COMMENTARY

When maternal periconceptional diet affects neurological development, it's time to think

Kevin D. Sinclair[a,1]

There is increasing awareness that the nutritional status of women at the onset of pregnancy can have a profound effect on the general health and well-being of children. However, recent analyses indicate that the majority of women from different socioeconomic backgrounds are ill prepared for the nutritional rigors of pregnancy, and that outcomes of dietary interventions once pregnancy has commenced are usually disappointing (1). It follows that current thinking is moving toward more targeted dietary advice for intending parents with the aim of improving nutritional status by the time of conception. There is certainly compelling evidence from animal studies to identify this as perhaps the most critical stage of mammalian development: one that is acutely sensitive to subtle alterations in maternal diet with far-reaching consequences for the development of late-onset noncommunicable diseases (2, 3). However, to date, animal studies have focused, for the most part, on aspects of cardiometabolic health. In PNAS, Gould et al. (4) report that modest protein restriction (a low-protein diet) in mice limited to the period of preimplantation embryo development (termed Emb-LPD) initially leads to a reduction in the population of neural stem cells but then, upon dietary realignment, subsequently results in an enhancement of neuronal differentiation within higher regions of the fetal brain. Furthermore, they report that this apparently early adaptive response ultimately leads to deficits of short-term memory in young-adult offspring. These observations therefore greatly extend those of previous studies on brain function, which have generally explored the effects of dietary restriction throughout gestation and lactation (5), to highlight the importance of nutrition during the periconceptional period.

Dietary Interventions: Adaptive vs. Maladaptive Responses

The specific nature and composition of the diets offered by Gould et al. (4) are almost of secondary importance to the discovery that a transitory period of moderate malnutrition lasting just 3 d from shortly after conception is sufficient to induce a profound effect on brain development. Equally important, these authors report that these effects are distinct from those associated with moderate malnutrition extended throughout gestation, and that they originate from a stage of embryo development that precedes the onset of neurogenesis. The study design and data presented in this article, however, cannot lead us to conclude that the effects reported were induced solely during the preimplantation period. Rather, the distinct neurological features and behavioral characteristics of Emb-LPD offspring may be attributed, in part, to the subsequent period of adaptation (or "maladaptation") that occurred during maternal realignment to the normal-protein diet (NPD) and/or to some form of "carryover" from the preimplantation period. Ultimately, however, this led to subsequent overproduction of cortical neurons and associated memory problems in adulthood.

The mechanism(s) that mediate such effects were not explored in that study and await further investigation. To this end, separating putative adaptive responses on the part of the developing embryo from transitionary changes in maternal intermediary metabolism following dietary realignment will be important to understand fully the effects of maternal diet. Nevertheless, from previous studies undertaken by this group (6), there is good reason to believe that compensatory ribosomal DNA (rDNA) transcription and ribosome biogenesis specific to embryonic lineages could account for at least some of these (maladaptive?) effects during the transition from an Emb-LPD to an NPD. Mediated by rDNA methylation and Pol I transcription factor Rrn3 expression, ribosomal biogenesis is suppressed initially during dietary (LPD) restriction but then appears to overcompensate following release from challenge, with persistent excessive rDNA transcription in a number of somatic cell types thereafter. This would certainly be a line worthy of inquiry in neural lineages. On the other hand, the discovery that transcript expression by the *fragile X mental retardation* (*Fmr1*) gene and its two autosomal

[a]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom
Author contributions: K.D.S. wrote the paper.
The author declares no conflict of interest.

See companion article on page E7398.
[1]Email: kevin.sinclair@nottingham.ac.uk.
Published online July 5, 2018.

paralogues (*Fxr1* and *Fxr2*) was reduced in the cerebral cortex of LPD offspring compared with NPD offspring (4) possibly points to more targeted epigenetic gene dysregulation during neurogenesis. This also represents an exciting line of inquiry, not least because there is considerable interest currently in the methylation status of the microsatellite (CGG repeat) in the 5′ UTR of *FMR1* in patients with fragile X syndrome (FXS). Recently, targeted demethylation of this expansion by dCas9-Tet1/CGG single guide RNA-mediated methylation editing reactivated *FMR1* expression in induced pluripotent stem cells from patients with FXS, an effect that persisted for at least 3 mo following injection of edited neuronal precursor cells into the brains of mice (7). At present, however, there is no precedent that maternal periconceptional diet is linked to this condition.

Metabolic Responses: A Tale of Three Organic Acids

On first inspection, it may seem implausible that one could extrapolate from these observations in mice in a manner that could aid in the development of nutritional advice for intending mothers. However, the dietary model used in the study of Gould et al. (4) is widely employed, and therefore well characterized. These diets also incorporate specific ingredients that are found commonly in human foodstuffs and that could underpin at least some of the metabolic and developmental responses reported. They are based on AIN-93 (8), a standard formulation used in many rodent studies, where the principal carbohydrate source is corn starch, to which either sucrose or dextrose (D-glucose) is added. Casein represents the principal source of protein, but it is deficient in cysteine and other sulfur-containing amino acids. Therefore, methionine (normally a mixture of D and L isomers) is included, which the maternal liver converts to cysteine by transsulfuration (2). Typically, fatty acids are derived from either corn or soya oil. The former is rich in n-6 polyunsaturated fatty acids (PUFAs), whereas the latter contains a high proportion of n-3 PUFAs. Finally, to ensure that LPD diets remain isocaloric, casein is partially replaced with additional corn starch and either sucrose or dextrose. From this dietary description emerges three classes of organic acid, each of which is implicated to a greater or lesser extent in mediating some of the more general metabolic and developmental responses observed when LPDs are fed during pregnancy.

Branched-Chain Amino Acids. The feeding of LPDs during the periconceptional period reduces circulating levels of insulin and uterine luminal concentrations of branched-chain amino acids (BCAAs) (9). These effects, in turn, invoke a series of apparently compensatory mechanisms in the blastocyst, mediated, in part, by the mTORC1-signaling pathway, whereby trophectoderm expansion and endocytosis are increased (9, 10). Significantly, similar effects on fetal development and adult offspring health are observed when mouse embryos are cultured in media containing low levels of insulin and BCAAs (11). However, consequences for neurological development and memory in offspring derived from such embryos remain to be explored. It is also not clear to what extent insulin and BCAAs may contribute directly to the neuropathology observed by Gould et al. (4).

Sulfur-Containing Amino Acids. Maternal serum concentrations of homocysteine (Hcy) increase threefold on day 3 of gestation in both pregnant mice and rats fed LPDs (12). This arises because activity of the final enzyme in the transsulfuration of methionine to cysteine (cystathionine γ-lyase; EC 4.4.1.1) declines to around 25% of its initial activity by around day 3 of gestation in LPD-fed rodents, presumably to spare amino acid oxidation in support of the increasing demands for fetal growth (13). However, dietary-induced disturbances of this nature to one-carbon (1C) metabolism during the periconceptional period lead to genome-wide alterations to DNA methylation in multiple cell types in the developing fetus (2), which, importantly, can be averted when LPD-fed mice are supplemented with folic acid (14, 15). The consequences for epigenetic regulation of neurological development in this model, however, remain to be determined.

PUFAs. Finally, although not measured in their study, Gould et al. (4) propose that reduced levels of docosahexaenoic acid (DHA; C22:6, n-3) could contribute to their observations. This is certainly plausible, given the known effects of DHA on neurosphere formation and on neural stem cell differentiation (16), and that their diets included n-3 PUFA-deplete corn oil. In previous studies, similar LPDs fed throughout gestation led to reduced levels of DHA within the phospholipid fraction of the fetal rat brain (17). The threefold increase in Hcy observed around day 3 (12) may also serve to compound this deficiency, as plasma DHA is negatively (and functionally) related to this intermediary metabolite of 1C metabolism (18). Again, periconceptional supplementation with B vitamins (folate, B6, and B12) and/or choline could ease the situation, subsequently leading to increased DHA within the phospholipid fraction of the fetal brain. This also represents a further and important line of inquiry.

Periconceptional Diet: When to Intervene

The foregoing discussion highlights the importance of a number of dietary components (e.g., PUFAs, B vitamins) and intermediary metabolites (e.g., Hcy) familiar to those offering nutritional guidance to intending parents, and provides some thoughts as to how the intriguing findings reported by Gould et al. (4) could lead to future research endeavors. The most striking finding from that study, however, and one that forms the basis of the message that this article seeks to convey, is that very subtle deviances in maternal nutrition around the time of conception can have important long-lasting consequences for the well-being of children. It would appear that dietary interventions initiated during pregnancy may be too late (1), and the study of Gould et al. (4) suggests that they could even be detrimental. The challenge faced by health care professionals relates to the definition of the "periconceptional period" within a clinical context so that appropriate and timely nutritional guidance can be provided. To that end, the article by Stephenson et al. (1) presents a very useful conceptual framework whereby they defined this period from biological, individual, and public health perspectives.

Acknowledgments

My research is supported by Biotechnology and Biological Sciences Research Council Grants BB/K017810/1 and BB/R007985/1 and a Society for Reproduction and Fertility Academic Scholarship Fund award.

1 Stephenson J, et al. (2018) Before the beginning: Nutrition and lifestyle in the preconception period and its importance for future health. *Lancet* 391:1830–1841.
2 Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD (2013) The periconceptional period, reproduction and long-term health of offspring: The importance of one-carbon metabolism. *Hum Reprod Update* 19:640–655.

3 Fleming TP, et al. (2018) Origins of lifetime health around the time of conception: Causes and consequences. *Lancet* 391:1842–1852.
4 Gould JM, et al. (2018) Mouse maternal protein restriction during preimplantation alone permanently alters brain neuron proportion and adult short-term memory. *Proc Natl Acad Sci USA* 115:E7398–E7407.
5 Amarger V, et al. (2014) Protein content and methyl donors in maternal diet interact to influence the proliferation rate and cell fate of neural stem cells in rat hippocampus. *Nutrients* 6:4200–4217.
6 Denisenko O, et al. (2016) Regulation of ribosomal RNA expression across the lifespan is fine-tuned by maternal diet before implantation. *Biochim Biophys Acta* 1859:906–913.
7 Liu XS, et al. (2018) Rescue of fragile X syndrome neurons by DNA methylation editing of the FMR1 gene. *Cell* 172:979–992.e6.
8 Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* 127(Suppl):838S–841S.
9 Eckert JJ, et al. (2012) Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. *PLoS One* 7:e52791.
10 Sun C, et al. (2014) Mouse early extra-embryonic lineages activate compensatory endocytosis in response to poor maternal nutrition. *Development* 141:1140–1150.
11 Velazquez MA, et al. (2018) Insulin and branched-chain amino acid depletion during mouse preimplantation embryo culture programmes body weight gain and raised blood pressure during early postnatal life. *Biochim Biophys Acta* 1864:590–600.
12 Petrie L, Duthie SJ, Rees WD, McConnell JM (2002) Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming. *Br J Nutr* 88:471–477.
13 Rees WD (2002) Manipulating the sulfur amino acid content of the early diet and its implications for long-term health. *Proc Nutr Soc* 61:71–77.
14 Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 135:1382–1386.
15 Altobelli G, Bogdarina IG, Stupka E, Clark AJ, Langley-Evans S (2013) Genome-wide methylation and gene expression changes in newborn rats following maternal protein restriction and reversal by folic acid. *PLoS One* 8:e82989.
16 Hamilton LK, Fernandes KJL (2018) Neural stem cells and adult brain fatty acid metabolism: Lessons from the 3xTg model of Alzheimer's disease. *Biol Cell* 110:6–25.
17 Burdge GC, Dunn RL, Wootton SA, Jackson AA (2002) Effect of reduced dietary protein intake on hepatic and plasma essential fatty acid concentrations in the adult female rat: Effect of pregnancy and consequences for accumulation of arachidonic and docosahexaenoic acids in fetal liver and brain. *Br J Nutr* 88:379–387.
18 van Wijk N, et al. (2012) Combined dietary folate, vitamin B-12, and vitamin B-6 intake influences plasma docosahexaenoic acid concentration in rats. *Nutr Metab (Lond)* 9:49.