

1 **THE DARK SIDE OF GLUCOSE TRANSPORTERS IN PROSTATE CANCER:**
2 **ARE THEY A NEW FEATURE TO CHARACTERIZE CARCINOMAS?**

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7 **SHORT TITLE: THE ROLE OF GLUT TRANSPORTERS IN PROSTATE CANCER**

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11 **KEY WORDS:** GLUT, PROSTATE CANCER, GLUCOSE METABOLISM, GLYCOLYSIS,
12 INSULIN

13 **ABBREVIATIONS:** 2DG: 2-deoxyglucose; AR: Androgen Receptor; AMPK: AMP-activated protein
14 kinase; ATM: Ataxia telangiectasia mutated; CaMKK β : Calcium/calmodulin-dependent protein kinase
15 kinase beta; CRPC: Castration Resistant Prostate Cancer; DHT: Dihydrotestosterone; FDG: Fluro-D-
16 glucose; G6PDH: Glucose-6-phosphate dehydrogenase; GLUT: Facilitative glucose transporter;
17 GSTP1: Glutathione-S-transferase pi 1; HGPIN: High-grade prostatic intraepithelial prostate; HIF:
18 Hypoxia-inducible factor; HK: Hexokinase; HMIT: H⁺/myo-inositol transporter; IGF: Insulin-like growth
19 factor; IGFR: Insulin-like Growth Factor Receptor; IR: Insulin Receptor; LKB1: Liver kinase B1;
20 OXPPOS: Oxidative phosphorylation; PCa: Prostate Cancer; PET: Positron Emission Tomography; PFK:
21 6-phosphofructo-2-kinase; PFKFB2: 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2; PGC1 α :
22 Proliferator-activated receptor gamma coactivator 1-alpha; PPP: Pentose Phosphate Pathway; PSA:
23 Prostate Specific Antigen; SGLT: Sodium/glucose transporters; SHBG: Sex-Hormone Binding
24 Globuline; SLC: Solute Carrier; SRC2: Steroid Receptor Coactivator 2; T2DM; Type 2 Diabetes
25 Mellitus; TCA: Tricarboxylic acid; TP53: Tumor Protein p53; TRAMP: Transgenic Adenocarcinoma of
26 Mouse Prostate; TXNIP: Thioredoxin-interacting protein; ZIP1: Zinc-regulated transporter/iron-regulated
27 transporter-like protein 1.

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30

ABSTRACT

31 One of the hallmarks of cancer cells is the increased ability to acquire nutrients,
32 particularly glucose and glutamine. Proliferating cells need precursors for cell growth
33 and NADPH reducing equivalents for survival. The principal responsible for glucose
34 uptake is facilitative glucose transporters (GLUTs), which usually are overexpressed in
35 cancer cells. Besides their role in glucose uptake, GLUT transporters are able to
36 transport other compounds such as dehydroascorbic acid or uric acid. They play a major
37 role in tumor progression and cellular processes such as regulated cell death. The
38 prostate gland has the particular characteristic of being more glycolytic than other non-
39 pathological tissues given an accumulation of citrate in the seminal fluid and the
40 inhibition of m-aconitase that affects to Tricarboxylic Acid Cycle. In prostate cancer
41 (PCa), androgens increase glucose uptake, upregulate GLUT transporters such as
42 GLUT1 and GLUT3 and stimulate AMPK pathway, suggesting a possible connection
43 between glycolytic and androgenic signaling. Interestingly, diabetes is not a risk factor
44 of PCa, as it is in other cancers, while insulin stimulates progression and IGF1 pathway
45 plays an important role in PCa progression. It was recently found that PCa cells
46 overexpress GLUT4 and, more importantly, that it seems to be related to the castration-
47 resistant phenotype, though little is known about its participation in tumor progression.
48 This review will focus on the role of GLUT transporters along with PCa progression,
49 and the involvement of GLUT4 on castration-resistant phenotype transition would be
50 considered.

51 **INTRODUCTION**

52 In the 20's, Otto Warburg described a phenomenon in tumors that it was called
53 the "Warburg effect". This discovery took place even before ATP was discovered or
54 glycolysis was formulated¹. It is based on the enhancement of lactate production
55 because of the increment of glycolysis independently of oxygen concentration².
56 Although Warburg related this effect with defects in mitochondria of tumor cells, later
57 studies showed that mitochondria were not altered in tumor tissues and the phenomenon
58 was related to proliferation and growth³.

59 The interest in cancer metabolism has increased during the last few years and not
60 only for its self-importance but also for its connection with other signaling pathways⁴.
61 Before being considered a hallmark of cancer in 2011 by Hanahan and Weinberg⁵,
62 Kroemer and Poysegeur suggested that all hallmarks of cancer have somehow relation
63 with metabolism⁶. Recently, six hallmarks of cancer metabolism have been proposed by
64 Pavlova and Thompson in 2016⁷. Metabolic alterations of cancer cells include an
65 increased ability to acquire nutrients, assigned preferred metabolic pathways and
66 alteration of differentiation pathways.

67 The increase of glycolysis carries a higher glucose uptake by glucose
68 transporters (GLUT)⁸. Therefore, GLUT levels are usually related to tumor progression.
69 Although most cancers drive with the traditional Warburg effect, there are differences
70 among them, including those concerning GLUT transporters. The prostate is
71 metabolically unique since the differentiated tissue is glycolytic instead of oxidative.
72 Prostate cancer (PCa) transformation involves a metabolic switch to oxidative
73 phosphorylation (OXPHOS) then, later, in the advanced castration-resistant phenotype
74 turns again to glycolytic^{9,10}.

75 **PROSTATE CANCER: AN HORMONE-SENSITIVE CANCER WITHOUT A**
76 **DIAGNOSTIC BIOMARKER**

77 PCa is the most common malignancy among men and the second leading cause
78 of cancer death¹¹. In 2012, more than 1.1 million cases were recorded (data are taken
79 from GLOBOCAN, the most recent statistics from WHO)¹². This means an 8% of total
80 new cancer cases and a 15% of all affecting in men. Its prevalence is higher in the west
81 (about 68% of new cases) perhaps due to lifestyle and environmental factors. However,
82 age and race are also well-recognized risk factors¹³. Despite high incidence, PCa is
83 usually characterized by a slow growth and unpredictable outcome. PCa is a disease
84 with a mixed origin, and the absence of a biomarker impedes to know how to anticipate
85 the outcome of the disease.

86 Still, Prostate Specific Antigen (PSA) screening is the only predictive method
87 employed in the clinic. However, its levels are dependent, among others, of obesity or
88 age, being not always reliable¹⁴. A potential alternative is Prostate Cancer Antigen 3
89 (PCA3), overexpressed in primary PCa and metastases¹⁵.

90 PCa is sensitive to hormones, mainly androgens. The active form of testosterone,
91 dihydrotestosterone (DHT), mediates androgen receptor (AR) classical activation.
92 Androgen responsive genes are involved in normal prostate architecture, homeostasis,
93 and physiology but, in PCa, androgens promote proliferation and survival of cancer
94 cells. Antihormonal therapy is, at first, a successful therapeutic approach¹⁶. However,
95 PCa frequently becomes resistant to androgen deprivation, reaching a castration-
96 resistant (CRPC) phenotype difficult to handle.

97 Contrary to other cancers, there is not a single pathway implicated in PCa
98 progression nor a clear candidate as a biomarker. The homeobox gene *HOXB13* was

99 found as a predisposition gene¹⁷. Also, seventy-seven single nucleotide polymorphisms
100 close to a noncoding region of the oncogene *c-MYC* and with the capacity to alter its
101 expression, are also considered a prognosis marker¹⁴. In the 90% of PCa, Glutathione-S-
102 transferase pi 1 gene (*GSTP1*) is hypermethylated, and it can be detected in urine¹⁸.

103 More than half of the cases of PCa drive with androgen-driven ETS gene
104 expression because of genomic rearrangements. However, ETS gene fusions need other
105 events such as activation of PI3K/AKT pathway, which is mainly related to PTEN loss.
106 Then, ETS-positive tumors are different from ETS-negative tumors, but *PTEN* and
107 *TP53* mutation occurs in both types¹⁴.

108 In CRPC, there are additional oncogenic pathways involved. The RAS/MAPK
109 pathway and TGFβ3 are upregulated in patients with metastatic CRPC. In addition to
110 AR signaling, the WNT/β-catenin and the Insulin-like growth factor (IGF) 1 pathways
111 seem to play a major role in the most aggressive phenotype of CRPC¹⁹.

112 Still, there is not a clear biomarker, as there is neither an effective treatment.
113 Anti-hormonal therapies employed when cancer is still androgen-sensitive, and they go
114 together with radiotherapy in localized or locally advanced carcinoma¹⁶. For CRPCs,
115 docetaxel has been the preferred and the first-option chemotherapy during the last
116 decade, but recent-discovered compounds have also been proposed as succesful²⁰.

117 Then, PCa is still a tumor without an effective prognostic and predictive
118 biomarker and a curative treatment. However, targeting glucose metabolism has the
119 potential to provide prognostic information and to treat PCa, since several glycolytic
120 pathways are altered in the disease.

121 **THE CURIOUS CASE OF GLYCOLYTIC METABOLISM IN**
122 **PROSTATE**

123 In differentiated cells, glucose in the presence of oxygen is predominantly
124 employed to get their energetic requirements from OXPHOS in mitochondria.
125 However, under hypoxia, lactic glycolysis is favored rendering only two ATP molecules
126 from one molecule of glucose, instead of the 36 molecules obtained by OXPHOS²¹. In
127 cancer cells, and in other proliferative cells, the rates of glycolysis and lactate
128 production are enhanced. Even in the presence of oxygen, cancer cells select for
129 glycolysis instead of OXPHOS to metabolize glucose in a process called “aerobic
130 glycolysis”³.

131 Aerobic glycolysis favors glycolytic pathways to produce the building blocks
132 necessary to cell growth. Several oncogenic signaling pathways promote aerobic
133 glycolysis and the increase of lactate secretion²².

134 PCa, like other tumors, progresses with molecular alterations that cause an
135 increase in glucose, glutamine and lipid metabolism. However, PCa is characterized by
136 a particular metabolism of glucose that differs from the rest of carcinomas.

137 The prostatic fluid contains high levels of citrate because of the inhibition of m-
138 aconitase, a tricarboxylic acid (TCA) cycle enzyme that converts citrate to isocitrate.
139 This inhibition is driven by the overexpression of the zinc-regulated transporter/iron-
140 regulated transporter-like protein 1 (ZIP1) in prostatic epithelial cells. Since the TCA
141 cycle is somehow compromised in the prostate gland, glycolysis is favored (Fig 1).
142 During tumor transformation, ZIP1 levels decrease, and OXPHOS is promoted⁹.
143 However, this may only happen during the first steps of carcinogenesis. One study
144 shows that the mitochondrial content does not change during carcinogenesis, but
145 OXPHOS decreases with invasiveness²³.

146 Lactate production is usually associated with tumor progression in PCa. In
147 addition to its catabolic products, pyruvate, and alanine, lactate measurement has been
148 considered urine biomarker for non-invasive detection of PCa²⁴. On the other hand,
149 factors related to reverse Warburg effect were recently proposed as a marker to
150 distinguish Gleason grades²⁵. PCa cells employ interleukin-6 secretion to activate
151 glycolysis in cancer associated fibroblasts, which, in turn, increase lactate secretion²⁶.
152 Lactate is consumed by OXPHOS-dependent PCa cells, having a role in redox
153 homeostasis and angiogenesis²⁷.

154 Glucose metabolism has not been considered as important as glutamine or lipid
155 metabolism in PCa progression, being the reason why it has been less studied. Several
156 candidates have been proposed as potential metabolic targets. Multiple studies are
157 underway employing inhibitors of lipogenesis, cholesterol metabolism, and glutamine
158 metabolism²⁸. Recently, the upregulation of the steroid receptor coactivator 2 (SRC2),
159 which drives glutamine-dependent de novo lipogenesis, was proposed as an important
160 co-regulator for PCa survival and metastases²⁹, and Sarcosine, an N-methyl derivative
161 of glycine, is considered an important regulator of progression and metastases³⁰.

162 However, glucose metabolism in PCa is different to other tumors given their
163 close relation to AR signaling. Glycolysis differs between androgen-sensitive and
164 insensitive cells, being tumors more aggressive more glucose-dependent^{31,32}. Also, AR
165 regulates several genes that are closely related to glucose consumption and biomass
166 production³³. Thus, an increased activity of several glycolytic enzymes by androgens
167 has been found. Hexokinase-2 (HKII) phosphorylation is stimulated by androgens via
168 protein kinase A signaling while 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase
169 2 (PFKFB2) is stimulated by direct binding of AR to *PFKFB2* promoter. Activation of
170 PFKFB2 causes a constitutive activation of 6-phosphofructo-2-kinase (PFK2), which is

171 involved in the second irreversible reaction of the glycolytic pathway³⁴. Another
172 isoform of PFK2, PFKFB4, was considered an important regulator for PCa survival³⁵.
173 HKII is also involved in the increase of glucose metabolism after androgen deprivation
174 in PTEN/Tumor protein p53 (TP53) deficient PCa cells³⁶. Thus, HK inhibitors such as
175 3-bromopyruvate or ionidamine are being tested in clinical trials³⁷. Moreover, Pentose
176 Phosphate Pathway (PPP) is promoted by an AR-mTOR mediated mechanism,
177 maintaining Glucose-6-phosphate dehydrogenase (G6PDH) levels higher during PCa
178 progression³⁸. Overall, androgen signaling stimulates both glycolysis and anabolic
179 metabolism

180 Androgens positively regulate glycolysis via Calcium/calmodulin-dependent
181 protein kinase kinase beta (CamKK β), which activates AMP-activated protein kinase
182 (AMPK)³⁹. AMPK is a metabolic regulator that promotes migration, cell growth and
183 survival of PCa cells^{40,41}. Because of AMPK activation, androgens activates peroxisome
184 proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α), which is connected
185 with mitochondrial biogenesis (Fig 2). Although PGC1 α is overexpressed in patient
186 samples, it was proposed as an antimetastatic factor⁴². Since PGC1 α favors an oxidative
187 metabolism, its loss could be related to the acquisition of a glycolytic and more
188 aggressive phenotype⁴³.

189 Interestingly, high blood glucose drives with low levels of AR⁴⁴. Thus, this
190 might explain the surprising inverse relation between diabetes and PCa that will be
191 discussed below.

192 **ARE GLUT SUFFICIENT TO SUPPORT PROSTATE CANCER**
193 **GROWTH AND SURVIVAL?**

194 Since cancer cells show a high demand for nutrients for cell growth, the uptake
195 has to be higher. In non-pathological tissues, cells become quiescent when the resources
196 are scarce, but cancer cells lose this control, being always addicted to nutrients.
197 Principal nutrients are glucose and glutamine³.

198 There are two different types of transporters for glucose: Na⁺/glucose
199 transporters (SGLTs, *SLC5A*) and facilitative glucose transporters (GLUTs, *SCL2A*).
200 Among the 12 members of SGLTs, only SGLT1 and SGLT2 are proposed as
201 responsible for glucose uptake in some cancer cells⁴⁵.

202 GLUT transporters internalize glucose by a mechanism of facilitated diffusion.
203 There are 14 members (GLUT1-12, GLUT14 and H⁺/myo-inositol transporter –HMIT-
204). They transport other compounds in addition to glucose, and that circumstance
205 establishes the differences between members. They also differ in their affinity for
206 substrate and tissue location. They are divided into three classes: Class I includes
207 GLUT1-4 and GLUT14, class II are GLUT5, GLUT7, GLUT9 and GLUT11 and class
208 III consist of GLUT6, GLUT8, GLUT10, GLUT12, and HMIT. Class I is the best
209 characterized, and its members share the same bacterial ancestor –XylE-⁴⁶. Class II is
210 able to transport fructose and, class III is structurally different. They share common
211 elements as 12 transmembrane α -helixes while the cytoplasmic N-terminus and C-
212 terminus are less conserved among members⁴⁷.

213 GLUT transporters respond to metabolic and hormonal regulation, and several
214 transcription factors are able to increase glucose uptake by overexpressing or locating
215 GLUTs on the cell membrane. Furthermore, under hypoxia or nutrient deprivation,
216 tumor cells overexpress at least one of the isoforms, being predominantly GLUT1⁴⁸.

217 GLUT1 is overexpressed under growth factor withdrawal, which makes cancer cells
218 more resistant to apoptosis⁴⁹.

219 GLUT transporters are regulated by glycosylation or phosphorylation. It was
220 well studied that GLUT transporters are N-glycosylated, which is associated with its
221 ability to increase glucose uptake^{50,51}. Phosphorylation was recently described, opening
222 a new paradigm of GLUT regulation. It was shown a phosphorylation site at serine 490
223 for ataxia telangiectasia mutated (ATM) that promotes surface GLUT1⁵² and at serine
224 226 for protein kinase C which is related with GLUT1 deficiency syndrome⁵³.

225 The important role of GLUT transporters is also linked to the uptake of other
226 compounds than glucose. The best well known is dehydroascorbic acid⁵⁴, the oxidized
227 form of vitamin C, and recently our group opens the possibility that melatonin might
228 also enter into the cell via GLUT transporters⁵⁵. The affinity for the different substrates
229 can be dependent on the interaction of other transmembrane proteins^{56,57}.

230 GLUT1 is usually associated with poor prognosis in tumors⁵⁸. However, not
231 always this transporter is found overexpressed in cancer, and other GLUTs are instead
232 involved in increasing glucose uptake⁸. GLUT-dependent glucose uptake has an
233 important role in diagnosis by positron emission tomography (PET) imaging. The
234 uptake of 2-deoxy-2-[¹⁸F]-fluoro-D-glucose (¹⁸F-FDG) is employed in PET to follow
235 glucose uptake. This compound is phosphorylated by hexokinase (¹⁸F-FDG-6P), but it
236 cannot continue the glycolytic pathway, being accumulated into the cytoplasm. This
237 methodology is valid as a diagnostic tool particularly when cancer drives with the
238 classical Warburg effect⁵⁹.

239 **GLUT TRANSPORTERS AS CLINICAL TARGETS IN PROSTATE CANCER**

240 In diagnosis, classic ^{18}F -FDG-PET has not been considered useful in primary
241 PCa tumors, so the increased glycolysis and GLUT overexpression have not been
242 recognized as relevant as in other tumors. However, now it is assumed that its utility is
243 dependent on the stage of the disease. Also, the high activity by urine in the adjacent
244 urine bladder overlaps the signal in prostate⁶⁰. Thus, the assumption that ^{18}F -FDG-PET
245 is not valid because PCa is not a glycolytic carcinoma should be discarded.

246 In PCa, it seems that exists a possible balance among GLUT transporters, being
247 the majority-produced transporter dependent of the step of the disease (Table 1).
248 GLUT1 is found overexpressed in PCa cells, being the highest levels found in
249 androgen-independent cells³². Since GLUT1 is usually the transporter overexpressed in
250 tumors, it has been the most studied in PCa. Although higher GLUT1 levels were also
251 found in non-tumor tissues⁶¹, it seems that GLUT1 is related to aggressiveness because
252 it is usually overexpressed in poorly differentiated tumours⁶². Also, GLUT1 is
253 connected with recurrence after radical prostatectomy⁶³. This overexpression seems to
254 be dependent of hypoxia more than androgenic regulation. Also, the stromal levels of
255 GLUT1 have been employed to classified PCa by Gleason score and to indicate the
256 presence of a tumor area undetected by biopsy²⁵. Furthermore, GLUT1 is involved in
257 the increase of glucose uptake by inflammatory cells in PCa⁶⁴ and it was recently
258 described that GLUT1 overexpression in PCa might be mediated by the reduced levels
259 of microRNA-132⁶⁵. Regarding its intracellular localization, GLUT1 was also found in
260 Golgi Apparatus where it could have a role in supplying glucose to the prostatic fluid⁶⁶.

261 On the other hand, GLUT3 is activated via caveolin-1 in AR-negative cells⁶⁷
262 and GLUT12, which is also considered an insulin-dependent transporter, was found in
263 PCa but not in non-tumor samples⁶⁶, suggesting a similar role than other insulin
264 dependent transporters as GLUT4 in tumorigenesis.

265 Fructose consumption in the modern diet is increasing, and it is considered a
266 cancer risk factor. In tumor cells, fructose is differently metabolized than glucose, being
267 mainly employed for nucleic acid synthesis⁶⁸. However, in PCa, fructose was not
268 considered as a risk of metastasis⁶⁹. Fructose is secreted in seminal vesicle, so high
269 concentration is found in the dorsal prostate and coagulating glands (rodent anatomy)⁷⁰.
270 GLUT5, the main fructose transporter in addition to GLUT2, is produced in the apical
271 membrane of secretory cells in normal tissue and high-grade intraepithelial neoplasia
272 (HGPIN). Furthermore, *SLC2A7*, *SLC2A9*, and *SCL2A11* mRNAs were found in PCa
273 but only *SCL2A11* mRNA levels increased in PCa tissue respect benign prostate⁶¹.
274 Altogether, fructose uptake might have a role in PCa progression, particularly at early
275 stages.

276 In PCa cells, androgen stimulation increases both GLUT1 and GLUT3,
277 increasing glucose uptake and the secretion of lactate⁷¹. However, these transporters,
278 particularly GLUT1, are downregulated by DHT in non-tumor Sertoli cells⁷². Thus,
279 hormonal regulation of GLUT1/3 seems to be tissue-dependent.

280 Interestingly, androgens and antiandrogens are able to interact with GLUT1 at
281 the external opening since GLUT1 and the ligand-binding domain of AR share
282 sequence homologies⁷³, establishing the idea that regulation is not only via signaling
283 pathway. Other members such as the fructose transporter GLUT5 also seems to be
284 under androgenic regulation. Using the antiandrogenic flutamide in *Scotophilus healthy*,
285 GLUT5 production is reduced in testis⁷⁴.

286 As previously discussed, androgen signaling activates AMPK. As a metabolic
287 regulator, AMPK regulates GLUT transporters⁷⁵. Activated AMPK inhibits activation
288 of thioredoxin-interacting protein (TXNIP), which binds to GLUT1 avoiding its

289 expression and translocation (Fig 2)⁷⁶. Consequently, the insulin-independent GLUT1,
290 and consequently glucose uptake, is also hormonal regulated.

291 Besides AMPK, PI3K/AKT/mTOR pathway has a major role in glucose
292 metabolism through its activation by insulin and IGF1. AKT1 stimulates glycolysis by
293 an increase in both the expression and translocation of GLUT transporters in addition to
294 the phosphorylation of glycolytic enzymes such as hexokinase and
295 phosphofruktokinase^{8,77}. On the other hand, PTEN, the inhibitor of PI3K/AKT pathway,
296 is able to reduce SLC2A1 expression directly⁷⁸.

297 Hypoxia-inducible factors HIF1 and HIF2 are involved in the cellular response
298 to low oxygen concentration in PCa⁷⁹. HIF1 promotes the transcription of the majority
299 of glycolytic enzymes and GLUT transporters, mainly GLUT1 and GLUT3⁸.

300 *KRAS* is frequently mutated in PCa, and its rearrangements were mainly
301 involved in metastases^{80,81}. Tumors with mutations in this gene drive with an increasing
302 rate of glycolysis and higher use of glycolytic products in other anabolic pathways⁸².
303 Mutation of *KRAS* and *BRAF* are related with GLUT1 overexpression in cancer⁸³.

304 TP53, known as “genome guardian” has a dual role in glucose metabolism. On
305 the one hand, it activates HKII expression but, on the other hand, it inhibits glycolysis
306 by the overexpression of TP53-inducible glycolysis and apoptosis regulator TIGAR⁸⁴.
307 TP53 reduces *SLC2A1* and *SLC2A4* transcription⁸⁵, and it interacts with *SLC2A12*⁸⁶. On
308 the other hand, the loss of TP53 upregulates GLUT3⁸⁷. *P53* mutations are usually found
309 in PCa and as a consequence, a higher expression of GLUT transporters was
310 described⁸⁸.

311 Since the inhibition of glycolysis by 2DG (2-deoxyglucose) causes an
312 activation of autophagy in patients with CRPC, its use in the clinic has to be considered

313 in combination with autophagy inhibitors^{89,90}. Then, targeting glucose metabolism could
314 be an option to treat PCa. However, consulting the current clinical trials in prostate
315 (data are taken from www.clinicaltrials.gov), only 1% has a direct association with
316 glucose metabolism, being one directly related with GLUT transporters (Fig 3).

317 Nevertheless, most studies are focused on inhibiting glucose uptake by
318 blocking GLUT transporters. Currently, the recent crystallization of GLUT transporters,
319 the better knowledge of the mechanism of inhibition and the development of GLUT-
320 specific inhibitors open a new approach for the treatment of cancer that develops with
321 increasing glucose uptake. Glucose deprivation kills cancer cells by different
322 mechanisms⁹¹. The consequences of blocking glucose transporters, besides the
323 downregulation of glycolysis, are the inhibition of cell growth, cell cycle arrest and
324 FAS-induced cell death^{92,93}. In fact, fasinin, a compound that inhibits GLUT1,
325 increases apoptosis by sensitizing cells to FAS-ligand death receptor signaling in PCa
326 cells⁹⁴.

327 Although there are not clinical trials focused on specific compounds that block
328 GLUTs in PCa, some compounds that inhibit progression are well-known blockers of
329 GLUT transport, particularly flavonoids. They play an important role in PCa prevention
330 since their phytoestrogen activity and have a promising application as adjuvant
331 treatment⁹⁵.

332 **THE INSULIN-DEPENDENT GLUT4 TRANSPORTER IN PROSTATE** 333 **CANCER: A LINK BETWEEN DIABETES AND PROSTATE CANCER?**

334 Although the insulin-dependent glucose transporter GLUT4 has not been
335 considered as important as GLUT1 in cancer, recent studies show the critical role that
336 might play in several types of tumors. This transporter was described by our group in

337 PCa culture cells⁹⁶ for the first time. We found that phytoestrogens regulate GLUT1⁹⁶ in
338 androgen sensitive LNCaP cells GLUT4 in androgen-insensitive PCa cells while
339 showing a possible balance between both transporters dependent on the phenotype of
340 the cells. We have found an increase production of GLUT4 by androgen-insensitive
341 cells (unpublished data). In fact, GLUT4 has been already detected in the prostate of
342 Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) mice⁹⁷. However, in this
343 model, the relevance of GLUT4 in tumor progression is under investigation.

344 It has been described that testosterone stimulates GLUT4-dependent glucose
345 uptake in human skeletal muscle cells, cardiomyocytes, and 3T3-L1 adipocytes
346 independently of AR signaling⁹⁸⁻¹⁰¹. In adipocytes, it was confirmed that this regulation
347 occurs through Liver kinase B1 (LKB1)/AMPK signaling. As previously described,
348 AMPK phosphorylates the Rab-GTPase TBC1D1, which triggers GLUT4
349 externalization¹⁰². On the other hand, endometrial GLUT4 levels decrease after DHT
350 treatment, and they are inversely related to AR expression in polycystic ovary
351 syndrome¹⁰³.

352 Since GLUT4 is regulated by androgen-independent mechanisms in other
353 tissues, its regulation in PCa might not be dependent on androgens or AMPK signaling.
354 On the other hand, IGF1/insulin pathway regulates insulin-dependent transporter, which
355 suggests its possible role in the regulation of GLUT4 in PCa. In this sense, it implies
356 that this transporter could be more relevant in androgen-insensitive phenotype as our
357 group previously suggested. In addition, GLUT4 might be an intermediate in the effect
358 of insulin on PCa and might have some role in the inverse relationship between diabetes
359 and PCa.

360 PCa risk is related to lifestyle being high-fat diet connected to its progression
361 and aggressiveness. Hyperinsulinemia, which is usually associated with insulin
362 resistance, is also related with a higher PCa risk^{104–106}. Interestingly, insulin levels are
363 higher in PCa patients¹⁰⁷, and insulin receptors (IR) have been found in PCa epithelial
364 cells¹⁰⁸. Moreover, epidemiological studies show a significantly decreased risk of PCa
365 in long-standing type 2 diabetes (T2DM)¹⁰⁹. However, it has also been reported that
366 diabetic men have a more aggressive PCa but their PSA levels remain low, avoiding its
367 early detection¹¹⁰.

368 Circulating insulin and testosterone levels are correlated in male¹¹¹. On the one
369 hand, higher insulin levels decrease the production of sex-hormone binding globulin
370 (SHBG), increasing free and biologically active testosterone^{112,113}. Moreover, the sex
371 hormone promotes insulin production in beta-cells by the extranuclear activity of AR¹¹⁴
372 (Fig 4A). Since this positive feedback, insulin signaling would be related to androgen
373 promotion of tumor growth. However, the IGF1 pathway is usually overexpressed in
374 PCa and, this pathway promotes AR hormone-independent activation which supports a
375 role of insulin-dependent glucose transporters, as GLUT4, in androgen-independent
376 tumor growth (Fig 4B).

377 Under hyperinsulinemia, T2DM is usually treated using metformin, which
378 activates AMPK in the liver¹¹⁵. Most of the current clinical trials connecting glucose
379 metabolism and PCa are focusing on the treatment with metformin in androgen
380 deprivation therapy. In several cancers, metformin itself is being considered a potential
381 treatment. Since metformin inhibits the mitochondrial complex I, it was reported a
382 metabolic switch towards glutamine metabolism in PCa¹¹⁶. However, it was shown that
383 metformin induces apoptosis in the presence of other compounds such as the

384 antiglycolytic 2DG¹¹⁷ or the anti-androgen bicalutamide¹¹⁸, and it is particularly
385 effective in CRPC¹¹⁹. Interestingly, metformin also inhibits androgen-induced IGF1
386 receptor (IGF1R) overexpression¹²⁰ so that GLUT4 transporter might be altered by this
387 treatment.

388 **CONCLUDING REMARKS**

389 After almost 100 years, the metabolic switch to aerobic glycolysis is still under
390 study in oncology. This change is accepted in most of the cancers, but PCa has been
391 considered particular from the metabolic point of view because the role of glucose
392 metabolism in the first steps of progression was less important. Now, several studies are
393 showing that glucose metabolism plays an important role also in prostate carcinogenesis
394 but in a different way than in the rest of cancers. Androgens, despite increasing
395 glycolysis, are involved in anabolism promoting PPP and mitochondrial biogenesis.
396 Thus, androgen dependent tumors are oxidative at first, shifting to glycolysis only in the
397 latest stages of the disease. Then, contrary to other tumors, glucose transporters, and in
398 particular GLUT1, are only overexpressed in the most aggressive tumors that usually
399 drive with hypoxia and with a higher glycolytic activity (Fig 5). Since insulin signaling
400 is related to PCa progression, it seems possible that insulin-dependent glucose
401 transporters might play a relevant role in the progression of the disease. A role of
402 insulin in PCa cancer is suggested by clinical observation since diabetes is inversely
403 related to PCa and some clinical trials are ongoing using the antidiabetic metformin.
404 This could be a good reason to look into the possibility of insulin-dependent glucose
405 transporters act as principal players in those stages of the disease that do not depend on
406 androgens. Glucose transporters have not been considered relevant in prostate cancer
407 progression because glucose metabolism is different in prostate gland than in other
408 differentiate tissues. However, the importance of these transporters has been recently

409 considered since the relevance of increasing nutrients uptake including glucose is
410 clearly demonstrated in prostate cancer. From now on, the employment of components
411 of glucose metabolism, including metabolites, enzymes or transporters to characterize
412 carcinomas will be an area of interest that should be exploited and carefully considered.

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Table 1: Expression of GLUT transporters in non-malignant and tumor prostate

Glucose transporter	Expression in prostate	Location	References
GLUT1	Non-malignant prostate/Aggressive tumors	Plasma membrane, cytoplasm, Golgi system. Secretory and luminal epithelial cells/Basal cells	61,62,63,66
GLUT3	CRPC		7
GLUT4	PCa	Plasma membrane, cytoplasm	97
GLUT5	Non-malignant prostate/ Overexpression in HGPIN	Plasma membrane (apical zone of epithelial cells)	61
GLUT7	Non-malignant prostate/ Overexpression in PCa (mRNA levels)		61
GLUT9	Non-malignant prostate/PCa without overexpression (mRNA levels)		61
GLUT11	Non-malignant prostate/PCa with overexpression (mRNA levels)		61
GLUT12	PCa	Plasma membrane, cytoplasm	66

809 **FIGURE LEGENDS**

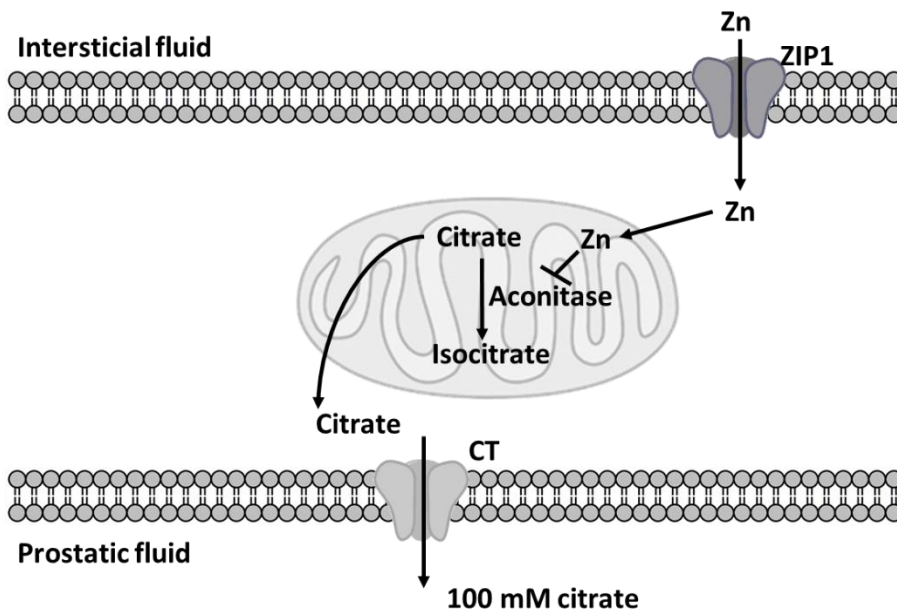
810 **Figure 1: Zn accumulation in the mitochondria of non-malignant prostatic**
811 **epithelial cells.** High Zn uptake in prostatic epithelial cells is due to the overexpression
812 of ZIP1. Zinc is accumulated in mitochondria where it inhibits aconitase, TCA-cycle
813 enzyme that converts citrate to isocitrate. Citrate excess is secreted into the prostatic
814 fluid.

815 **Figure 2: Androgen regulation of AMPK in prostate cancer cells.**
816 Androgen stimulation activates CAMKK β , which phosphorylates AMPK. AMPK is
817 responsible for promoting glucose uptake via GLUT1, glycolysis, and mitochondrial
818 biogenesis in PCa cells.

819 **Figure 3: Clinical trials related to glucose metabolism in prostate cancer.**
820 (A) Number of clinical trials focused on metabolism and glucose metabolism in cancer
821 and, particularly, in prostate cancer. (B) Number of current clinical trials (recruiting and
822 active) focused on metabolism and glucose metabolism in cancer and, particularly, in
823 prostate cancer.

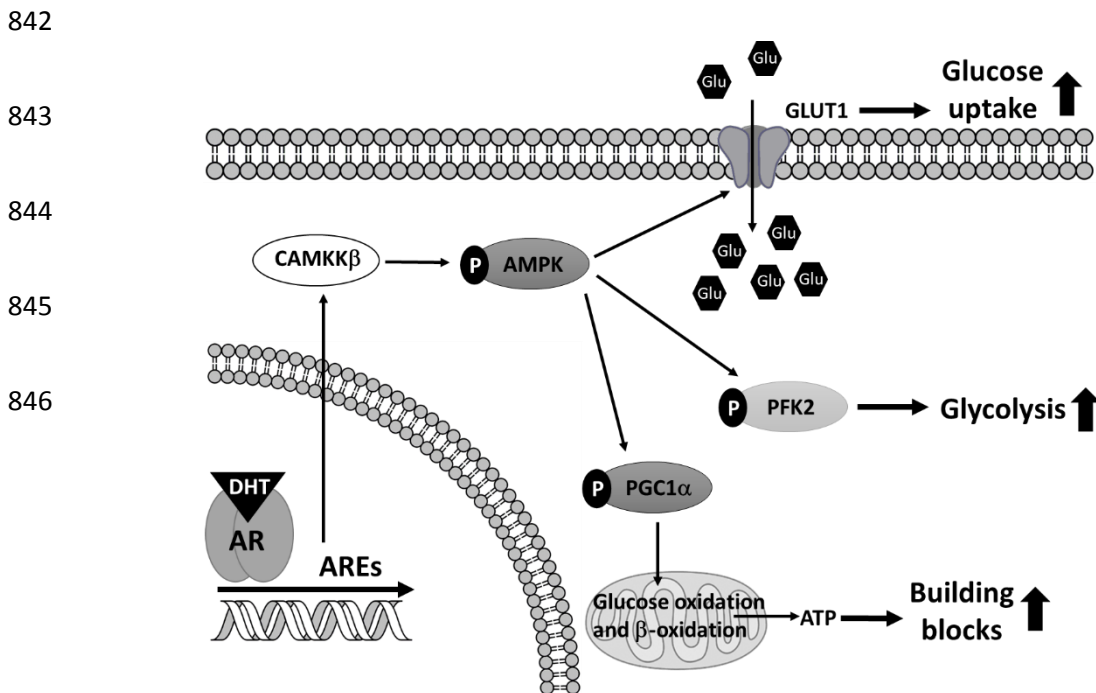
824 **Figure 4: Possible molecular pathways regulated by insulin in prostate**
825 **cancer.** (A) Positive feedback regulation between insulin and testosterone. Since insulin
826 reduces SHBG synthesis in the liver, circulating active testosterone levels increase and
827 stimulates insulin release by beta-cells in pancreas via extranuclear AR activity (B)
828 Insulin stimulates testosterone production, which may activate AR signaling, and
829 activates PI3K/AKT pathway by itself or through IGF pathway. This last activation
830 might lead GLUT4 regulation in PCa cells. SHBG = Sex-hormone binding globulin.
831 Test =Testosterone.

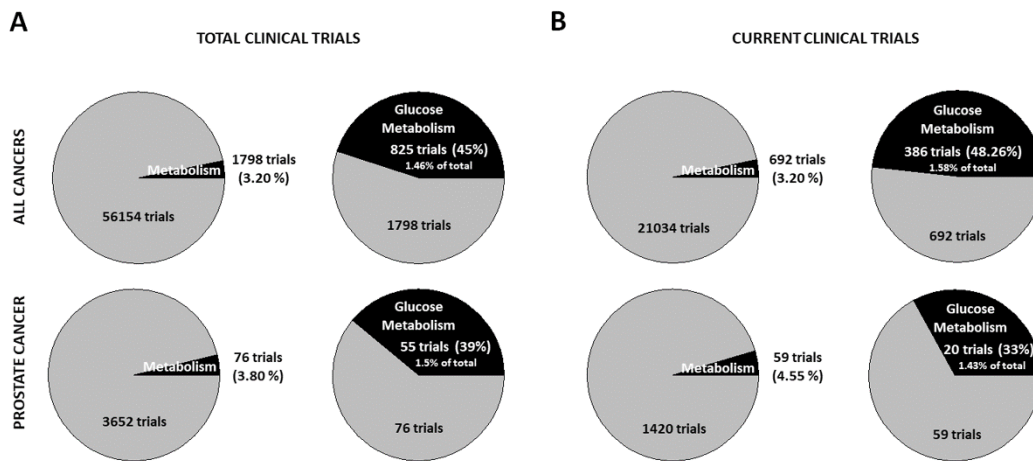
832 **Figure 5: Tumor progression in prostate and GLUT expression along it.**
833 The non-pathological prostate is characterized by high glycolytic activity and GLUT1
834 expression because of impairment in TCA cycle. In the first stages of tumorigenesis,
835 OXPHOS is favored, and circulating insulin levels increase in comparison with the non-
836 tumorigenic prostate. This may be connected with the expression of the insulin-
837 dependent transporter GLUT4. In poorly differentiated PCa tumors, glycolysis and
838 GLUT1 overexpression are again promoted, being correlated with low oxygen levels.
839



840 **Figure 1**

841 **Figure 2**





847 **Figure 3**

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849 **Figura 4**

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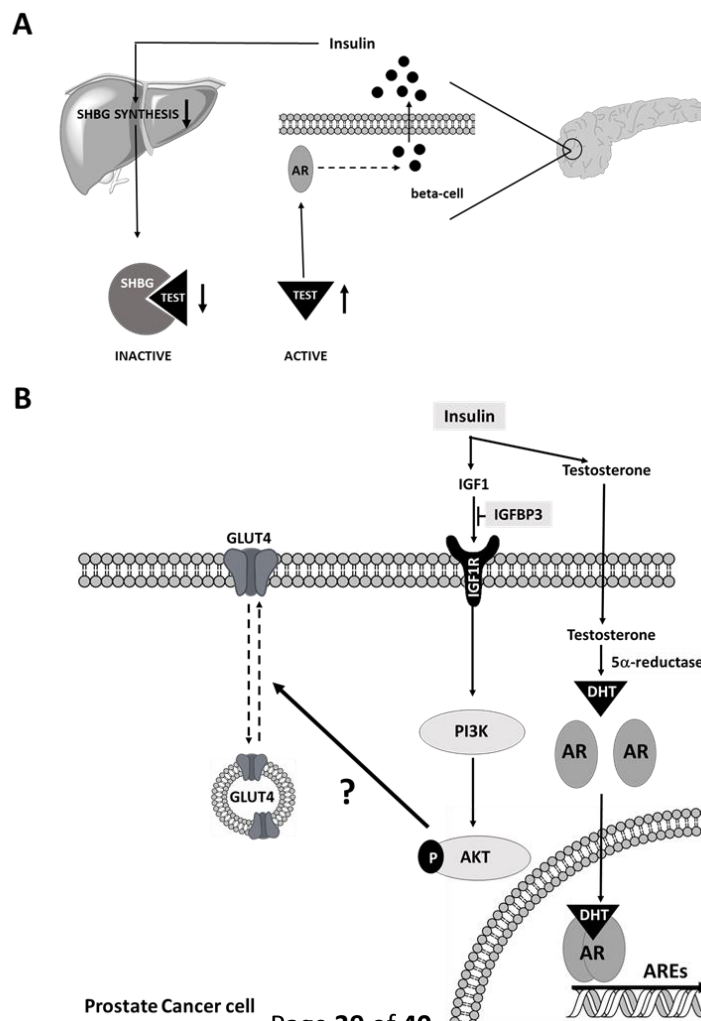
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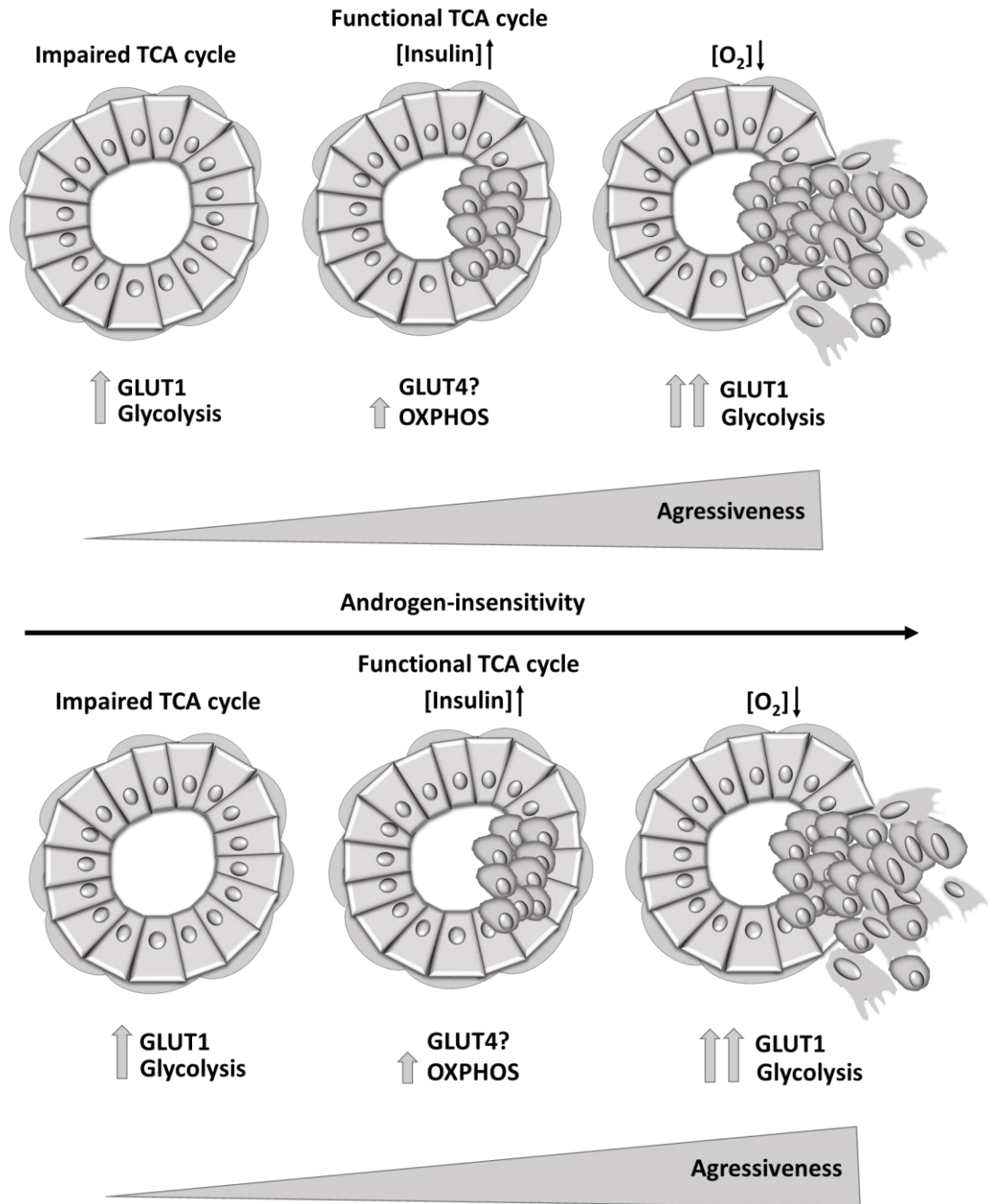


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Figura 5



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