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At the Cutting Edge

Arguments raised by the recent discovery that insulin and leptin are expressed in and secreted by human ejaculated spermatozoa

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Abstract

The recent findings demonstrating that insulin and leptin are expressed in and secreted by human ejaculated spermatozoa raise the controversial issue related to mRNA function in male gamete. Capacitated sperm display an increased metabolism and overall energy expenditure presumably to affect the changes in sperm signaling and function during capacitation. However the relationship between the signaling events associated with capacitation and the change in sperm metabolism energy is poorly understood. It emerges from the findings here reported that both leptin and insulin may be crucial in ejaculated spermatozoa to manage their energy status. Immunoistochemical analysis revealed that in uncapacitated sperm insulin was located at the subacrosomial level, in the midpiece and through the tail while leptin was immunodetected at the equatorial segment and at the midpiece. Capacitated sperm display an overall decrease and a more uniform distribution in the signal for both hormones and this is in agreement with their enhanced release in the medium.

Both hormones in ejaculated sperm somehow recapitulate the cross-talk between their signalling transductional pathways in somatic cells, resulting in the increase of phosphoinositide 3-kinase (PI3K) activity, AKT S473 and Glycogen synthase kinase 3 (GSK-3)-S9 phosphorylations. During capacitation GSK-3 phosphorylation was abolished suggesting how in capacitating sperm there is a block in glycogen synthesis. This reasonably indicates how during capacitation glycogen reserve is mobilized and this makes the glucose as energy substrate available. For instance insulin dismissed by ejaculated spermatozoa up-regulates Glucose 6-Phosphate Dehydrogenase (G6PDH), the rate-limiting enzyme in the pentose phosphate pathway (PPP), which has be shown to be crucial in the acquisition of fertilizing capability as well as to mediate gamete fusion. Insulin immunoneutralization or blockage of its release, dramatically down regulated G6PDH. Interestingly, in the presence of a disruptor of insulin signaling wortmannin, an inhibitor of PI3K, the intrinsic activity of G6PDH drops. Leptin appears to play similar action to that of insulin on G6PDH in sperm (data in progress). The enhanced activity of this enzyme induced by both hormones produces an increase of NADPH that is essential for fatty acid synthesis from acetyl CoA. These fatty acids have two possible fates: β-oxidation to produce ATP or reesterification back into triacylglycerol. Inter-relationships of the classes of substrates of free fatty acids (FFA) and glucose utilized for energy, has been long established [Randle, P.J., 1964. The interrelationships of hormones, fatty acid and glucose in the provision of energy. Postgrad. Med. J. 40, 457–463]. The authors observed in ejaculated spermatozoa what it occurs in somatic cells: FFA β-oxidation tested utilizing the octanoil-CoA as substrate, appears to be stimulated by leptin and down-regulated by the contemporaneous presence of insulin in uncapacitated sperms. FFA β-oxidation activity dramatically increases when capacitation starts, so it may be assumed the possibility that leptin may work to stimulate such enzymatic activity providing additional metabolic fuel to triggering capacitation process. The autonomous capability of sperm to release insulin and leptin suggests that they through an autocrine short loop may provide the recruitment of energy substrate according to sperm metabolic needs. This occurs independently by the systemic regulation and may represent a protective mechanism which preserves sperm fertilizing capability by any detrimental effects produced by long calorie restriction or by alterations occurring in the energy homeostasis at systemic level.

Keywords: Insulin; Leptin; Human sperm; Lipid metabolism; Glucose metabolism; Energy homeostasis

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Contents

1. About the controversy of mRNAs in ejaculated spermatozoa

The recent discovery that the ejaculated spermatozoa contain both insulin and leptin transcripts as well as proteins, raises the still controversial issue related to the existence of transcriptional activity and latent translation in male gamete. Although it is difficult to reconcile the latter finding in the virtual absence of 28 S and 18 S ribosomal RNAs (Ostermeier et al., 2002) in these cells. About the first issue it deserves to be pointed out how the DNA packaging within the human sperm nucleus appears to be unique because about 15% of chromatin remains bound to somatic type histones (Tanphaichitr et al., 1978; Gatewood et al., 1987). Therefore it is tempting to speculate that DNA regions linked to somatic type histone in sperm head may represent site of active transcription, preparing the paternal genome for providing specific transcripts into fertilized oocyte before zygote genomic activation (Wykes et al., 1997; Dadoune et al., 2004, 2005). For instance some of thousands of cDNAs that have been detected in ejaculated spermatozoa encode proteins known to participate in fertilization and embryonic development (Ostermeier et al., 2004). These sperm-specific transcripts are absent in the oocyte, indicating that spermatozoa provide novel and specific transcripts to the fertilized oocytes (Ostermeier et al., 2004). Supporting this view, the identified transcripts associated with fertilization, embryogenesis and morphogenesis have been found to be present in embryos that have failed at in vitro fertilization (Ostermeier et al., 2002).

Additionally, in order to explain the substantial store of RNAs still present in the sperm, it remains to be fully explored whether the identified mRNAs may have specific interaction at 3′UTR with cytoplasmatic proteins involved in the regulation of translation, degradation and polyadenylation of mRNA, similar to that demonstrated for transition protein and protamin up to the stage of elongated spermatids (Steger, 1999). The binding of these repressors proteins to nucleic acids depends on phosphorylation (Ceman et al., 2003; Kwon and Hecht, 1991, 1993; Kwon et al., 1993; Ladomery and Sommerville, 1994). Dephosphorylation may be one means to release these proteins from mRNAs thereby activating the stored transcripts and allowing translation.

On the latter scenario lays the second issue which warrants particular relevance when the potential translated proteins appear to be already available tools to sustain spermatozoa survival against the adverse environmental conditions encountered during their journey in the genital tracts. For instance, human spermatozoa produce mRNA and protein of antimicrobial factors (defensins) addressing the evidence of an important innate organ defense system (Com et al., 2003) to be properly exploited against pathogens. In the same vein, several transcripts and proteins appears to sustain the capability of sperm to respond to female environment when they move within the female tract, particularly to estrogen (Aquila et al., 2004) and aromatizable androgens (Aquila et al., 2002) that are known to increase the metabolic and flagellar activity of spermatozoa (Aquila et al., 2003) as well as their ability to penetrate ovocite (Adeoya-Osiguwa et al., 2003).

Despite the fact the above reported findings have covered for quite a while following the first visualization and quantization of mRNA molecules in ejaculated sperm (Pessot et al., 1989; Dadoune et al., 1991) and the first identification of mRNA through the localization of c-myc mRNA (Kumar et al., 1993), it still persists the opinion to consider mRNA in human ejaculated spermatozoa somehow remnant of stored mRNA from post-meiotically transcribed genes. This assumption lays on the view of the majority of biologists of reproduction to consider the sperm as a tool for transporting the paternal genome to the waiting egg and nothing more (Miller et al., 1999, 2005; Miller, 2000). All these matters raise again when mRNA transcripts coexist with codified proteins involved in cell signaling producing rapid change in sperm energy and metabolic rate as it occurs during capacitation. This is the case of insulin and leptin both expressed in and secreted by human spermatozoa as we recently discovered (Aquila et al., 2005a,b).

2. Are insulin and leptin important modulators of sperm energetic metabolism independently of the systemic regulation?

The existence of both hormones in male gamete has been demonstrated through their transcripts evaluated by RT-PCR, their protein content evidenced by Western blotting and through their localization by immunostaining analysis (Aquila et al., 2005a,b). A potential role for leptin in influencing sperm fertilizing capability raises by the evidence that the ob/ob mice (lacking of functional leptin) or OB-R/OB-R mice (lacking of functional leptin receptor) are infertile and fail to undergo normal sexual maturation. Importantly, fertility of ob/ob mice is restored by leptin and not by simply reducing body weight, indicating an effect of the hormone per sè on reproductive function (Hileman et al., 2002; Mounzih et al., 1997). As it concerns insulin, it may be evidenced that in men affected by insulin dependent diabetes, sperm do exhibit severe structural defects, significantly lower motility (Baccetti et al., 2002), and lower ability to penetrate hamster eggs (Shrivastav et al., 1989).

Immunocytochemical analysis by immunofluorescence of insulin showed an heterogeneous expression pattern, in unca-