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A New Role for Pancreatic Insulin in the Male Reproductive Axis

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The recent discovery that insulin is expressed by the rat testis (1) raises questions about whether and how locally produced insulin regulates testicular function. It also raises the question whether pancreatic insulin regulates testicular function, and whether fertility might be impaired as a consequence of insulin deficiency in type 1 diabetes. As reported in this issue of *Diabetes*, Schoeller et al. (2) have found that pancreatic insulin regulates the male hypothalamic-pituitary-gonadal axis and is essential for fertility. In contrast, insulin produced in the testes may not be essential for testicular function. The article by Schoeller et al. provides an important advance toward understanding why fertility may be diminished in men with type 1 diabetes.

It has only recently been recognized that diabetes can reduce sperm quality and that the female partners of diabetic men have lower pregnancy rates (3,4). It is not well understood whether hyperglycemia or abnormal insulin signaling is responsible, and what stage(s) of spermatogenesis is affected. Increased nuclear and mitochondrial DNA damage in sperm samples from diabetic men suggests that hyperglycemia-induced oxidative stress may be responsible (3,5). However, it is also possible that the adverse effects of diabetes may be due to abnormal insulin signaling in the testis, systemic effects of insulin, or both, and may be separate from insulin's effects on blood glucose levels.

Schoeller et al. (2) have taken advantage of the Akita mouse model of insulin-deficient diabetes to cleverly sort out these possibilities. The dominant Akita phenotype is caused by a mutation in the *Ins2* allele. The resulting misfolded protein product causes endoplasmic reticulum (ER) stress, leading to pancreatic β-cell death (6). Akita mice develop hyperglycemia, although the age at onset and severity of hyperglycemia depends on the sex, strain background, and whether the mice are heterozygous or homozygous for the mutant *Ins2* allele (M.R.L., personal observations; Schoeller et al. [2]). Notably, whereas primates carry only one insulin gene, rodents carry two functional insulin genes, *Ins1* and *Ins2*. *Ins1* arose from a duplication of the ancestral *Ins2* gene approximately 20 million years ago (7); therefore, the mouse *Ins2* gene is orthologous to the human insulin gene. Thus, if *Ins2* is expressed in the mouse testis, it is very likely that the human testis expresses insulin as well. The beauty of using the Akita model is that it has the potential to distinguish between the roles of testicular and pancreatic insulin on male reproductive function. This is not possible with other models, such as streptozotocin-induced diabetes or the NOD mouse, in which testicular insulin production would not be interrupted.

The Moley laboratory previously observed that sperm from homozygous Akita males fertilizes fewer oocytes and that the resulting blastocysts are developmentally impaired (8). In this study (2), they used males that were either heterozygous or homozygous for the Akita allele; heterozygous Akita males develop hyperglycemia (>300 mg/dL) by 5 weeks of age, whereas homozygous Akita males develop hyperglycemia by 3 weeks of age (i.e., prior to puberty) and die by 8–12 weeks of age unless treated with insulin. Fertility diminishes by 4–6 months of age in heterozygous Akita males, whereas homozygous Akita males are completely infertile. RT-PCR demonstrated that, unlike the pancreas in which both *Ins1* and *Ins2* are transcribed, only *Ins2* was transcribed in the testis. Immunolocalization showed that insulin was detected predominantly in Sertoli cells. However, there was no associated ER stress. Thus, while the resulting Ins2 protein may be hypomorphic or nonfunctional in the testis, infertility in homozygous Akita males does not appear to be due to ER stress-induced apoptosis of testicular cells.

Although treatment of the homozygous Akita males with exogenous insulin using subcutaneous insulin implants restored spermatogenesis and fertility, this was not due to restoration of insulin within the testes because insulin did not cross the blood-testis barrier. There could be effects on Leydig cells, which contain insulin receptors and are located outside the blood-testis barrier (9). Therefore, they would presumably not be responsive to insulin produced by Sertoli cells. However, because circulating levels of luteinizing hormone and testosterone, which were significantly reduced in Akita homozygotes, were restored by insulin treatment, the primary effects of exogenous insulin appear to be on the hypothalamic-pituitary axis.

This study by Schoeller et al. (2) is of clinical relevance to men with type 1 diabetes because it demonstrates that pancreatic insulin is crucial for the male reproductive axis. The infertility in homozygous Akita males appears to be due to insulin deficiency, not hyperglycemia. This is because heterozygous Akita males become as severely hyperglycemic as the homozygotes at only a slightly older age, and they are fertile at least until they are 4–6 months of age. On the other hand, it is possible that severe hyperglycemia before puberty interferes with the function of the hypothalamic-pituitary-testicular axis, whereas hyperglycemia occurring during or after puberty only interferes with the function of the reproductive axis after several

months of chronic exposure. However, the ability to restore fertility in homozygous Akita males with exogenous insulin suggests that if prepubescent hyperglycemia interferes with the function of the reproductive axis, it is not irreversible.

This article (2) opens the door to future studies that aim to understand how insulin regulates the male reproductive axis, and whether insulin is regulating the pituitary, the hypothalamus, or higher central nervous system nuclei. Knockout of the insulin receptor gene in the central nervous system impairs luteinizing hormone production and spermatogenesis (10). There was no significant difference in follicle-stimulating hormone levels between wild-type, heterozygous, and homozygous Akita males, suggesting that the effects of insulin are on the hypothalamus or on the responsiveness of the pituitary gonadotropes to the hypothalamic gonadotropin–releasing hormone.

It should be noted that insulin has long been recognized to play a role in the ovary. Insulin is detectable in ovarian follicular fluid and synergizes with gonadotropins for oogenesis, ovulation, and luteinization (11,12). Because the ovarian follicle is permeable to circulating hormones, it has been thought that follicular fluid insulin is derived from the pancreas. However, in light of this current study, whether insulin (and *Ins2* in particular) is expressed by ovarian cells ought to be examined. Although this study did not find an essential role for testicular insulin, additional experimentation, perhaps with testis-specific *Ins1* or *Ins2* knockout or transgenic strains, is necessary to further understand the function of testis-derived insulin. It will also be important to study the effects of insulin and insulin deficiency that are conserved, as well as those that are distinct, between the hypothalamic-pituitary-gonadal axes of females and males.

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