Looking Farther Afield

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This Little Piggy Went to (Fish) Market...

There are several health benefits to eating fish, including possibly a reduction in cancer risk. Most benefits are attributed to ω-3 fatty acids. However, there is growing concern regarding high levels of mercury and other chemical contaminants in fish. In contrast to fish, livestock have very little ω-3 fatty acids due to the extensive use of feed grain rich in ω-6 (the ''bad'' type of fatty acid) and the lack of enzymes necessary to convert ω-6 to ω-3. Here, Lai et al. (1) report on transfecting a humanized *fat-1* gene found in the roundworm, *Caenorhabditis elegans*, into early-passage porcine male primary fetal fibroblast cells to produce piglets rich in ω-3 fatty acids. Selected colonies of the transfected porcine cells were used to clone *hfat-1* transgenic pigs by nuclear transfer. Of 1,633 constructed embryos, 12 early pregnancies were established. Of these, 10 were born alive including six piglets positive for the *hfat-1* transgene (four piglets were negative for the gene, suggesting that colony selection was not complete for transfected cells). Only three of six piglets with a successful gene transfer had higher levels of ω-3 fatty acids than their nontransgenic littermates. Three piglets, irrespective of gene transfer, developed symptoms of a heart defect that appeared to be caused by the cloning process. In a subsequent experiment, eight healthy piglets were cloned from muscle fibroblasts from one of the transgenic piglets. All eight had significantly higher levels of ω-3 fatty acids and none had any obvious defect. The authors indicate that these transgenic animals could provide an alternative source for ω-3 fatty acids in the diet.

COMMENT: Genetic manipulation is fascinating from the standpoint of creating important scientific breakthroughs with regard to disease treatment. There is, however, something unappetizing and a bit unethical regarding the need to create a ''heart-healthy'' pig (let alone what the implications are for our dietary questionnaires!). Foods that are naturally high in ω-3 fatty acids include flax seeds, walnuts, soybeans, and winter squash. It would seem a lot easier and healthier to encourage consumption of these foods if increasing ω-3 intake is the goal. Furthermore, the authors did not comment on whether they ate their experiments—thus, the jury is still out on whether all this work will result in palatable pork.—Julie A. Ross.

Soy and Coat Color: Another Changing of the Guard

As reported in a previous article (2), there is increasing interest in understanding whether maternal diet during pregnancy can permanently alter the expression of genes in offspring—an epigenetic phenomenon. The most visible example involves coat color change in the offspring of mice due to manipulation of the maternal diet. The protein product of the wild-type murine agouti allele (*A*) regulates the change of pigment production in follicular melanocytes from black eumelanin to yellow pheomelanin leading to a brown (agouti) coat color. The mutant, nonagouti allele (*a*), results in loss of expression of the agouti gene and mice that are black. The A^{vy} allele arose naturally from the 5′ insertion of the intracisternal A particle retrotransposon in the *A* allele and is known to be epigenetically regulated by methylation of the long-terminal repeat of the intracisternal A particle (3, 4). Isogenic littermates that are heterozygous for the A^{vy} allele (A^{vy}/a) show phenotypic variation in coat color ranging from yellow to pseudoagouti (black). At least three studies have examined the effect of prenatal methyl supplementation (e.g., diets high in folic acid) on coat color in A^{vy}/a offspring. In the first study, offspring of mothers who were on methyl-supplemented diets during pregnancy compared with those on control diets had greater black mottling of their coats (5, 6). In the second study, using a similar experimental design, offspring in the methyl-supplemented group had increased methylation of the agouti long-terminal repeat and tended to be healthier than offspring in the control group (7). Finally, the third study (6) built on the work of the other two and showed that the shift in coat color distribution in the offspring of the methyl-supplemented dams resulted from increased methylation at the A^{vy} locus (6).

Now, some of these same authors have evaluated whether genistein, an isoflavone found in soy, also affects methylation of the A^{vy} locus in offspring (8). Prior to mating, female mice were randomized to receive either a phytoestrogen-free diet or the same diet modified with 250 mg/kg diet of genistein (a dietary level that is comparable to humans consuming high levels of soy; ref. 9). Maternal genistein supplementation shifted the coat color of the offspring toward the pseudoagouti phenotype; 50% of the supplemented group versus 23% of the unsupplemented group were either pseudoagouti or heavily mottled. As in their previous experiment (6), the authors demonstrated that coat color was dependent on the amount of methylation of the promoter region of the A^{vy} intracisternal A particle. Furthermore, average body weight for the pseudoagouti mice was significantly lower at 60 weeks (35.6 versus an average >54 g for the other coat color classes), suggesting that genistein supplementation via methylation reduces obesity among offspring. Notably, as genistein is not a methyl donor, the mechanism must be independent of the one-carbon metabolism pathway. Because of an epigenetic influence on obesity, the authors suggest that their results may help explain the reduced incidence of certain cancers among Asians compared to Westerners.

COMMENT: Although the overall message of this work is that genistein might be beneficial during pregnancy due to hypermethylation and reduction of obesity, it is important to be cautious with interpretation. This work, like the studies with respect to folic acid, again raises the issue with regard to timing. In animal studies, maternal exposure to genistein during pregnancy has been shown to increase the risk of chemically induced mammary tumors in offspring (10). It is thought that this action occurs through the estrogenic effect of phytoestrogens. It is not implausible that hypermethylation at key tumor repressor sites may also play a role in increasing mammary cancer risk. The difficulty with interpreting animal experiments is extrapolating them to a real-life setting. Infants who are exposed to high levels of genistein *in utero* typically come from countries where soy foods are ubiquitous. Thus, these children tend to continue to be exposed to high levels throughout their lives. Epidemiological studies suggest that

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prepubertal exposure to high soy foods reduces the risk of breast cancer (11, 12). Animal studies are also pointing to the possible protective effect of prepubertal exposure (10, 13). The question remains whether start/stop cycles of exposure during critical time points in development could permanently alter genes or affect the pathways involved in proliferation. The classic example is folic acid, which appears to have beneficial and deleterious consequences depending on the timing of the exposure (14). Additional animal studies that take into account some of these epidemiological observations may help shed further light on these questions.—Julie A. Ross.

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