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# Influence of free fatty acids on glucose uptake in prostate cancer cells ☆

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ABSTRACT

*Introduction:* The study focuses on the interaction between glucose and free fatty acids (FFA) in malignant human prostate cancer cell lines by an *in vitro* observation of uptake of fluoro-2-deoxy-D-glucose (FDG) and acetate.

*Methods:* Human prostate cancer cell lines (PC3, CWR22Rv1, LNCaP, and DU145) were incubated for 2 h and 24 h in glucose-containing (5.5 mM) Dulbecco's Modified Eagle's Medium (DMEM) with varying concentrations of the free fatty acid palmitate (0–1.0 mM). Then the cells were incubated with [$^{18}$ F]-FDG (1 μCi/mL; 0.037 MBq/mL) in DMEM either in presence or absence of glucose and in presence of varying concentrations of palmitate for 1 h. Standardized procedures regarding cell counting and measuring for $^{18}$ F radioactivity were applied. Cell uptake studies with $^{14}$C-1-acetate under the same conditions were performed on PC3 cells.

*Results:* In glucose containing media there was significantly increased FDG uptake after 24 h incubation in all cell lines, except DU145, when upper physiological levels of palmitate were added. A 4-fold increase of FDG uptake in PC3 cells (15.11% vs. 3.94%/$10^6$ cells) was observed in media with 1.0 mM palmitate compared to media with no palmitate. The same tendency was observed in PC3 and CWR22Rv1 cells after 2 h incubation. In glucose-free media no significant differences in FDG uptake after 24 h incubation were observed. The significant differences after 2 h incubation all pointed in the direction of increased FDG uptake when palmitate was added. Acetate uptake in PC3 cells was significantly lower when palmitate was added in glucose-free DMEM. No clear tendency when comparing FDG or acetate uptake in the same media at different time points of incubation was observed.

*Conclusions:* Our results indicate a FFA dependent metabolic boost/switch of glucose uptake in PCa, with patterns reflecting the true heterogeneity of the disease.



## 1. Introduction

Prostate cancer (PCa) remains a major health concern being the most common malignancy among men in the United States, accounting for approximately one third of all cancer diagnoses [1]. For most tumors glucose appears to be the most important energy substrate with metabolic changes during malignant transformation being well-described, as both the Warburg [2] and the Crabtree [3] effect suggest that an altered metabolic control and/or enzymes play a role in tumor glucose metabolism. However, contrary most malignancies, a fraction of PCa are characterized by a relatively low rate of glycolysis and thus low glucose uptake. This reflects the fact that PCa vary widely in their rate of growth, aggressiveness, and tendency to metastasize. The biology of this disease evolves from a small, slow-growing, androgen-dependent 'indolent' carcinoma toward a more and more aggressive, androgen-independent tumor during the course of progression [4,5]. With this in mind, focus has switched to fatty acids (free fatty acids and endogenous lipid esters from both tumor and normal tissues) as the potentially dominant bioenergetic source in the malignant prostate, as well as to the interaction between the different energy sources available [6–8].

The interaction between glucose and free fatty acid (FFA) metabolism was first demonstrated in a perfused rat heart [9] and has thereafter been well-established in normal tissues and healthy subjects [10–13]. The picture is different in cancer patients who show tumor-associated changes in host metabolism, PCa patients included, and a relatively scarce amount of data exist. However, the possibility of a metabolic switch in terms of the preferred energy source has been proposed, as a reversed glucose and fatty acid transporter expression in the cell membrane has been demonstrated in human endometrial cancer [14]. This protein-mediated transport probably plays an essential role in facilitating glucose and FFA movement across the plasma membrane into the cells. Additionally, the phosphatidylinositol 3-kinase (PI3K)/Akt signal transduction pathway has been of

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main attention due to its supposed essential role in the regulation of glycolysis through controlling the expression of glucose transporters at the cell surface as well as the activity of the enzymes which catalyzes phosphorylation of glucose [15–18], with the FFA palmitate stimulating Akt phosphorylation in a time- and dose-dependent manner in rat muscle cells [19].

Even though no such data exist regarding PCa, our study group recently demonstrated both an incremental uptake of the glucose analog fluoro-2-deoxy-D-glucose (FDG) in androgen-independent PCa xenografts (PC3) implemented in mice, as well as higher tumor-to-background ratios after acute administration of the antilipolytic drug Acipimox, and thereby a lowering of the amount of FFA available in the bloodstream [20]. No such effect was seen in a more androgen-responsive xenograft (CWR22R*v*1). Our results suggested a strong interaction between the amounts of energy sources available and that a metabolic switch or boost in specific prostate cancer xenografts (PC3) could be induced by antilipolysis and/or reduced fatty acid oxidation. The present study focuses and adds knowledge on the interaction between glucose and FFA in different malignant human PCa cell lines by an *in vitro* observation of uptake of FDG and acetate. It embraces different concentrations (within the physiological range) of energy sources available in the cell media as well as the time course of uptake. We hypothesize the possibility of demonstrating a boost or switch in metabolism in malignant prostate cells.

## 2. Methods

### 2.1. Cell lines

The CWR22R*v*1 cell line is originally derived from a human primary prostate tumor and expresses androgen receptors. Its growth is androgen responsive but with elements of androgen insensitivity. The LNCaP cell line is androgen sensitive and originates from a lymph node metastasis of PCa. DU145 cells are androgen independent and are derived from a brain metastasis. The PC3 cell line is established from bone metastasis of PCa. It is androgen independent and defined to be androgen receptor negative.

### 2.2. FFA/BSA complex solution preparation

A palmitate (sodium salt) stock solution was prepared by dissolving and heating (90 °C) to equal molar amounts of NaOH and fatty acids to a concentration of 100 mM. A 5% (wt/vol) fatty acid free BSA solution was prepared in deionized water. A 10 mM FFA/5% BSA stock solution was made by adding the palmitate stock solution dropwise to the 5% BSA solution at 55 °C in a water bath, then vortex mixed for 10 sec, followed by further 10 min incubation at 55 °C. The FFA/BSA complex solution was cooled to room temperature and sterile filtered (0.45 μm pore size membrane filter). Each FFA/BSA complex solution was freshly prepared before each experiment.

### 2.3. Acipimox

The antilipolytic drug Acipimox is a long-acting ($t_{½plasma}$ ~ 2 h) nicotinic acid derivative, which through its actions is used to inhibit the release of non-esterified fatty acids from adipose tissue. Consequently, Acipimox enhances glucose and decreases lipid oxidation *in vivo*. Acipimox also decreases glucose utilization for gluconeogenesis [21]. As there are no adipocytes in the cell media applied in this study (as described below), the effect of Acipimox *in vivo* cannot be transferred to *in vitro* conditions. In this study the addition of Acipimox in the cell media was used as a control to baseline conditions, as there theoretically should be no differences in PCa cell uptake of neither FDG or acetate in media with or without Acipimox. According to reports by Carballo-Jane et al. [22], the concentration of Acipimox used in the cell media was set to 0.1 mM.

### 2.4. [$^{18}$ F]-FDG cell uptake study

Human prostate cancer cell lines – PC3, CWR22R*v*1, LNCaP, and DU145 – were obtained from the American Type Culture Collection and cultured under conditions provided by the supplier. Cells from each tumor cell line were seeded in eight 6-well cell culture plates each. Media was aspirated and dispatched at a cell confluency of approximately 70–80%, followed by addition and subsequent aspiration of 1x phosphate buffered saline (PBS). The tumor cells then underwent incubation/equilibration at 37 °C for 2 h (4 plates for each cell line) and 24 h (4 plates for each cell line) in regular glucose-containing (5.5 mM) Dulbecco's Modified Eagle's Medium (DMEM), glucose-containing DMEM with 0.1 mM Acipimox, glucose-containing DMEM with 0.5 mM palmitate, and glucose-containing DMEM with 1.0 mM palmitate, respectively. After incubation/equilibration media was aspirated, followed by addition and subsequent aspiration of 1x PBS. Then 1 mL of both no-glucose- and glucose-containing DMEM (regular, with 0.1 mM Acipimox, 0.5 mM palmitate, and 1.0 mM palmitate) with fluorine-18 ($^{18}$ F) labeled FDG (1 μCi/mL; 0.037 MBq/mL) were added to 3 wells each and incubated at 37 °C for 1 h. 1 mL standards of all the described media containing $^{18}$ F-FDG were collected. Media and subsequent addition of 1× PBS was collected before adding 0.5 mL trypsin to the wells. After 8 min of trypsinisation 0.5 mL of media (no-glucose- and glucose-containing DMEM – regular, with 0.1 mM Acipimox, 0.5 mM palmitate, and 1.0 mM palmitate) was added to the same 3 wells, respectively, and the cells were harvested. 200 μL of the cell suspension was used for cell counting (5× dilution - Vi-Cell Automated Cell Viability Analyzer; Beckman Coulter, Inc.). The rest of the cell suspensions, as well as the media suspensions and standards were measured for $^{18}$ F radioactivity in a calibrated γ-counter (2480 Wizard$^2$ Automatic Gamma Counter; PerkinElmer, Inc.) using a dynamic counting window (peak 511 keV; threshold 20%) and decay correction.

### 2.5. $^{14}$C-1-acetate Cell Uptake Study

Supplemental cell uptake studies of carbon-14-1-acetate ($^{14}$C-acetate) were performed in PC3 cells only. The procedure was the exact same as described above with the following exceptions: Media contained $^{14}$C-acetate (0.1 μCi/mL; 0.0037 MBq/mL) instead of $^{18}$ F-FDG, and cell counting was performed using the INCYTO C-Chip Neubauer Improved DHC-N01-5 haemocytometer (Fisher Scientific, Inc.). All samples were solubilized (Soluene-350; Packard Instrument Co., Inc.) and a scintillant agent (Bioscint; National Diagnostics, Atlanta, GA, USA) was added. $^{14}$C radioactivity was determined by liquid scintillation counting (Tri-Carb 2910TR Liquid Scintillation Analyzer; PerkinElmer, Inc.) using external standard quench corrections.

### 2.6. Statistics

The Student *t*-test was made using IBM® SPSS® Statistics 19.0 (SPSS Inc., IBM, Somers, NY, USA). All *P*-values were calculated as two-sided and differences were considered significant at $P < 0.05$.

## 3. Results

### 3.1. [$^{18}$ F]-FDG cell uptake study

When comparing glucose containing media with different concentrations of palmitate (Table 1), there was a significant tendency of increased FDG uptake after 24 h incubation in all cell lines, with the exception of DU145, when upper physiological levels of palmitate (1.0 mM) was added in media. An almost 4-fold increment of FDG uptake in PC3 cells (15.11% vs. 3.94%/$10^6$ cells) was observed in media with 1.0 mM palmitate compared to media with no palmitate added.