The facilitated glucose transporter GLUT12: What do we know and what would we like to know?

Authors: Jonai Pujol-Giménez, Jaione Barrentxe, Pedro González-Muniesa*, Maria Pilar Lostao *

* Both authors have contributed equally to this review

Short title: GLUT12, present and future directions

Key words: GLUT12, *Xenopus laevis* oocytes, GLUT, Cancer, Diabetes, Glucose transport

Address:

Department of Nutrition, Food Science, Physiology and Toxicology.

University of Navarra

Irunlarrea 1

31008 Pamplona (Spain)

Corresponding author:

M.P. Lostao

E-mail: plostao@unav.es

Phone: 34-948425649

Fax: 34-948425740

Abstract

Human GLUT12 was isolated from the breast cancer cell line MCF-7 by its homology with GLUT4. Glucose has been described as its main substrate, but it also can transport other sugars. In humans, GLUT12 protein is expressed mainly in insulin sensitive tissues. Functional analysis has showed that GLUT12 transports sugars down its concentration gradient, but it can also work as a proton-coupled symporter. Studies from our laboratory, performed in *Xenopus laevis* oocytes expressing GLUT12, show that glucose uptake increases in the presence of Na^+ and induces inward current. These findings suggest a transport mechanism never described for other GLUTs, which would indicate a distinct functional role for GLUT12. In relation with its physiological and pathophysiological function, GLUT12 has been mainly studied due to its role as a secondary insulin-sensitive glucose transporter and its possible implication in impaired insulin signalling pathologies. Its expression in some tumour tissues has been described and recently, it has been proposed as one of the key proteins in the glucose supply to malignant cells. Overall, even though a lot of information about GLUT12 has been released during the last years, its functional characteristics, physiological role or implication in the development of some diseases is still unclear. Therefore, this review of the literature can help to address further investigations needed to elucidate these issues that, in our view, are of great interest mainly due to the direct GLUT12 relation with cancer and probably with diabetes development.

Introduction

Glucose, one of the most abundant molecules in nature, is used by most of the mammalian cells as their main energy source. Obtained from the diet, glucose is absorbed in the small intestine, incorporated into the circulating blood and stored as glycogen, mainly in the liver and muscle. Due to its hydrophilic nature, glucose cannot cross the plasma membrane by simple diffusion; instead, glucose enters the cell by specific membrane transporters. There are two families of glucose transporters, distinguished by their functional and structural properties: the Na^+/ glucose cotransporter family SGLT/SLC5A [47] and the facilitative glucose transporter family GLUT/SLC2A [11, 22].

GLUT protein family members transport monosaccharides across the plasma membrane without energetic requirement, using the favourable concentration gradient of the hexose generated in some physiological situations. GLUTs share a common structural feature of 12 transmembrane domains, with both amino and carboxy terminal domains located on the cytosolic side, and a N-linked

oligosaccharide site present either on the first or on the fifth extracellular loop [37]. At present, 14 different members of this family, divided in three classes according to its sequence homology, have been identified [11]. Class I is constituted by the well characterized GLUT1, GLUT2, GLUT3, GLUT4 and GLUT14 (gene duplication of GLUT3); Class II comprises the fructose transporter GLUT5, and GLUT7, GLUT9 and GLUT11; and class III includes GLUT6, GLUT8, GLUT10, GLUT12 and GLUT13 (HMIT) [12]. In relation to this classification, a recent phylogenetic analyses proposes that the proteins belonging to Class III could be separated into three different groups and, therefore, suggest five structurally and/or functionally distinct GLUT classes (Figure I) [43].

Location, expression and regulation of the GLUT transporters are specific for each tissue and cellular type and is related to the cell metabolic needs. In many cases, the up or down-regulation of the GLUT proteins is directly linked to the development of diseases (Table I). GLUT1, 2, 3, 4 and 5 were the first members of the GLUT family cloned and their physiological function has been well characterized. GLUT9 seems to be an urate transporter, newly described as electrogenic [44], while GLUT13 (HMIT) is a H^+-dependent myoinositol cotransporter [36]. However, the physiological significance of the rest of GLUT transporters of class II and III still needs to be elucidated. Therefore, it is important to continue investigating the location and cellular function of recently discovered GLUT transporters, not only to determine their function in the organism, but also to understand their contribution in some diseases, thus giving the possibility of using them as therapeutic targets.

Consequently, the objective of the present review is to summarize the current information about GLUT12, including some new data from our laboratory, and give some proposals for future studies that could help to better understand its relevance in the organism both in health and disease.

GLUT12

Structure and Tissue Distribution

Human GLUT12 cDNA was isolated from the breast cancer cell line MCF-7 by its homology with GLUT4 [30]. GLUT12 gene has five exons and encodes a 617 amino acid protein with a predicted molecular mass of 50 kDa. Its structure comprises 12 transmembrane domains, with the conserved features of the glucose transporters family for the substrate-binding site and catalytic activity [43]. As the other members of Class III, its membrane topology predicts an exofacial loop between helices 9 and 10 with a N-linked glycosilation site, and the absence of the exofacial loop between the first two

helices described for Class I [11] (Figure 2). Interestingly, in helix 7 GLUT12 does not present the QLS motif, which is highly conserved in GLUT1, GLUT3 and GLUT4; however it contains an isoleucine in the third position of the NXI/V motif like GLUT2, GLUT5 and GLUT7 [22]. The absence of this QLS motif in GLUT2 and GLUT5 and the presence of a key hydrophobic residue in position 314, such as isoleucine in GLUT2, GLUT5 and GLUT7, have been described as essential for fructose transport [1, 21]. Finally, it is remarkable that GLUT12, as the rest of class III transporters and GLUT4, possesses the internalization di-leucine motifs at the N- and C-terminal ends of the protein, that retain these transporters into intracellular compartments in the absence of stimulus [30] (Figure 2). In fact, in the absence of insulin, GLUT12 was localized in the perinuclear region of MCF-7 cells [30].

GLUT12 cDNA has also been cloned from mouse and cow, presenting respectively 83 and 87 % homology with the human isoform [24, 49], and expression of GLUT12 has been reported in rat [18], horse [38] and dog [41].

In relation to its tissue distribution, in human GLUT12 protein is expressed in skeletal muscle, adipose tissue, small intestine [30] and placenta [10]. Its mRNA is abundant in skeletal muscle, heart and prostate, and it is present at low levels in brain and kidney [30]. In rat, expression of GLUT12 protein has been described during foetal development in skeletal muscle, brown adipose tissue, heart, foetal chondrocytes, lung, kidney [18], mammary gland during pregnancy [19] and kidney distal tubes and collecting ducts [15]. In cow, its mRNA expression is abundant in spleen and skeletal muscle, less expressed in kidney, testis and mammary gland, and even lower expressed in liver, lung and intestine [24]. GLUT12 protein expression has also been found in Chinese hamster ovary cells [7], horse skeletal muscle [38] and dog heart muscle [41].

Functional characteristics

Analysis of glucose transport in GLUT12 cRNA injected *Xenopus laevis* oocytes, showed that radiolabeled 2-deoxy-glucose (2-DOG) uptake was 6-fold higher than in water-injected oocytes. Uptake of 2-DOG was also measured in the presence of different sugars, showing the following substrate selectivity: D-Glucose > 2-DOG > D-Galactose > D- Fructose > L-Glucose. Transport of 2-DOG into the oocytes was inhibited by cytochalasine B, a GLUTs specific inhibitor [28]. Further analysis of the functional glucose transport in canine kidney epithelial cell line (MCDK), showed that GLUT12 can work as a proton-coupled symporter, transporting glucose against its concentration gradient. This transport mechanism was also inhibited by cytochalasine B and was pH dependent [43].

Preliminary studies from our laboratory, using *Xenopus laevis* oocytes as expression system, have shown that glucose transport by GLUT12 increases in the presence of Na^+ (Figure 3). Furthermore, studies using electrophysiological techniques showed that GLUT12 mediated glucose transport induce electrical currents (Figure 4), confirming that the GLUT12 transport mechanism is coupled to ions movement.

Physiological role

GLUT12 has been mainly studied due to its role as a secondary insulin-sensitive glucose transporter in insulin dependent tissues. This physiological function was demonstrated in normal human skeletal muscle, where it was described that GLUT12 is translocated to the plasma membrane together with GLUT4 in response to insulin [32]. This fact was recently confirmed in a GLUT12 over-expressing mice, where the whole body insulin sensitivity and the glucose clearance rate in insulin sensitive tissues was higher than in control mice [26]. In normal human skeletal muscle, it has been described that GLUT12, GLUT4 and GLUT5 account for 98 % of the total GLUT mRNA, with an expression ratio for GLUT4: GLUT12 of 12:1, and being GLUT12 predominantly co-expressed with GLUT4 in type I fibers [33]. In another study, it has been shown a protein expression ratio for GLUT4: GLU12 of 8:1, representing for GLUT12 around 12 % of the potentially insulin translocable GLUT. Moreover, activation of PI3-K was involved in GLUT12 translocation, as has been described for GLUT4 [32].

Regarding adipose tissue, GLUT12 mRNA is expressed in all the major adipose tissue depots in rat, but not in the 3T3-L1 murine adipocytes cell line [45]. In humans, GLUT12 mRNA has been found in omental and subcutaneous depots and also in the human SGBS adipocyte cell line [45]. Further studies from the same group have demonstrated in human adipocyte primary culture, an increase of 2-DOG uptake due to hypoxia, without modification of GLUT12 mRNA levels [46].

In addition to its role as a secondary insulin sensitive transporter, GLUT12 has been also studied in other physiological conditions as foetal development and mammary gland during pregnancy and lactation.

GLUT12 protein expression has been largely investigated during rat foetal development, when glucose delivery is particularly important because cells are rapidly dividing and differentiating. The studies showed that during gestational period, from day 15 to 21, GLUT12 is expressed in insulin sensitive tissues as heart, skeletal muscle and brown adipose tissue. During similar gestational period, GLUT12

was also detected in chondrocytes, kidney distal tubules and collecting ducts, and in lung bronchioles. According to these data, authors propose that GLUT12´s physiological role during rat foetal development could be related to the uptake of different hexoses, other than glucose, and the glucose delivery to insulin sensitive tissue before GLUT4 appearance [18]. In extracts of human placenta from the first trimester and at term, a spatio-temporal difference in GLUT12 protein expression was described. This different expression pattern matches with the location of placenta insulin receptors during foetal development, and authors propose that GLUT12 could be responsible of insulin regulated glucose uptake in that tissue [10]. In human term placenta, GLUT12 mRNA and protein have been detected in omnion and choriodecidua cells [9].

Glucose is very important for the mammary gland function during lactation, as glucose is used as an energy source and milk precursor. Presence of GLUT12 protein has been described in the cytoplasm, likely associated with vesicles and/or the Golgi apparatus, of rat mammary epithelial cells during the final period of pregnancy and postpartum. In contrast, during lactation, GLUT12 is present at the apical membrane of those cells. As it is the only GLUT isoform described in the apical membrane of mammary glands, authors suggest that GLUT12 is the responsible for glucose transport into the milk [19]. Supporting these findings, GLUT12 was also identified in the cytoplasm of cow mammary epithelial cells and in a bovine mammary epithelial cell line (MAC-T). It is interesting to mention that cow GLUT12 does not have a dileucine motif at the C-terminal region, suggesting different function and/or regulation of GLUT12 in this animal [24].

Implication in Cancer and Other Diseases

Malignant cells are characterized by uncontrolled cellular division and accelerated metabolism that implies high glucose requirement and, therefore, increased glucose uptake. To satisfy this glucose need, GLUTs are overexpressed in malignant cells [17]. Therefore, as glucose transport across the plasma membrane is the first rate-limiting step for glucose metabolism, and GLUTs overexpression is related to accelerated metabolism, GLUTs are widely studied in cancer for diagnosis and even treatment purposes. In this regard, GLUT12 protein expression has been described in the MCF7 malignant breast epithelial cell line [30], in human breast tumours [29] and in human prostate carcinoma cell lines and tissue samples [5]. On the other hand, GLUT12 protein expression is increased by stimulators of breast cancer cell growth such as estradiol and epidermal growth factor, which may indicate that GLUT12 contributes to increase glucose uptake in breast cancer [17].

Differential expression of GLUTs mRNA in normal and tumour kidney tissue suggests that GLUT4 and GLUT12 could be possible candidates for the increased glucose uptake in chromophobe renal cell carcinoma [35].

More interestingly, recent experiments performed to develop strategies for anticancer therapy have shown that GLUT12 together with GLUT1, are the main glucose transporters involved in the glycolytic metabolism characteristic of human cancers, known as the Warburg effect [40]. In those studies, authors showed that p53 transcription factor, a tumour development suppressor, specifically binds, among other genes, to the GLUT12 and GLUT1 promoters and repress their expression. In addition, the pharmacological activation of p53 inhibits the glycolytic metabolism, even under hypoxic conditions, in different cancer cell lines [48].

As an insulin sensitive transporter, GLUT12 expression has been largely studied in insulin sensitive tissues in different physiological and pathophysiological conditions and mainly in those with impaired insulin signalling, as in type II diabetes. In human muscle biopsies, GLUT12 mRNA levels decreased (40 %) in endurance-trained compared to sedentary individuals, whereas GLUT4 mRNA increases (78 %) [31]. In contrast, other studies indicated that cycle training increases protein expression of GLUT4 (66%) and GLUT12 (104%), compared to sedentary subjects, together with the activation of the mTOR muscle hypertrophy pathway [34]. In an equine model of insulin resistance (IR) an increase of GLUT4 basal cell-surface expression has been found, but not for GLUT12, without modification of GLUT4 and GLUT12 total protein levels [38]. In adipose tissue from the same IR equine model, cell-surface GLUT4 content was lower in visceral and subcutaneous depots compared to insulin sensitive animals, while total GLUT4 content was only reduced in omental tissue. However, neither total nor cell-surface GLUT12 protein content was affected in any adipose depot in the IR animals [39]. In a Ren-2 rat model of diabetic nephropathy, GLUT12 overexpression was described in distal tubules and collecting ducts compared to control animals, suggesting that GLUT12 could be involved in the regulation of glucose reabsorption in distal renal tubules in response to elevated glucose levels [15]. Further analysis of GLUT12 cellular location and targeting in the MDCK cell line demonstrated apical targeting of GLUT12 from the perinuclear location, in response to elevated glucose concentration, in which mTOR signalling pathway was involved. Activity of mTOR signalling pathway has been associated with diabetic kidney nephropathy [42]. Finally, in a canine model of chronic heart failure, increase of

GLUT4 and GLUT12 protein and mRNA expression was described in comparison to control heart samples [41].

Summary and Future directions

GLUT12 was one of the last glucose transporters to be cloned [30]. During the past years, research has focused on the study of GLUT12 role as a secondary insulin sensitive transporter [26, 32] and its role on impaired insulin signalling pathologies [15, 38, 39]. Its expression in some tumour tissues has been described [5, 29, 35] and recently it has been proposed as one of the key proteins in the glucose supply to malignant cells [48] (Figure 5).

Taking into account the information presented in this review and our preliminary experimental data, we would like to propose some questions in order to address future investigations about GLUT12 physiology.

1) Functional features described for GLUT12 are very different from those of GLUT4. GLUT12 can transport various hexoses other than glucose [28], it is able to transport glucose against its concentration gradient through a proton-coupled symport mechanism [43] and, according to our preliminary data, presents electrogenic properties and its transport mechanism is modified in the presence of extracellular Na^+. Therefore, instead of being an evolutionary GLUT4 ancestor, maintained as a backup [32], could GLUT12 complement GLUT4 activity?

2) Changes in GLUT4 and GLUT12 expression pattern have been described both during physical training [31] and in the development of insulin resistance [38]. If there is a loss of function of GLUT4, as described in human adipose tissue in insulin resistance [37], could this loss be counter-balanced with an increase in GLUT12 expression? Would the response to insulin be the same?

3) GLUT12, together with GLUT1, has been described as the main glucose transporters in the glycolytic metabolism of malignant cells [48]. In agreement with this, in the tumours where the expression of GLUT12 has been reported (prostate, breast and kidney) [5, 29, 35], can be the expression of GLUT12 considered as tissue specific or is a consequence of a predominant glycolytic metabolism? And, if a glycolytic metabolism is a common feature of most tumours, can we expect GLUT12 expression in all of them?

4) GLUT12 expression has been reported in small intestine, but its location is still unknown. Assuming that in basal conditions it would be located in cytoplasm vesicles, could it be possible that at high

luminal glucose concentration, as described for MCDK cells [42], GLUT12 would be translocated to the apical plasma membrane during intestinal glucose absorption?

5) Recently, phylogenetic studies have reported that GLUT10 and GLUT12 may constitute a new GLUT family class [43], and as they are evolutionary close to the proton-myoinositol transporter HMIT (GLUT13), could it be possible that GLUT10 share with HMIT and GLUT12 some of their particular functional features?

In conclusion, even though a lot of information about GLUT12 has been released during the last years, still little is known about its functional characteristics, and its physiological role is not clear yet. Therefore, further studies are needed to elucidate these issues in order to establish a solid body of knowledge of GLUT12. In our view, this is of great interest due to GLUT12 direct relation to the glycolytic metabolism of malignant cells, its role as insulin sensitive transporter and maybe, as a possible therapeutic target to fight against diseases of high prevalence in our society as cancer and diabetes.

Acknowledgment:

We thank Asunción Redín for technical assistance and Dr. Alejandra Pérez and Alejandro Reyes for its scientific collaboration. This work has been supported by a grant from "Fundación Marcelino Botín".

References

1. Arbuckle MI, Kane S, Porter LM, Seatter MJ, and Gould GW (1996) Structure-function analysis of liver-type (GLUT2) and brain-type (GLUT3) glucose transporters: expression of chimeric transporters in Xenopus oocytes suggests an important role for putative transmembrane helix 7 in determining substrate selectivity. Biochemistry 35(51):16519-27.
2. Augustin R (2010) The protein family of glucose transport facilitators: It's not only about glucose after all. IUBMB Life 62(5):315-33.
3. Birnir B, Loo DD, and Wright EM (1991) Voltage-clamp studies of the Na+/glucose cotransporter cloned from rabbit small intestine. Pflugers Arch 418(1-2):79-85.
4. Burant CF, Takeda J, Brot-Laroche E, Bell GI, and Davidson NO (1992) Fructose transporter in human spermatozoa and small intestine is GLUT5. J Biol Chem 267(21):14523-6.
5. Chandler JD, Williams ED, Slavin JL, Best JD, and Rogers S (2003) Expression and localization of GLUT1 and GLUT12 in prostate carcinoma. Cancer 97(8):2035-42.
6. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GM, Kambouris M, Gardella R, Facchetti F, Willems PJ, Forsyth R, Dietz HC, Barlati S, Colombi M, Loeys B, and De Paepe A (2006) Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet 38(4):452-7.

7. Flessner LB and Moley KH (2009) Similar [DE]XXXL[LI] motifs differentially target GLUT8 and GLUT12 in Chinese hamster ovary cells. Traffic 10(3):324-33.
8. Gaster M, Handberg A, Schurmann A, Joost HG, Beck-Nielsen H, and Schroder HD (2004) GLUT11, but not GLUT8 or GLUT12, is expressed in human skeletal muscle in a fibre type-specific pattern. Pflugers Arch 448(1):105-13.
9. Gude NM, Stevenson JL, Murthi P, Rogers S, Best JD, Kalionis B, and King RG (2005) Expression of GLUT12 in the fetal membranes of the human placenta. Placenta 26(1):67-72.
10. Gude NM, Stevenson JL, Rogers S, Best JD, Kalionis B, Huisman MA, Erwich JJ, Timmer A, and King RG (2003) GLUT12 expression in human placenta in first trimester and term. Placenta 24(5):566-70.
11. Joost HG and Thorens B (2001) The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol Membr Biol 18(4):247-56.
12. Joost HG, Bell GI, Best JD, Birnbaum MJ, Charron MJ, Chen YT, Doege H, James DE, Lodish HF, Moley KH, Moley JF, Mueckler M, Rogers S, Schurmann A, Seino S, and Thorens B (2002) Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. Am J Physiol Endocrinol Metab 282(4):E974-6.
13. Kellett GL, Brot-Laroche E, Mace OJ, and Leturque A (2008) Sugar absorption in the intestine: the role of GLUT2. Annu Rev Nutr 28:35-54.
14. Larrayoz IM, Casado FJ, Pastor-Anglada M, and Lostao MP (2004) Electrophysiological characterization of the human Na(+)/nucleoside cotransporter 1 (hCNT1) and role of adenosine on hCNT1 function. J Biol Chem 279(10):8999-9007.
15. Linden KC, DeHaan CL, Zhang Y, Glowacka S, Cox AJ, Kelly DJ, and Rogers S (2006) Renal expression and localization of the facilitative glucose transporters GLUT1 and GLUT12 in animal models of hypertension and diabetic nephropathy. Am J Physiol Renal Physiol 290(1):F205-13.
16. Lostao MP, Hirayama BA, Loo DD, and Wright EM (1994) Phenylglucosides and the Na+/glucose cotransporter (SGLT1): analysis of interactions. J Membr Biol 142(2):161-70.
17. Macheda ML, Rogers S, and Best JD (2005) Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. J Cell Physiol 202(3):654-62.
18. Macheda ML, Kelly DJ, Best JD, and Rogers S (2002) Expression during rat fetal development of GLUT12--a member of the class III hexose transporter family. Anat Embryol (Berl) 205(5-6):441-52.
19. Macheda ML, Williams ED, Best JD, Wlodek ME, and Rogers S (2003) Expression and localisation of GLUT1 and GLUT12 glucose transporters in the pregnant and lactating rat mammary gland. Cell Tissue Res 311(1):91-7.
20. Maher F, Vannucci SJ, and Simpson IA (1994) Glucose transporter proteins in brain. Faseb J 8(13):1003-11.

21. Manolescu A, Salas-Burgos AM, Fischbarg J, and Cheeseman CI (2005) Identification of a hydrophobic residue as a key determinant of fructose transport by the facilitative hexose transporter SLC2A7 (GLUT7). J Biol Chem 280(52):42978-83.
22. Manolescu AR, Witkowska K, Kinnaird A, Cessford T, and Cheeseman C (2007) Facilitated hexose transporters: new perspectives on form and function. Physiology (Bethesda) 22:234-40.
23. Matsuo H, Chiba T, Nagamori S, Nakayama A, Domoto H, Phetdee K, Wiriyasermkul P, Kikuchi Y, Oda T, Nishiyama J, Nakamura T, Morimoto Y, Kamakura K, Sakurai Y, Nonoyama S, Kanai Y, and Shinomiya N (2008) Mutations in glucose transporter 9 gene SLC2A9 cause renal hypouricemia. Am J Hum Genet 83(6):744-51.
24. Miller PJ, Finucane KA, Hughes M, and Zhao FQ (2005) Cloning and expression of bovine glucose transporter GLUT12. Mamm Genome 16(11):873-83.
25. Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, and Lodish HF (1985) Sequence and structure of a human glucose transporter. Science 229(4717):941-5.
26. Purcell SH, Aerni-Flessner LB, Willcockson AR, Diggs-Andrews KA, Fisher SJ, and Moley KH (2011) Improved insulin sensitivity by GLUT12 overexpression in mice. Diabetes 60(5):1478-82.
27. Rea S and James DE (1997) Moving GLUT4: the biogenesis and trafficking of GLUT4 storage vesicles. Diabetes 46(11):1667-77.
28. Rogers S, Chandler JD, Clarke AL, Petrou S, and Best JD (2003) Glucose transporter GLUT12-functional characterization in Xenopus laevis oocytes. Biochem Biophys Res Commun 308(3):422-6.
29. Rogers S, Docherty SE, Slavin JL, Henderson MA, and Best JD (2003) Differential expression of GLUT12 in breast cancer and normal breast tissue. Cancer Lett 193(2):225-33.
30. Rogers S, Macheda ML, Docherty SE, Carty MD, Henderson MA, Soeller WC, Gibbs EM, James DE, and Best JD (2002) Identification of a novel glucose transporter-like protein-GLUT-12. Am J Physiol Endocrinol Metab 282(3):E733-8.
31. Seki Y, Berggren JR, Houmard JA, and Charron MJ (2006) Glucose transporter expression in skeletal muscle of endurance-trained individuals. Med Sci Sports Exerc 38(6):1088-92.
32. Stuart CA, Howell ME, Zhang Y, and Yin D (2009) Insulin-stimulated translocation of glucose transporter (GLUT) 12 parallels that of GLUT4 in normal muscle. J Clin Endocrinol Metab 94(9):3535-42.
33. Stuart CA, Yin D, Howell ME, Dykes RJ, Laffan JJ, and Ferrando AA (2006) Hexose transporter mRNAs for GLUT4, GLUT5, and GLUT12 predominate in human muscle. Am J Physiol Endocrinol Metab 291(5):E1067-73.
34. Stuart CA, Howell ME, Baker JD, Dykes RJ, Duffourc MM, Ramsey MW, and Stone MH (2010) Cycle training increased GLUT4 and activation of mammalian target of rapamycin in fast twitch muscle fibers. Med Sci Sports Exerc 42(1):96-106.

35. Suganuma N, Segade F, Matsuzu K, and Bowden DW (2007) Differential expression of facilitative glucose transporters in normal and tumour kidney tissues. BJU Int 99(5):1143-9.
36. Thorens B and Mueckler M (2010) Glucose transporters in the 21st Century. Am J Physiol Endocrinol Metab 298(2):E141-5.
37. Uldry M and Thorens B (2004) The SLC2 family of facilitated hexose and polyol transporters. Pflugers Arch 447(5):480-9.
38. Waller AP, Burns TA, Mudge MC, Belknap JK, and Lacombe VA (2011) Insulin resistance selectively alters cell-surface glucose transporters but not their total protein expression in equine skeletal muscle. J Vet Intern Med 25(2):315-21.
39. Waller AP, Kohler K, Burns TA, Mudge MC, Belknap JK, and Lacombe VA (2011) Naturally occurring compensated insulin resistance selectively alters glucose transporters in visceral and subcutaneous adipose tissues without change in AS160 activation. Biochim Biophys Acta 1812(9):1098-103.
40. Warburg O (1956) On the origin of cancer cells. Science 123(3191):309-14.
41. Ware B, Bevier M, Nishijima Y, Rogers S, Carnes CA, and Lacombe VA (2011) Chronic heart failure selectively induces regional heterogeneity of insulin-responsive glucose transporters. Am J Physiol Regul Integr Comp Physiol 301(5):R1300-6.
42. Wilson-O'Brien AL, Dehaan CL, and Rogers S (2008) Mitogen-stimulated and rapamycin-sensitive glucose transporter 12 targeting and functional glucose transport in renal epithelial cells. Endocrinology 149(3):917-24.
43. Wilson-O'Brien AL, Patron N, and Rogers S (2010) Evolutionary ancestry and novel functions of the mammalian glucose transporter (GLUT) family. BMC Evol Biol 10:152.
44. Witkowska K, Smith KM, Yao SY, Ng AM, O'Neill D, Karpinski E, Young JD, and Cheeseman CI (2012) Human SLC2A9a and SLC2A9b isoforms mediate electrogenic trasnport of urate with different characteristics in the presence of hexoses. Am J Physiol Renal Physiol.Doi:10.1152/ajprenal.00134.2012
45. Wood IS, Hunter L, and Trayhurn P (2003) Expression of Class III facilitative glucose transporter genes (GLUT-10 and GLUT-12) in mouse and human adipose tissues. Biochem Biophys Res Commun 308(1):43-9.
46. Wood IS, Wang B, Lorente-Cebrian S, and Trayhurn P (2007) Hypoxia increases expression of selective facilitative glucose transporters (GLUT) and 2-deoxy-D-glucose uptake in human adipocytes. Biochem Biophys Res Commun 361(2):468-73.
47. Wright EM, Loo DD, and Hirayama BA (2011) Biology of human sodium glucose transporters. Physiol Rev 91(2):733-94.
48. Zawacka-Pankau J, Grinkevich VV, Hunten S, Nikulenkov F, Gluch A, Li H, Enge M, Kel A, and Selivanova G (2011) Inhibition of glycolytic enzymes mediated by pharmacologically activated p53: targeting Warburg effect to fight cancer. J Biol Chem 286(48):41600-15.
49. Zhou Y, Kaye PL, and Pantaleon M (2004) Identification of the facilitative glucose transporter 12 gene Glut12 in mouse preimplantation embryos. Gene Expr Patterns 4(6):621-31.

Figure Legends

Figure 1. Dendogram of multiple alignments of all members of the facilitative glucose transporters family GLUT/SLC2A. Alignment was performed using the CLUSTAL W online tool from the European Bioinformatics Institute (EBI). The division of GLUT/SLC2A family proposed in [43] is indicated with dashed lines boxes, while classic division is indicated with solid bold lines.

Figure 2. Schematic model of GLUT12 protein with its predicted 12 trans-membrane domains. N-glycosylation site is located between trans-membrane domains 9 and 10 (N). Characteristic di-leucine motifs (LL) are located at the amino and carboxy terminal ends and NXI motive in trans-membrane domain 7.

Figure 3. Effect of Na^+ on 5 mM glucose uptake in *Xenopus laevis* oocytes expressing GLUT12. Stage VI oocytes from *X. laevis* were obtained and microinjected with 50 nl of GLUT12 mRNA as previously described [14]. After 3-5 days, 5 mM glucose uptake was determined after 15 min incubation, using the radiotracer method [16] in the presence of Na^+ buffer (NaCl 100 mM, KCl 2 mM, MgCl2 1 mM, CaCl2 1mM, Hepes 10 mM, pH 7.5) or Na^+-free buffer (ChCl 100 mM, KCl 2 mM, MgCl2 1 mM, CaCl2 Hepes 10 mM, pH 7.5). Values are the mean of 8-10 oocytes and error bars indicate standard error. In the presence of Na^+ uptake increased ~ 45 %. Similar results were obtained with oocytes from at least three different animal donors. NI, non-injected oocytes.

Figure 4. Current induced by glucose in *Xenopus laevis* oocytes expressing GLUT12. Currents associated to the transport of glucose mediated by GLUT12 were recorded using the two-electrode voltage-clamp technique [3]. **A** GLUT12 expressing oocyte was perfused with Na^+ buffer (solid box) and a stable base-line current was recorded. The perfusion of 100 mM glucose (↓) induced a current of

65 nA. After the perfusion of glucose, oocyte was washed out with Na^+-free buffer (white box) and the base-line current and glucose induced current disappeared. **B** For control, the same experiment was performed in a non-injected oocyte from the same batch and no response to glucose was obtained. Similar results were obtained with oocytes from more than 10 different animal donors.

Figure 5. **Scheme of the most relevant features described about GLUT12 and some of the questions still without response**. Glut12 is expressed in insulin sensitive tissues (muscle and adipose tissue) where its function is related with glucose transport in response to insulin stimulation. It is also expressed in small intestine but its role in this tissue is still unknown. In relation to its functional characteristics, GLUT12 could transport glucose down its concentration gradient (equilibrative transport) or against its concentration gradient (concentrative transport) in cotransport with H^+ or, according to our preliminary data, Na^+. GLUT12 transports different sugars as glucose, fructose and galactose. GLUT12 expression has been described in prostate and breast tumours and its role as one of the main glucose suppliers to glycolytic metabolism has been proposed. As secondary insulin sensitive transporter, GLUT12 possible relation with diabetes has been studied, but its role is still unclear.