Effects of excess dietary fructose on liver pathology study have significant methodologic limitations

Dear Sir:

The role of abnormal or disturbed nutrition in humans is often studied in animal models, with a hope of using the findings to inform human well-being. An article by Kavanagh et al. (1), in a recent issue of the Journal, describes results of such a study conducted in non-human primates. The role of excess dietary fructose was examined and linked to hepatic injury. The human clinical questions are timely and have great significance for the general human population. However, as described below, there are significant methodologic issues with this study that limit any translatability of the results and argue against a scientifically valid interpretation of the study.

Study subjects

The authors noted that 2 different species of monkeys were used in the study, Macaca fascicularis and Chlorocebus aethiops, yet provided no explanation or precedent articles to support collapse of these 2 species into a single statistical group. These species are native to separate continents: M fascicularis to Asia and C aethiops to Africa. Recent estimates suggest species divergence occurred ~7 million years ago (2). Although these species are similar in size and appearance, the duration of time since the species split could have led to significant differences in metabolism that were not discussed by the authors. There was no description of age, sex, or numerical breakdown of each species. Furthermore, there is no description of previous research studies that involved these monkeys. All of these factors could have significant effects on the current study outcomes.

Study design

In study 1, liver samples were obtained from 17 male and female monkeys, who died of causes unrelated to the study. The authors did not describe the cause of death in any of the monkeys. Because the cause of death, timing of sample collection, and clinical history might have a significant impact on the liver histology, it is impossible to determine whether any of the liver findings are related to excess fructose or other, underlying pathologies. In study 2, only female monkeys were used, thus limiting any conclusions to a single sex. The description of stratification of middle-aged to aged monkeys into groups is vague and does not give any sense of how groups were actually determined. Furthermore, because diet quantity was adjusted weekly, there does not appear to be any control of the diet.

Study diet

The composition of the high fructose (HFr) diet was substantially different from that of the control diet. Fructose content of the HFr diet was twice that of a typical Western diet (3), whereas the control diet was nearly devoid of fructose, making interpretation of the results difficult to put into context. In addition to having the planned higher fructose content, the HFr diet had different sources of carbohydrates, fats, and proteins. For example, the sources of carbohydrates in the control diet were grain starches and plant fiber, whereas the sources of carbohydrates in the HFr diet were wheat flour and fructose. The potential effects of different sources of carbohydrates, proteins, and fat were not addressed, or even recognized, in the article. Fat content, as a percentage of energy, was 30% higher in the HFr diet. Increased fat intake has been associated with endotoxemia in healthy humans (4). Finally, because fructose-fed monkeys were heavier than controls, the volume of diet received by these monkeys was up to 80% greater than in the control group.

Understanding the role of increased fructose consumption is certainly an important issue, because fructose accounts for 10–15% of all energy consumed (3). The role of diet and lifestyle on hepatic steatosis and injury has been extensively described in the human population with the use of large clinical trials (4–6). Thus, further awareness of the roles of specific dietary components on human liver pathology is most likely to come from other human-based studies.

Neither of the authors had a conflict of interest.

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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.

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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.

Prenatal 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.

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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 decreases 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 retire 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.
As the NIH has now recognized by the many funding mechanisms it has initiated to obtain carefully controlled scientific data such as those we report, developmental programming is a major contributor to disease susceptibility. Leading researchers in the field have stated that “The interaction of the clinical, epidemiological, and basic science communities is essential in evaluation of study outcomes and in defining future directions and needs. New mechanisms and models developed at the bench will inform clinicians as to potential markers and targets to be examined at the bedside, with novel clinical observations then characterized for underlying mechanisms in animal models” (7). Our study is a part of this essential collaborative effort, and we are extremely pleased to be able to disseminate and further clarify our findings in the Journal.

The authors reported no conflicts of interests.

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Belief beyond the evidence: using the proposed effect of breakfast on obesity to show 2 practices that distort scientific evidence

Dear Sir:

Recently, Brown et al (1) highlighted 2 practices that distorted scientific evidence: “research lacking probative value” and “biased research reporting.” They conducted a cumulative meta-analysis on the “proposition that skipping breakfast causes weight gain” and concluded that J this “proposed effect of breakfast on obesity” is presumed true despite the conflicting evidence, 2) observational studies have shown a clear direct association since 1998 that should have prevented further “gratuitous observational studies” from being conducted, and 3) there was evidence of reporting bias in one’s own research and others’ research. Whereas there may be some validity to these conclusions, a more useful meta-analysis would not just have examined the overall association without conducting any tests for heterogeneity, subgroup analyses, sensitivity analyses, and assessment of quality of studies as per the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist (2) for observational studies and the Quality of Reporting of Meta-analyses (QUOROM) statement (3) for randomized controlled trials.

Undeniably, there are myriad settings in which observational data can be useful, not just to get an overall estimate but also to examine various factors such as the following:

1) Consistency of evidence in diverse populations and settings, which constitutes one of the firmest criterions for causality outside of randomization.

2) Methodologic issues to discern whether the association is robust after controlling for confounding variables.

3) Sources of heterogeneity or interaction—eg, by sex, age, overall dietary pattern, overall diet quality, types of food consumed at breakfast, physical activity habits, smoking habits, and baseline BMI.

4) Duration relation (in many settings), because most, if not all, of the randomized trials conducted on this topic were of short duration; thus, it becomes crucial to evaluate this association over longer periods of follow-up (years instead of months) through observational studies and to scrutinize whether the frequency and duration of skipping breakfast is linked to weight gain.

5) Consistency of association over time as well as time sequence to show the exposure (breakfast consumption pattern) happened before the outcome (weight gain). Notably, the authors mentioned that 86% of the studies were cross-sectional; this justifies the necessity of conducting longitudinal studies, which take a long time to conduct and publish. The authors, however, generalized their statement about studies being conducted “gratuitously” to all observational studies without discerning between the different types.

6) Precision for magnitude of association, which could be mainly detected if studies were replicated over time and in different settings.
We agree that randomized trials may be required to establish causality. A careful extensive analysis of the observational data coupled with a better understanding of the underlying physiology would help justify (or not) randomized trials and could aid in their designs. For example, contextual factors, such as the influence of the type of diet, accompanying physical activity level, and whether to keep total caloric intake constant, would be important in the design of randomized trials.

Neither of the authors had a conflict of interest to declare.

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Reply to RA Mekary and E Giovannucci

Dear Sir:

We thank Mekary and Giovannucci for their thoughts and welcome the continued dialogue about research and research reporting. We see 2 overarching points in their letter. The first, and seemingly more minor, point is their belief that our meta-analysis would have been more useful if it addressed the checklists from guidelines such as the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) or Quality of Reporting of Meta-analyses (QUORUM) statements and explored heterogeneity in the association. Although we agree with the importance of these ideas in some contexts, the point is tangential to the purpose of our analysis. Our meta-analysis (1) aimed solely at determining I whether an association of skipping (compared with eating) breakfast with increased obesity had been unequivocally shown, and if so, 2) whether association studies continued to be conducted for many years after such a demonstration occurred. Our analysis, as designed and reported, clearly answered those questions in the affirmative, and it was beyond our scope of interest to explore heterogeneity or other aspects of that association.

The second and more fundamental point from Mekary and Giovannucci relates to the value of observational studies. We agree with the general sentiment that observational data can be useful, as we acknowledged in our article. We further agree, and acknowledged in our article, that additional studies may be valuable for confirmation of the association or for identifying mediators of the association. In particular, novel associations or mediators proposed by observational evidence can push research in new directions to subsequently conduct the appropriate studies to determine causation. However, we seem to disagree about the point at which the marginal scientific knowledge gained from additional observational studies becomes negligible. In our example, the associations were established in a wide range of populations and settings, and it is unlikely that continuing to conduct observational analyses, longitudinal or otherwise, would meaningfully improve our ability to determine whether a causal relation exists.

Mekary and Giovannucci invoke some of Hill’s (2) 9 viewpoints as a means of establishing causality from observational evidence, specifically consistency and temporality. Consistency of evidence across many observational analyses still results in the possibility of a pervasive confounder (eg, obese individuals choose to skip breakfast; individuals with a particular genotype are predisposed to a phenotype of consuming calories later in the day). Furthermore, conducting additional longitudinal studies to establish temporality is of questionable value, especially considering the 11 longitudinal analyses already conducted. At the end of a longitudinal study, we are still left with an association and the question of whether making a change in the putative causal factor will, in fact, influence the outcome. We posit that the "entry ticket" to using Hill’s viewpoints for establishing causation from observational evidence is that randomization of humans to the postulated causal exposure and follow-up on the outcome of interest is infeasible, impossible, or unethical. Randomly assigning people to eat or skip breakfast and observing effects (or lack of effects) on weight is none of these.

We agree with Mekary and Giovannucci that a careful evaluation of observational evidence can be important in designing randomized controlled trials. Well-designed and well-executed observational studies, in which well-defined estimates of exposure are compared against reliable measurements of outcomes, can help guide experimental designs that are more likely to establish a causal relation. However, we showed that these observational analyses are too often used to state causal relations between an observed or self-reported estimate of the exposure and an observed or self-reported estimate of the outcome, independent of the whole of the literature. Meanwhile, conducting well-controlled experiments that test the influence of the exposure on the outcome is often feasible and relatively cheap for topics such as the breakfast-obesity hypothesis. Unfortunately, when a body of scientific evidence is distorted to produce the patina of a demonstrated causal conclusion from insufficient data, it may be difficult to convince funding agencies, fellow scientists, and the public that such well-controlled experiments are even needed.

None of the authors declared a conflict of interest.

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