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Single-Subject Studies in Translational Nutrition Research

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Abstract

There is a great deal of interest in personalized, individualized, or precision interventions for disease and health-risk mitigation. This is as true of nutrition-based intervention and prevention strategies as it is for pharmacotherapies and pharmaceutical-oriented prevention strategies. Essentially, technological breakthroughs have enabled researchers to probe an individual's unique genetic, biochemical, physiological, behavioral, and exposure profile, allowing them to identify very specific and often nuanced factors that an individual might possess, which may make it more or less likely that he or she responds favorably to a particular intervention (e.g., nutrient supplementation) or disease prevention strategy (e.g., specific diet). However, as compelling and intuitive as personalized nutrition might be in the current era in which data-intensive biomedical characterization of individuals is possible, appropriately and objectively vetting personalized nutrition strategies is not trivial and requires novel study designs and data analytical methods. These designs and methods must consider a very integrated use of the multiple contemporary biomedical assays and technologies that motivate them, which adds to their complexity. Single-subject or N-of-1 trials can be used to assess the utility of personalized interventions and, in addition, can be crafted in such a way as to accommodate the necessarily integrated use of many emerging biomedical technologies and assays. In this review, we consider the motivation, design, and implementation of N-of-1 trials in translational nutrition research that are meant to assess the utility of personalized nutritional strategies. We provide a number of example studies, discuss

appropriate analytical methods given the complex data they generate and require, and consider how such studies could leverage integration of various biomarker assays and clinical end points. Importantly, we also consider the development of strategies and algorithms for matching nutritional needs to individual biomedical profiles and the issues surrounding them. Finally, we discuss the limitations of personalized nutrition studies, possible extensions of N-of-1 nutritional intervention studies, and areas of future research.

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INTRODUCTION

The belief that one can tailor interventions, including nutritional interventions, to an individual's often nuanced and potentially unique genetic, biochemical, behavioral, and exposure profile is receiving a great deal of attention. Although some unmitigated success has been observed for specific targeted and individualized pharmacotherapies, especially those designed to treat cancers (13, 106), less success has been observed for personalized, individualized, or precision nutritional interventions. There are at least three interrelated reasons for this lack of success. First, it is likely that not enough time has elapsed since the introduction of high-throughput, data-intensive assays characterizing unique physiologic and exposure profiles (such as DNA sequencing, wireless glucose monitoring, smartphone application-driven diet diaries, etc.) for researchers to have identified definitive connections between the activities or benefits of specific nutrients, diets, and/or nutritional supplements and individual profiles, except in the context of rare, often genetically mediated overt nutritional deficiencies (7, 8). Second, identifying and characterizing the molecular and physiologic processes and deficiencies forming the basis for such connections is difficult and

may be much more complex than making connections between, e.g., highly contrived pharmaceutical products and specific gene products. Third, testing or vetting the utility of a personalized dietary intervention is also nontrivial and likely requires study designs, analytical methods, and overall strategies that differ from those used in the past.

The third reason is the actual focus of this review, although we argue that studies can be designed to simultaneously assess the benefits of personalized nutritional interventions for an individual and identify factors that solidify the connection between a specific dietary intervention and an individual's biochemical, physiological, behavioral, and exposure profile. In addition, despite the lack of a large number of success stories proving that personalized nutrition works on a large scale, there is now motivation for testing the benefits of personalized nutrition given the availability of high-throughput assays, such as DNA sequencing, proteomics, and wireless monitoring, as well as a growing number of insights into how fundamental molecular physiologic processes respond to or require specific nutrients (62). Thus, questions surrounding how one can best prove that personalized nutritional interventions benefit individuals are crucially important to address in order to advance personalized nutrition. One set of study designs, those falling under the heading of single-subject or N-of-1 studies, is highly appropriate in that their focus is on testing whether an individual exhibits any evidence, in a well-designed and controlled study, that they responded to a particular intervention. These studies can be extended and modified in a number of important ways. In this light, we ultimately argue that, in an era where personalization is emphasized (in, e.g., medicine, nutrition, advertising, general service industries, finance, etc.), nutrition-based clinical trials need to focus on intraindividual variations in responses exhibited by each participant over the course of the trial as much as interindividual variations in responses across participants in the trial. The former may help identify factors that influence responses in participant-specific ways, and the latter can shed light on whether a factor shared among others helps solidify its role in mediating responses. Note that we use the term personalized nutrition (as opposed to individualized or precision nutrition) in what follows to refer to attempts to match specific diets, nutrients, or natural-product-based supplements to an individual's profile except in very specific instances.

The remainder of the review contains nine broad sections. The first section provides a general background on the state of clinical trials in nutrition and on why different trial designs are needed to advance personalized nutrition. The second considers the biological motivations for personalized nutrition and the need for more appropriate clinical trial designs. The third section discusses basic N-of-1 trial designs and their extensions. The fourth considers how one can aggregate the results of N-of-1 trials to make broader claims about the utility of a nutritional intervention in the population at large. The fifth discusses the problem of determining what to measure to assess success when designing a study to test, e.g., a diet's effects on an individual. The sixth considers monitoring individuals for health status changes in the wake of providing them an intervention or for determining their general vulnerability to disease. The seventh section considers the increasing interest in vetting or testing matching strategies that relate specific diets to individual profiles rather than simply vetting the specific diets themselves. The eighth section focuses on a few of the more intriguing and relevant recently published studies that motivate N-of-1 trials for nutrition and how future studies like them could be modified along the lines discussed in this review. The ninth and last section provides a brief summary and discussion of N-of-1 trials in nutrition as well as areas of future research.

PERSONALIZED NUTRITION AND HUMAN CLINICAL STUDIES

Traditional Population-Based Clinical Trials

Most clinical trials are designed to address questions about the utility or health benefits of a drug or intervention in the population at large and do not necessarily address questions about

the unequivocal health benefits for any single individual participating in the trial. In broad terms, population-based trials typically involve providing a particular intervention to a group of individuals while a comparator intervention, often a placebo, is provided to another group of individuals (36, 43, 48, 52, 89, 105). The average benefit of the intervention across those individuals provided the intervention (e.g., average weight loss, average drop in blood pressure, or average cholesterol level) is compared to the average benefit of those individuals provided a placebo or comparator intervention. It is rare in such trials that enough data is collected on any one participant to state unequivocally that the benefit observed for that participant can be attributed to the intervention itself. Although of extreme value in the nutritional sciences (see, e.g., **Table 1** for some examples of large-scale nutritional intervention studies), such studies do not often accommodate the quantification of the degree to which the subjects exhibit variations in response within the intervention and comparator groups. Population-based clinical trials

Table 1 Example large-scale nutritional intervention studies

Authors (year)	Reference number	Diet and contrast	Number	Study design	Outcome
Guasch-Ferré et al. (2013)	43	Mediterranean diet with nuts, olive oil, or control group	7,216	Randomized control trial	Hazard ratio for death in group eating nuts: 0.61 (0.45–0.83)
Lippman et al. (2009) SELECT	69	Selenium and vitamin E versus placebo	35,533	Randomized control trial	No difference in prostate cancer incidence between intervention and control group
Qiao et al. (2009)	93	Selenium, beta-carotene, vitamin E	29,584	Randomized trial	Decreased overall mortality in group taking vitamins
Gaziano et al. (2012) Sesso et al. (2008) Physician's Health Study II	36 105	Vitamin E and C versus placebo	14,641	Randomized placebo controlled trial	No decrease in risk of cancers or heart disease
Prentice et al. (2006) Howard et al. (2006) Women's Health Initiative	89 48	Intervention-directed reduced fat diet versus no intervention	48,835	Randomized control trial	No change in the incidence of breast cancer in low-fat diet group No change in risk of stroke or coronary heart disease
McCullough et al. (2003) American Cancer Society Cancer Prevention Study II—Nutrition Cohort	79	Calcium, vitamin D, dairy intake	127,749	Longitudinal cohort study	Intake of calcium supplements and dietary and supplemental vitamin D inversely associated with risk for colorectal cancer
Menotti et al. (1999) Seven Countries Study	80	18 different diets	12,763	Longitudinal cohort study	Legumes, fish, and alcohol intake had a negative correlation with mortality from coronary heart disease
Giovannucci et al. (1998) Nurses' Health Study	39	Multivitamins and folate	88,756	Longitudinal cohort study	Fifteen years of multivitamin use and dietary folate alone associated with decreased risk of colon cancer

Abbreviation: SELECT, Selenium and Vitamin E Cancer Prevention Trial.

can actually be designed to explore the benefits of personalized nutritional interventions using, for example, some of the methods discussed in the section titled Basic Study Designs and the section titled Aggregated Single-Subject Studies, which are meant to assess the overall benefit of personalized versus nonpersonalized interventions. In general, however, traditional designs used in population-based trials are not appropriate if the goal is to ultimately evaluate the utility of personalization in medicine and nutrition. This is especially important because many of the most often used interventions have been documented not to work in a large fraction of the individuals provided them in population-based studies, and the reasons for this are largely unknown but could be explored with appropriate study designs [see, e.g., the editorial by Schork (101)].

Post Hoc Identification of Responders and Nonresponders

One practice that is pursued often in the context of large-scale population-based clinical trials involves the pursuit of post hoc analyses that explore the relationships between different factors (i.e., covariates measured on the trial participants) and responses. Despite the potential insights that could arise from such analyses, they are often frowned upon unless they are pursued as a way of generating hypotheses that could be tested in a more sophisticated way in a future clinical trial (116). Many researchers have considered using post hoc analyses involving large-scale clinical trial data to identify genetic variants or other biomarkers that may predict responses (45). However, pharmacogenetic analyses of these sorts are complicated by the fact that there are rarely additional studies that can be used to replicate the findings arising from these post hoc analyses, and replication is considered the sine qua non of genetic association studies (5, 49, 59, 83). Despite this, many studies have been pursued to identify genetic variants that influence responses to nutrients, diets, or dietary supplements (i.e., nutrigenomics studies; see **Table 2**). Nutrigenomic findings can provide motivation for focused N-of-1 trials on individuals harboring genetic variants associated with a nutrient or diet, as discussed in the Section titled Biological Motivation for Individualized Nutrition, but also suffer from replication issues.

One important component of studies designed to determine if an individual has responded to a particular intervention, whether in the context of a pharmacological or nutritional intervention, is the need for internal, individual-specific controls. This can be achieved through the use of cross-over study designs wherein individuals are provided an intervention and then purposefully provided a comparator intervention (which could be a placebo or sham intervention) to generate an appropriate contrast. **Tables 3** and **4** list many studies investigating the benefits of a nutritional intervention. Some used a cross-over design, even though they were conceived and designed as traditional population-focused studies. Although there are many issues with the design and conduct of cross-over trials (53, 104), not including a cross-over component in a trial can be highly problematic for making claims about an individual's unique (if any) response to the intervention of interest. Essentially, in a trial without a cross over, claims about whether a change in the health status of an individual can actually be attributed to the intervention of interest would be based entirely on population-based statistics comparing the health status of that individual to others in the trial. This makes claims about individual rather than group responses to interventions problematic because any individual may exhibit an equivalent response to other interventions (including a placebo or sham intervention), which undermines both confidence that the intervention is working through a unique mechanism and that it is an appropriate intervention for an individual relative to other interventions that could have been chosen.

Single-Subject Trials and Determining Individual Responses

If the goal of a study is to truly determine whether a particular individual is responding to a specific intervention, then for the reasons discussed in the section below entitled Biological Motivation for

Table 2 Example nutrigenomics studies leveraging a nutritional intervention

Authors (year)	Reference number	Diet and contrast	Number of participants	Study design	Outcome
Konstantinidou et al. (2010)	58	Traditional Mediterranean diet (TMD) with virgin olive oil versus TMD with washed virgin olive oil versus habitual diet	90	Randomized control trial	TMD with olive oil resulted in downregulation of proatherogenic genes *INFγ*, *IL7R*, *ADRB2*, *POLK*
Pu et al. (2016) COMIT study	90	Canola oil High oleic canola oil High oleic canola oil enriched with DHA Flax/safflower oil Corn/safflower oil	170	Cross-over randomized control trial	Allele carriers at snp rs324420 in *FAAH* gene had significantly higher DHEA levels than the CC genotype carriers, indicating a possible beneficial effect on circulating fatty acid levels
Frankwich et al. (2015)	33	Nutrigenetic four guided diets: balanced, low fat, low carbohydrate, and Mediterranean Standard versus a nonguided balanced diet	51	Randomized control trial	Participants with low-risk obesity polymorphisms lost significantly more weight
Wojczynski et al. (2015)	122	High-fat diet with 83% fat, 14% carbohydrate, and 3% protein	872	Genome-wide association study	Two SNPSs identified as significant near the *APOA1/C3/A4/A5* gene cluster in lipid metabolism
Shab-Bidar et al. (2015)	107	Vitamin D–fortified yogurt drink Plain yogurt drink	60	Randomized control trial	Carriers of the AA genotype of VDR-Cdx-2 had significant decreases in obesity indices compared with carriers of GA and GG genotypes
Goni et al. (2014)	40	Personalized nutrition diet based on genotype Diets similar in the total amount of protein, carbohydrate, and vegetables	167	Longitudinal cohort	Carriers of variant alleles in *FTO*, *MC4R*, and *MTNR1B* had lower weight loss than noncarriers Women carriers of variant in *MTNR1B* who ate high total protein and high animal protein diets lost less weight than wild-type carriers
Renda (2012)	96	Caffeine consumption	110	Observational cohort study	Variants in the *ADORA2A* and *ADRA2B* genes were associated with increased blood pressure after caffeine consumption

Abbreviations: COMIT, Canola Oil Multicenter Intervention Trial; DHA, docosahexaenoic acid; DHEA, dehydroepiandrosterone; SNPs, single nucleotide polypmorphisms.

Table 3 Example nutritional intervention studies focusing on different measures and outcomes

Category	Measures	Author (year)	Reference number	Number[a]	Nutritional component	Study design	Outcome
Cognitive, mental, behavioral health	Mood, quality of life, sleep, sexual function	Martin et al. (2016)	75	218	Caloric restriction of 25% versus ad lib diet for 2 years	Randomized control trial	Caloric restriction group had significantly reduced tension and improved mood, sleep duration, sexual drive, and general health
	Depression scale	Rahe et al. (2014)	94	16 studies	Mediterranean diet (MD) Western diet (WD)	Systematic review	MD had lower risk of depression WD had higher risk of depression
		Tiemeier et al. (2002)	114	3,884	Vitamin B_{12} and folate deficiency	Cross-sectional survey	Higher risk of depression
		Jacka et al. (2010)	50	1,046	Traditional diet with fish, vegetables, and fiber	Cross-sectional survey	Lower risk of depression
	Quality of life, Karnofsky performance status, cognitive function, global health status	van der Meij (2012)	117	40	Supplements with polyunsaturated fatty acids	Double-blind randomized control trial	Improvements in all categories for lung cancer patients taking supplements versus those not taking supplements
	Cognition scores	Lemaire et al. (2010)	65	20	Frequent small healthy meals versus baseline diet	Cross-over trial	Score on cognitive testing significantly improved with healthy meals
	Sleep	Afaghi et al. (2007)	1	12	High-carbohydrate meals	Cross-over trial	High-carbohydrate meal given 4 h versus 1 h before sleep shortened sleep-onset latency
		Killer et al. (2015)	56	13	High-versus moderate-carbohydrate diet	Cross-over trial	Sleep time was significantly higher in moderate-carbohydrate group

(*Continued*)

Table 3 (*Continued*)

Category	Measures	Author (year)	Reference number	Number[a]	Nutritional component	Study design	Outcome
Physiologic and metabolic function	Body weight, sleep, and energy levels	Gill & Panda (2015)	37	8	Time-restricted eating	Nonrandomized observational cohort study	Decreased body weight, improved sleep and energy levels when eating restricted to 10 time periods of an hour each
	Insulin resistance, highly sensitive C-reactive protein (hs-CRP), adiponectin	Jennings et al. (2014)	52	1,997	Flavonoid intake	Cross-sectional survey	High-flavone and anthocyanin intake associated with lower peripheral insulin resistance Higher anthocyanin associated with lower hs-CRP Higher-flavone intake associated with improved adiponectin concentrations
	Weight loss	Look AHEAD Research Group et al. (2013)	70	5,145	Caloric restriction with meal replacement for diabetics versus normal diet	Randomized control trial	Greater weight loss in intervention group
		Brehm et al. (2003)	11	53	Very low-carbohydrate versus low-fat diet	Randomized control trial	Very low-carbohydrate group lost more weight
	Weight loss, fat-free mass, fat mass	Lopes Gomes et al. (2016)	71	34	Whey protein supplementation versus not	Randomized control trial	Intervention group lost more weight than control group
	Blood pressure, blood glucose, and lipids	Domenech et al. (2014)	23	235	Mediterranean diet, olive oil or nut supplements	Randomized control trial	Reduced blood pressure, blood glucose, and lipids in intervention group
	Non-alcoholic fatty liver disease	Elias et al. (2010)	27	31	Caloric restriction	Nonrandomized observational cohort study	Significant improvements in liver function tests, insulin resistance, visceral fat, liver density, and high-density lipoprotein levels
	Blood gene expression levels	Leonardson et al. (2010)	66	40	Fasting state versus fed state	Cross-over trial	Significant differences in gene expression between fasted and fed states

[a]Number of participants unless the entry states it is the number of studies.
Abbreviation: AHEAD, Action for Health in Diabetes.

Table 4 Examples of nutritional intervention studies focusing on the gut microbiome

Category	Author (year)	Reference numbers	Number of partici-pants	Nutritional component	Study design	Outcome
Micro-biome	Dao et al. (2016)	19	90	Energy-restricted high-protein diet versus weight maintenance diet	Cross-over trial	Participants with higher levels of *Akkermansia muciniphila* showed greater improvement in metabolic health parameters, e.g., decreased insulin resistance
	Brahe et al. (2015)	10	58	*Lactobacillus paracasei* versus flax seed mucilage versus placebo	Three-arm, single-blind, randomized control trial	Significant alterations in microbiota in flax seed group but not *L. paracasei* arm compared with placebo Improved insulin sensitivity in flax seed group only
	David et al. (2014)	20, 21	11	Animal based versus plant based	Nonrandomized observational cohort study	Significant diet-based alterations in microbial communities
	Dewulf et al. (2013)	22	30	Prebiotics (inulin/oligofructose) versus placebo	Double-blind, randomized control trial	Prebiotic use lead to: Increased *Bifidobacterium* and *Faecalibacterium prausnitzii* and associated decreased serum lipopolysaccharides Decreased *Bacteroides* associated with slight decrease in fat mass
	Cotillard et al. (2013)	17	49	Energy-restricted high-protein diet versus weight maintenance diet	Cross-over trial	Microbiome with greater diversity (high gene count) associated with eating more fruits and vegetables Individuals with low-diversity microbiome changed to high diversity after eating energy-restricted diet
	Walker et al. (2011)	120	14	Resistant starch, versus nonstarch polysaccharides versus low-carbohydrate weight loss	Cross-over trial	Significant alterations in bacterial phylotypes occur rapidly (within 3 to 4 days of change in diet)

Individualized Nutrition, classical population-based clinical trials are not appropriate. Classical population-based designs simply do not accommodate the collection of enough information on any one individual over the time the intervention is being administered to allow for unequivocal inferences about that individual's unique response to be made. This, of course, could be changed as emerging, simple, cost-effective, and convenient ways of collecting appropriate data, e.g., through wireless devices, could facilitate such studies (28, 86, 87). However, the actual design of such studies is as crucial as collecting enough data on an individual because appropriate contrasts that exploit

that data must be made to draw compelling inferences about the unique response of an individual to an intervention. This suggests that studies focusing on individuals, i.e., N-of-1 studies, do indeed have their place if emphasis is on assessing those individuals' unique and nuanced responses to an intervention. We consider specific study designs for N-of-1 studies below in the Section titled Basic Study Designs but believe that it is important to provide additional historical and biological perspectives on the motivation for such designs.

The origins of N-of-1 clinical trials have been discussed by many authors (47, 67, 101), but have been implemented most often in education, behavioral assessment, and pain research settings (67). Of most relevance to this review is the consideration of N-of-1 trials in personalized, individualized, or precision medicine, including disease prevention and management settings. In this light, a paper by Hogben & Sim (47) published in the early 1950s described N-of-1 trials as logical extensions of actual clinical practice. The authors argued that physicians often take into consideration the unique and nuanced profile, in terms of medical history, behaviors, and environmental exposures, of patients in making decisions on how to treat them. Essentially, they argued, physicians are accustomed to dealing with patients as individuals with unique characteristics that could affect their care, but rarely end up proving to themselves that the nuanced way in which they approach each patient actually worked for that patient, or at least worked better than another approach they could have taken, for example, by treating everyone in exactly the same way. Rather, in traditional clinical care, the information about whether an intervention worked, or is working, on an individual patient is collected informally in the context of return or follow-up visits, dialog with other hospital staff, or perhaps mail-in records. The ultimate question Hogben & Sim raised was whether this process could be formalized and made more objective. They ultimately argued that one could bring principles of experimental design and data collection into this process in two important ways: (*a*) by providing the patient with charts she could use to track her symptoms over time that may identify important features of her treatment earlier and in a more objective way than standard practice would; and (*b*) by using control mechanisms and purposeful, possibly prespecified, data analyses to statistically assess whether the patient's improvement, or lack thereof, could be attributed to the actual intervention in question and not to something else (e.g., the placebo effect, a measured or unmeasured covariate, noncompliance, or other issue).

The belief that one can make objective claims about an individual response to an intervention using information collected on just that person is backed by the very intuitive notion that it is the number of measures taken on an individual, not the number of individuals being studied, that is important, as well as how, and under what conditions, those measures have been collected to enable statistically and clinically meaningful conclusions to be drawn from them. Consider the fact that many in vitro studies involving, e.g., cellular systems or cell lines, make replicate measures on the cells they are studying under different conditions to draw inferences about the relationships between various factors measured on them despite the fact that those cells came from a single individual. Of course, it could be the case that different results would have been observed had a different set of cells been used, perhaps from a different individual, but this possibility actually solidifies the point that there may be individual differences between units of observations (e.g., cells, cell lines, inbred mouse strains, individual humans) that could only be brought to light if those individual units were studied in isolation to identify the phenotypes or outcomes they may not share with others. In other words, one can be just as careful and sophisticated in their thinking in an approach to making objective claims about an individual's response to an intervention as they could in making claims about the utility of an intervention in the population at large. The actual need for studying individuals in isolation and making claims about their unique and nuanced responses to nutritional interventions is also supported by studies leveraging data-intensive, high-throughput assays, such as DNA sequencing, which clearly shows molecular physiologic differences between

individuals that likely influence their responses to diets, nutrients, and supplements. In addition, historical and traditional clinical studies of nutritional interventions often document the very wide variations individuals exhibit in response to nutritional factors (see, e.g., **Table 1** and note the fact that the participants in those studies did not seem to exhibit identical responses to the interventions given that the standard deviations associated with response metrics for the studies were never 0.0).

BIOLOGICAL MOTIVATION FOR INDIVIDUALIZED NUTRITION

The recognition that individuals, whether as patients in a clinical setting or as individuals in the population at large, may exhibit unique responses to nutritional interventions that could only be teased out by studying each of them directly has its roots in a great deal of historical and emerging scientific studies. In fact, many reviews have been written on the biological motivation for personalized nutrition (2, 41, 60–62), and we therefore provide just a brief overview with a few examples to help put into context the need for N-of-1 studies and more appropriate N-of-1 study designs. Archibald Garrod (35) is typically attributed with the introduction of the notion of the biochemical individuality of humans. He basically argued that the unique genetic profiles each individual possesses create subtle, if not overt, differences between individuals in the way they respond to the environment, including pharmacological and nutritional interventions. This idea paved the way for the emerging field of pharmacogenetics, whose goal is to identify genetic variants some people possess that influence their unique responses to pharmacologic agents (88, 100). The insights from pharmacogenetics studies, it is argued, could lead to clinical practices in which pharmacologic interventions for preventing and treating a disease are tailored (or personalized) to patients based on their genetic profiles. Pharmacogenetics research has benefited enormously from the recent and rapid advances of molecular genetic assays, such as DNA sequencing and high-throughput proteomics, and the routine application of those technologies in association studies, especially genome-wide association studies that could lead to connections between genetic variants and phenotypes of all sorts, and may have clinical utility (12, 44, 45, 98).

The variation individuals exhibit in their responses to pharmacologic agents that may be attributable to genetic or other (e.g., exposure profile) differences between individuals is certainly consistent with the emerging field of nutrigenomics (54, 55, 60, 61, 73, 81, 118). Many researchers have identified associations between specific genetic variants and responses to diets, nutrients, and dietary supplements of all sorts (see **Table 2**), suggesting that individual responses to nutritional interventions could be as nuanced as responses to pharmacologic agents. Consider, for example, the rare disease phenylketonuria, which is caused by genetic mutations in the *PAH* gene and treatable by manipulating the amount of phenylalanine and protein levels in the diet of an individual with the condition (8). It is known that there is a complex relationship between mutations in the *PAH* gene, other genes, and genetic variants in other genes, that could modify the effect of *PAH* mutations, the severity of the condition, and the response to dietary manipulations to treat the condition (102). Another very detailed and recent study showed that the indigenous people of Greenland, the Inuit, exhibit evolutionarily mediated genetic variants at several loci that influence the levels of the omega-3 polyunsaturated fatty acids they exhibit. Furthermore, these genetic variants were found to be associated with multiple metabolic and anthropometric phenotypes, to have large effects on weight and height, and to modulate fatty acid composition (34). Complexities in the relationship between various genetic and biochemical factors, as well as behavioral and exposure factors, and responses to nutritional interventions are also consistent with many studies involving comparisons of different strains of model organisms (32, 84, 95, 110).

Finally, there is ample evidence for individual variation in response to nutritional factors emerging from the study of highly contrived interventions such as the oral glucose tolerance test and related tests (112). In fact, these tests are designed to determine if, in fact, an individual may possess an inability (or enhanced ability) to process and control products (such as insulin) that are provoked by a specific nutritional challenge relative to other individuals (9, 77, 82).

BASIC STUDY DESIGNS

Objective Data Collection Strategies

The mounting data, consistent with the notion that individuals exhibit variations in response to nutritional interventions, have broad implications for determining the best ways to optimize nutrition and maximize health for an individual. If these variations are attributable to inherent differences between individuals at the genetic and biochemical or behavioral and exposure levels, then obvious strategies for optimizing nutrition can be framed. For example, researchers can identify these factors and test how well they can predict an individual's response to a nutritional intervention. Clinicians and dieticians can then leverage this information in deciding how best to deal with a particular patient. Unfortunately, there are few instances where a direct, unequivocal relationship between a single (or even set of) identified factor(s) and a nutritional response is known. Therefore, researchers must empirically and directly test an individual's response to a nutritional intervention using objective and scientifically sound criteria. To do so requires sophisticated N-of-1 study designs.

Block and Period Structure of Single-Subject Trials

There are many possible N-of-1 clinical trial study designs that can be used to test a nutritional intervention or compare multiple interventions on a single individual. The design of any N-of-1 study, however, should be rooted in the biological issues associated with the primary hypothesis of interest as well the practicalities of the study's implementation. **Figure 1** provides a graphic depiction of 10 different N-of-1 study designs assuming that two different interventions, denoted 1 and 2 (e.g., a restricted or supplemented diet versus an ad libitum or comparator diet), are compared on an individual with respect to a particular quantitative health-related outcome (e.g., lean weight, levels of a particular metabolite, relative mood, etc.). It is further assumed that a crossover design is exploited over a total of 16 periods (e.g., 16 days or weeks) in which observations on the health-related outcome are made on the individual while that individual is either not receiving any intervention or receiving one of the two interventions of interest. Of course the total number of periods, the number and type of measures collected during each period, the length of the periods, the order in which interventions are provided during the various periods, and other factors are all important to consider. In addition, the design of an N-of-1 study must take into consideration practical, scientific, and ethical issues as well (e.g., is it practical or ethical to measure something on someone every day for a year? Is the measurement device technically sound enough to warrant its use in collecting multiple measurements?).

The first two columns of **Figure 1** simply index and label the example trial designs. A crucial question for N-of-1 designs concerns how to distribute the interventions across the 16 periods, e.g., provide one intervention for eight weeks and then the other for eight weeks? A related question concerns how often to make measurements within these periods, and this bears on the power of the study, which is briefly addressed in the Section titled Issues Affecting the Power of Single-Subject Designs. With 16 periods, one could, as noted, provide intervention 1 for eight consecutive periods

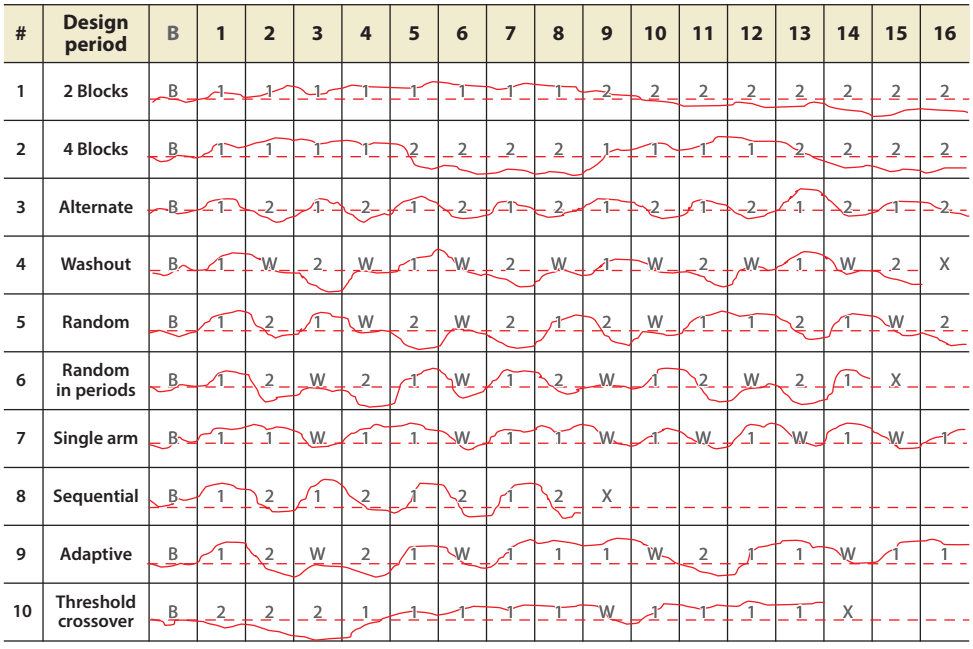

Figure 1

Hypothetical depiction of 10 different N-of-1 study designs comparing two interventions, denoted 1 and 2. The leftmost columns number and name the different designs. The gray numbers and letters in each cell describe the intervention administered during 16 different measurement periods in addition to a baseline period, denoted B. The entries in the cells correspond to the following: 1, intervention 1; 2, intervention 2; W, washout period; and X, termination of the study prior to completing all 16 periods. The dashed red line corresponds to values of a measure that are not associated with a favorable or unfavorable response to the interventions but are ambiguous with respect to response. The solid red lines provide the values of hypothetical continuous measures made on an individual, with the values above the red dashed line indicating a positive (preferable) response and values dipping below the dashed line indicating a negative response.

and then intervention 2 for eight periods. This is the first example study design (study design 1), labeled 2 blocks because the interventions were provided in two blocks of eight periods each. The second design (study design 2, labeled 4 blocks) assumes the interventions are provided in four blocks of four periods each, such that intervention 1 is provided for four consecutive periods, intervention 2 is provided for four periods, then intervention 1 is provided again for four periods, and finally intervention 2 is provided for four periods. Study design 3 considers simply alternating the interventions over the 16 periods. This type of design is most often associated with N-of-1 trials but does suffer from a few issues that are considered in greater detail in the Section titled Issues Affecting the Power of Single-Subject Designs, such as carry-over and order effects.

Randomizing the Order of Interventions and the Use of Washout Periods

Study design 4 in **Figure 1** depicts a trial that makes use of washout periods, i.e., periods in which the individual is taken off all interventions to let their body reset or reacclimate before testing another intervention. The use of washout periods is motivated by many factors (see the Section titled Issues Affecting the Power of Single-Subject Designs), and although they add to the length and complexity of a study, they help greatly with the interpretation of the study results. Studies 5

and 6 introduce randomization into the designs either with respect to the overall sequence in which the interventions are provided (study design 5) or within specified blocks of periods flanked by washout periods (study design 6). Randomization can be used to avoid a number of thorny issues such as order and carryover effects (see the Section titled Issues Affecting the Power of Single-Subject Designs).

Single-Arm, Sequential, Adaptive, and Multivariate Designs

There are a number of extensions to the basic N-of-1 designs. For example, interest may be in a single intervention in which the comparison between the values of the measure taken during the intervention period involves assessing differences with the values taken in the preintervention or baseline period, or during washout periods (study design 7). Obviously, using a placebo or sham intervention against a single intervention has advantages in reducing biases and confounding that might result if the question of interest is whether an intervention has any overall utility or not. Another design involves pursuing the study in a sequential manner in which stopping boundary rules are set a priori such that if the measure of interest reaches a level outside those bounds the trial is halted, as this would be indicative of overwhelming evidence that one or another of the interventions of interest has a compelling positive, or negative, effect (study design 8). Sequential trials are often pursued with only one intervention. Other studies can be designed and pursued in an adaptive manner, whereby the intervention exhibiting the best evidence for a positive benefit in the trial is applied more often—possibly by changing (e.g., increasing) the probability that the individual will receive that intervention as part of a randomization scheme going forward (study design 9). Adaptive study designs of this sort are often referred to as play-the-winner designs (109), and they are thought to be more ethical than many other designs because they minimize the amount of time an individual spends on what the evidence suggests is an inferior intervention and yet still retain the statistical power to make definitive claims about the utility of the interventions relative to one another. Finally, studies can be pursued that combine elements of adaptive and sequential trials in that they minimize the amount of time an individual spends on what appears to be an inferior treatment and are stopped if the data is overwhelmingly in favor of a better benefit for one or another intervention (study design 10).

Other study designs could involve multivariate outcomes; for example, monitoring weight, mood, microbiome species abundance, and blood chemistries simultaneously to assess the more global impact of the intervention, or involve testing the combined effects of interventions to determine their synergy. For example, one might design a study to see if a behavioral intervention when coupled with a dietary intervention leads to a better health profile for an individual than either of these interventions alone. Such designs would have to devote certain periods in the study to the individual being assigned each intervention alone to complement the periods when the interventions are combined to assess the nonadditive or synergistic effects of the interventions.

Issues Affecting the Power of Single-Subject Designs

There are a number of issues that could affect the yield of an N-of-1 trial. For example, the number of measurements made on a subject has a profound influence on the power to detect a difference between interventions or a statistically significant change in a measure during an intervention. Obviously, the number of measurements made during any period in an N-of-1 trial is dictated by the expense of, as well as any logistical issues surrounding, the collection of those measurements. The duration of each period in which an intervention is provided undoubtedly contributes to the total amount of time one has to make relevant measurements. This duration is dictated to a large

degree by the half-life, or the amount of time one would likely see an effect, of an intervention. Thus, if it is known that it will take weeks before specific diets will affect the body weight of an individual, then having the periods in which the interventions are applied last only a few days would not work.

Another crucial factor affecting the power of N-of-1 studies is the serial correlation between the observations. Because the measures are made on a single individual, they will be correlated over time, especially if the measures are made either continuously or with short intervals between them. This is unlike measures made on a large number of unrelated individuals in free-living populations, where the correlations between them are likely nonexistent or negligible. Strong serial correlations between observations can have a profound effect on the power of a study because the lack of independence of the observations reduces the statistical information provided by them (26, 113). The hypothetical examples in **Figure 1** assume that the measurements could be made continuously, which may be possible with wireless devices, such as an actigraph or continuous glucose monitor (111), but this would be difficult in other settings (e.g., blood draws or whole-body imaging to explore body fat distribution).

The likelihood of carryover and order effects is also important to consider in the design of an N-of-1 study. Carryover effects occur when the effect of one intervention lingers over some period of time after that intervention is stopped or changed. This can occur with many pharmaceutical and behavioral interventions as the amount of drug- or specific behavior-induced changes, and the effects of that drug or behavior, affect the body going forward. Carryover effects can confound claims about the biological effects of a specific intervention because it becomes difficult to distinguish the effects of the intervention of interest from a previous intervention. Washout periods are often used to avoid carryover effects, but their use adds to the time and complexity of a study. To avoid certain biases even further, one could leverage blinding in a study, such that the individual receiving the interventions would not know to which intervention they were being subjected. This might be very difficult to achieve in practice given, e.g., food tastes, textures, the physiologic effects of certain supplements, etc. because the individual receiving them may recognize which intervention they are being provided based on these features. Blinding could also be applied to the research team and medical overseers by not letting them know which intervention an individual in a trial might be on. Such blinding could avoid conscious or unconscious biases a research group might have about the effects of an intervention.

Order effects are related to carryover effects and occur when one intervention is systematically provided before another. This can create the illusion that one intervention is superior when in fact there could be a learning effect (i.e., an individual recognizes when he or she is on one or another intervention and that leads to biases in terms of behaviors that might impact interpretation of measures used to assess the differences between interventions), tolerance to one intervention, or a carryover effect that confounds an ability to attribute differences in a measure to an actual intervention and not a bias in the measurements. Order effects can be avoided by randomizing the order in which interventions are provided to an individual.

Finally, two very important considerations in an N-of-1 trial (or any trial for that matter) involve covariate effects and the statistical analysis methods used. Essentially, if there are factors that could influence a primary measure independently of an intervention, then they must be measured and taken into consideration when analyzing and interpreting the results of the trial. For example, physical activity influences weight and therefore should be considered in a trial investigating the influence of different diets on weight. In this light, it is important to consider whether to explicitly control for covariate effects in the design of the trial (e.g., stipulate that activity levels remain constant or are built into the trial as an additional intervention) or simply measure the covariate and accommodate it as such in analyzing the data. In addition, there are a variety of statistical

methods that can be leveraged for drawing inferences from N-of-1 trial data, including simple linear models (99); time series analyses (72); simple comparisons and contrasts using t-tests (42); and bootstrap, permutation, and randomization tests (68). A complete assessment of the statistical methods than can be used is beyond the scope of this review but is an incredibly important component of any N-of-1 trial (6, 15, 25, 26, 91).

AGGREGATED SINGLE-SUBJECT STUDIES

It is possible to aggregate the results of N-of-1 studies and thereby draw more general conclusions about the utility of an intervention in the population at large. Methods for aggregating N-of-1 clinical trial data have been proposed that are based on mixed effects models, which can take into consideration population averages as well as individual-specific variations (92). In this light, aggregated N-of-1 studies may be interpreted in a way that is analogous to population-based trials of the type discussed in the Subsection titled Traditional Population-Based Clinical Trials, except that they have been designed to ensure that enough data is collected on each individual to make unequivocal claims about their responses (or lack thereof) to the interventions of interest. Such analyses have advantages because they explicitly model and account for regression to the mean, measurement error, cryptic or latent variable effects, and other population-level phenomena when drawing inferences about the response of an individual to a specific intervention.

Aggregating N-of-1 trials can facilitate a number of important additional analyses. For example, one could identify, with great precision, individuals who share a response profile and then consider what these individuals might have in common (e.g., a specific set of genetic factors, exposures to certain environmental conditions, etc.). **Figure 2** provides a graphical depiction of this concept. This type of analysis suggests that N-of-1 trials can be used to bring out a response phenotype in a sophisticated way that could be explored further.

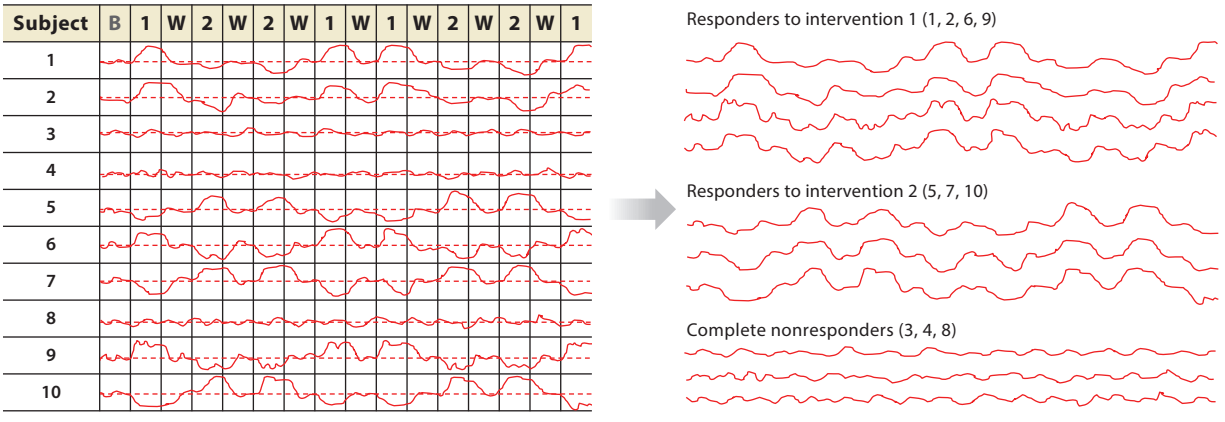

Figure 2

A graphical depiction of the result of aggregating the outcomes of 10 different N-of-1 studies. For the left panel, it is assumed that each individual underwent an N-of-1 trial with a similar design in which interventions were alternated after baseline and include washout periods. As with **Figure 1**, the dashed red line corresponds to values of a measure that are not associated with a favorable or unfavorable response to the interventions but are ambiguous with respect to response. The solid red lines provide the values of hypothetical continuous measure made on an individual, with values above the red dashed line indicating a positive response and values dipping below the dashed line indicating a negative response. The right panels depict the results of a clustering of the individual responses, with some individuals exhibiting a greater response to intervention 1 (the upper set of response profiles), some individuals exhibiting a greater response to intervention 2 (middle set of response profiles), and some individuals exhibiting a lack of response to either intervention.

The ability to aggregate the results of N-of-1 trials ultimately suggests that it is wrong to argue that N-of-1 trial results cannot be generalized. In addition, one could always conduct a study to test the hypothesis that conducting N-of-1 trials on individuals leads to better health outcomes for those individuals than not conducting N-of-1 trials on them or providing them interventions based on standard practices. Such a study may simply randomize individuals to a group that is subjected to an N-of-1 trial for identifying an optimal intervention and to a group that is simply provided interventions based on standard and legacy practices (103).

WHAT DOES IT MAKE SENSE TO MEASURE?

There are many different phenotypes or outcomes one could measure in the context of an N-of-1 study. Ultimately, the choice of which phenotype to study would depend on the nature of the condition for which the intervention is being considered (i.e., the reason the individual may be undergoing an N-of-1 study in the first place) as well as the nature of the intervention. **Tables 3** and **4** list a number of studies exploring the impact of a nutritional intervention on different phenotypes and outcomes. None of these studies was pursued in the context of an N-of-1 or aggregated N-of-1 study, but clearly each could have been pursued as such with appropriate changes in their design and execution. The nature of the nutritional interventions listed in **Tables 3** and **4** is also broad and includes overall caloric restriction (75); the timing of food consumption on a daily basis (37); food-based diets (Mediterranean, high carbohydrate, etc.) (1, 94); vitamins and supplements (folate, $B\text{-}_{12}$, omega-3 fatty acids) (50, 69, 114); bioactive compounds (phenols such as resveratrol, phytoestrogens such as isoflavone, carotenoids such as lycopene) (52, 74, 121); and, of increasing interest, probiotics (10).

There are seemingly endless ways in which the impact of a nutritional intervention can be measured as **Tables 3** and **4** make clear. Some of the more obvious measures include body weight and body composition (e.g., fat distribution) (11, 70, 71). Many physiological measures have been studied, for example, blood pressure or heart rate (23). Often considered in nutritional intervention studies are markers obtained from easily accessible tissues such as blood. Blood-based biomarkers that have been considered in published nutritional clinical trials include gene expression levels (66) and specific factors, such as C-reactive protein and adiponectin (52), as well as glucose and insulin (123), in addition to standard clinical chemistries, such as cholesterol and triglyceride levels (78). Many investigations have considered the influence of nutritional interventions on psychological factors, such as mood (75), cognition scores (65), depression scales (50), as well as sleep (75). As noted in **Table 4**, yet another measure that a number of studies have considered involves the microbiome (20, 21). Given the ease with which fecal samples can be obtained, studies of the gut microbiome are of particular interest, especially considering its role in digestion, and will likely continue to be pursued (85).

One additional area where there is growing interest is in postintervention monitoring to assess the outcomes and impact of using wireless devices (46, 86). There are many devices that can measure activity, mood, sleep quality and length, and related phenotypes; all of these could be used in N-of-1 nutritional intervention studies. In addition, there is no reason one could not consider multiple phenotypes in N-of-1 and aggregated N-of-1 studies (e.g., for sleep, blood pressure and heart rate, body composition, and mood) as noted in the Section titled Single-Arm, Sequential, Adaptive, and Multivariate Designs. The statistical methods for the analysis of the data from such a trial may be more complicated, but they would not be unprecedented. Also, such studies may provide a more complete picture of how an individual is responding to a particular nutritional intervention and hence provide more insight into how to refine or optimize that individual's nutritional and health profile.

MONITORING AND PERSONAL THRESHOLDS

Sequential N-of-1 trials can also be framed as ways of monitoring an individual for a health status change in the wake of taking on an intervention. However, determining the levels of a measure collected over time on an individual that would be indicative of a change is not trivial. Traditionally, this was done by determining the levels of a measure that are associated with poor outcomes in the population at large, i.e., determining and exploiting population-based thresholds. For example, if the cholesterol level of an individual was monitored while on a diet meant to influence cholesterol levels, then if the individual exhibited cholesterol readings greater than 200 mg/dl at a certain point, an argument could be made that the individual was exhibiting signs of poor health given that epidemiologic studies have shown that cholesterol levels >200 mg/dl are associated with an increased risk of heart disease. However, there are issues with the use of population thresholds for this kind of monitoring that are rooted in the potentially unique physiology each of us possesses. For example, the use of population thresholds ignores the fact that changes in certain measures or biomarkers might reflect physiological disruptions that are simply not consistent with population-based threshold criteria if the change in the biomarker is significantly different from biomarker values collected previously on an individual despite the fact the biomarker value did not cross the population threshold. Thus, the use of individual-specific or personal thresholds for identifying health status changes may be more appropriate.

Figure 3 provides a graphical depiction of the concept of personal thresholds for 25 hypothetical individuals, who have undergone measurements on a phenotype measuring an aspect of health (e.g., cholesterol level or other biomarker). The values of these 25 individuals are ranked and are made at 10 different time points going forward. Please note that these values could (and should in many instances) be adjusted for certain covariates. Note that some individuals have values that are above a population threshold and others exhibit fluctuations in values over time that may be biologically meaningful despite not crossing the population threshold.

The use of personal thresholds to guide inferences about health status changes has been shown to be useful in monitoring CA-125 levels in the blood of individuals at risk for ovarian cancer (24). It has been argued that most biomarkers of relevance to health are likely to be better assessed and utilized with personal thresholds rather than population thresholds (3). Such monitoring could be done with multiple markers simultaneously in a multivariate analysis setting. One important issue with the use of personal thresholds is that if the monitoring is truly done in real time, and not done in a retrospective manner after samples have been collected over time and then processed together to obtain biomarker values for the different time points, then the biomarker assay results must not suffer from assay drift or temporal technical variations (e.g., show great variation depending on the technician performing the biomarker assay). This ensures that the values of the measures are comparable. This is not necessarily easy to achieve, as most studies involving longitudinal measurements are done retrospectively, as the samples collected over time are processed within a single batch and as such the resulting measures avoid having overt technical variation complicate the interpretation of the temporal variation exhibited by those measures.

MATCHING STRATEGIES: VETTING ALGORITHMS VERSUS VETTING SPECIFIC NUTRITIONAL INTERVENTIONS

As an alternative to aggregated N-of-1 trials that combine the results of individual N-of-1 trials in order to make broad claims about the utility of individualized interventions, one could leverage extensions and offshoots of what have been referred to as basket, bucket, or umbrella trial methodology in the cancer clinical trials literature (97). Essentially, bucket trials assume that there may

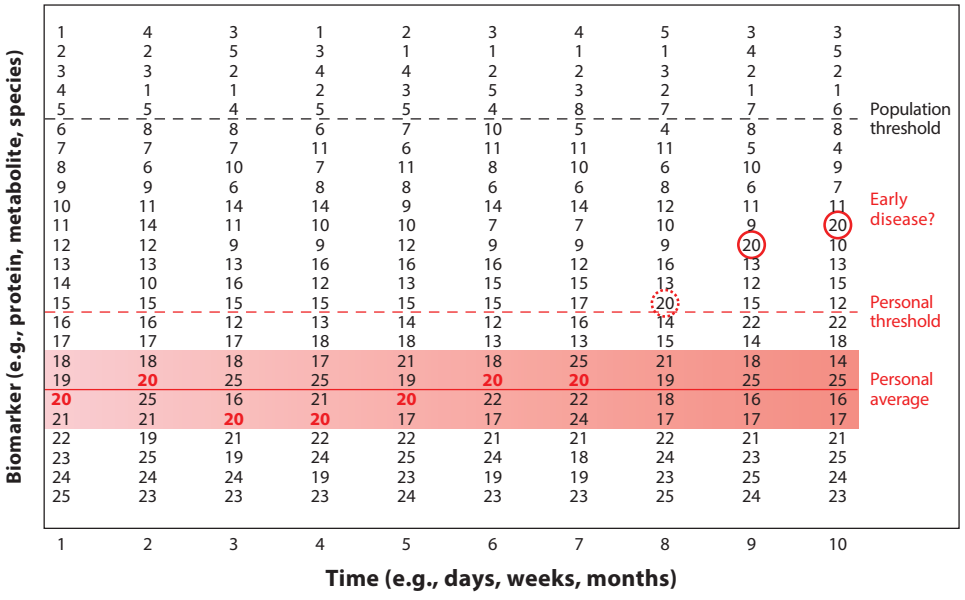

Figure 3

Graphical depiction of the concept of personalized thresholds for making claims about a health status change for an individual. Twenty-five hypothetical individuals have undergone measurements on a phenotype measuring health (e.g., cholesterol level or other biomarker). Their values are ranked and are made at 10 different time points. A population threshold (e.g., cholesterol level >200 units) is depicted (*dashed black line*). The rankings and values of a single individual, number 20, are highlighted (*red highlights*). After enough measures are collected over time, one can calculate a personal average for individual 20 (denoted by the *solid red line* as well as error bars representing variation in that individual's values (*red shading*). Based on the variation exhibited by individual 20, a personal threshold can be established for which any value beyond that limit has a low probability of occurring randomly given the prior values collected on the individual. This is depicted by the dashed red line. The dashed red circle indicates a value outside the personal threshold, and at later time points, two additional values (*circled in red*) get progressively higher. This deviation from historic or legacy values on the individual that have a low probability of occurring by chance could be an indication of a health status change despite being lower than the established population threshold.

be many interventions to choose from for an individual based on that individual's profile, whether genetic, microbiome, metabolic, behavioral, some combination of these, or something else. If a strategy for matching the individual profiles with the different interventions (e.g., low-fat diet for individuals genetically predisposed to heart disease) is set up a priori, then as individuals are enrolled in the trial and their profiles assessed, they are placed into the appropriate intervention buckets and provided that intervention. The goal is to then see if the scheme for providing the individuals interventions based on their profiles results in better outcomes compared to a group of individuals that was either not provided any intervention, provided a sham intervention (i.e., a placebo), or provided a single common one-size-fits-all intervention. Obviously, as in any trial, the nature of the outcomes and measures used to assess the success of the interventions is of crucial importance to such trials.

Bucket and related trials conceived in this way suffer from a few major issues. First, they are actually not focusing on the interventions themselves but rather on the strategy or algorithm for matching the interventions to the individual profiles. One could imagine a situation in which an intervention works particularly well—just not for the individuals it was assigned to in the bucket

trial because the matching scheme used in the trial was faulty. This could lead to the rejection of a perfectly good intervention. Second, the matching scheme used is only as good as the biological insights it is based on. Third, and a bit more complicated, such trials often lock down the strategies for matching the interventions to the patients at the start of the trial to determine how well the strategies in question work. This may compromise one's ability to incorporate new information about, e.g., the likely effects of a specific nutritional intervention for individuals with a certain profile because the evidence for this may arise after the initiation of the trial. New insights arising after the initiation of a trial are problematic for any clinical trial but possibly more so for bucket trials because such trials may be more wide ranging than a trial focusing on a single, very specific, intervention. The issues plaguing attempts to incorporate the new insights into the trial would vary. For example, statistical issues could arise if the new insight was incorporated into the trial, as it may lead to the creation of a new bucket whose contribution to the overall effect of the set of interventions being tested would have to be considered. In addition, statistical analysis of the trial data may have to accommodate weighting of the individuals. When individuals are enrolled later in a trial after new insights are incorporated into the strategy for assigning individuals to intervention buckets, the newcomers may benefit from a better intervention strategy than individuals enrolled early in the trial. Finally, ignoring new insights that are truly compelling may create ethical problems if they could really enhance health because the individuals in the trial could be perceived as receiving inferior interventions.

There are three important extensions of trials seeking to match individuals to nutritional interventions based on their profiles, however defined. First, one may not have to predefine the intervention buckets corresponding to specific features in individual profiles but rather address or test a much broader question concerning whether or not the profiling itself has any merit for identifying appropriate and effective nutritional interventions. For example, one could genetically profile a group of individuals and use that profiling to determine the best nutritional intervention based on a panel of experts' opinions in assessing that profile, as with tumor boards in the cancer treatment setting (63) or an adaptive machine learning strategy whose calculations consider that kind of profiling (123). The idea would then be to compare how the individuals responded to the interventions provided on the basis of the genetically guided profiling versus those that may have received expert advice or information resulting from a machine learning strategy that did not consider genetic profiles in their deliberations or as part of the calculations.

Second, one could consider the results of assays and response profiles using biospecimens from the individuals participating in a trial in ex vivo or in vitro settings of particular phenomena to determine what the most appropriate intervention might be for those individuals. For example, establishing cell lines, induced pluripotent cell lines, or organoids from individuals and then exploring how they respond to different nutritional interventions could lead to insights into the best intervention for the individual. Such studies are used routinely to identify treatments for individuals with rare congenital diseases (115). Obviously, the relevance of the assay system and the measures used to assess the nutritional responses in vitro to the in vivo setting is a crucial concern with such studies. Fenech and colleagues (30, 31) have written extensively on this type of strategy in the context of DNA repair capacity and nutritional schemes to minimize cancer and other disease susceptibility.

Third, an issue related to strategies for assigning, or optimizing, nutritional interventions to individuals on the basis of ex vivo or in vitro assays that make use of biospecimens obtained from those individuals involves the media in which the cellular assays are performed and what, if any, insights might be obtained from the choice of that media. Consider the fact that when cell lines are created and passaged, or when organoids are grown and maintained, they require cell culture media. Oftentimes this media contains a number of factors, such as hormones, growth factors,

vitamins, or other things (51, 76). A relevant question in the context of this review is whether one could personalize the media used to empirically grow cells or create organoids from an individual by testing different media constituents and comparing the viabilities and functional capabilities of the cells and organoids, and then use the resulting insights to craft better nutritional interventions for the individuals from whom those cells or organoids were harvested and created.

RECENT STUDIES MOTIVATING SINGLE-SUBJECT TRIALS IN NUTRITION

There have been a number of recently published studies investigating the impact of nutritional factors on indicators of health that, although not technically N-of-1 studies, motivate N-of-1 studies because of what they showed and how they were pursued. The study by Alm and coworkers (20) in which daily microbiome measures were obtained for two individuals over the course of a year is one good example of the potential of N-of-1 studies. Alm and his colleagues (20) also described how the daily eating patterns of the two participants were kept and then correlated with the constituent species of the microbiome. A number of very interesting correlations were found that gave insight into what the two individuals may want to avoid or encourage in the future with respect to food consumption to optimize the balance of constituent species in their microbiomes. Although no purposeful nutritional intervention was pursued in the study, it was clear that the results generated some obvious hypotheses about how certain dietary substances may impact the gut microbiome of the two individuals, setting the stage for a bona fide randomized, blinded (to the degree possible) cross-over N-of-1 study of the type emphasized in this review for testing those hypotheses.

Another study that has received considerable attention and is truly reflective of studies exposing nuanced, individual responses to nutritional interventions was the continuous time glucose monitoring study by Segal and coworkers (123). Essentially, Segal and his colleagues (123) continuously monitored week-long glucose levels in an 800-person cohort, which ultimately considered these individuals' responses to over 46,000 meals. They found overwhelming evidence for variability in the response to identical meals between individuals, suggesting that individual dietary needs must be identified from objective empirical studies-based measures of an individual's response to a change in diet, and these needs may be hard to anticipate from any prior information on those individuals. However, the authors did devise a machine learning algorithm that leveraged blood-based biomarker profiles, dietary habits, anthropometrics, physical activity, and gut microbiota measured on the 800 individuals in the study and showed that it could accurately predict postprandial glycemic response to real-life meals for individuals in the study. The authors went on to validate these predictions in an independent 100-person cohort. To top things off, the authors pursued a blinded, randomized controlled dietary intervention based on the algorithm, which resulted in significantly lower postprandial responses and consistent alterations to gut microbiota for the participants who were provided the intervention. The authors concluded that their results suggest that personalized diets may successfully modify elevated postprandial blood glucose and its metabolic consequences. These findings could easily be explored in N-of-1 and aggregated N-of-1 studies of the type envisioned.

CONCLUSIONS

The concepts of individualized medicine and individualized nutrition are not likely to be proven useless and disappear soon. Rather, their worth can be evaluated in studies designed to determine the optimal intervention for individuals. The results of these studies can then be aggregated to show that they could not have been anticipated in other ways or at least that they had to be performed

to bring out nuanced responses to an intervention whose determinants would have to be explored in the future. N-of-1 trials and aggregated N-of-1 trials of this sort thus have an obvious role to play in the identification of factors that influence responses to nutrition that might be shared among a set of individuals. In other words, it could be argued that most variation in response to nutritional factors is likely to be explained by a set of identifiable (but as yet unidentified) genetic, environmental, and behavioral factors, with the remaining amount of variation being very individual specific, the clinical significance of which is in doubt. To date it is not clear how much interindividual variation in nutritional response can be attributed to identifiable, shared factors, so more research into individual variation in response to nutritional factors is needed. In addition, because we cannot make confident predictions about an individual's response to all nutritional interventions until we identify the factors that might be used for such predictions, an individual's response must be evaluated empirically to explore variations that could be attributable to those factors in the future, and N-of-1 trials are one vehicle for doing this.

There are a number of trends that could both motivate and enhance N-of-1 trials in nutrition beyond a general interest in personalized health care. First, there is tremendous interest in self-monitoring for health purposes given the changing health care system, a new focus on disease prevention, and the availability of cheap and relatively sophisticated biomarker and wireless data collection devices. This interest is taken to the extreme by individuals within the quantified self-movement in which participants knowingly experiment on themselves to determine optimal ways of living (29, 38, 57, 108, 119). However, most studies pursued by people within the quantified self-movement are anecdotal and lack the scientific rigor of N-of-1 trials, although this could change if the individuals within the movement were exposed to N-of-1 trial methodologies. Second, there is tremendous interest in improving the sophistication of data collection devices for health monitoring purposes, making them more reliable, cost-efficient, and transparent to the user (14, 124). Such devices can easily enable N-of-1 studies if they collect appropriate information. Third, there is growing interest in big data and the use of large databases to mine information that might be useful for some purpose (4, 16, 18, 64). One could imagine designing and implementing systems to facilitate the conduct of N-of-1 trials and aggregating their results for pattern discovery and data mining. Ultimately, even though they will never be a panacea for all nutritional ills, given these trends, and the biological intuitions behind personalized nutrition, N-of-1 trials are likely to become very relevant approaches to optimizing individual health and advancing health care generally.

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