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Leading Edge
Essay

Putting the Balance Back in Diet

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The notion of dietary balance is fundamental to health yet is not captured by focusing on the intake of energy or single nutrients. Advances in nutritional geometry have begun to unravel and integrate the interactive effects of multiple nutrients on health, lifespan, aging, and reproduction.

Diet Balance Problem

One of the most important and prominent public health messages is to eat a healthy, balanced diet. But what does that mean? Balanced with respect to what—and when during the life course? What are the consequences of failing to achieve a balanced diet? These are fundamental questions that remain less well answered than is necessary to devise effective public health policy to combat the pandemic of obesity and metabolic disease (Simpson and Raubenheimer, 2012). Here, we show that advances from nutritional ecology are providing new ways to address these problems.

The classical approach to understanding diet balance has been painstakingly to derive individual estimates for required intakes of each of the dozens of macro- and micronutrients that are needed for health and wellbeing. Such "one variable at a time" (OVAT) approaches (Box et al., 1978) have provided the foundations of nutrition science. The evidence-base has been built from a combination of animal studies in which single constituents have been manipulated in experimental diets, epidemiological analysis of the associations between intakes of single nutrients and health outcomes in human populations, and single-variable clinical trials. These data have in turn informed national dietary guidelines with associated recommended daily intakes (RDIs) for micro- and macronutrients. Clinical practice, food labeling policies, and public health strategies have followed.

There is an abundant literature showing that fats, sugars, salt, vitamins, etc. contribute to health outcomes, but one consequence of taking a single-variable approach has been to promote adversarial debate between proponents of single-nutrient causes (or solutions) to diet-related health problems. This is nowhere better illustrated than in the long running debate over the roles of sugar and saturated fats in obesity and metabolic disease (Feinman, 2011; Willett, 2011). As a result, public confusion reigns—even (perhaps especially) among the well-educated populace—fuelled by commercial interests in the food sectors and the fad diet industry (Simpson and Raubenheimer, 2014).

The fundamental problem with OVAT approaches is that they fail to capture the multidimensional essence of nutrition (Ruohonen and Kettunen, 2004). It is axiomatic that diets are more than the sum of their components; they are combinations of foods, each comprising mixtures of nutrients and other constituents. Changing the concentration of one component in the diet can alter the character of the entire blend. In simple statistical terms, OVAT looks only at the main effects of single nutrients and does not account for the interactions between nutrients within diets—neither the non-independence of dietary constituents within mixtures nor the interactive effects of nutrients on health outcomes.

We need an approach that explicitly takes account of the interactions among nutrients within foods and diets and is able to define and quantify the consequences of different diet compositions on multiple measures of health across the life course. In this essay we illustrate such an approach, known as the geometric framework, which originated from the field of nutritional ecology (Raubenheimer et al., 2009). Nutritional geometry integrates not only multiple diet components, but also scales across molecules, cells, organs, organisms, populations, and ecosystems (Simpson and Raubenheimer, 2012). Starting with the ideas of nutrient-specific appetites and regulatory priorities, we introduce the concept of nutritional response landscapes using model organisms including *Drosophila* and mouse, and then discuss the application of nutritional geometry in humans.

Geometry of Nutrient-Specific Appetites

A fundamental requirement for considering the multilayer interactive effects of nutrients is to establish the extent to which the intakes of different nutrients are specifically regulated by the animal. In other words, are there so-called "nutrient-specific appetites" distinct from intake control merely based on total dietary energy or volume? Nutritional geometry provides a series of simple yet powerful concepts and experimental designs for addressing this question. One example has been to explore whether an animal has the capacity to regulate its intake of two nutrients simultaneously when challenged with different pairwise combinations of nutritionally complementary foods varying in their ratio and/or concentrations of the two focal nutrients. If animals converge upon the same ratio and amounts of the nutrients eaten

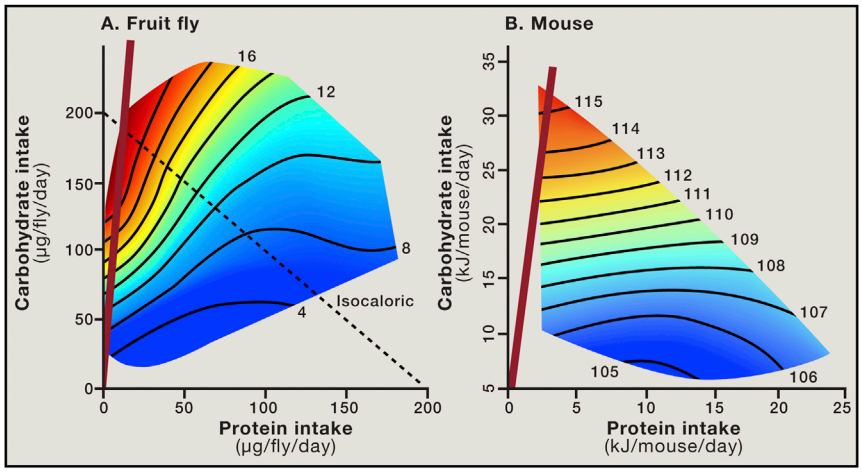

Figure 1. The Relationship between Protein and Carbohydrate Dietary Intake versus Lifespan in Flies and Mice

(A and B) Flies (Lee et al., 2008) (A) and mice (Solon-Biet et al., 2014) (B). In both cases, lifespan was maximized by diets with low ratios of protein to carbohydrate (red lines).

("intake target") across experimental food pairings, in each case ingesting the unique amount of each food required to do so on that particular pairing, it is then evident that the animal has separate regulatory systems controlling intake of the two nutrients. Similar types of experimental design have been used to show that organisms from acellular slime molds all the way to primates possess nutrient-specific appetite systems for macronutrients, such as proteins, carbohydrates, and fats, as well as for at least two micronutrients, sodium and calcium (Simpson and Raubenheimer, 2012). However, most micronutrients do not seem to be specifically regulated; rather, their intakes are maintained within healthy limits by a combination of correlation in foods with other regulated nutrients and non-specific mechanisms such as learned aversion to foods associated with development of a micronutrient deficiency, coupled with heightened attraction to novel foods (Simpson and Raubenheimer, 2012).

Having demonstrated that specific appetites exist for certain nutrients, the question arises as to how these are prioritized when the animal is restricted to a diet composition that does not allow the intake target to be reached for all regulated nutrients simultaneously. Under such circumstances, the animal must balance eating too little of some nutrients against overconsuming others relative to the intake target. Understanding how animals prioritize different nutrients under these circumstances is of considerable importance for appreciating or predicting the health impacts of shifts in diet (Lihoreau et al., 2014; Raubenheimer and Simpson, 1997). As a premise, we need first to be able to map nutritional response landscapes.

Mapping Nutritional Outcomes in *Drosophila melanogaster*

Drosophila provides a simple system for illustrating how to map the consequences of nutrition in multiple, potentially interacting nutrient and response dimensions. Lee et al. (2008) used nutritional geometry to disentangle the effects of calories from those of macronutrients in the context of increased lifespan upon caloric restriction (Curtis and de Cabo, 2013; Everitt et al., 2010; Mercken et al., 2012; Speakman and Mitchell, 2011) and also explored the basis for the frequently reported trade-off between aging and reproduction (Tatar, 2011). Flies offer several advantages for this type of analysis. First, their dietary calories come principally from two macronutrient sources—protein and carbohydrate (lipids, although essential, provide only a small caloric contribution)—thereby defining a two-dimensional nutrient space. Second, flies are small and short-lived, making large numbers of dietary treatments in a longevity study feasible.

In this study, flies were confined throughout their lifetime with ad libitum access to one of 28 diets, comprising seven protein to carbohydrate ratios (P:C), each at one of four total concentrations. Response landscapes for longevity and reproductive output were mapped onto an array of individual P:C intakes recorded for more than 1,000 flies, thereby allowing the consequences of nutrient and energy intakes to be visualized and analyzed. The results were striking (Figure 1A). Low-calorie intake per se was not associated with prolonged lifespan in *ad libitum*-fed flies; rather, lifespan was a function of the ratio of protein to carbohydrate ingested, declining as P:C increased. Second, lifespan and reproduction had differently shaped response landscapes with peaks in different places on the protein-carbohydrate intake plane—the diet composition that sustained longest life led to a lower intake of protein than needed to support maximal reproductive success. When allowed to compose their own diet by selecting among complementary food pairings, flies chose to mix a diet maximizing reproductive output rather than lifespan. Subsequent studies have shown that the trade-off between lifespan and reproduction is not obligatory or causal, but simply reflects differing nutritional optima for the two traits (Grandison et al., 2009; Tatar, 2011).

From Flies to Mice

A similar experiment has been conducted in mice (Solon-Biet et al., 2014). Here, the aim was to extend the use of nutritional geometry to quantify, *inter alia*, the impacts of macronutrients on food intake, body composition, lifespan, reproductive potential, cardio-metabolic health, immune status, mitochondrial function, gut microbiota, and nutrient signaling pathways. Nine hundred mice were confined from weaning with *ad libitum* access to one of 30 diets. These comprised ten protein to carbohydrate to fat ratios (P:C:F), which systematically sampled the 3D macronutrient mixture space, each ratio provided at one of three total energy densities by dilution with cellulose. Of the 30 diets, five that were very low (5%) in protein, high in fat, and low in energy density failed to sustain growth in young mice and were discontinued. Food intake was recorded throughout the experiment.

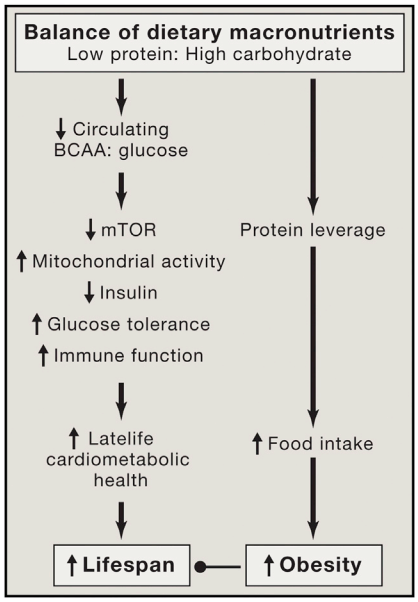

Figure 2. A Low Dietary Protein to Carbohydrate Ratio Has Counterposing Effects on Food Intake and Late Life Cardiometabolic Health

Mice, like other animals, possess separate macronutrient appetites (Sørensen et al., 2008), and when these were forced to compete by restricting animals to a single diet composition, total food intake was driven principally by protein, increasing as percent protein in the diet fell (consistent with compensatory feeding to stabilize protein intake). Compensatory feeding for carbohydrate was also apparent, with intake increasing as percent carbohydrate fell in the diet but to a somewhat lesser degree than for protein. Unlike protein and carbohydrate, however, the concentration of dietary fat had little influence over total food intake. Consequently, total food and energy intakes were maximal on diets combining low percent protein with high percent fat.

Energy intakes in turn corresponded to the body composition of mice, with adiposity increasing as a function of energy intake. Even though mice on low P:C diets were moderately adipose (although not to the extent of low-protein, high-fat fed mice), they lived longest (Figure 1B). Indeed, longevity mirrored the pattern seen in flies, being greatest on low P:C diets. Markers of metabolic health (insulin, glucose tolerance) and immune function at 15 months of age were consistent with the longevity data, being best on low P:C diets and worst on high-protein and high-fat diets (Le Couteur et al., 2014; Solon-Biet et al., 2014; Figure 2). By contrast, measures of reproductive potential in both males and females were highest on a higher-protein diet, consistent with results from flies.

There was no evidence for prolongation of lifespan on *ad libitum* diets that restricted calorie intake by reducing the energy density of the diet. The standard regime for restricting calorie intake that is well known to extend lifespan involves providing mice with a daily aliquot of food, which is soon eaten, leaving the animal deprived for the rest of the day (Curtis and de Cabo, 2013; Everitt et al., 2010). By inference, then, the results of Solon-Biet et al. (2014) imply that extension of lifespan with standard caloric restriction protocols may not entirely be secondary to reduction in calories; rather, other factors may contribute such as periods of fasting (Mattson et al., 2014), and reduction in protein intake that ensues once the mouse has eaten its daily food allocation.

A major conclusion from the geometric experiments on flies and mice is that the balance of macronutrients in the diet has a profound impact on food and energy intake, metabolic health, lifespan, immune function, and reproduction. The diet composition that best supports longevity is not the same as that which sustains maximal reproductive output or leanness. The question arises as to whether these conclusions apply to humans. The evidence suggests that they do.

Humans Behave Like Mice and Flies

For mice on diets differing in the ratio and concentrations of protein, carbohydrate, and fat, food intake was driven most strongly by the concentration of protein in the diet, but with a strong competing feedback emanating from signals associated with the specific appetite for carbohydrate. The data from population survey analyses (Austin et al., 2011; Austin and Krueger, 2013; Martinez-Cordero et al., 2012), compendia of controlled trials (Gosby et al., 2014), and detailed clinical studies involving foods formulated to disguise their macronutrient composition (Gosby et al., 2011) indicate that prioritization of protein intake may be even stronger in humans. Humans compensate for reduction in the available proportion of dietary energy contributed by protein by increasing food intake, and in so doing over-ingest fats and carbohydrates. Since the percentage of energy from protein in the diet is always smaller than that from fats and carbohydrates combined, compensatory adjustments in intake that redress relatively small deficits in protein "gear up" to relatively large excess of fats and carbohydrates, and thus energy intake overall—what we have termed "protein leverage" (Simpson and Raubenheimer, 2005). Studies have shown that total energy intake is, indeed, a negative function of percent protein in the diet across the range seen in all human populations measured to date with food sufficiency, namely 10%–25% protein of total energy. Above ca. 20%–25% protein the reduction in intake with rising percent protein becomes attenuated (Gosby et al., 2014), presumably because of increasingly strong opposing feedbacks arising from deficiency of other nutrients, notably carbohydrate, driving increased intake. At the other extreme, clinical trials using 5% protein (Martens et al., 2013; Martens et al., 2014a, b) failed to show increased energy intake relative to 15% protein diets, indicating that, as in mice and other animals, there is a lower limit to compensatory responses to dietary protein. Five percent protein approximates the composition of French fries from fast-food outlets, and is insufficient to maintain lean mass. Maintaining protein intake at adequate levels on such a diet would require ingesting an unfeasible quantity of food.

Gosby and colleagues (2011) showed that the 12% increase in *ad libitum* energy intake among subjects confined to a 10% protein diet relative to 15% or 25% protein diets was due to increased consumption of savory-flavored foods between meals. The seeking of savory cues is indicative of protein hunger, and is reflected in increased activity in brain regions associated with reward, such as the inferior orbitofrontal cortex and striatum (Griffioen-Roose et al., 2014). These results indicate that protein status influences gustatory pathways in a way that affects protein intake in humans. In insects, feedbacks onto gustatory

responses occur at the periphery, through direct modulation of taste receptors, as well as via learning of nutrient-specific cues (Simpson and Raubenheimer, 2012). The mediating nutrient signaling systems controlling protein appetite are thought to involve both circulating free amino acids and lean hormonal signals such as FGF 21 (Laeger et al., 2014).

Controlled, prospective experiments testing the effects of multiple diets, equivalent to those performed in animals, are not feasible in humans. Nevertheless, there is growing evidence from observational studies and quasi-interventional trials indicating that health and lifespan are influenced by the balance of macronutrients and can be best interpreted using nutritional geometry. In a systematic review of human dietary studies (Pedersen et al., 2013), it was concluded that long-term, high-protein, low-carbohydrate diets and increased mortality are associated. In addition, long-term, high-protein, high-fat and low-carbohydrate diets increased the risk of type 2 diabetes mellitus. Consistent with this notion, Fung and colleagues (Fung et al., 2010) reported that high-protein, low-carbohydrate diets were associated with increased mortality over 20–26 years in the Nurses' Health Study and the Health Professionals' Follow-up Study. Similar results linking low-carbohydrate, high-protein diets with increased mortality and/or cardiovascular disease have been reported in the Swedish Women's Health and Lifestyle cohort (Lagiou et al., 2012; Lagiou et al., 2007) and the Greek cohort of the European Prospective Investigation into Cancer and Nutrition (Trichopoulou et al., 2007). These studies have specifically reported the balance of two macronutrients, protein and carbohydrate, and consistently indicate that low-carbohydrate, high-protein diets increase mortality. Such conclusions are consistent with results in animals where the balance of macronutrients, rather than the intake amount of either, is a key determinant of lifespan, and that diets with high-carbohydrate and low-protein were associated with increased lifespan and improved cardiometabolic outcomes in late life (Lee et al., 2008; Solon-Biet et al., 2014). These conclusions are indirectly supported by associations between increased mortality and low-carbohydrate diets in humans (Noto et al., 2013) and a recent study showing increased mortality and cancer on high-protein diets (Levine et al., 2014).

In demonstrating that both high and low P:C diets have benefits and risks, these data clearly illustrate the importance of dietary balance. But a conundrum remains (Figure 2). Whereas a low P:C diet appears beneficial for longevity and late life health, protein leverage on such a diet tends to drive overconsumption of total energy and risk of obesity, thereby mitigating the health benefits of low-protein intake. Another consideration is that overweight in humans might be associated with poor outcomes if caused by low-protein, high-fat diets, but better outcomes when low-protein, high-carbohydrate diets apply. Managing these counterposing effects might include reducing the intake of proteins with high concentrations of sulfur- and branched chain amino acids linked to pro-aging and disease pathways (Hine et al., 2015; Solon-Biet et al., 2014), decreasing dietary P:C by replacing dietary fats with healthy carbohydrates, periods of intermittent fasting, and drug development targeting nutrient-sensing pathways (Le Couteur et al., 2012; Baur et al., 2012; Mattson et al., 2014).

Age itself is a major determinant of what constitutes an optimal diet. Hence, whereas low P:C diets benefit late life health and longevity (Levine et al., 2014), they are not optimal for somatic growth and reproduction earlier in life, which require higher protein intakes. In addition to age, a network of interacting factors need to be considered to determine an optimal diet, including genotype, epigenotype, sex, health, and immune status, commensal ecology, societal context, physical environment, and the level of activity.

Nutritional Geometry at the Cellular and Molecular Level

Mapping response landscapes as a function of multiple nutrient dimensions offers a step-change in understanding the nutritional phenotype of an animal, compared to energy or single-nutrient-based single-dimensional approaches. The same potential applies to deciphering cellular and molecular pathways. The concept that appetite and metabolism respond to specific nutrients and nutrient ratios is transformative for dissecting cellular mechanisms for these processes, evidenced by the recent discovery of FGF 21 as the first known candidate endocrine signal in the control of protein intake (e.g., Laeger et al., 2014). A geometric analysis can also better aid interpretation of the effects of genetic or pharmacological manipulations (Piper et al., 2011).

As an example of the use of nutritional geometry, a number of interacting nutrient-sensing pathways are considered to mediate the link between diet and aging, including mTOR, AMPK, insulin/IGF1/GH, and SIRT1. The effects of dietary P:C on lifespan in mice and flies led to the prediction that these pathways, either individually or in combination, are responsive to P:C ratio rather than to energy or single nutrients (Simpson and Raubenheimer, 2009). This hypothesis was supported by response surface analyses indicating that circulating insulin levels were strongly influenced by dietary P:C, and that hepatic mTOR activation was a positive function of the ratio of circulating branched chain amino acids and glucose (Solon-Biet et al., 2014).

Food for Thought

Here we have focused on the relationships among diet composition, intake, and health, but nutritional geometry has also been used to investigate the broader causes of variance in diet composition of humans and other animals, including developmental, economic, evolutionary, and ecological (Raubenheimer et al., 2015). This intake-focused approach is not an alternative to theories of human nutrition that center on variation in biological responses to ingested nutrients, for example the propensity to store fat (Wells, 2006). Rather, as stressed by Speakman (2014), nutrient intake and its consequences are best modeled as part of the same system, enabling the understanding, prediction, and management of organism- and population-level responses to different environments (Lihoreau et al., 2014). We stress, further, that nutrient combinations entered into a geometric model should be considered on a case-by-case basis. To date many questions have been addressed by modeling interactions among the macronutrients (Simpson and Raubenheimer 2012), but in other cases mineral micronutrients and

vitamins (e.g., Blumfield et al., 2012) or specific amino acids (Solon-Biet et al., 2014) have been integrated into the model. The quality of macronutrients (types of fats, carbohydrates, and proteins) is another important aspect of diet that is amenable to geometric analysis, yet remains uncharted. It is only through acknowledging the complexity of nutrition and systematically charting its implications from the food environment to dietary choices and health consequences that we can hope to tame the epidemic of obesity-related diseases that has arisen over recent decades.

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Note Added in Proof

Since the submission of this manuscript, an additional paper has shown that reproductive function is best supported in male and female mice on a higher-protein diet.

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