Title

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Authors

Rucker, Robert B
Watkins, Bruce A

Publication Date

2019-05-01

DOI

10.1016/j.nutres.2019.03.007

Peer reviewed

NUTRITION RESEARCH 65 (2019) 1–3

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Editorial

Inadequate diet descriptions: a conundrum for animal model research

Experimental animal models are indispensable in endeavors to understand the processes that link diet as an external regulatory factor to virtually all aspects of physiological regulation and function. Accordingly, it is regrettable that many nutrient-related observations are compromised because of an inability to reproduce or validate reported research observations [1]. In this regard, inadequate dietary descriptions are a significant problem that persists in the scientific literature. Herein, we describe the importance of animal diets in research and the need for authors to fully describe diet compositions, type of ingredients, formulations relative to research objectives, and administration of the diets in their scientific publications to facilitate subsequent replications and confirm results.

The admonition for diet guidelines used in animal research stated by Greenfield and Briggs [2], nearly a half century ago, remains relevant today; the use of poor dietary formulations or inadequate diet descriptions sets the stage for equivocal conclusions. Similar concerns also have been noted in publications that have focused on the use of and periodic refinements made to the AIN series of diets (eg, the AIN-76 diet and AIN-93 diets), which were designed in part to provide improved consistency in nutritional studies [3-5]. In a recent editorial for *Trends in Endocrinology and Metabolism*, Mandić and Blaut [6] ask the question, "Do we choose control diets wisely?" Using data by Dalby et al [7], they noted that using differing control diets in animal studies designed to investigate the interaction between diet, the gut microbiota, and obesity development leads to differing conclusions that are dependent on the type of control diet used. In this case, the gut microbiota responds differently to commercial pelleted diets (most often cereal-grain based) compared to a fixed formula diet with a defined composition. As an extension of this example, another consideration is the acknowledgment that commercial diets differ markedly in texture because of the degree of milling or the formulations were assembled on a least-cost component basis. Commercial animal diet formulations can lead to varying ingredient compositions because primary formulation constraints focus on macronutrients often without regard to the micronutrient variations. Furthermore, inconsistencies in composition and other batch to batch variations occur. These diets are suitable for maintenance of animal colonies but not reliable for a nutrition-focused research experiment. As a consequence, the use of ill-defined commercial diets can easily lead to difficulties related to experimental replication and validation. When interpreting nutritional, physiological, biochemical, and behavioral observations, reproducibility of basal dietary complexity is, without doubt, an essential requisite.

As an additional example of diet composition and animal response, there is usually a downregulation of the hundreds of genes involved in the metabolism of secondary metabolites and reactive oxidative species when elemental or chemically defined diets are used. Rudolf et al [8] described the impact of dietary complexity on the relative expression of phase I and II biotransformation enzymes by measuring the enzymatic activity and mRNA levels for the cytochrome P450 monooxygenase isoforms (CYP1A1, CYP1A2, and CYP2B1/2) and glutathione-*S*-transferase isoforms (GSTA, GSTM, and GSTP). Changes in the expression of the isoforms were measured following exposure of mice and rats to basal diets with or without the addition of flavone. The basal diets used for the comparisons were a near chemically defined amino acid–based diet, a semipurified egg white–based diet, and a commercially prepared laboratory chow diet. In both rats and mice, the increase in phase I and II enzyme expression observed in response to flavone exposure was most dynamic when using the amino acid–based diet; often >20-fold for given isoform enzymatic activities and >200-fold for the expression of corresponding mRNAs relative to the basal chow diet. Flavone exposure resulted in a 10- to 20-fold increase for given isoform enzymatic activities and a >100-fold increase for the expression of corresponding mRNAs using the egg white–based formulation as the basal diet. The commercially prepared laboratory chow diet resulted in a higher level of background expression of CYP and GST isoforms, and as a consequence, the observed fold increases in CYP and GST isoforms were substantially less (1- to 10-fold for given isoform enzymatic activities and highly variable changes in the expression of corresponding mRNA levels, 1- to 150-fold). The point is that the composition of the basal diet can have significant impact on the relative magnitude of a

Robert B. Rucker, Ph.D., is a Distinguished Professor Emeritus in the Department of Nutrition, University of California, Davis. He has over 50 years of experience in areas important to early growth and development and the physiological roles of various biofactors in foods (http://nutrition.ucdavis.edu/people/faculty/rucker_robert.html).

biological response (eg, induction or expression of an enzyme) that results from exposure to a given test agent. Other examples include changes in the relative intestinal absorption rates for various micronutrients or, as noted above, changes in the microbial composition of the microbiome in response to changes in diet composition [2,6,7,9-14]. When a specific type of basal diet is chosen to enhance a given experimentally induced signal or response relative to the basal background signal or response, to be salient and transparent, a clear rationale for its use is essential.

Moreover, knowledge of the chemical nature and composition of the various ingredients that compose the diets and their impact on potential dependent variables is essential. If the choice is to use a commercially prepared diet, details regarding the ingredient composition should not be the sole responsibility of suppliers but also determined by the investigator to be appropriate for the goals of research to avoid problems with interpretation of the data collected. There are dozens of well-established interactions between dietary constituents that can influence an observational outcome [9,10]. Meeting physiological requirements often requires adjustments in dietary requirements to account for differences in bioavailability of the given nutrients affected. Indeed, the same attention should be conferred to ingredient formulations as given to analytical methodologies that are chosen or developed to provide high sensitivity and precision for biological endpoint measurements resulting from a change in dietary composition.

Diet is perhaps the most important external factor in the modulation of epigenetic-related events. When DNA methylation of given genes is associated with distinct developmental profiles or phenotypes, the downstream consequences can be complicated and long-lasting. Examples range from observations that gestational zinc deprivation in mice may persist up to 3 filial generations even when the second and third generations are fed Zn-repleaded diets [11] to short-term gestational and neonatal vitamin or related biofactor deficiencies wherein impairment of immune, neurological, and other systemic functions may persist well into adult stages of development [12,13]. The multifaceted interaction of nutrients on a given physiological and biochemical response is an inherent feature of nutritional research. In many instances, there is a high likelihood that the same or different research groups will not easily or precisely reproduce individual experiments.

It is noteworthy that investigators in disciplines other than nutrition are increasingly recognizing the importance of how diet ingredient composition and nutrient content affect critical endpoints. As examples, in journals that report on topics such as the importance of the microbiome to neurobehavior, cancer-related cachexia, or epigenetics, high priority is given to accurate and adequate descriptions of the macronutrient and micronutrient composition of the diet and the levels as a prerequisite to publication [6,9,12-15].

When complexity of the biological and physiological response is an inherent feature of nutrition questions and research, there is a high likelihood that individual experiments will not be easily or precisely reproduced when diet is not adequately described [9]. Consequently, all who engage in nutrition research have an obligation to provide documentation of methods and materials that are sufficient to permit direct replication or modification of a given nutrition protocol. When journals do not require investigators to report complete ingredient composition of diets, a vital common point of reference, the results from animal studies may become suspect and lead to the conundrum of trying to determine how the findings can be interpreted or confirmed. Accordingly, *Nutrition Research* is updating its requirements for dietary descriptions beyond listing the ingredients used in animal studies. The new Journal requirements will require information about diet types, proper units, description of diets, ingredient composition of diets in grams per kilogram, chemical form of amino acids, minerals and vitamins, and administration of diets. These requirements have been adapted by investigators and recommended [15] by the *Journal of Nutrition*. The ultimate goal is to describe better experimental approaches that use nutrition as a focus and allow research to be confirmed.

Robert B. Rucker
Bruce A. Watkins*
Department of Nutrition, University of California, Davis, Davis, CA
*Corresponding author
E-mail address: bawatkins@ucdavis.edu

https://doi.org/10.1016/j.nutres.2019.03.007

Bruce A. Watkins, Ph.D., is Professor Emeritus of Nutrition, Purdue University, and Professional Researcher at the University of California, Davis. His work has focused on lipid biochemistry, with emphasis on endocannabinoids in metabolism, disease, and aging. (https://nutrition.ucdavis.edu/people/bruce-watkins).

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