You Are What Your Dad Ate

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Maternal nutrition and metabolism are critical determinants of adult offspring health. Recent reports describe adverse offspring outcomes associated with the *father's* diet, indicating nongenetic inheritance of paternal experience. Determining underlying mechanisms may require reconsideration of our understanding of the heritability of epigenetic states.

We are in the midst of unparalleled epidemics of obesity and type 2 diabetes, complex phenotypes originating at the intersection of genetic and environmental risk. The environmental component of this may initiate during prenatal life (Gluckman et al., 2008). For example, exposure of the developing fetus to maternal undernutrition or obesity increases risk of obesity and diabetes during adult life. Several lines of evidence suggest epigenetic mechanisms underlie developmentally mediated phenotypes. First, offspring exposed to environmental stressors during early life have sustained alterations in cellular function and transcriptional regulation, suggesting developmental or epigenetic mechanisms that correlate with alterations in DNA methylation and/or histone marks in rodent models (Park et al., 2008). Second, metabolic risk can be "transmitted" to subsequent generations even in the absence of further environmental stressors. For example, nutrition during intrauterine life and childhood influences risk for diabetes and cardiovascular disease in both children and grandchildren (Pembrey et al., 2006).

Other mechanisms may contribute to intergenerational transmission. Maternal transmission can arise from inheritance of both nuclear and mitochondrial DNA, effects of maternal metabolism (e.g., diabetes or obesity), or epigenetic mechanisms mediated by female germ cells. The beauty of paternal transmission models is that sperm transmit solely genetic and epigenetic factors (and possibly small noncoding RNAs), allowing epigenetic hypotheses to be cleanly tested experimentally.

Two recent publications (Ng et al., 2010; Carone et al., 2010), together with a prior publication (Jimenez-Chillaron et al., 2009), provide experimental evidence for nongenetic, intergenerational, paternal transmission of metabolic phenotypes to offspring. In their recent *Nature* study, Ng and colleagues bred control female rats with males with high-fat-diet-induced obesity (Figure 1). Female offspring of these obese males had glucose intolerance, reduced insulin secretion, and altered pancreatic islet gene expression, potentially linked to reduced methylation near *IL13ra2*. Carone and colleagues also demonstrate that paternal diet can modulate offspring metabolism, gene expression, and epigenetic marks (Figure 1). In this study, male mice were fed a reduced-protein or chow diet and bred with chow-fed females; both male and female offspring of low-protein males demonstrated increased hepatic expression of lipid and cholesterol synthesis genes. The authors asked whether expression patterns were associated with altered epigenetic modifications. Overall DNA methylation in offspring was unchanged; however, modestly increased methylation was observed in an intergenic CpG island between *PPARα* and *Wnt7b*. Interestingly, differential methylation was previously observed at the *PPARα* promoter in offspring of female rats fed a low-protein diet during pregnancy (Lillycrop et al., 2008). Whether site-specific changes in methylation at this locus are functional is an important avenue for future studies. Alternatively, differences in developmental trajectories or systemic metabolism in low-protein offspring could also contribute secondarily to expression patterns. Prior mouse studies from our laboratories (Jimenez-Chillaron et al., 2009) also demonstrated that male mice with a history of intrauterine exposure to maternal undernutrition could influence metabolism in their offspring (Figure 1). Together, these data independently and strongly support that the dietary or metabolic history of males affects metabolism in offspring, even in cases of normal diet at breeding. These phenotypes are likely mediated by sperm, potentially via epigenetic marks in germ cells.

For epigenetic perturbations in sperm to be transmitted to offspring and influence adult phenotypes, they must first encounter developmentally important epigenetic reprogramming events immediately postfertilization in preimplantation embryos. For example, the paternally inherited genome undergoes dramatic, genome-wide, replication-independent DNA demethylation (Morgan et al., 2005). An environmentally induced change in epigenetic state present in sperm must either survive this process or influence it in a lasting manner. There is precedent for resistance to genome-wide epigenetic reprogramming at regions regulating genomic imprinting (Edwards and Ferguson-Smith, 2007); imprinting is controlled by differential DNA methylation on male and female genomes acquired during gametogenesis and subsequently maintained. We do not know the extent of "genome-wide" demethylation in preimplantation embryos. Like imprints, there may be regions that can inherit and maintain heritable DNA methylation from the male germline, and these may be particularly vulnerable to paternal environmental compromise. Carone and colleagues indicate that imprinted genes are not significantly perturbed in offspring of low-protein fathers.

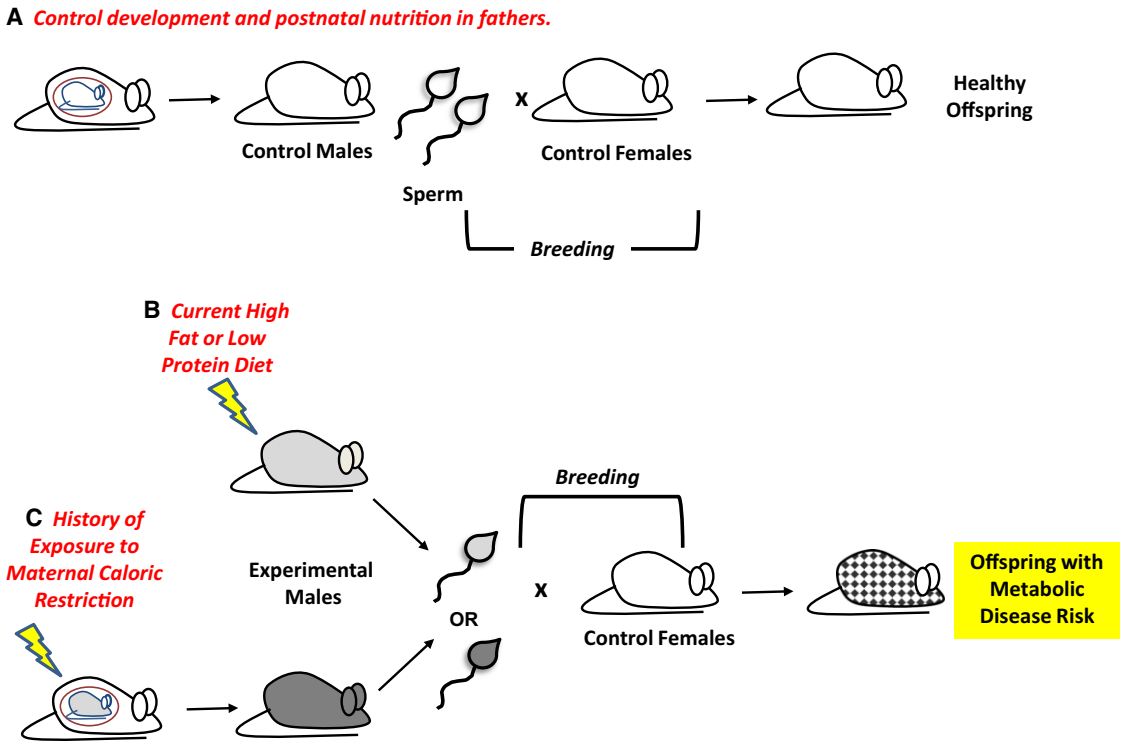

Figure 1. Metabolic Risk Can Be Conferred via the Paternal Lineage
(A) Males with normal developmental history and postnatal nutrition yield healthy offspring.
(B and C) Alterations in current paternal diet, including high-fat or low-protein diets (B), or prior history of intrauterine exposure to maternal caloric restriction, even with normal postnatal nutrition (C), can result in increased metabolic risk in offspring. Despite different stages of exposure (in utero, influencing primordial germ cells, or postweaning, influencing the spermatogonial and subsequent stages) such paternal-lineage risk must be conferred via sperm, potentially via alterations in DNA methylation, chromatin properties, or small noncoding RNAs. In turn, alterations in gene expression and metabolic risk in offspring indicate either the possible persistence of epigenetic marks or effects on early postimplantation embryos, modulating developmental trajectories.

Perhaps of greater importance, they did not find global alterations in sperm methylation in low-protein-fed mice. It remains to be seen whether more sensitive assays of sperm methylation will uncover key differences and/or whether other mechanisms may contribute to nongenetic paternal inheritance.

The influence of paternal diet on offspring metabolism could also be mediated posttranscriptionally via the action of small RNAs such as microRNAs. MicroRNA levels were not measured in sperm by Carone et al., and some differences in histone marks and RNA expression were observed, suggesting that paternal diet or metabolism alters chromatin modulation. For example, some transcription factors and chromatin remodelers were found to be downregulated. Since chromatin organization and the sperm epigenome are important for postfertilization developmental regulation (Hammoud et al., 2009), perturbations in establishment of normal chromatin properties might contribute to heritable abnormalities. Finally, sperm production and fertility may be altered by nutritional modification, so an influence on spermatogonia and/or semen cannot be excluded.

In distinct nutritional models of paternal inheritance of metabolic phenotype (Figure 1), the developmental stage of male germ cell exposure differs, yet both influence offspring metabolic outcomes. It will be interesting to compare developmental expression and epigenetic correlates in these models to determine whether common mechanisms are responsible and to determine specific nutritional components contributing to paternally inherited metabolic phenotypes. The described models are examples of paternal germline effects rather than transgenerational effects. A true transgenerational effect would be manifest in offspring from sperm never exposed to dietary modification; it remains to be determined whether paternal grandchildren of low-protein-diet males also exhibit and transmit metabolic phenotypes. If so, the effect would have to survive genome-wide epigenetic reprogramming both in embryonic primordial germ cells (Morgan et al., 2005) and postfertilization. Regardless, these experimental models of metabolic disease tell us that the environmental burden on offspring phenotype is not only just maternal territory: the father's nutritional and metabolic status certainly merits our attention, too, if we are to optimize health of his children and grandchildren.

REFERENCES

Carone, B.R., Fauquier, L., Habib, N., Shea, J.M., Hart, C.E., Li, R., Bock, C., Li, C., Gu, H., Zamore, P.D., et al. (2010). Cell *143*, 1084–1096.

Edwards, C.A., and Ferguson-Smith, A.C. (2007). Curr. Opin. Cell Biol. *19*, 281–289.

Gluckman, P.D., Hanson, M.A., Cooper, C., and Thornburg, K.L. (2008). N. Engl. J. Med. *359*, 61–73.

Hammoud, S.S., Nix, D.A., Zhang, H., Purwar, J., Carrell, D.T., and Cairns, B.R. (2009). Nature *460*, 473–478.

Jimenez-Chillaron, J.C., Isganaitis, E., Charalambous, M., Gesta, S., Pentinat-Pelegrin, T., Faucette, R.R., Otis, J.P., Chow, A., Diaz, R., Ferguson-Smith, A., and Patti, M.E. (2009). Diabetes *58*, 460–468.

Lillycrop, K.A., Phillips, E.S., Torrens, C., Hanson, M.A., Jackson, A.A., and Burdge, G.C. (2008). Br. J. Nutr. *100*, 278–282.

Morgan, H.D., Santos, F., Green, K., Dean, W., and Reik, W. (2005). Hum. Mol. Genet. *14* (Spec No 1), R47–R58.

Ng, S.F., Lin, R.C., Laybutt, D.R., Barres, R., Owens, J.A., and Morris, M.J. (2010). Nature *467*, 963–966.

Park, J.H., Stoffers, D.A., Nicholls, R.D., and Simmons, R.A. (2008). J. Clin. Invest. *118*, 2316–2324.

Pembrey, M.E., Bygren, L.O., Kaati, G., Edvinsson, S., Northstone, K., Sjostrom, M., Golding, J., and ALSPAC Study Team. (2006). Eur. J. Hum. Genetics *14*, 159–166.

Can Dietary Nitrates Enhance the Efficiency of Mitochondria?

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A decline in mitochondrial function occurs in many conditions. A report in this issue of *Cell Metabolism* (Larsen et al., 2011) shows that dietary inorganic nitrates enhance muscle mitochondrial efficiency. It is an attractive hypothesis that dietary changes enhance energy efficiency, but its potential application depends on long-term studies investigating net benefits versus adverse effects.

Mitochondrial are critical for oxidizing fuels (i.e., glucose, fatty acids, amino acids) and converting them to ATP, the chemical energy required for cellular functions. A vast body of literature demonstrates that mitochondria are responsive to a variety of environmental stimuli. For example, they are responsive to aerobic exercise (Holloszy and Coyle, 1984), insulin and amino acid infusion (Stump et al., 2003), and thyroid hormones (Short et al., 2007). In this issue of *Cell Metabolism*, Larsen et al. (2011) add to this body of literature by demonstrating that several facets of skeletal mitochondrial physiology are influenced by dietary intake of inorganic nitrates.

Larsen and coworkers present the findings from a placebo-controlled, crossover study of nitrate supplementation in healthy humans. Dietary inorganic nitrate increased the capacity for ATP synthesis in mitochondria isolated from muscle biopsy tissue. This increase in ATP production capacity appears to occur in the absence of any increase in mitochondrial content. Although long-term studies are needed to rule out the influence of nitrates on mitochondrial biogenesis, these observations suggest that nitrates enhance mitochondrial coupling efficiency (Larsen et al., 2011). Mitochondrial efficiency is determined by a variety of factors such as the macronutrient source (e.g., glucose versus free-fatty acids) of electron flow into the cytochrome chain and various ways in which the transmembrane proton gradient is dissipated or uncoupled, liberating potential energy rather than coupling it to ATP synthesis (Figure 1). The influence of nitrates on mitochondrial coupling was thoroughly examined using high-resolution respirometry in isolated mitochondria. With nitrate supplementation, mitochondrial proton leak was reduced and P:O ratio was increased, supporting the notion that nitrates decrease energy "wastage," effectively increasing the amount of ATP generated per unit of oxygen consumed.

Armed with this evidence from in vitro experiments, Larsen et al. next showed that whole-body oxygen cost during steady-state exercise decreases with nitrate supplementation while the mechanical work output to oxygen cost ($Watt/VO_2$) concomitantly increases, providing in vivo confirmation of experiments in isolated mitochondria. Exercise economy can be affected by changes in mechanical efficiency, mitochondrial coupling efficiency or both. The results in the current study suggest that nitrate-induced improvements in exercise economy are due in part to enhanced mitochondrial coupling efficiency; this differs from another study that reported that such improvements were due to enhanced mechanical efficiency (and reduced ATP turnover) (Bailey et al., 2010). Further studies are needed to determine whether both of these occur simultaneously.

The current study provides strong evidence that short-term dietary nitrate supplementation enhances mitochondrial efficiency, decreases mitochondrial proton leak, and enhances exercise performance. While the underlying mechanisms are not clear, nitrate supplementation reduced the expression of adenine nucleotide translocase, which liberates protons in the process of exchanging ADP and ATP across the inner mitochondrial membrane (Figure 1). Furthermore, Larsen et al. found a tendency for lower uncoupling protein 3 (UCP3) expression, which dissipates the mitochondrial proton gradient during