INSULIN STIMULATES GLUT8 EXPRESSION IN HUMAN SPERMATOZOA

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1. INTRODUCTION
Glucose uptake and metabolism are essential for proliferation and survival of cells and is usually carried out through glucose transporters (GLUTs). It is largely known that the fertility of germ cells is directly associated with the glucose metabolism of these cells and that spermatogenesis is disturbed in insulin dependent diabetes mellitus (IDDM), thereby causing infertility [1, 2].

The principal glucose transporter that mediates its uptake in regulating whole body glucose homeostasis is GLUT4 [3]. GLUT4 is insulin stimulated and is mainly expressed in insulin-sensitive cells such as adipocytes and muscles cells [4, 5]. However, GLUT4 is not present in the human spermatozoa [6]. It has been assumed previously that the major sugar transporter of the sperm cell is GLUT5 [7]. GLUT5 is a specific fructose transporter [8] and does not transport glucose to a significant extent. Because GLUT5 was not detected in rat testis, it was suggested that other sugar transporters, presumably the GLUT3, catalyze the fuel supply of the rat sperm cell [9].

In recent years, a novel 447-amino-acid glucose transporter protein, GLUT8 was discovered [10, 11, 12]. GLUT8 is expressed to some extent in insulin-sensitive tissues, e.g., brain, adrenal gland, spleen, adipose tissue, muscle, heart, and liver [10, 11, and 13]. GLUT8 mRNA expression is greatest in the testicular tissue and its expression was linked to circulating gonadotrophin levels [11, 14].

GLUT8 was found to be specifically located in the head of mouse and human spermatozoa and that it is predominantly located within the head of mature sperm cells in the region of the acrosome [15]. Coincidentally, insulin has also been reported to be predominant in these areas of human spermatozoa [16]. The intracellular localization of GLUT8 is similar to that of insulin-sensitive GLUT4, and it has indeed been described that insulin could produce a translocation of GLUT8 to the plasma membrane of blastocysts [12]. In addition, it has been shown that GLUT8 translocates between internal membranes and the plasma membrane in rat adipocytes and COS-7 cells. This study was aimed at investigating if insulin has an effect on GLUT8 expression and translocation in human spermatozoa [17].

2. MATERIALS AND METHODS
2.1. Chemicals
Human insulin was purchased from Lilly France S.A.S (Federsheim, France). GLUT8 goat polyclonal IgG primary
antibody and donkey anti-goat IgG-Texas Red conjugated secondary antibody were purchased from Santa Cruz Biotechnology, California, USA. Donkey serum was from Sigma Aldrich Inc., St Louis, MO, USA. DakoCytomation Fluorescent Mounting Medium was bought from Dako North America Inc., CA, USA, and Hoechst was purchased from Invitrogen, California, USA.

2.2. Preparation of sperm samples
Semen was collected from normozoospermic donors according to the World Health Organisation (WHO) criteria. Samples were left to liquefy for 30 minutes before processing. Motile sperm fractions were retrieved from the samples using a double wash in HAMS-F10 (Sigma-Aldrich, Cape Town, South Africa) medium (400 x g, 5 min) swim-up technique (3% Hams-bovine serum albumin, 37°C, 5% CO2). After 1 hour, the supernatant containing motile sperm was collected and divided into aliquots.

2.3. Experimental procedure
Human spermazoa were washed 3 times in PBS before fixed and permeabilized with 1:1 methanol/acetone fixative in an eppindorf on ice and incubated at 4°C for 10 minutes. The fixative was washed out 3 times by PBS. The sperm sample was layered on a slide and airdried for 20 minutes. The slide was rinsed with 1.5 mL PBS before addition of 10% donkey serum (100 μl) for 20 minutes at room temperature.

The serum was drained from the slide before the addition of GLUT8 goat polyclonal IgG primary antibody (100 μl; 1:200) and incubated for 90 minutes at room temperature. The slides were then carefully rinsed with PBS before the addition of donkey anti-goat IgG-Texas Red conjugated secondary antibody (100 μl; 1:200) and incubated for 30 minutes at room temperature in the dark. This was followed by the addition of 100 μl Hoechst (1:200) and incubated for 10 minutes. The slides were washed 3 times with PBS and mounted with DakoCytomation Fluorescent Mounting Medium. Fluorescence was detected by means of fluorescence microscopy.

3. RESULTS
Figure 1 shows the GLUT8 immunoreactivity with and without insulin stimulation in human spermatozoa. The blue colour represents the Hoechst staining whereas the red colour represents the GLUT8 Texas-Red conjugated secondary antibody immunofluorescence. There was no immunofluorescence detection of GLUT4 expression in the negative control cells observed under light and fluorescent microscope.

The cells which were stimulated with insulin produced an increased immunoreactivity when compared with the unstimulated cells. More GLUT8 immunoreactivity in the insulin stimulated cells was observed to be localized in the plasma membrane whereas that in the unstimulated cells was localized more in the intracellular region of the midpiece. GLUT8 immunoreactivity was also observed in the acrosome and midpiece regions of the insulin stimulated spermatozoa.

4. DISCUSSION
Studies have shown that glucose is necessary for sperm function, and it has to be metabolized by spermatozoa to ensure that tyrosine phosphorylation occurs during capacitation, zona pellucida penetration and sperm-oocyte fusion [18].
The process of sperm capacitation requires a significant amount of energy and glucose seems to be a major energy source needed to maintain in vitro capacitation in mice and human spermatozoa since this sugar has been shown to induce much higher penetration rates and capacitation-like changes than do other monosaccharides such as fructose or mannose [19]. GLUT4 is the most important and studied insulin-regulated transporter but it has not been detected in the testis or spermatozoa [15, 20, 21], therefore making GLUT8 the best candidate for insulin-stimulated glucose up-take in human spermatozoa. Studies have reported that GLUT8 expression in mouse testis first appears when round spermatids are formed, persists during spermiogenesis, and is present in spermatozoa isolated from the epididymis. However, it is not present in spermatogonia or spermatocytes [22]. Our study has found that GLUT8 is constitutively expressed in the mid-piece region of mature human spermatozoa (Figure 1B2). As far as we are aware, this is the first study to report an increase in expression of GLUT8 in the midpiece region when spermatozoa are stimulated with insulin as well as its translocation to the acrosomal region. It is not yet clear why GLUT8 translocates to the acrosomal region. Previously it has been reported that GLUT8 translocates from subcellular compartments to the cell membrane in insulin-treated blastocysts.

Figure 1: GLUT8 Texas-red conjugated immunofluorescence in human spermatozoa as visualized by light and fluorescence microscopy. Negative control observed under light microscope (A1), Negative control with Hoechst staining observed under fluorescent microscope (A2); Unstimulated spermatozoa observed under light microscope (B1); Unstimulated spermatozoa observed under fluorescent microscope showing the localization of GLUT8 in the midpiece region (B2); Insulin stimulated spermatozoa observed under light microscope (C1); Insulin stimulated spermatozoa observed under fluorescent microscope showing increase in GLUT8 localization in the midpiece region and its translocation to the acrosome region (C2).
We therefore speculate that insulin through PI3K activation, leads to PKB/Akt phosphorylation, which in turn activates GLUT8’s translocation and insertion into the cell membrane. This allows increased glucose uptake, thereby fueling glucose metabolism necessary for increased motility and the acrosome reaction.

5. REFERENCES


