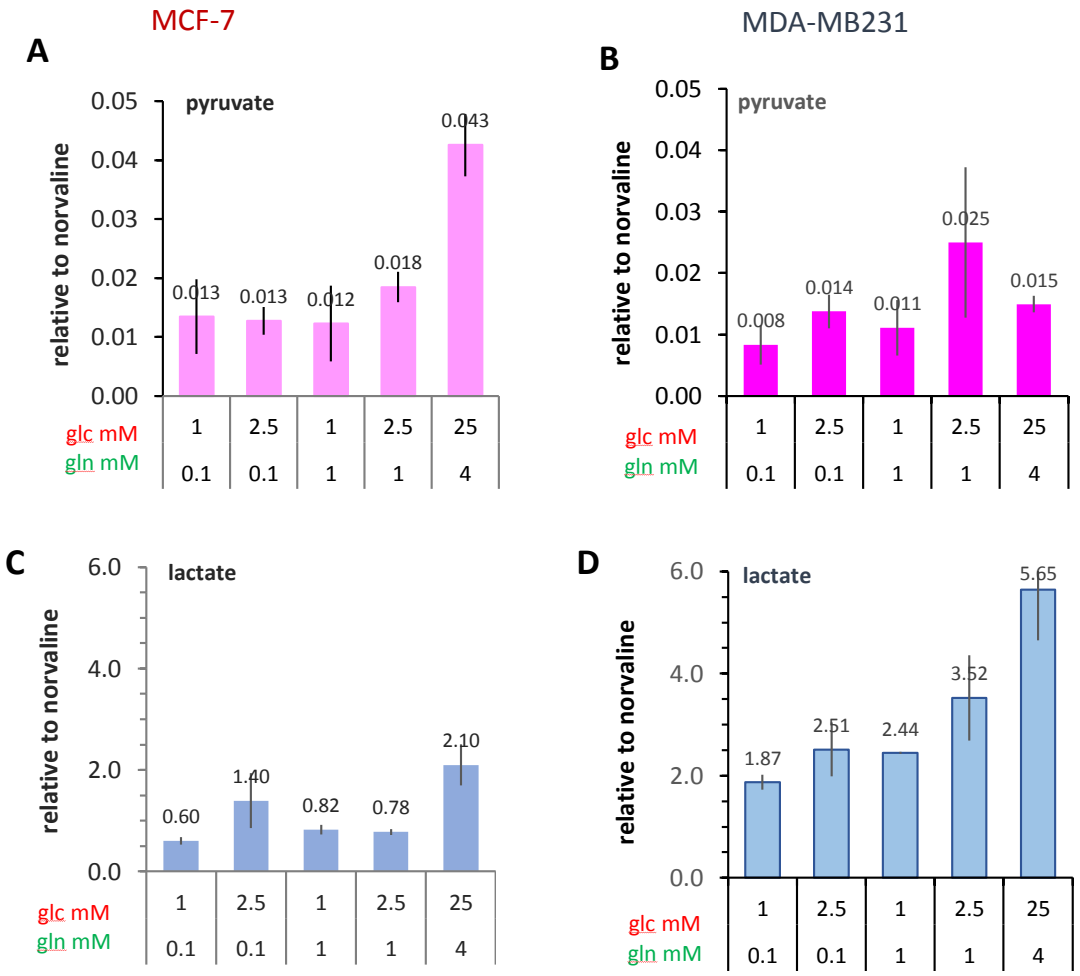


**Figure S3.** Intracellular pyruvate and lactate levels in MCF-7 and MDA-MB231 cells in different glucose/ glutamine conditions as determined by GC-MS.

Cells were incubated for 2 h with medium containing the indicated [U-$^{13}C_6$]glucose and glutamine concentrations and prepared for metabolite analyses by GC-MS (Material and Methods). Following chromatographic separation, metabolites were quantified relative to the GC-reference compound norvaline. Shown are averages and s.e.m. of two experiments for (A, C) MCF-7 and (B, D) MDA-MB231-MB231