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Review article

Conducting dietary intervention trials in people with multiple sclerosis: Lessons learned and a path forward

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ABSTRACT

Disease course in people with multiple sclerosis (MS) is heterogeneous. The impact of dietary and nutritional factors on MS prognosis is of interest to both patients and clinicians; differences in diet are hypothesized to contribute to disease evolution over time. However, studying diet, especially in people with MS, introduces methodologic complexity that should be recognized. In this review, we focus on methodological aspects relevant to the conduct of dietary interventions in people with MS, given our experience in leading such studies and the challenges we encountered in the realization of this work. We summarize key aspects of study design and important considerations, regardless of the specifics of the actual study (e.g. the particular diet of interest, target MS population, etc.). We discuss strategies for the design of the intervention as well as the selection of appropriate study endpoints. Finally, we provide an overview of strategies to improve the rigor of conducting dietary studies in people with MS.

1. Introduction

The prognosis of multiple sclerosis (MS) is highly variable. While several environmental influences like vitamin D status and smoking are consistently associated with the disease course (Ascherio and Munger, 2016; Ascherio et al., 2012), much of the heterogeneity remains unexplained. Interest in diet as a possible environmental disease-modifying intervention for MS is rapidly rising. Many dietary components modulate mechanisms (e.g. immune and mitochondrial function, oxidative stress, gut microbiota diversity) that are hypothesized to influence disease evolution over time (Kau et al., 2011; Dai et al., 2008; Bjørklund and Chirumbolo, 2017; Fan and Zhang, 2019; McMurray et al., 2016; Tremlett et al., 2017). In a previous review, we, the Wellness Research Group, convened by the National MS Society, concluded that the state of knowledge concerning the relation of diet to outcomes in people with MS was suboptimal (Motl et al., 2018). At that time, scientific evidence evaluating a possible role of diet in MS disease modification was sparse, though some anecdotal evidence had supported this hypothesis.

Since the publication of our initial report, several dietary intervention trials assessing a variety of types of diets in people with MS have been completed or will soon be completed (Fitzgerald et al., 2018; Brenton et al., 2019; Yadav et al., 2016). As several of these studies were conducted by members of our group, our experiences have led to the identification of some of the challenges and logistical complexities an investigator faces in designing and conducting interventional diet studies, regardless of the specifics of the study design (e.g. number of study arms, exact intervention chosen). In what follows, we discuss some of our "lessons learned," including potential pitfalls and considerations to address these challenges as they relate to the study of diet in MS. Specifically, we discuss (1) aspects of the design of diet interventions, (2) considerations of study endpoints, and (3) various

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Table 1
Summary of advantages and disadvantages of key aspects of study design for dietary intervention trials in people with MS.

Delivery method	Advantages	Disadvantages
1. Controlled feeding	Relative ease at which adherence can be monitored (for on-site controlled feeding studies)	Expensive
	Financial constraints on participants are minimized.	Often short in duration
	Collection of biospecimens can be completed at meal times (potentially reducing missing data) for on-site controlled feeding studies	High participant burden
	Diet is standardized across participants (e.g. everyone eats a nutritionally identical meal)	Requires access to kitchen and/or cafeteria setting in which to prepare (for remote and on-site controlled feeding studies) and serve foods (for on-site controlled feeding studies)
		Diet is relatively inflexible, and participants may grow tired of eating the prepared foods by the study team.
2. Self-directed study	Flexibility	Difficult to monitor adherence
	Relatively low participant burden (relative to controlled feeding study)	May impose financial constraints on participants
	Participants can select specific foods catered towards his or her liking	Actual nutrient composition or amount of consumption may vary across individuals
Potential outcomes in Dietary Intervention Trials		
1. Traditional "hard" MS outcomes (clinical or radiographic outcomes)	Can demonstrate affects MS-specific disease processes	Requires large sample size
		Requires longer duration
		Because of high costs, long duration and large sample size, may not be appropriate for pilot interventions
2. Immunologic / MS-biomarker outcome	May be more sensitive to change than traditional MS outcomes	May be difficult to determine effect size
	Some biomarkers can demonstrate effects MS-specific disease processes	Requires expertise in specific laboratory methods
	Study will likely be smaller and/or shorter in duration	Requires standardized processing and collection of specimens (some types may be more challenging than others)
	May be more appropriate for pilot interventions	
3. Patient-reported outcomes (e.g. mood, fatigue)	Study will likely be smaller and/or shorter in duration	Relevance to MS-related disease processes is not straightforward and their implications for long-term MS outcomes is less clear
	May be more sensitive to change	
	May be more appropriate for pilot interventions	
4. Comorbidity-relevant outcomes (e.g. weight loss, improved blood pressure	Study will likely be smaller and/or shorter in duration	Relevance to MS-related disease processes is not straightforward and their implications for long-term MS outcomes is less clear
	May be more sensitive to change	
	May be more appropriate for pilot interventions	

strategies for improving rigor of dietary studies intervention in dietary interventions in people with MS.

2. Design of the dietary intervention mechanism

A major decision point in the design of dietary intervention studies is whether to use a controlled-feeding or self-directed/educational approach to deliver the intervention. Advantages and disadvantages of each approach as well as suggestions for optimization are outlined here and are summarized in Table 1.

2.1. Controlled feeding study

2.1.1. Traditional (e.g. on-site) controlled feeding study

From a scientific and practical perspective, on-site, controlled feeding studies offer an array of advantages over other study designs. On-site feeding trials allow for the study of diet in a controlled setting, thereby minimizing potential confounding due to differences in diet that can occur where participants are asked to follow a recommended diet but choose the specific foods which to consume. In addition, food and nutrient intake (including intake timing, if applicable) are standardized across study participants, lessening between-participant variability in caloric, food, and nutrient consumption. Another key strength of this design is that it allows for relatively straightforward adherence monitoring. Study staff can weigh food not consumed as well as encourage participants to consume provided meals in entirety.

There are also some noteworthy disadvantages of controlled feeding studies. On-site controlled feeding studies are resource-intensive (and costly) for both study investigators and participants. Most traditional controlled feeding studies require a registered dietician to design dietary interventions as well as appropriate study staff to prepare and serve meals. Increasing the variety of prepared meals may increase study participant engagement and retention and may be more consistent with a real-world scenario, however it creates additional work for dieticians developing the diet as well as staff in preparing meals, thus adding expense. Additional screening measures before enrollment may also need to be employed to ensure participants are able (e.g. no food allergies) and willing to eat food included on the menus. Moreover, while a participant may initially be able and willing to consume a prescribed diet, he or she may not like specific foods or become bored with the provided diet, such that ongoing support from dieticians may be necessary to provide equivalent substitutions that maintain adherence and study integrity as best as possible. Most controlled feeding studies also require participants to consume the majority of meals on site; the study team must have access to a location that supports study visits (e.g. a cafeteria setting), and study participants must agree to visit such a location routinely (typically, twice a day). This requirement may reduce the generalizability of the study, as likely only a subset of people with MS who live close enough to such a center and have the capacity to visit this often are likely to be able to participate. Moreover, though onsite adherence monitoring can be conducted with relative ease, controlled feeding studies still need to consider additional calories/foodstuffs consumed away from the study site. Participants should be encouraged to remove tempting foods from their pantries and cupboards to minimize their off-study consumption. While participants may build a sense of community with other participants with whom they interact at the study site, they may also experience some loss of social aspects of eating (e.g. consuming family meals, eating out at restaurants). Other logistical aspects that may impede participant participation and the study's success include variables such as weather concerns, participant travel, holidays/special occasions, family issues, during which participants may not be able or willing to

come to the feeding center. Finally, and perhaps, most importantly, because of the high cost and intense study team and participant involvement, most controlled feedings studies are typically short in duration. The duration of the study has implications for the selection of endpoints; these considerations are discussed in more detail in Section 2. Selection of Study Endpoints and Table 1.

2.1.2. Remote (e.g. off-site) controlled feeding study

Remote or off-site feeding studies share many of the same advantages as traditional controlled feeding studies; participants are shipped standardized, pre-set prepared food for all meals for the duration of the study. As such, diet is also evaluated under highly controlled circumstances which, like traditional controlled feeding studies, minimizes (1) potential differences in diet due to overall health behavior status and (2) extraneous between-subject variation in nutrient or food intake. This design also reduces participant burden, likely easing recruitment and reducing drop-out rates; participants receive all meals at their homes and are not required to visit the study cafeteria multiple times per day. It also allows for participant travel; shipments can be redirected to his or her destination and participants can remain on-study during this time.

Similar to traditional controlled feeding studies, this design also requires significant participant and study team engagement. Study dieticians and meal service staff are required to design and prepare meals. This format additionally requires staff to prepare meal shipments on top of actual food preparation. It also necessitates the purchase of packaging materials to ensure food arrives fresh and intact. For example, we conducted an eight-week remote controlled feeding study of intermittent calorie restriction in people with MS (Fitzgerald et al., 2018). Participants received shipments of pre-prepared meals twice per week (on Tuesdays and Thursdays). For our study, two study team members needed a full day to prepare shipments for 12 participants (in addition to two full-day additional staff to prepare all foods). Each week, packaging materials were estimated to cost approximately $1000 (in 2016–2017). Weather-related issues may impact shipping logistics; snow or other inclement weather emergencies may lead to unexpected delays in shipments, while hot weather requires consideration of adding a greater number ice packs and/or changing the distribution thereof within each shipment box to ensure food safety. To that end, in our team's experience, ice bricks were most useful for shipments in that they are wide and flat and tend to stay consistent, thus seemingly moving less. To mitigate concerns of weather-related issues impacting the integrity of the study, we created “shelf-stable” or freezable back-up meals (two days’ worth for each representative “day” in the study). Educating study participants about assessing food for evidence of breakage of containers and inadequate refrigeration on the ice packs is critical.

2.2. Self-directed study

The other option for delivery of a dietary intervention is through education, such that participants follow prescribed guidelines to prepare food on their own rather than receive prepared meals throughout the study. Like a controlled feeding study, there are advantages and disadvantages to this approach. Perhaps the largest advantage is the resulting ease of applicability of the intervention and accessibility by interested clinicians and patients. Instructions for following an educational dietary program can be disseminated in a widespread fashion more easily than access can be granted to a controlled feeding program. Another advantage is that while educational sessions and availability of a study nutritionist to answer questions require some personnel investment, overall this approach is significantly less costly to administer. This reduction in cost will allow a greater number of participants and/or increased study duration as compared to a similar study employing a controlled feeding approach. An educational program also allows for increased flexibility regarding individual dietary restrictions and preferences and palatability of the intervention. Logistic complications with respect to on-site feeding or food shipping are entirely avoided.

However, compared to a controlled feeding approach, this approach also comes with several disadvantages, the largest of which relates to difficulty measuring participant adherence. Though there are some nutritional biomarkers available (discussed below, “Issues related to the collection of biospecimens” section), these are suboptimal with regard to precise adherence measurements in a dietary intervention study and therefore, the self-directed approach necessitates relying heavily on participant report through some combination of self-assessment tools and intake assessments. In addition, the self-directed approach involves a higher level of engagement/commitment due to the need for participants to shop for and prepare compliant meals on their own. Participants’ financial concerns may influence their ability to purchase foods that are recommended by study personnel. Finally, family needs and dynamics also need to be considered more carefully than in a controlled feeding study, though as discussed, the controlled feeding approach brings its own social issues.

We recommend beginning a self-directed dietary intervention study with an initial educational session led by a nutritionist who is familiar with the principles of the intervention as well as local options for food shopping (this will be more challenging in a multicenter study). This session could also potentially involve additional input from other MS experts who may provide some background on the MS and how diet might be beneficial in order to facilitate improved dietary adoption and adherence, as both are important for studies that rely on participants to change his or her dietary behavior significantly. The time required for this initial session as well as the timing of subsequent follow-up sessions will be driven by the complexity of the intervention. Preparing individuals to follow a ketogenic diet will be much more time-intensive than preparing individuals to follow a single component intervention such as a low-sodium diet. Educational sessions and materials should be flexible enough to allow for a wide range of food preparation proficiency. In our experience, allowing those who already cook to be creative with the plan while providing the option for high-level, specific guidance for those who do not, permits greater flexibility for recruitment and adherence and reduces anxiety. Providing guidance with regard to general food preparation principles, menu planning (including examples of compliant daily menus for several meals, along with recipes), and sample grocery lists can enhance participant knowledge, satisfaction, and adherence. Optimally, the amount of “extra” support given to each participant should be quantified in order to account for the possibility that this co-intervention may influence adherence and, therefore, results. Participants must be educated on navigating the grocery store and reading food labels as well. Stocking the pantry with staples and removing food items that are not compatible with the plan should be recommended.

Guidance on eating outside the home is also important to promote maintenance of enjoyment of the social aspects of eating and to allow for travel. Participants should be encouraged to view restaurant menus prior to selecting a destination, or to speak with family/friends responsible for choosing the restaurant about their dietary plan to maximize the likelihood that they will be able to enjoy the meal while remaining adherent to the program. Participants should discuss specific concerns with restaurant staff and should feel comfortable asking that their questions or requests be relayed to the chef if needed. Most restaurants are accustomed to dealing with food allergies and other dietary restrictions and in our experience, study participants were pleasantly surprised at how their requests were received, although whether regional differences exist for such accommodations is not clear. These guidelines are also helpful in travel scenarios where meals will be consumed in restaurants. When visiting an unfamiliar city and staying in a hotel, it may be helpful to ask family or friends familiar with the location for recommendations as well as to make use of the hotel concierge. This is also helpful for identifying a local market to purchase fresh items that can be kept in the room. When staying with family and

friends, our participants have found it helpful to discuss their diet plan in advance so that their host has the opportunity to properly shop ahead of time or can direct the participant to food shopping on arrival. It is also important to bring along snacks/meals for the travel time itself, including a few extras in the case of travel delays, to avoid the need to purchase food in an airport or at a fast food restaurant.

We highly recommend inclusion of partners/significant others in educational sessions, as we have found this to be effective with respect to improving engagement and understanding the requirements of commitment to the study at home. In our studies, participants whose partners agreed to change their dietary habits to accommodate study recommendations reported greater ease of implementing the dietary intervention, as it increased the likelihood that a single meal could be prepared for the household rather than requiring separate meal preparation; these observations are largely consistent with findings from qualitative studies from other groups (Neate et al., 2019). Depending on the specifics of the intervention, the family dynamic, and prior dietary habits, it may or may not be feasible to extend the diet plan to include children or other household members. The effect of study participation on others in the household and likelihood that the whole household will be able to incorporate the dietary intervention into daily life should therefore be considered as a factor of importance when weighing potential dietary recommendations for inclusion in a study program.

3. Selection of study endpoints

As described above, selection of appropriate study endpoints for dietary intervention trials coincide with key aspects of the proposed study design (e.g. the size and length of the study). Answers to these questions will determine the selection of traditional MS-specific outcomes (e.g. change in disability or the development of new lesions), biomarker, surrogate, or patient reported outcomes. Furthermore, the body of existing evidence or level of preliminary data motivating the dietary intervention can also provide insight into the most appropriate study endpoint (e.g. development of new lesions is likely not the most appropriate outcome for the first study of a novel dietary intervention). Key advantages and disadvantages of selected study endpoints are summarized in Table 1.

4. Improving rigor in dietary studies in MS

4.1. Issues related to recruitment, retention, and adherence

Although many people with MS express an interest in dietary modification as a potential for disease modification, participating in a dietary intervention study may present considerable burden for participants, thus presenting challenges to recruitment, retention, and adherence. It is imperative that prior to enrollment, potential participants fully understand the design of the study and the potential dietary interventions they may be asked to follow, what will be expected of them with respect to time, finances, and travel (to controlled feeding sites, training sessions, other study visits, etc.).

There are many threats to retention in dietary intervention trials, some of which are generalized and others of which are specific to dietary and other behavioral studies. In randomized dietary intervention trials, in which blinding may be difficult, members of a placebo arm may be more likely to lose interest in completing study-related assessments if they don't perceive some benefit of participation. A common strategy to address this includes a “wait-list control” option, in which the participants initially randomized to usual care/placebo ultimately gain access to the (in this case, dietary) intervention (Yadav et al., 2016). However, the study's outcome should be considered if the wait-list control option is employed, especially if longer-term outcomes like disability are desired as these participants may have a prolonged wait-list period in which drop-out or other temporal changes may occur. Another option involves an active control arm, in which participants learn, for example, about some other aspect of MS other than diet or other lifestyle interventions. The possibility of this leading to greater engagement in healthy eating or other behavioral changes that may impact the outcomes of interest (e.g. a control intervention improving adherence to medications and, therefore, reducing subsequent MS activity) must be strongly considered. It may be best to account for this possibility when calculating sample size estimates, as well as to deliver similar education to the active arm participants in order to minimize differences in the arms of the study other than the dietary intervention. Retention rates in dietary interventions are often variable, which also should be considered when calculating sample size estimates that adequately account for potential dropout.

Several strategies are often employed in dietary intervention studies to promote adherence and retention. These strategies include maintaining regular contact with study participants (assessing and encouraging adherence), ensuring study staff are readily available to answer questions quickly (by email and/or phone), and continuously encouraging and expressing appreciation for participants. Whether such strategies are truly beneficial in the MS population is not known. For example, in one of our pilot studies, participants found regular phone calls assessing adherence to a self-directed dietary intervention to be burdensome (Fitzgerald et al., 2018) and in another small pilot study, regular text messages as a strategy to improve adherence did not, in fact, appear to be associated with an improvement therein (Roman Fox et al., 2017).

Additional activities that may improve adherence and retention include providing ongoing education regarding the scientific rationale for conducting the study, both to participants and, potentially, to other members of the household who may be affected by the changes required of the study participant. Group-based study activities may provide additional motivation, but the composition of the groups must be considered carefully. For example, participants or study staff may be blinded to the specifics of the intervention, or active arm or control participants may become dissuaded from continuing in their assigned group based on interacting with members of the opposite arm. In all such endeavors, care must be taken to minimize any differential treatment of the study arms while not dissuading one group (typically, the control group) from becoming less interested in continued participation.

Even if participants do not adhere to the diet as dictated by the study, rigorous studies will attempt to continue to engage participants to complete study-related assessments so as to minimize bias due to loss to follow-up. Randomized trials typically assess the outcome from such participants using an “intention to treat” methodology, though sensitivity analyses may consider including the degree of adherence to the prescribed intervention in order to assess the impact of the intervention in those individuals who followed the plan more consistently.

Measuring adherence to prescribed diet plans, whether in a controlled feeding setting or in a self-directed study, can be challenging. Even subjects participating in a traditional controlled feeding study in which consumption of most study-provided foods is monitored may still deviate from the prescribed intervention in the off-site setting. While no true gold standard for assessing dietary intake exists, a widely accepted dietary intake assessment method involves the collection of multiple 24-hour recalls by a trained research dieticians (Willett, 2012; Thompson and Byers, 1994; Buzzard and Sievert, 1994). This methodology introduces expense and requires coordination of schedules between the study dietician and the participant, as well as time for the actual interview (e.g. 30 to 40 min), which in our experience was burdensome for many participants. A web-based, automated 24-hour recall is an alternative to the in-person interview (Subar et al., 2001), however, some research suggests they may have reduced accuracy when compared to other validated dietary assessment methods (Yuan et al., 2018). Asking participants to complete detailed diet records for specified period of time is another widely-accepted dietary assessment method (Willett, 2012; Thompson and Byers, 1994).

However, a study dietician with access to nutrient databases is still required to analyze recorded intakes, which introduces additional expense. With diet records, it is possible that participants will incompletely record dietary intake (especially if the record-keeping period extends for a long period of time). Repeated food frequency questionnaires (FFQs) (Subar et al., 2001; Willett et al., 1985; Block et al., 1986; Rimm et al., 1992) may reduce the need for substantial study staff involvement and may improve timing flexibility for study participants, but they still do require 30–60 min to complete. FFQs can be administered online or as a paper hardcopy. Keep in mind that participants may lose paper forms if they take them home, and study staff may need to remind participants repeatedly to complete the surveys. Hardcopy FFQs are often completed on scantron forms (e.g. machine-readable answer sheets for multiple-choice surveys), which can be challenging for people with reduced manual dexterity or limited upper limb mobility, and study staff may need to assist some participants with data collection. Brief FFQs or screeners are designed to capture the foods that contribute to > 90% of a particular nutrient, such as vitamin D, sodium, or dietary fat. These can reduce participant burden but may also limit functionality of the measure.

Whether smartphone apps or other technology-enabled measures are valid remains to be determined but may, in theory, offer improved affordability and patient satisfaction. Additionally, for each type of self-reported dietary assessment method, participants may be less likely to report intake of "undesirable" foods (e.g. foods which are not consistent with the study diet). While biological markers of nutritional intake may be reasonable to consider for some types of dietary interventions (e.g. 24-h urine sodium measures in a low-salt dietary intervention or detection of urinary ketones in a ketogenic dietary intervention (Brenton et al., 2019)), studies of broad dietary change often do not have a "gold standard" biological measure that is useful as a marker of adherence.

Of note, how an investigator would like to monitor adherence can also help guide investigators in choosing among the different dietary assessment methods. Theoretically, 24-hour recalls and diet records measure actual consumption, while FFQs measure typical intake over a period of time; the reference intake timeframe for biological markers can vary depending on the marker (Willett, 2012). Thus, the investigator's choice of how to monitor adherence (e.g. measuring average consumption of a macronutrient over time vs. following a prescribed diet plan) will determine choice of measure.

Because FFQs ask people to reflect on intakes over long periods of time, they are less susceptible than the 24-hour recall or the food record to errors that arise from day-to-day variability in diet (Willett, 2012). However, attempting to average a respondent's intake over longer time intervals introduces new errors of estimation; it can be difficult for respondents to conceptualize average intakes over long periods of time.

In addition to measuring adherence, it is important to measure potential confounders, both at baseline and, when relevant, throughout the course of the study. For example, timing of meal intake may influence some outcomes and thus, depending on the specific study, measuring typical intake timing may be useful. Other lifestyle factors, such as exercise habits, body weight at baseline, cigarette smoking, alcohol intake, vitamin D supplement use, and sleep, all of which may vary with diet, should be assessed so that differences in such behaviors can be assessed and, if needed, accounted for in statistical analyses.

In summary, well-designed research studies that employ optimal methods to improve retention and measure adherence are needed to advance our understanding of the impact of diet on MS. People with MS are more likely to experience depression, anxiety, fatigue, and mobility restrictions, challenges that must be considered in the design of intervention administration and follow-up. The insights gained from such studies may also help to promote incorporation of an identified beneficial diet, or of a more broadly healthy diet, in the real-world setting for people with MS.

4.2. Issues related to the collection of biospecimens

Many dietary intervention studies, particularly those of short duration, may seek to assess the impact of the intervention on biological outcomes, whether as a primary aim of the study or after acquiring funding for future, secondary investigations. Careful planning and conduct of specimen collection will enhance the quality of subsequent studies using previously collected biological samples. In addition, investigators should thoughtfully balance the potential "ideal" of repeated collections with the impact of this decision on study participants. For example, collecting samples too frequently may deter recruitment, reduce retention, increase missing data, and/or diminish the representativeness of the study sample (i.e. enrolling those willing to incur more invasive data collection can introduce selection bias).

4.2.1. Blood

Collecting blood is a nearly ubiquitous feature of studies that investigate biological outcomes. In MS, processing blood to obtain serum or plasma is common, and these subcomponents are often used to conduct metabolomic and lipidomic analyses. Many MS diet studies also report serum cytokine levels, however it may be more informative to assess peripheral blood mononuclear cells (PBMCs) in order to characterize an immunophenotype and document change related to a dietary intervention (Jason et al., 2001). Collecting PBMCs, however, requires a substantial investment of time and expertise in order to process the specimens and conduct subsequent studies. Some studies may wish to collect genetic material, such as DNA or RNA, particularly if the sample size is large enough to support analyzing genetic contributions to the effects of the diet or change in gene expression as a result of dietary intervention.

Standardization of blood collection and processing is critical to minimizing heterogeneity that may make results less interpretable. Study personnel should record subject-specific information and be aware that this information may influence future studies using the samples, including the sample collection date and time and the time of the last meal/fasting status. Ideally, single-center studies will use the same personnel to collect and process blood, although this is not always feasible. Whether collecting blood at one or multiple sites, it is good practice to use the same blood collection and processing materials and to minimize heterogeneity in sample handling (e.g. create standards for collection, processing, transfer, and storage of specimens). Multisite studies should also standardize the timing and methods for specimen shipping, considering the weather (changes in temperature may cause shipping delays and/or sample degradation) and the timing of weekend and holiday schedules for shippers and receivers.

4.2.2. Urine

Urine samples can provide a quantitative measure of adherence for some dietary intervention studies (e.g. low-sodium diet). Collecting 24-h samples is often ideal (e.g. quantifying sodium intake), but whether to combine urine samples into one collection kit or to collect each sample in a separate container so that circadian changes can be assessed may depend on the anticipated use of the samples. Of additional consideration for people with MS is that urinary frequency, retention, and nocturia may have implications for studies seeking to take into account the time of day in which samples are collected. Urinary symptoms or their treatment should be considered in the study design phase and documented at the time of sample collection (Mauruc et al., 2017).

4.2.3. Stool

Stool collection for gut microbiota analysis may help elucidate mechanisms of dietary interventions and aid in adherence assessment. The first step in planning stool collection involves making decisions regarding anticipated sample use. For example, if the anticipated use is solely 16S sequencing for bacterial identification, a simple dry collection swab will likely be sufficient and the least costly option. If,

however, experiments are planned that require the microbes to function upon thawing (for example, experiments in germ-free mice), a larger sample using a "wet" kit is needed. Thorough review with collaborators should direct kit selection. The same holds true for planning sample storage conditions. Samples should be stored and processed together to avoid batch effects, when feasible.

Once a kit has been selected, it is important to continue with the same exact kit through study completion. Uniformity is crucial for participant collection and shipping. Providing participants a detailed yet simple and clear instruction sheet with illustrations will increase the likelihood that the sample is collected correctly. Instructions should include acceptable days of the week for sample shipment to avoid arrivals to the lab on weekends and holidays. Depending on anticipated use of the samples, instructions should include preferred time of day for sample collection (often first bowel movement of the day is recommended) and considerations for constipation, which is often an issue for people with MS (Gulick, 2010). A member of the research team should review the instructions with each participant and remain available for questions. This is particularly important for participants reporting cognitive issues. Providing participants with a pre-paid label affixed to the return shipping envelope or box can increase the return rate. When selecting a shipment method, cost and the preservation of sample quality must be considered. Prior to settling on a shipment mechanism, investigators should explore different options with a "dummy" package to discern the length of transit time and ensure the sample arrives at the correct temperature (for most kits this means the included ice packs are still cold and the sample is still frozen though, there are some room temperature kits available). Many institutions have access to discounted rates with particular shipping providers, which should be investigated prior to the initiation of the study.

5. Conclusions

There are many logistical issues that an investigative team faces in conducting dietary intervention studies, some of which are common to any population and others of which may be unique to individuals with MS. This manuscript identified some of the challenges and decisions that future investigators may anticipate when designing a dietary intervention study for MS, regardless of the type of dietary change planned or the specifics of study design (e.g. randomized or single-arm, specifics of subpopulation targeted, or outcomes of interest). We recommend that investigators consider and address some of the practical issues discussed herein during the design phase of their own studies, carefully balancing the goals of their studies while being mindful of the impact of each decision on the feasibility and generalizability of participant recruitment and retention. It would be ideal, as additional studies are completed, for investigators in the MS community to contribute their own "lessons learned" to the literature in order to promote continuous refinement and improvements in MS dietary intervention trials.

Declaration of Competing Interest

We certify that no party having a direct interest in the results of the research supporting this article has or will confer a benefit on the authors or on any organization with which they are associated and we certify that all financial and material support for this research and work are clearly identified in the Acknowledgments Section.

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