Application of *n*-of-1 Clinical Trials in Personalized Nutrition Research: A Trial Protocol for Westlake N-of-1 Trials for Macronutrient Intake (WE-MACNUTR)

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ABSTRACT

Personalized dietary recommendations can help with more effective disease prevention. This study aims to investigate the individual postprandial glucose response to diets with diverse macronutrient proportions at both the individual level and population level, and explore the potential of the novel single-patient (*n*-of-1) trial for personalization of diet. Secondary outcomes include individual phenotypic responses and the effects of dietary ingredients on the composition of gut microbiota. Westlake N-of-1 Trials for Macronutrient Intake is a multiple crossover feeding trial consisting of 3 successive 12-d dietary intervention pairs including a 6-d washout period before each 6-d isocaloric dietary intervention: a 6-d high-fat, low-carbohydrate diet, and a 6-d low-fat, high-carbohydrate diet. The results will help provide personalized dietary recommendations for macronutrients in terms of postprandial blood glucose responses. The proposed *n*-of-1 trial methods could help in optimizing individual health and advancing health care.This trial was registered with clinicaltrials.gov (NCT04125602). *Curr Dev Nutr* 2020;4:nzaa143.

Keywords: personalized nutrition, dietary intervention, *n*-of-1, single patient trial, high-fat diet, low-carbohydrate diet, postprandial blood glucose, gut microbiome

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Abbreviations used: CGM, continuous glucose monitoring; HbA1c, glycated hemoglobin; HF-LC, high-fat, low-carbohydrate; LF-HC, low-fat, high-carbohydrate; MAGE, mean amplitude of glycemic excursions; PMG, postprandial maximum glucose; RCT, randomized controlled trial; WE-MACNUTR, Westlake N-of-1 Trials for Macronutrient Intake; %E, percentage of total energy.

Introduction

Diet and nutrition are key to maintain human health. Previous studies have shown much interest in the metabolic effects of different ratios of dietary fat to carbohydrate intake (1–5). Some studies suggest that a high-fat, low-carbohydrate (HF-LC) diet can improve glycemic control by reducing glycated hemoglobin (HbA1c) and fasting glucose concentrations, whereas others support the beneficial effect of a low-fat, high-carbohydrate (LF-HC) diet with particular focus on the quality of carbohydrate (1, 6–10). One important interpretation of these inconsistent results is that they reflect individualized or personalized responses to dietary macronutrient intake, also called "personalized nutrition" (11).

Personalized nutrition focuses on an individual's potentially unique dietary needs instead of assuming a "one-size-fits-all" approach where everyone is thought to benefit from the same diet (12). The general aim of personalized nutrition is to improve health using nutritional, genetic, phenotypic, and other information about individuals to develop targeted nutritional advice, services, or other products (13–15). Although specific dietary recommendations have been made for pregnant women, infants, children, adults, or the elderly, they are still subgroup recommendations that are far from the stage of "personalization" or "precision."

The application of "*n*-of-1" clinical trials, or "single-patient" studies, represents a new direction in personalized nutrition research (**Figure 1**). It can capture intraindividual variability in health behaviors over time, aiming to identify individual responses to a given intervention in a controlled trial, which provides a great opportunity to assess the personalization potential of different diets, nutrients, or nutrition supplements (16–18). The idea of *n*-of-1 has been applied in special education, psychotherapy, psychology, and pharmaceutical studies for decades to test the individual response to specific drugs or treatments (19–23). A previous study reported the efficacy of mexiletine on reducing muscle stiffness in patients with nondystrophic myotonia using a series of *n*-of-1 trials (24). However, there are no published *n*-of-1 studies in the nutrition field so far. The Westlake N-of-1 Trials for Macronutrient Intake (WE-MACNUTR) study will be a novel series of clinical trials that use macronutrient intake as an exemplar for recent progress in the field.

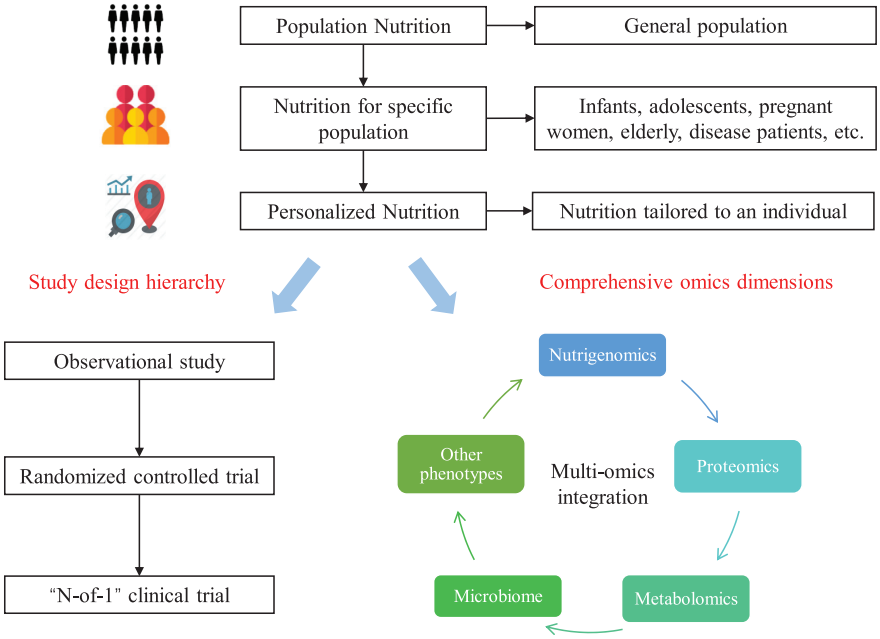

FIGURE 1 The development of personalized nutrition. Personalized nutrition was born in the context that a conventional "one-size-fits-all" approach usually fails to meet an individual's nutritional requirements. An "*n*-of-1" clinical trial is a novel study design for the investigation of personalized nutrition, contrasting with traditional designs such as the observational study or randomized controlled trial. Integration of multiomics data, including nutrigenomics, proteomics, metabolomics, microbiome, and other phenotypes, is key for the development of personalized nutrition.

We describe the trial protocol of the WE-MACNUTR, a series of *n*-of-1 clinical feeding trials in adults. The feeding trial will be composed of 2 dietary interventions, an HF-LC diet and an LF-HC diet. The primary objective is to investigate the postprandial glycemic responses to the dietary interventions, and the primary outcomes include the postprandial maximum glucose (PMG), the AUC24 of postprandial glucose from 0:00 to 24:00, and the mean amplitude of glycemic excursions (MAGE) obtained by measuring the arithmetic mean of the differences between consecutive peaks and nadirs. Secondary objectives of this study include evaluating different phenotypic responses, such as circulating lipid profile changes and evaluating the impact of different dietary components on the composition and structure of the gut microbiota.

Methods

Study design

An *n*-of-1 trial is a multiple crossover feeding trial conducted in a single participant, comparing her/his response to different interventions and assessing the variability in these responses (25). In the WE-MACNUTR study, a series of *n*-of-1 trials will be employed simultaneously and a common regimen of interventions is applied to all participants (**Figure 2**). Participants will experience 3 successive 12-d intervention pairs including a 6-d washout period between each intervention. The diets will be isocaloric and all provided by the researchers, with the fat and carbohydrate contents (an HF-LC diet and an LF-HC diet) as their primary distinguishing features. Prior studies have shown that dietary patterns can have rapid influences on glycemic control, and dietary intervention studies successfully observed a significant effect of high-carbohydrate diets (compared with high-fat diets) on various blood glucose measurements after a 5-d intervention (26). In addition, the gut microbial community was also reported to change substantially within 4 d in response to a dietary change (27). Therefore, we will set a washout period of 6 d between each intervention arm (HF-LC and LF-HC), after balancing the outcomes of the study (postprandial glycemic responses and gut microbiota changes), and feasibility of the designed feeding trials. Each 12-d intervention pair will comprise 6 d of HF-LC (3 meals daily) and 6 d of LF-HC (3 meals daily) diets in a random order. The order of the diets in each pair will be determined using block randomization. Major investigators and laboratory personnel responsible for the measurements will be masked to group allocation. Meal providers will be aware of the group and diet allocation, but they will not get involved in the rest of the trial.

Participants will be asked to complete a basic questionnaire on a daily basis after dinner to summarize their eating behaviors and multiple lifestyle factors including physical activity, mood, and sleep patterns throughout the day. Any adverse events reported from the participants will be evaluated by a physician. Telephone follow-up will be performed until the intervention-related adverse events are resolved. Biological samples including blood, saliva, urine, and fecal samples will be collected every 6 d for metabolomics profiling using an untargeted metabolomics strategy (i.e., collection of the metabolite data without pre-existing knowledge). Saliva will also be collected for oral microbiota investigation (**Figure 3**). The fasting venous blood samples will be collected every 6 d only during the first set of interventions of the study so

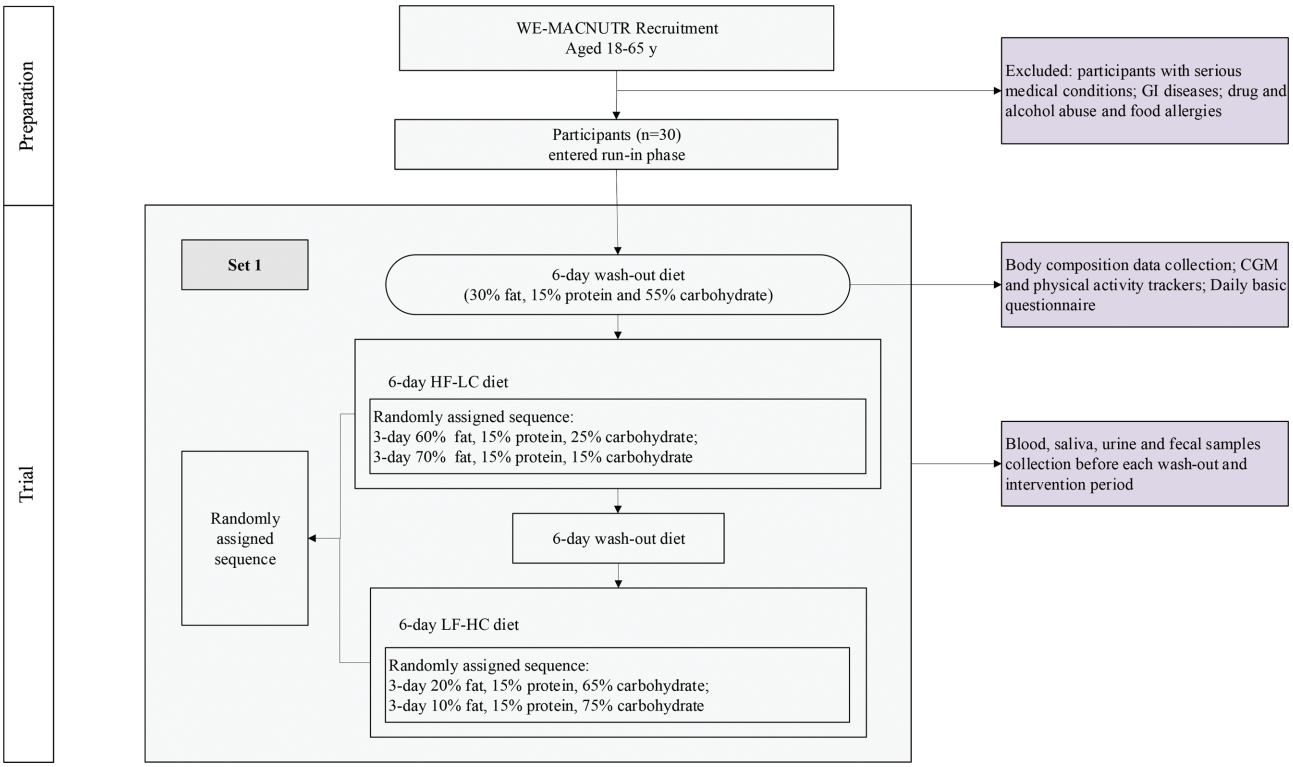

FIGURE 2 Flow diagram of the Westlake N-of-1 Trials for Macronutrient Intake (WE-MACNUTR) trial. The flowchart summarizes the preparation phase and Set 1 of the trial. Sets 2 and 3 share the same trial design as Set 1 except for no blood sample collection for washout or intervention period. The sequence of 2 types of 6-d dietary interventions in each set is randomized using a block randomization as LF-HC and HF-LC diets in Set 1; HF-LC and LF-HC diets in Set 2; and HF-LC and LF-HC diets in Set 3. CGM, continuous glucose monitoring; GI, gastrointestinal; HF-LC, high-fat, low-carbohydrate; LF-HC, low-fat, high-carbohydrate.

as to reduce the burden and increase the compliance of the participants. The parti-cipants will be asked to wear a continuous glucose monitoring (CGM) device (Freestyle Libre Pro System), which measures interstitial glucose every 15 min. The same CGM system was used by a recent personalized nutrition study (PREDICT 1) to monitor individual postprandial glycemic responses (28). Because most individuals do not resemble "the average" in the context of precision nutrition, using data sets generated from digital wearable devices, such as CGM, will potentially help inform individualized food choices (29). In the study, participants will be asked to wear the sensor 2 d before the start of each intervention period, and remove it by the end of each 6-d intervention period. CGM will not be used during the washout periods. In addition, participants will be asked to continue their regular daily activities and exercise throughout the study period, and wear a wrist-based triaxial accelerometer (AX3; Axivity) on the nondominant wrist to monitor the physical activity and exercise intensity during the intervention period.

The study has been approved by the Westlake University Internal Ethical Review Board in Hangzhou, China, and registered with clinical trials.gov (identifier: NCT04125602). Written informed consent will be obtained from all study participants, and then a random ID number will be assigned to each participant. The link between the ID numbers and identity of the participants will be accessible only by a data manager.

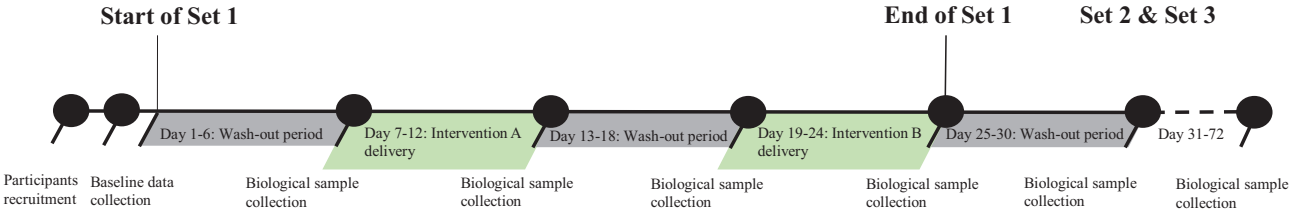

FIGURE 3 The timeline of the Westlake N-of-1 Trials for Macronutrient Intake (WE-MACNUTR) trial. The timeline illustrates a preparation period for participant recruitment, a baseline data collection period, and 3 feeding trial periods. The first set of the trial consists of 2 washout periods (highlighted in gray) and 2 randomized dietary intervention periods (highlighted in green). In all 3 sets of the trial, both washout and intervention periods last for 6 d.

TABLE 1 Outcome and assessment points[1]

Variable	Measure	Assessment point		
		Set 1	Set 2	Set 3
Primary outcomes				
Continuous glucose concentration				
PMG	FreeStyle Libre Flash glucose monitoring system	•	•	•
AUC24		•	•	•
MAGE		•	•	•
Secondary outcomes				
Lipid metabolism	Blood samples			
Inflammation	Blood samples			
Oral microbiota	Saliva	√	√	√
Gut microbiota profiling	Fecal samples	√	√	√
Fecal metabolites	Fecal samples	√	√	√
Metabolomics profiling	Blood samples			
	Fecal samples	√	√	√
	Urine samples	√	√	√
Physiological characteristics				
Weight	Kubei height scale	√	√	√
Blood pressure	YUWELL YE660D upper arm sphygmomanometer	√	√	√

[1]AUC24, total area under the continuous glucose monitoring curve from 0:00 to 24:00; MAGE, mean amplitude of glycemic excursions; PMG, postprandial maximum glucose. • = per day during intervention periods; √ = before and after intervention periods.

Dietary intervention

A dietitian will design the diet for the intervention and the washout period based on the Chinese Dietary Guidelines (2016) and Chinese Dietary Reference Intakes (2013) as well as the participants' demographic information, eating habits, and physical activity levels (30, 31). Besides, factors such as local food availability, the cooking methods of the canteen kitchen of Westlake University, and the current recipes are all important factors in the meal planning.

Washout diet.

Prior to each dietary intervention, all participants will be provided with the same standardized diets for 6 d as the "washout diet" to reduce potential sources of bias and to eliminate any carryover effects of the previous intervention. The washout diet consists of 30% total energy (%E) from fat, 15%E from protein, and 55%E from carbohydrate based on the acceptable macronutrient distribution range (32).

HF-LC diet intervention.

Throughout the 6-d HF-LC intervention, participants will be provided with an HF-LC diet, including a 3-d diet consisting of 60%E from fat, 15%E from protein, and 25%E from carbohydrate, and another 3-d diet consisting of 70%E from fat, 15%E from protein, and 15%E from carbohydrate.

LF-HC diet intervention.

Throughout the 6-d LF-HC intervention, participants will be provided with an LF-HC diet, including a 3-d diet consisting of 20%E from fat, 15%E from protein, and 65%E from carbohydrate, and another 3-d diet consisting of 10%E from fat, 15%E from protein, and 75%E from carbohydrate.

Participants

Participants will be recruited among students and staff from Westlake University, Hangzhou, China. Inclusion criteria are: *1*) adults aged between 18 and 65 y; *2*) able to provide written informed consent; and *3*) have access to smart phones or computers. Exclusion criteria include: *1*) long-term gastrointestinal disease; *2*) neurological conditions and cognitive impairment; *3*) other clinically diagnosed medical conditions including type 2 diabetes, hypertension, cardiovascular diseases, liver/kidney diseases, and/or other systemic diseases; *4*) taking antibiotics within the last 2 wk; *5*) hospitalization or surgery planned within the next 3 mo; *6*) pregnant or lactating women; *7*) tobacco, alcohol, or illicit drug abuse; *8*) vegan or food allergies; *9*) no access to a smart phone or computer with an internet connection; *10*) enrolled in concurrent intervention study; and *11*) non–Chinese-speaking. This study emphasizes assessing individual responses to the intervention of interest rather than drawing a general conclusion at the population level, and therefore study participants will cover a broad age range and will not be balanced by sex.

Measures

The primary outcomes will be: *1*) the PMG: the peak value of CGM within 3 h after the first bite of a meal or the maximum value of CGM between 2 meals when the interval is <3 h; *2*) the AUC24: the total area under the CGM curve from 0:00 to 24:00; and *3*) the MAGE: obtained by measuring the arithmetic mean of the differences between consecutive peaks and nadirs, provided that the differences are >1 SD around the mean glucose values. Secondary outcomes will include different phenotypic responses, such as circulating lipid profile and gut microbiome profile, to a specific diet among individuals (**Table 1**).

Sample size calculation

A total of 30 participants will be enrolled in the WE-MACNUTR study, and Bayesian hierarchical model meta-analysis will be applied to combine the results from each *n*-of-1 trial to generate pooled effect estimates at the population level. At present, no formula-based methodology exists for sample size calculation for such a design at the population level (33). Referring to the method reported previously, we performed

a simulation-based statistical power calculation at the population level (24). In brief, a prior distribution for the mean intervention effect (HF-LC compared with LF-HC) on the PMG was prespecified based on results of a previous randomized controlled trial (RCT) (26). In that study, a 1.6-mmol/L (6.2 mmol/L compared with 7.8 mmol/L) difference in the PMG was established between participants receiving high-fat compared with high-carbohydrate meals. We drew a random realization and simulated an individual-specific virtual mean intervention effect for each participant. At the next level of simulation, longitudinal measurements for each participant (3 sets, 2 intervention periods per set, and 18 observations per intervention period) were simulated assuming a normal distribution of the measurements centered around the individual-specific virtual mean effect size, with a common within-participant residual variance. Thereafter, all the simulated data from these *n*-of-1 trials were used to perform a multilevel Bayesian meta-analysis, specifying a linear mixed model with flat noninformative priors at the population level.

A previous study found that the difference in PMG between healthy participants aged 25–45 y and those older than 45 y was 3 mg/dL (0.167 mmol/L) (34, 35). Therefore, we considered this magnitude of difference to be clinically meaningful and determined the posterior probability on an intervention effect of $\geq$0.167 mmol/L from the simulation-based Bayesian meta-analysis. To keep a balance between the power of detecting a meaningful difference and false positive rate, a relatively strict 90% posterior probability on an intervention effect of 0.167 mmol/L was treated as a clinically positive result at the population level, whereas a stricter 99% posterior probability on an intervention effect of not being equal to zero was treated as a statistically positive result. The above procedures were repeated 1000 times (corresponding with data from 1000 aggregated *n*-of-1 trials) and the fraction of meta-analysis that returned a positive conclusion was treated as a measure for the power. Consequently, the power was estimated to be 100% for detecting the intervention effect of 0.167 mmol/L in PMG.

To determine the false positive rate, we performed another set of simulations under the assumption of no intervention effect. For each virtual participant, we simulated data that allowed the participant to have an individual-specific virtual mean intervention effect that centered around the true population-level mean effect size (zero) with a normal distribution. At the next level of simulation, the same method as that used in power calculation was applied. A posterior probability of >90% on an intervention effect of 0.167 mmol/L and >99% on an intervention effect of not being equal to zero were treated as clinically and statistically false positive results, respectively. After repeating the procedures 1000 times, the fraction of meta-analyses that returned a clinically or statistically false positive conclusion was determined as a measure of the type I error rate (2.3% and 4.8% for clinically and statistically false positive rate, respectively) at the population level. Thus, this simulation-based sample calculation indicates that with 30 participants completing the trial (3 sets, 2 intervention periods per set, and 18 observations per intervention period), we will have a satisfactory type I error rate and enough power to detect the prespecified intervention effect.

Statistical analysis plan

Data management.

Even though all participants will be students/staff who routinely have meals on campus, we anticipate that some participants will skip some meals that we provide in the dining room and will eat other foods instead, which adds uncertainty to the effects on postprandial blood glucose concentrations. Therefore, participants will be instructed to sign in before each meal, to consume only the provided foods or beverages, and to report any extra intake in the daily questionnaires for compliance evaluation. In addition, the graphs and trends available with CGM will be used to assess potential subject bias and tendency for any misreported data. Other major violations, such as failure to complete $\geq$1 set of interventions, will prevent statistical analysis at the individual level and lead to exclusion of the participants from meta-analysis at the population level.

Primary analysis.

The primary analysis of the intervention effect will be the comparison of the effect of HF-LC diets with that of LF-HC diets on postprandial blood glucose concentrations. At the individual level, we will use each individual's intervention effects to guide dietary decisions for each participant. Moreover, we generate estimates of an intervention effect at the population level by combing the *n*-of-1 results with meta-analysis.

Analysis of baseline data.

Descriptive statistics with demographics and baseline characteristics will be presented for each participant.

Analysis of individual n-of-1 trials.

Statistical analysis will be performed separately for each *n*-of-1 trial to estimate the intervention effect at the individual level. Bayesian models will be applied to estimate the intervention effects (**Figure 4**). Posterior probabilities of outcomes will be calculated using an interface that incorporates open-source R Foundation software (3.6.1) and open-source OpenBUGS (version 3.2.3). The results will be reported for primary variables (e.g., the peak concentration of postprandial blood glucose and the AUC of postprandial glucose concentrations over 24 h), and participants will be provided with an estimate of differences in the variables and the probabilities that the differences are induced by the different interventions (HF-LC compared with LF-HC).

Meta-analysis of n-of-1 trials.

A Bayesian multilevel model will be used to combine the results of the multiple *n*-of-1 trials (24, 36, 37). Participant will be treated as a random effect and a common within-participant residual variance will be assumed. Noninformative priors will be applied for all model parameters, with mean parameters using normal prior distributions with very large SDs and variation parameters using inverse γ distributions with both shape and scale parameters equal to 0.01. Using the interface that incorporates R software (3.6.1) and OpenBUGS (version 3.2.3), combining the data from the individual *n*-of-1 trials will obtain posterior distributions for the mean intervention effect at the population level. Secondary and exploratory outcomes will be analyzed similarly.

Discussion

To advance the field of personalized nutrition, the *n*-of-1 clinical trial appears to be a promising study design to advocate, although real-world examples are rare. We will use the WE-MACNUTR trial as an exemplar to showcase the study design of *n*-of-1 trial so as to test the

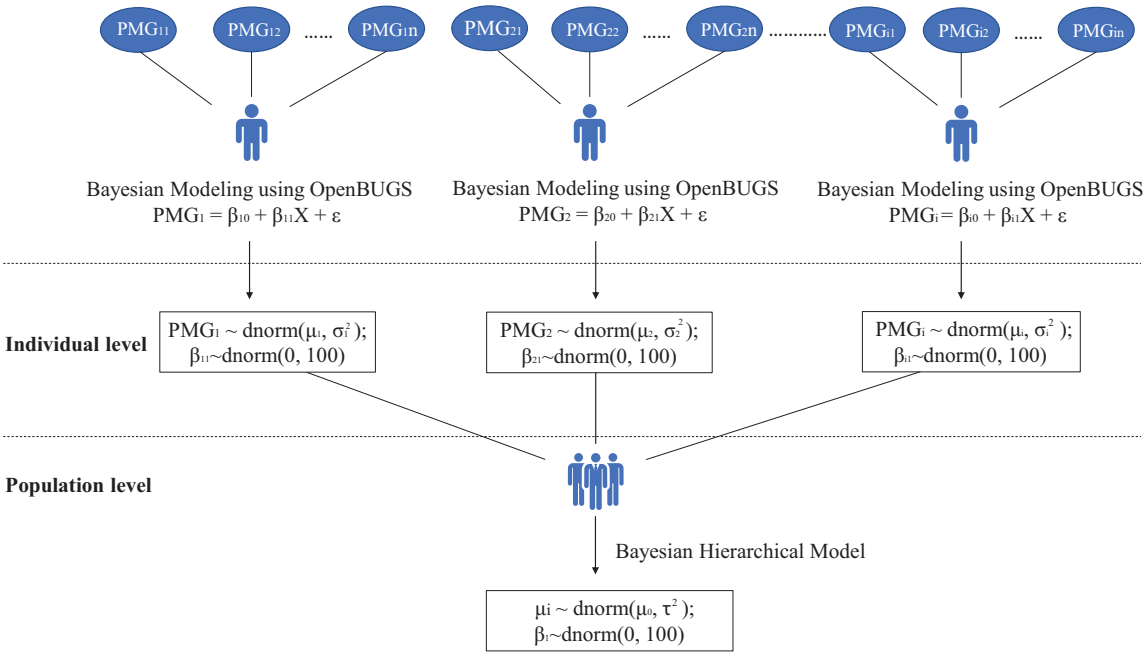

FIGURE 4 Representation of the hierarchical Bayesian estimation for the primary outcomes at both individual and population level (a combination of single-patient studies). The observed repeated measurements of the peak postprandial glucose concentrations for a given patient are combined into a sample mean and a sample variance. The model assumes that the patient's measurements follow a normal distribution centered about that patient's true mean effect (μ_i) with variance $\sigma_1{}^2$. At the population level, the various patients' true means (μ_i) are assumed to follow a normal distribution centered about an overall population mean (μ_0) with between-patient variance τ^2. For the Bayesian specification, prior distributions are assigned for β, μ_0, $\sigma_1{}^2$, and τ^2. In the present study, these prior distributions are standard noninformative prior distributions. X represents the independent variable: dietary patterns (high-fat and low-carbohydrate compared with low-fat and high-carbohydrate). Secondary and exploratory outcomes will be analyzed similarly. PMG, postprandial maximum glucose.

individualized responses to different macronutrient intakes in adults. The study, if successful, will provide insights into the feasibility of *n*-of-1 approaches for personalizing or tailoring a dietary intervention to individuals.

Previous studies have suggested that the magnitude of postprandial responses to mixed meals depended largely on the total amount of fat and carbohydrate intake (1, 5, 6). The American Diabetes Association recommends monitoring carbohydrate intake to achieve better glycemic control in patients with type 2 diabetes, which is based on studies showing reduced postprandial glucose concentrations and triglyceride responses in individuals consuming HF-LC diets (38–40). However, previous systematic reviews discussed the effects of HF-LC and LF-HC diets on metabolic risk factors and showed inconsistent results (5, 7, 41). LF-HC diets with high-fiber contents showed beneficial effects on glycemic control and insulin sensitivity in both Asian Americans and Caucasian Americans (42). Several studies have reported that both HF-LC and LF-HC diets reduced HbA1c and fasting glucose concentrations in obese adults with type 2 diabetes, whereas HF-LC diets achieved greater improvements in glycemic control (1, 2). A previous study in Japanese diabetic patients suggested that changes in HbA1c and fasting plasma glucose did not differ significantly between HF-LC and LF-HC diets (43). The health benefits and drawbacks of different dietary patterns are under intensive study these days despite the lack of a standardized definition regarding the macronutrient contents (44–46). Therefore, the *n*-of-1 trial has huge potential to help explore the main effects of a specific dietary intervention, and identify the factors that influence individual responses to nutritional factors. In the present study, it is expected that the trial will provide information on the responses of individuals' postprandial blood glucose concentration to different dietary interventions, namely HF-LC and LF-HC diets, enabling a better understanding of intraindividual differences in absorption, distribution, and metabolism of macronutrients.

Individual humans are not only unique with respect to the host genome, but also in respect of the gut microbiome that represents the combined influence of the diet and lifestyles, as well as host genetics (47, 48). Both animal and human studies have demonstrated that the composition of the gut microbiome can be rapidly affected, within 4 d, by a specific dietary component exposure (27, 49). Furthermore, integration of machine-learning algorithms with gut microbiome features has shown powerful potential to predict one's response to different dietary patterns in terms of postprandial glycemic responses (50). The researchers monitored the postprandial glucose responses in a cohort of 800 participants in Israel in response to identical meals. Multidimensional data, including gut microbiome features, anthropometrics, blood parameters, and physical activities, were integrated into a machine-learning algorithm that was capable of predicting personalized postprandial glucose responses with the gut microbiota (51). These new approaches have stimulated more research on the application and

integration of the gut microbiome into the personalized nutrition field. Results from our current proposed study could provide further evidence suggesting that the *n*-of-1 trial is feasible in characterizing individual microbiome profiles.

With the aggregated data from isocaloric meals but different carbohydrate-to-fat ratios, our study will facilitate deeper investigations of the underlying interactions between specific food components and microbiota species. Therefore, future methodological studies on developing and implementing effective evaluation of personalized dietary interventions could assist individuals in promoting a healthy gut microbiota profile and preventing cardiometabolic diseases. Another strength of the study with the *n*-of-1 method is its flexibility, which enables the study design to be personalized to individuals' interests and requirements, and its high level of evidence for making clinical decisions for individuals alongside systematic reviews of RCTs.

Limitations

The *n*-of-1 study does have limitations. Participation in feeding trials like WE-MACNUTR will require time and effort so the trial cannot be conducted in an ideally controlled setting. Compliance of the participants with the intervention over time will be challenging, because they will be required to eat the provided foods with no extra food intake throughout the feeding trial. Any extra snack or beverage intake could affect individual blood glucose concentrations. Besides, slight changes in cooking methods, food groups, or food ingredients under inevitable circumstances will also jeopardize the final results.

Conclusions

In summary, the WE-MACNUTR trial, as an exemplar of a nutritional *n*-of-1 trial, will address the call for a new method to advance the field of personalized nutrition. WE-MACNUTR will potentially help clarify the individual postprandial glucose response to diets with diverse macronutrient proportions, and help design and optimize the macronutrient composition in long-term dietary intervention studies. The results of WE-MACNUTR will also be helpful for understanding the individual response of the gut microbiome to macronutrients.

Acknowledgments

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