Reply to KN Litwak and S Levin

Dear Sir:

We agree with Litwak and Levin that clinical trials are the best way to determine human health effects of dietary components. However, nonhuman primates offer experimental control and an anatomically and physiologically representative animal model and have inherent value in translational research. Details regarding study methodology are available on request, as they were at the time of review. Studies were conducted appropriately with regard to animal selection, known caloric intakes, and comparable dietary volume (our Table 1: caloric density was only 5% different). Species were not collapsed in any statistical analyses, because study 1 was conducted in cynomolgus macaques and study 2 in African green monkeys. Both species showed liver pathology with consumption of the same diet, which upholds the commonality in primate physiology that imparts value to dietary studies in monkeys. We believe that our results indicate that microbial translocation is an understudied factor in the epidemic of fatty liver and that the role of fructose deserves further investigation.

None of the authors had any conflicts of interest with respect to the work presented.

Kylie Kavanagh
Ashley T Wylie
Kelly L Tucker

Department of Pathology
Section on Comparative Medicine and Lipid Sciences
Wake Forest University Health Sciences
Medical Center Boulevard
Winston-Salem, NC 27127
E-mail: kkavanag@wakehealth.edu

Timothy J Hamp
Raad Z Gharibeh
Anthony A Fodor

Department of Bioinformatics and Genomics
University of North Carolina at Charlotte
Charlotte, NC

John M Cullen

College of Veterinary Medicine
North Carolina State University
Raleigh, NC


Study examining effects of poor nutrition during pregnancy and lactation in a primate lacks translatability

Dear Sir:

The recent article by Keenan et al (1) intends to describe, in a nonhuman primate, the effects of poor nutrition during pregnancy and lactation on future neurodevelopment of the offspring. Certainly, this is a relevant topic to study because many epidemiologic studies have associated insufficient intakes of calories, proteins, and micronutrients by the mother with poor developmental outcomes in the child. However, beyond the ethical issues with regard to the use of animals for questions better answered in human investigations, there are important scientific flaws in the study design, which contains multiple confounding variables that have not been adequately described and likely have a significant effect on the outcomes, interpretation, and utility of the data.

Preandual characteristics

The maternal nutrient restriction (MNR) diet was based on consumption of the control monkeys at the time of pregnancy assessment. Thus, the amount of food that was consumed by the MNR group, as a percentage of their baseline consumption, could have been quite variable. In fact, as described previously by this group (2), using the control group to set the diet of the restricted group resulted in a 41% decrease in calories offered. Because the weight of the monkeys was determined frequently and they were fed individually, it should have been possible to better control the caloric value of the restricted diet. Although the diet was certainly restricted from baseline levels, it is unclear if the decrease represents undernutrition. Pursuant to that point, data previously published by this group describe minor weight loss (<10% of baseline) in nutrient-restricted females but no fetal size difference compared with controls. Finally, the pregnant females were housed in 2 groups of up to 16. Maternal social status and associated stress play a significant role in fetal development and have been shown to have long-term effects on the neonate (3).

Postpartum description

The authors’ Table 1 describes the morphologic characteristics of the MNR and control offspring. Whereas there appears to be a difference in weight between groups (MNR compared with control: 0.74 ± 0.05 compared with 0.88 ± 0.04 kg), both are within the normal range of baboon birth weights (4). The authors describe the juveniles being moved in cohorts of 5–7 over a period of 9 mo to a different facility, where they were singly housed. However, there is no description of when juveniles were removed from their mother and their initial age at the time of single housing, both of which could have had a much greater effect on neurodevelopmental outcomes than gestational nutrition. In fact, many studies have shown the deleterious effects of social impoverishment on physiologic and behavioral processes (5). Finally, there was no mention of other studies to which the juvenile monkeys were exposed over the intervening time between separation from their mother and conduct of the current study.

Diet

The term “poor nutrition” defines a broad category of nutritionally incomplete diets. In the context of this study, it was used to define a lack of calories; however, the term can also be used for overly high-calorie diets, high-fat diets, diets lacking in nutrients, etc. Use of the appropriate descriptive terms will help readers assess the value of the data. Because no data were presented on the weights of the mothers during pregnancy, it is impossible to assess if the amount of diet consumed by the mothers was lacking in calories, the central tenet of the article.

The authors state that in all human studies poor nutrition occurs in the context of psychosocial stressors and genetic risk factors. They then state that a controlled study in a well-established
nonhuman primate model of maternal undernutrition would allow determination of whether the variance in developmental outcomes could be explained by prenatal nutrition. In fact, the many confounding variables in a nonhuman species produced data that could not be translated into the human experience. Ultimately, this study would have been much better conducted in carefully conceived human studies.

Neither of the authors had a conflict of interest.

Kenneth N Litwak
Susan Levin

Physicians Committee for Responsible Medicine
5100 Wisconsin Avenue NW
Suite 400
Washington, DC 20016
E-mail: klitwak@pcrm.org; slevin@pcrm.org

REFERENCES

do: 10.3945/ajcn.113.075507.

Reply to KN Litwak and S Levin

Dear Sir:

In their letter regarding our article entitled “Poor nutrition during pregnancy negatively impacts neurodevelopment of the offspring: evidence from a translational primate model,” Litwak and Levin claim that study flaws and multiple confounding variables negatively affected the impact of our study. Furthermore, they questioned whether hypothesis testing with regard to the impact of poor nutrition on neurodevelopmental outcomes in nonhuman primates is sufficiently translatable to humans. In terms of the concerns about study design and interpretation, Litwak and Levin have misunderstood the structure and study aims.

The study goals were to compare the development of offspring of control and maternal nutrient restriction (MNR) mothers fed 70% of the controls—a 30% caloric reduction throughout pregnancy and lactation. As stated in our previous article (1), to which they refer, in a comparable period of gestation (before 50% of gestation) control mothers ate 64.3 kcal·kg⁻¹·d⁻¹, whereas MNR mothers were fed 45.7 kcal·kg⁻¹·d⁻¹. Thus, MNR reduction in intake was 29%, exactly on target, and not 41% as stated in their letter, which is actually the reduction from the prepregnancy feeding. Our nutrition reduction model provides very precise control with a 5% CV for MNR group food intake.

The authors question whether this amount of reduction “represents undernutrition.” We have published several articles showing that this regimen produces fetal undernutrition. First, as stated in the previous methods article (1) to which Litwak and Levin refer, ad libitum–fed mothers always leave food in the cage, whereas MNR mothers always eat all food provided. Furthermore, we have shown clear changes in the MNR mother, placenta, and/or fetus. For example, whereas circulating maternal amino acids are unchanged, key fetal amino acids are somewhat paradoxically increased in MNR fetal blood, potentially reflecting a lowering of fetal amino acid metabolism to adjust for the decreased nutrient availability (2). MNR produces decreased activity in the maternal (1), placental (1), and fetal insulin-like growth factor systems (3). In our article published in the Proceedings of the National Academy of Sciences (4) we describe considerable delay in fetal frontal cortex neurogenesis, again with signs of compensatory changes such as increased cell division in an attempt to compensate for increased apoptosis. Perhaps the strongest proof that the fetus is undernourished in this paradigm is evidence we provided, for the first time in a primate, showing that the fetus responds to this moderate nutrient deficiency by decreasing methylation of the phosphoenolpyruvate carboxykinase gene in the liver, an epigenetic modification leading to an increase in this key enzyme in the gluconeogenesis pathway (5). These findings of nutrient reduction–induced changes in the developing fetus were the basis of our current work to determine whether persistent postnatal effects in behavior could be observed.

The last question posed by Litwak and Levin regarding the effectiveness of our nutrition restriction model was whether nutrition could be deficient in the absence of group differences in fetal weight. There are multiple reports in the literature of only minor increases in birth weight in the face of decreased fetal nutrition in animal models (6), an important indication that weight is a poor measure for body composition. Matthew Gilman, a leader of many studies of developmental programming in human epidemiologic cohorts stated, “We must retie weight as an indication of outcomes in response challenges in utero. It is body composition that matters” (PW Nathanielsz, personal communication, 2011).

In raising the issue of multiple confounding variables, Litwak and Levin actually do a good job of making the case for the important translational value of testing the impact of poor nutrition on neurodevelopmental outcomes in nonhuman primates. We agree with them that maternal social status, other environmental maternal stressors, and postnatal factors moderate effects of developmental programming. In human studies, these environmental and social factors are highly confounded with prenatal nutrition, and even a “well-conceived study” in pregnancy could not be ethically conducted to parse the variance in outcomes due to such effects. In our model, nonpregnant females were allocated at random to the control and MNR groups. Food consumption between the 2 groups before study onset did not differ. All offspring were housed in their home cage until weaning and socially housed in peer groups up until they were transferred to a separate facility for behavioral testing. The movement of juvenile offspring to individual cages was only for the behavioral testing and all animal movements were the same for both groups. Housing and all other resources were the same for both groups. Data from studies using these types of experimental controls build on patterns of associations shown in epidemiologic studies by providing evidence for a causal mechanism. We, and others, would argue that this is the safest and most direct and efficient route toward developing and testing preventive interventions in humans and improving the human condition. Such interventions may be among the most cost-effective approaches to reducing the risk of common childhood physical and mental health disorders, but evidence for the causal relation between deficient prenatal nutrition and health outcomes needs to be established.