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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, CWR22R*v*1, 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 CWR22R*v*1 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.

☆Conflict of interest. The authors declare that they have no conflict of interest.

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Keywords

Cell uptake; Free fatty acid; Glucose; Metabolism; Prostate cancer

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 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 1× 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 1× 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. The same tendency was observed in PC3 and CWR22R*v*1 cells after 2 h incubation. When looking at glucose free media (Table 1), no significant differences in FDG

uptake after 24 h incubation were observed. The FDG uptake data after 2 h incubation pointed in the direction of increased utilization of FDG when palmitate was added in media. Even though some significant differences were observed, there was no clear tendency when comparing FDG cell uptake in the same media at different time points of incubation (2 h and 24 h; Table 1). Addition of Acipimox in the media had no influence on FDG uptake (Table 1).

3.2. ^{14}C-1-acetate cell uptake study

Acetate uptake in PC3 cells was significantly lower when palmitate was added in DMEM without glucose both after 2 h and 24 h incubation (Table 2). No significant differences in acetate uptake were observed in glucose containing DMEM. Addition of Acipimox in the media did not influence on acetate uptake. Additionally, there were no significant differences in acetate uptake when comparing PC3 cells in the same media at different time points of incubation (2 h and 24 h).

4. Discussion

The present study adds knowledge to the interaction between glucose and FFA in different stages of PCa, as the cell lines examined are derived from a primary tumor (CWR22R*v*1), a malignant lymph node (LNCaP), as well as a bone (PC3) and brain metastasis (DU145), embracing different levels of androgen-dependency and aggressiveness. The study design also embraces available energy sources in terms of different concentrations of glucose and palmitate.

Our laboratory study finding of a metabolic boost/switch of glucose uptake in the majority of PCa cell lines (CWR22R*v*1, LNCaP, and PC3) examined when cell media contained upper normal physiological concentrations of the FFA palmitate has by our knowledge not been reported previously. This effect was more noticeable with increasing concentrations of FFA in cell media as well with a longer period of incubation. We believe that the observed effect, at least partly, could be related to alterations in the PI3K/Akt signal transduction pathway, as it is thought to be pivotal in the regulation of glycolysis by: 1) controlling glucose entry into the cell via the expression of glucose transporters (GLUT1 and GLUT4) at the cell surface and 2) regulating the activity of hexokinases, which catalyzes the phosphorylation of glucose [15–18]. A recent report by Pu and coworkers [19] demonstrated that rat skeletal muscle cells acutely exposed to palmitate stimulated GLUT4 translocation to the plasma membrane as well as basal glucose uptake in a time-dependent manner. The increased glucose uptake correlated with the localization of GLUT4 on the plasma membrane. Palmitate stimulated Akt phosphorylation in a time- and dose-dependent manner, and by inhibiting PI3K – which is upstream of Akt in the signal transduction pathway – glucose uptake was abolished by a blocking of Akt phosphorylation. Summarized, these results demonstrate the important role of this signaling pathway in the cell line examined, and similar results have been demonstrated in adipocytes [23]. It is tempting to suggest a similar effect of palmitate on the PI3K/Akt pathway in prostate cancer cells to explain the observations made in our study. However, further and more specifically designed studies are needed to potentially verify this.

One of the main challenges in administration of PCa is the heterogeneity of the disease, reflected by the fact that PCa vary widely in their rate of growth, aggressiveness, and tendency to metastasize. Our results underline this statement, as the most pronounced boost of glucose uptake was observed in the androgen-independent PC3 cell line, which is highly tumorigenic, while less distinct changes were seen in more androgen-dependent cell lines (LNCaP and CWR22R*v*1). Glucose uptake in the brain metastasis derived DU145 cell line was unaffected by different palmitate concentrations in cell media. One of the possible explanations to this – even though not widely accepted – could be that transport of energy sources across barriers in brain tissue and subsequently the preferred energy source for further metabolism exerts different characteristics compared to extra-cerebral locations [24,25].

No consistent significant differences in both FDG and acetate uptake at different time points of incubation (2 h and 24 h) indicate that saturation/equilibrium was achieved within 2 h in all examined cell lines. This supplements the results by Liu et al. [7], which reported that uptake of both glucose and palmitate increased in the malignant prostate cell lines PC3 and LNCaP between measurements 0.5 h, 1 h, and 2 h in a time dependent, dynamic manner. However, when looking closer at that study, statistically significant differences could only be demonstrated between 0.5 h and 1 h, and even though there was a tendency of increased uptake at 2 h compared to 1 h, the differences in uptake did not turn out to be significant. As no incubation periods shorter than 2 h were examined in our study, we cannot specify at which time within the 2 h incubation period saturation/equilibrium occurred.

Vavere and coworkers demonstrated a linear correlation between uptake of ^{11}C-acetate and fatty acid synthase expression levels in human prostate cancer xenografts [26]. Thus, the observed significant fall in acetate uptake in androgen-refractory PC3 cells – and consequently a fall in fatty acid synthase expression levels – when palmitate was added to glucose-free cell media, is likely to be related to a larger part of the total amount of palmitate needed for subsequent regulatory functions in a majority of signal transduction pathways in PCa [27] being covered by exogenous palmitate. This study design did not measure uptake of FFA, thus, it cannot give any direct information regarding transport of fatty acids across the membrane for subsequent intracellular oxidation nor the binding of the fatty acid to the cell membrane.

Possible misinterpretations of the results could be related to the fact that the rate of phosphorylation in malignant cells can be altered. Kaarstad and co-workers [28] demonstrated – besides ^{18}F-FDG-6-phosphate (P) – the formation of two secondary metabolites to ^{18}F-FDG-6-P (^{18}F-FD-PG1 and ^{18}F-FDG-1,6-P_2) in malignant tumor tissue after ^{18}F-FDG administration. However, they did not identify any of the nucleotide derivatives of ^{18}F-FDG-1-P or incorporation of ^{18}F-FDG into glycogen. The fact that ^{18}F-FDG-metabolites other than ^{18}F-FDG-6-P were found in tumor tissue may have implications for the interpretation of estimated kinetic rate constants in terms of the enzymatic processes. However, the finding does not in any way alter the conventional use of ^{18}F-FDG uptake in tumor cells as an indicator of the rate of glucose use, because the trapped label in cells still reflects the ^{18}F-FDG phosphorylation rate regardless of whether the label is associated with ^{18}F-FDG-6-P or with the products of ^{18}F-FDG-6-P. It would have been a completely

different story if the discovered metabolites were products of ^{18}F-FDG directly, that is, in parallel or in competition with the phosphorylation reaction. Additionally, time course studies of percentage tissue content of FDG-6-P and its secondary metabolites in mice bearing different carcinomas were relatively consistent from approximately 30 min and thereafter, justifying the time points chosen in the our study (cell uptake determined approximately 60 min after adding ^{18}F-FDG).

In conclusion, there are still mechanisms in the glucose-FFA cycle in prostate carcinoma cells which need to be further addressed by specifically designed study assays. Our results indicate a FFA dependent metabolic boost/switch of glucose uptake in PCa, with patterns reflecting the true heterogeneity of this disease. This study also supports observations stating that saturation/equilibrium in PCa cell uptake of glucose and acetate in a fixed setting is achieved within a period of 2 h.

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References

1. U.S. National Cancer Institute. SEER database. 2012 [Ref Type: Online Source].
2. Warburg, O. Stoffweschel der Tumoren. Berlin: Verlag Springer; 1926.
3. Crabtree HG. Observations on the carbohydrate metabolism of tumours. Biochem J. 1929; 23(3): 536–545. [PubMed: 16744238]
4. Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med. 2004 Jan; 10(1):33–39. [PubMed: 14702632]
5. Craft N, Chhor C, Tran C, Belldegrun A, DeKernion J, Witte ON, et al. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res. 1999 Oct 1; 59(19):5030–5036. [PubMed: 10519419]
6. Clarke NW, Brown MD. The influence of lipid metabolism on prostate cancer development and progression: is it time for a closer look? Eur Urol. 2007 Jul; 52(1):3–4. [PubMed: 17467164]
7. Liu Y, Zuckier LS, Ghesani NV. Dominant uptake of fatty acid over glucose by prostate cells: a potential new diagnostic and therapeutic approach. Anticancer Res. 2010 Feb; 30(2):369–374. [PubMed: 20332441]
8. Mistry T, Digby JE, Desai KM, Randeva HS. Obesity and prostate cancer: a role for adipokines. Eur Urol. 2007 Jul; 52(1):46–53. [PubMed: 17399889]
9. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963; 1(7285):785–789. [PubMed: 13990765]
10. Knuuti MJ, Maki M, Yki-Jarvinen H, Voipio-Pulkki LM, Harkonen R, Haaparanta M, et al. The effect of insulin and FFA on myocardial glucose uptake. J Mol Cell Cardiol. 1995 Jul; 27(7):1359–1367. [PubMed: 7473782]
11. Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teras M, Haaparanta M, et al. Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest. 1992 Jun; 89(6): 1767–1774. [PubMed: 1601987]
12. Nuutila P, Knuuti MJ, Raitakari M, Ruotsalainen U, Teras M, Voipio-Pulkki LM, et al. Effect of antilipolysis on heart and skeletal muscle glucose uptake in overnight fasted humans. Am J Physiol. 1994 Dec; 267(6 Pt 1):E941–E946. [PubMed: 7810638]
13. Thiebaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, et al. Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism. 1982 Nov; 31(11):1128–1136. [PubMed: 6752642]

14. Knapp P, Chabowski A, Harasiuk D, Gorski J. Reversed glucose and fatty acids transporter expression in human endometrial cancer. Horm Metab Res. 2012 Jun; 44(6):436–441. [PubMed: 22351475]
15. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. Genes Dev. 2001 Jun 1; 15(11):1406–1418. [PubMed: 11390360]
16. Hill MM, Clark SF, Tucker DF, Birnbaum MJ, James DE, Macaulay SL. A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol Cell Biol. 1999 Nov; 19(11):7771–7781. [PubMed: 10523666]
17. Kan O, Baldwin SA, Whetton AD. Apoptosis is regulated by the rate of glucose transport in an interleukin 3 dependent cell line. J Exp Med. 1994 Sep 1; 180(3):917–923. [PubMed: 8064240]
18. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol. 2003 Oct; 23(20):7315–7328. [PubMed: 14517300]
19. Pu J, Peng G, Li L, Na H, Liu Y, Liu P. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. J Lipid Res. 2011 Jul; 52(7):1319–1327. [PubMed: 21518696]
20. Andersen KF, Divilov V, Koziorowski J, Pillarsetty N, Lewis JS. Antilipolytic drug boosts glucose metabolism in prostate cancer. Nucl Med Biol. 2003 May; 40(4):524–528. [PubMed: 23454248]
21. Puhakainen I, Yki-Jarvinen H. Inhibition of lipolysis decreases lipid oxidation and gluconeogenesis from lactate but not fasting hyperglycemia or total hepatic glucose production in NIDDM. Diabetes. 1993 Dec; 42(12):1694–1699. [PubMed: 8243814]
22. Carballo-Jane E, Gerckens LS, Luell S, Parlapiano AS, Wolff M, Colletti SL, et al. Comparison of rat and dog models of vasodilatation and lipolysis for the calculation of a therapeutic index for GPR109A agonists. J Pharmacol Toxicol Methods. 2007 Nov; 56(3):308–316. [PubMed: 17643322]
23. Hebbachi A, Saggerson D. Acute regulation of 5′-AMP-activated protein kinase by long-chain fatty acid, glucose and insulin in rat primary adipocytes. Biosci Rep. 2013; 33(1):71–82. [PubMed: 23095119]
24. Johnson RC, Young SK, Cotter R, Lin L, Rowe WB. Medium-chain-triglyceride lipid emulsion: metabolism and tissue distribution. Am J Clin Nutr. 1990 Sep; 52(3):502–508. [PubMed: 2118303]
25. Linscheer WG, Blum AL, Platt RR. Transfer of medium chain fatty acids from blood to spinal fluid in patients with cirrhosis. Gastroenterology. 1970 Apr; 58(4):509–515. [PubMed: 5438002]
26. Vavere AL, Kridel SJ, Wheeler FB, Lewis JS. 1-11C-acetate as a PET radiopharmaceutical for imaging fatty acid synthase expression in prostate cancer. J Nucl Med. 2008 Feb; 49(2):327–334. [PubMed: 18199615]
27. Flavin R, Zadra G, Loda M. Metabolic alterations and targeted therapies in prostate cancer. J Pathol. 2011 Jan; 223(2):283–294. [PubMed: 21125681]
28. Kaarstad K, Bender D, Bentzen L, Munk OL, Keiding S. Metabolic fate of 18 F-FDG in mice bearing either SCCVII squamous cell carcinoma or C3H mammary carcinoma. J Nucl Med. 2002 Jul; 43(7):940–947. [PubMed: 12097467]

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Table 1

FDG uptake in different prostate cancer cells (comparison of media and incubation time).

Cell line	Media	Avg. FDG-uptake/10^6 cells (%) ± SD				
		2 h incubation	P (media)	24 h incubation	*P* (media)	*P* (IT)
PC3	DMEM No Glucose	34.90 ± 1.36		32.58 ± 2.24		0.20
	DMEM No Glucose/0.1 mM Acipimox	39.03 ± 4.04	0.17	29.26 ± 2.74	0.18	0.03*
	DMEM No Glucose/0.5 mM Palmitate	45.02 ± 5.38	0.03*	36.91 ± 1.51	0.05	0.07
	DMEM No Glucose/1.0 mM Palmitate	39.32 ± 3.62	0.12	39.50 ± 5.15	0.12	0.97
PC3	DMEM Reg. Glucose	4.13 ± 0.26		3.94 ± 0.43		0.54
	DMEM Reg. Glucose/0.1 mM Acipimox	3.85 ± 0.06	0.14	4.11 ± 0.50	0.69	0.47
	DMEM Reg. Glucose/0.5 mM Palmitate	5.38 ± 0.47	0.02*	4.28 ± 0.37	0.37	0.03*
	DMEM Reg. Glucose/1.0 mM Palmitate	5.89 ± 0.78	0.02*	15.11 ± 3.36	0.03*	0.01*
LNCaP	DMEM No Glucose	7.83 ± 1.89		9.25 ± 1.69		0.46
	DMEM No Glucose/0.1 mM Acipimox	6.48 ± 0.10	0.41	8.32 ± 0.72	0.44	0.04*
	DMEM No Glucose/0.5 mM Palmitate	5.86 ± 0.13	0.21	8.23 ± 0.83	0.42	0.01*
	DMEM No Glucose/1.0 mM Palmitate	8.41 ± 0.41	0.65	9.09 ± 0.14	0.92	0.05
LNCaP	DMEM Reg. Glucose	2.55 ± 0.70		1.42 ± 0.34		0.07
	DMEM Reg. Glucose/0.1 mM Acipimox	2.36 ± 0.28	0.68	1.54 ± 0.22	0.69	0.04*
	DMEM Reg. Glucose/0.5 mM Palmitate	2.14 ± 0.29	0.40	3.15 ± 0.39	0.004*	0.02*
	DMEM Reg. Glucose/1.0 mM Palmitate	2.67 ± 0.14	0.80	3.31 ± 0.12	0.001*	0.004*
CWR22Rv1	DMEM No Glucose	7.04 ± 0.33		8.21 ± 1.01		0.13
	DMEM No Glucose/0.1 mM Acipimox	7.90 ± 0.54	0.08	6.54 ± 0.30	0.05	0.02*
	DMEM No Glucose/0.5 mM Palmitate	11.44 ± 1.57	0.01*	9.12 ± 1.92	0.51	0.26
	DMEM No Glucose/1.0 mM Palmitate	10.45 ± 0.70	0.002*	10.81 ± 0.88	0.03*	0.61
CWR22Rv1	DMEM Reg. Glucose	2.56 ± 0.01		2.25 ± 0.40		0.26
	DMEM Reg. Glucose/0.1 mM Acipimox	2.21 ± 0.87	0.68	2.68 ± 0.65	0.39	0.54
	DMEM Reg. Glucose/0.5 mM Palmitate	3.54 ± 1.39	0.34	2.96 ± 0.12	0.04*	0.51
	DMEM Reg. Glucose/1.0 mM Palmitate	3.34 ± 0.29	0.04*	3.63 ± 0.42	0.01*	0.39

Cell line	Media	Avg. FDG-uptake/10^6 cells (%) ± SD				
		2 h incubation	P (media)	24 h incubation	*P* (media)	*P* (IT)
DU145	DMEM No Glucose	45.26 ± 1.02		38.35 ± 9.50		0.28
	DMEM No Glucose/0.1 mM Acipimox	41.76 ± 10.86	0.63	45.40 ± 8.96	0.40	0.68
	DMEM No Glucose/0.5 mM Palmitate	44.61 ± 2.22	0.67	55.74 ± 5.99	0.15	0.20
	DMEM No Glucose/1.0 mM Palmitate	49.70 ± 3.81	0.12	53.63 ± 12.81	0.12	0.43
DU145	DMEM Reg. Glucose	6.48 ± 1.34		4.94 ± 0.91		0.18
	DMEM Reg. Glucose/0.1 mM Acipimox	5.61 ± 0.43	0.35	5.97 ± 0.40	0.15	0.35
	DMEM Reg. Glucose/0.5 mM Palmitate	7.53 ± 0.84	0.31	6.31 ± 0.33	0.07	0.08
	DMEM Reg. Glucose/1.0 mM Palmitate	7.40 ± 0.83	0.37	5.52 ± 0.85	0.47	0.05

Abbreviations: IT: incubation time; DMEM: Dulbecco's Modified Eagle's Medium; SD: standard deviation;

$P < 0.05$ is considered significant (marked with *).

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Table 2

Acetate uptake in PC3 cells (comparison of media).

Cell line	Media	Avg. acetate-uptake/10^5 cells (%) ± SD			
		2 h incubation	*P*	24 h incubation	*P*
PC3	DMEM No Glucose	3.99 ± 1.18		3.19 ± 0.45	
	DMEM No Glucose/0.1 mM Acipimox	3.82 ± 0.98	0.86	4.54 ± 1.42	0.19
	DMEM No Glucose/0.5 mM Palmitate	1.21 ± 0.33	0.02 *	1.15 ± 0.28	0.003 *
	DMEM No Glucose/1.0 mM Palmitate	1.15 ± 0.21	0.01 *	0.96 ± 0.07	0.001 *
PC3	DMEM Reg. Glucose	1.64 ± 0.74		3.13 ± 1.88	
	DMEM Reg. Glucose/0.1 mM Acipimox	2.32 ± 1.20	0.45	3.23 ± 1.18	0.94
	DMEM Reg. Glucose/0.5 mM Palmitate	1.07 ± 0.28	0.28	1.37 ± 0.52	0.19
	DMEM Reg. Glucose/1.0 mM Palmitate	1.26 ± 0.38	0.47	1.06 ± 0.15	0.13

Specific activity of ^{14}C-acetate: 55 mCi/mmol. 0.1 μCi of ^{14}C-acetate ($1.8 * 10^{-3}$ μmol) was added to media (± glucose) with different concentrations of palmitate.

$P < 0.05$ is considered significant and marked with *.

No significant differences between different time points of incubation (2 h vs. 24 h) were observed.

Abbreviations: DMEM: Dulbecco's Modified Eagle's Medium; SD: standard deviation.