BIOLOGY OF REPRODUCTION (2012) **87**(3):72, 1–4
Published online before print 22 August 2012.
DOI 10.1095/biolreprod.112.104109

Commentary

Sperm Bioenergetics in a Nutshell[1]

P.E. Visconti

Department of Veterinary and Animal Science, Integrated Sciences Building, University of Massachusetts, Amherst, Massachusetts

Introduction

After ejaculation, mammalian sperm acquire energy from nutrient molecules found in the seminal plasma and in the female reproductive tract environment. As in other animal cells, most of this energy is transformed into ATP and other high-energy compounds and used for biological work. Among different functions, ATP in sperm is used for movement, for fusion events during the acrosome reaction, and to transport ions and other molecules through membranes against concentration gradients. In addition, ATP is used in multiple metabolic reactions, including regulatory signaling pathways such as the production of cAMP and phosphorylation by protein kinases.

One of the problems in the quantitative study of ATP generation and use in sperm is that these cells are highly polarized. By a simple microscopic examination, two main regions are easily visualized, the head and the flagellum. Each of these regions can be subdivided in different compartments (Fig. 1A). The head is composed of the acrosome, the equatorial segment, and the postacrosomal region; the flagellum is compartmentalized into the midpiece, the principal piece, and a short terminal endpiece [1]. Regarding ATP formation, the midpiece concentrates the mitochondrial machinery, and the principal piece is enriched by all the enzymes needed for glycolysis. Figure 1 summarizes these pathways in sperm. More information about the compartmentalization of metabolic pathways in sperm can be obtained in recent reviews [2–4].

How Do Sperm Produce ATP?

In this issue of *Biology of Reproduction*, Goodson et al. [5] used a battery of glycolysable and nonglycolysable metabolic substrates to analyze the contribution of glycolysis and oxidative phosphorylation in ATP formation, sperm motility, hyperactivation, and the capacitation-associated increase in protein tyrosine phosphorylation. First, the authors demonstrated that in the absence of metabolic substrates, ATP is rapidly consumed and, consequently, the sperm become immotile and unable to hyperactivate. Glucose, mannose, fructose, and sorbitol in the glycolytic pathway sustained a high percentage of motile sperm and supported the increase in tyrosine phosphorylation. However, fructose and sorbitol did not support hyperactivation. On the other hand, the nonglycolysable substrates, pyruvate, lactate, and hydroxybutyrate, maintained motility with only low levels of tyrosine phosphorylation and hyperactivation. In the presence of citrate, most sperm became immotile after a 2-h incubation.

To further investigate these pathways, the authors disrupted either glycolytic or mitochondrial ATP production using α-chlorohydrin or carbonyl cyanide 3-chlorophenylhydrazone (CCCP), respectively, without affecting the alternative pathway [6]. α-Chlorohydrin blocked neither the percentage of motile cells nor ATP production when sperm were incubated in the presence of the nonglycolysable substrates pyruvate and hydroxybutyrate. However, these parameters were significantly reduced when glucose and fructose were used. To inhibit oxidative phosphorylation, the authors used CCCP, a compound that collapses the H^+ gradient responsible for ATP production by ATP synthase (Fig. 1D). As expected, this compound completely blocked motility and ATP production in sperm incubated with pyruvate and β-hydroxybutyrate, without affecting these parameters when either glucose or fructose was used. Interestingly, hyperactivation in the presence of fructose was up-regulated by CCCP (see below). Overall, these data indicate that although both glycolytic and mitochondrial oxidative phosphorylation are functional in mouse sperm, only glycolysis is capable of fully supporting tyrosine phosphorylation and hyperactivated motility. These data are consistent with findings reported by other authors [7, 8], and they highlight the conclusion that ATP produced in different compartments plays different roles. Although motility can be supported by both ATP sources, the ATP produced in the principal piece is more efficiently used for processes such as hyperactivation.

What Can We Learn from the Study of Goodson et al. [5]?

In previous studies, the same group used alternative genetic approaches to investigate similar questions by knocking out the sperm-specific glyceraldehyde phosphate dehydrogenase, spermatogenic (GAPDHS) and phosphoglycerate kinase 2 (PGK2). In both cases, mice null for these enzymes were almost completely infertile [9, 10]. Considering that mitochondrial pathways are active in mouse sperm and that GAPDHS only participates in the glycolytic pathway, it is surprising that sperm from these mice are not capable of using pyruvate or lactate present in the incubation media to support ATP synthesis and motility. Despite these findings, GAPDHS-null sperm consume oxygen at wild-type levels, suggesting that the citric acid cycle (Fig. 1C) is functional. These data suggest that in addition to the expected problems in glycolysis, sperm from

[1]Supported by National Institutes of Health grants R01 HD44044 and HD038082 (to P.E.V.).
[2]Correspondence: E-mail: pvisconti@vasci.umass.edu

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

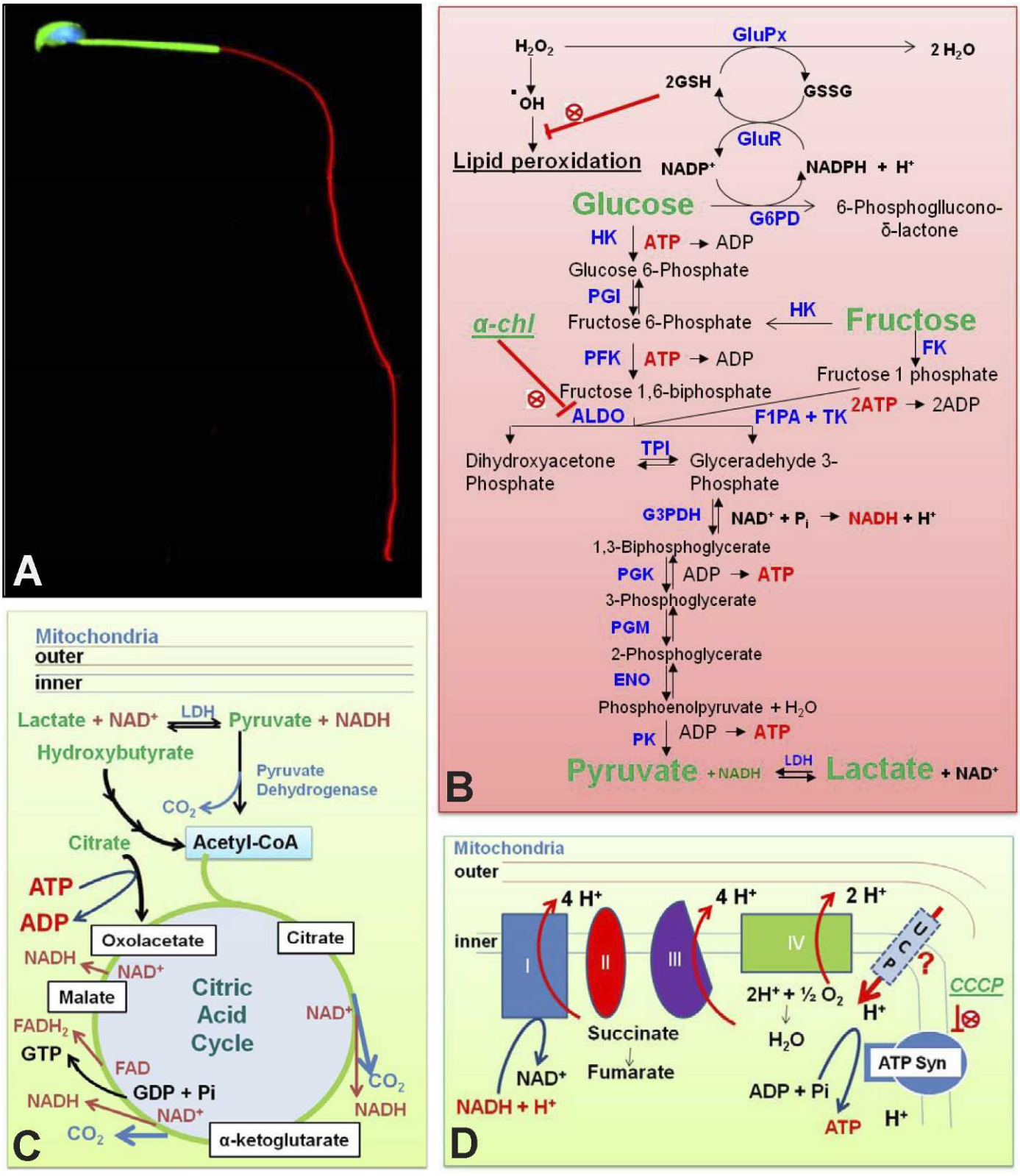

FIG. 1. Diagram of bioenergetics pathways in sperm. **A**) Mouse sperm was stained with anti-tubulin antibody (red), MitoTracker and peanut agglutinin (PNA) (green), and Hoechst (blue). Because of the strong MitoTracker signal in the midpiece, the tubulin was only observed in the principal piece. Original magnification ×60. **B**) Glycolysis. It is well established that all the enzymes for this process are found in the sperm principal piece. Interestingly, most of the steps in this process are catalyzed by sperm-specific isoforms of the respective proteins. During glycolysis, glucose is oxidized to pyruvate with a final net gain of 2 ATP and 1 e^- in the form of NADH. Fructose enters the glycolytic pathway after phosphorylation by hexokinase (HK) or, alternatively, through the Hers pathway initiated by fructokinase. Alpha-chlorohydrin has been shown to block sperm glycolysis [6]. The final product of glycolysis is pyruvate, which is used to regenerate NAD^+ from NADH in a coupled reaction in which this metabolite is converted into lactate by LDH. The NAD^+ regeneration is essential for continuing glycolysis. Note that in the upper section of **A**, the alternative use of glucose by the first enzyme of the pentose phosphate pathway is presented. Glucose is oxidized to 6-phosphoglucono-δ-lactone with reduction of $NADP^+$ to NADPH. In turn, NADPH can be used by glutathione

these mice have defects in coupling H^+ gradients to ATP production by ATP synthase (Fig. 1D). Although the presence of members of the uncouple protein (UCP) family have not been described in sperm, it is predicted that if present, up-regulation of their activity will be consistent with the lack of ATP production in sperm from these mice. Alternatively, the low ATP levels could be due to their consumption in the first half of glycolysis [4].

Pyruvate, lactate, and hydroxybutyrate were all able to sustain high ATP levels and sperm motility, but citrate was not. Despite being part of the citric acid cycle, citrate is not permeable to mitochondria. It needs to be converted to malate with the use of ATP to be able to enter the citric acid cycle. The use of citrate is therefore less efficient than the use of other nonglycolysable substrates. Whether this is the cause for the observed decrease in sperm motility and ATP production has not been defined. Alternatively, the enzymes responsible for the conversion of citrate to malate and pyruvate outside the mitochondria might not be present in mouse sperm, in which case this metabolite would not be able to enter the citric acid cycle.

The differential effects of fructose and glucose in the promotion of hyperactivation are also noteworthy. Both of these glycolysable compounds can serve as hexokinase substrates to form the respective hexose-6 phosphate; in addition, both can follow alternative metabolic pathways (Fig. 1B). Fructose can be phosphorylated by fructokinase to fructose 1-phosphate, which in turn can be converted to glyceraldehyde 3-phosphate through the Hers pathway and reenter glycolysis. On the other hand, glucose could be oxidized to 6-phosphogluconate by glucose 6-phosphate dehydrogenase in a reaction coupled to the formation of NADPH, and NADPH can then be coupled to a reaction with oxidized glutathione (GSSG) to form reduced glutathione (GSH) (Fig. 1B). Because GSH can react with peroxides, it can consequently decrease deleterious lipid peroxidation [4]. Therefore, through this alternative pathway, glucose offers protection for oxygen radicals and facilitates hyperactivation, whereas fructose does not. Goodson et al. [5] discuss this possibility and present results consistent with this hypothesis. The authors demonstrate that in the presence of fructose, sperm produced significantly higher levels of oxygen radicals than in the presence of glucose.

In many cell types, glycolysis is coupled to the citric acid cycle. In this way, the NAD^+ needed for glycolysis is recovered through oxidative phosphorylation. In part, the coupling between both processes is mediated by the affinity of the N-terminal domain of hexokinase (porin-binding domain) to mitochondrial porin. In sperm, the somatic hexokinase is not present; it is replaced by three differentially spliced variants that do not have the porin-binding region [11]. It is therefore not surprising that in these cells, glycolysis and the citric acid cycle function independently. It is also well established that the sperm-specific lactate dehydrogenase, LDH-C4, is essential for sperm function [12]. Mice lacking this enzyme are almost completely infertile both in vivo and in vitro. The rationale for this phenotype is that, similar to the case for anaerobic glycolysis, NAD^+ needs to be recycled to be used in the GAPDHS reaction. Although sperm from these mice have a reduced level of ATP, the level is not as low as in the case of the GAPDHS-null sperm. The role of LDH-C4 in glycolysis is further suggested by the finding that LDH-C4-null sperm are unable to consume glucose. However, these sperm remain capable of forming lactate from pyruvate, indicating that other LDH homologues (e.g., LDHA) are present in these cells. Interestingly, overexpression of LDHA in sperm is able to rescue the LDH-C4-null phenotype [12].

Conclusions

Sperm are capable of producing ATP by glycolysis and by oxidative phosphorylation; however, the glycolytic pathway is uniquely compartmentalized in the principal piece to allow the changes in motility pattern known as hyperactivation. The relevance of the study by Goodson et al. [5] is given by the thorough evaluation of the role of glycolysis and oxidative phosphorylation in several sperm functional parameters. That paper also provides strong evidence for the interconnection between sperm metabolism and other signaling pathways (e.g., tyrosine phosphorylation and oxygen radical formation). The study by Goodson et al. was conducted using the mouse as the experimental model; similar studies in sperm from other mammals might reveal species-specific differences, as is the case for bull sperm [4]. In addition, it will be important to determine where the ATP used for fusion and ion-channel regulation during the acrosome reaction in the sperm head is, in fact, produced. These questions, together with those mentioned above, warrant future studies in the field of sperm bioenergetics.

ACKNOWLEDGMENT

I thank Dr. Ana Maria Salicioni for critical reading of this manuscript and Dr. Julian Sosnik for Figure 1A.

REFERENCES

1. Inaba K. Sperm flagella: comparative and phylogenetic perspectives of protein components. Mol Hum Reprod 2011; 17:524–538.

reductase (GluR) to form GSH, and in the presence of glutathione peroxidase, GSH can reduce H_2O_2 and block oxidative damage of lipids, DNA, and proteins. **C**) Citric acid cycle. Pyruvate can enter the mitochondria through specific transporters. Once in the mitochondria, it can be oxidized to acetyl-coenzyme A (CoA) and CO_2. Acetyl-CoA then enters the citric acid cycle, which generates one high-energy bond in the form of GTP, four reduced compounds (3 NADH and 1 FADH2), to be used in the oxidative phosphorylation process taking place also in the mitochondria. The ketone body hydroxybutyrate can originate two acetyl-CoA molecules through a series of intermediate reactions. Regarding citrate, when it is obtained from oxaloacetate inside the mitochondria as part of the citric acid cycle, exogenous citrate (in green) cannot permeate the inner mitochondrial membrane. Thus, before entering the cycle, it should be first converted to malate and to pyruvate by a series of enzymes with the use of ATP. In addition, citrate is an allosteric inhibitor of glycolysis at the level of PFK enzyme (see **A**). The electrons conserved in NADH and FADH2 are then used to reduce oxygen in the oxidative phosphorylation process. **D**) Oxidative phosphorylation. Through a series of redox reactions catalyzed by protein complexes (I–IV), NADH is used to donate electrons to oxygen. For each pair of electrons transferred to O_2, 10 H^+ are pumped out through the inner mitochondrial membrane. The H^+ gradient formed favors reuptake of H^+ through the ATP synthase complex, which uses the proton-motive energy to generate ATP. Certain conditions and reagents can uncouple oxygen reduction from ATP synthesis. For example, the electron gradient can be dissipated through UCPs, as in brown adipose tissue. Also, weak acids such as CCCP can readily enter the inner mitochondrial membrane in the protonated form and collapse the H^+ gradient blocking the production of ATP. For more information on the pathways, see Nelson and Cox [13]. α-chl, α-chlorohydrin; ALDO, aldolase; ATPSyn, ATP synthase; ENO, enolase; F1PA, fructose 1-phosphate aldolase; FK, fructokinase; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; GluPx, glutathion peroxidase; PFK, phosphofructokinase; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglyceromutase; PK, pyruvate kinase; TK, triose kinase; TPI, triose phosphate isomerase.

2. Miki K. Energy metabolism and sperm function. Soc Reprod Fertil Suppl 2007; 65:309–325.
3. Mukai C, Travis A. What sperm can teach us about energy production. Reprod Domest Anim 2012; 47(suppl 4):164–169.
4. Storey BT. Mammalian sperm metabolism: oxygen and sugar, friend and foe. Int J Dev Biol 2008; 52:427–437.
5. Goodson SG, Qiu Y, Sutton KA, Xie G, Jia W, O'Brien DA. Metabolic substrates exhibit differential effects on functional parameters of mouse sperm capacitation. Biol Reprod 2012; 87(3):75, 1–15.
6. Bone W, Jones AR, Morin C, Nieschlag E, Cooper TG. Susceptibility of glycolytic enzyme activity and motility of spermatozoa from rat, mouse, and human to inhibition by proven and putative chlorinated antifertility compounds in vitro. J Androl 2001; 22:464–470.
7. Travis AJ, Jorgez CJ, Merdiushev T, Jones BH, Dess DM, Diaz-Cueto L, Storey BT, Kopf GS, Moss SB. Functional relationships between capacitation-dependent cell signaling and compartmentalized metabolic pathways in murine spermatozoa. J Biol Chem 2001; 276:7630–7636.
8. Mukai C, Okuno M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 2004; 71:540–547.
9. Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, Perreault SD, Eddy EM, O'Brien DA. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A 2004; 101: 16501–16506.
10. Danshina PV, Geyer CB, Dai Q, Goulding EH, Willis WD, Kitto GB, McCarrey JR, Eddy EM, O'Brien DA. Phosphoglycerate kinase 2 (PGK2) is essential for sperm function and male fertility in mice. Biol Reprod 2010; 82:136–145.
11. Mori C, Nakamura N, Welch JE, Shiota K, Eddy EM. Testis-specific expression of mRNAs for a unique human type 1 hexokinase lacking the porin-binding domain. Mol Reprod Dev 1996; 44:14–22.
12. Odet F, Duan C, Willis WD, Goulding EH, Kung A, Eddy EM, Goldberg E. Expression of the gene for mouse lactate dehydrogenase C (*Ldhc*) is required for male fertility, Biol Reprod 2008; 79:26–34.
13. Nelson D, Cox M. Lehninger Principles of Biochemistry, 5th ed. New York: W.H. Freeman and Company; 2008.