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Clinical Nutrition Studies: Unique Applications

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Nutrition research is the hallmark of establishing nutrient requirements and giving dietary guidance to promote health and wellbeing throughout life. Over the years it has been an active area of investigation, leading to the discovery of many important findings that have provided the basis for dietary guidelines and recommendations. Until recently, resources describing the design, implementation, and management of clinical nutrition studies were limited. Because of the growing interest and activities in clinical nutrition research, a number of important resources are now available that describe key aspects of conducting clinical nutrition studies. These resources (i.e., books and journal articles) are listed in Table 16.1. They provide detailed information about all aspects of conducting nutrition research with human participants. Collectively, they are a wealth of information for all researchers interested in and actively involved with human nutrition research. Indeed, these resources are a true "goldmine" to the field for standard studies, including those that employ either a single- or multicenter model. However, there are many studies that are uniquely different, presenting what may seem to be insurmountable challenges with respect to the experimental design, diet design, participant population studied, and feeding model utilized. For the most part, these have not been discussed in detail in the publications listed in Table 16.1.

Thus, the purpose of this section is to describe unique challenges in human nutrition research applications and provide guidance about how they can be effectively managed to maintain tight experimental control. First, we have included a variety of forms that were developed for different nutrition studies we have conducted that are not available in other resources. These forms deal with important aspects of conducting a well-controlled feeding study. They can be adapted to other human nutrition studies that vary in design from more typical protocols. Second, we provide descriptions of studies that illustrate challenges in the design and conduct of clinical nutrition studies, with notes on how these were handled.

Forms and Documentation for Assuring Dietary Protocol Compliance

Essential to all controlled feeding studies is recruiting participants who not only meet the eligibility criteria that have been defined, but are also willing to adhere to all aspects of

TABLE 16.1

Resources for Information on the Conduct of Clinical Nutrition Studies

Dennis, BH, Ershow, AG, Obarzanek, E, Clevidence, BA. *Well-Controlled Diet Studies in Humans.* The American Dietetic Association, Chicago, IL, 1999.

Dennis, BH, Kris-Etherton, PM. Designing and Managing a Small Clinical Trial. In: *Research, Successful Approaches,* pp. 151-170. Edited by E. Monsen. The American Dietetic Association, Chicago, IL, 1992.

Dennis, BH, Stewart, P, Hua-Wang, C, Champagne, C, Windhauser, M, Ershow, A, Karmally, W, Phillips, K, Stewart, K, Van Heel, N, Farhat-Wood, A, Kris-Etherton, PM. Diet design for a multicenter controlled feeding trial: The DELTA Program. *JADA* 1998; 98: 766-776.

Obarzanek, E, Moore, TJ. The Dietary Approaches to Stop Hypertension (DASH) Trial. *JADA* 1999; 8: S1-S104.

the experimental protocol. Hence, they serve as partners in research with the investigative team. Also important are the day-to-day operations that depend largely on the proper preparation, delivery, and consumption of the diets and adherence to study protocol by the participants. For many studies the diets are the treatments, and therefore must be strictly delivered as set forth by the protocol. Forms to assist with participant selection and documentation of diet adherence activities are needed to assure quality.

Recruiting Participants

The major goal of recruitment is to enroll the required number of participants necessary for the study within the projected timeline and within the budget constraints. The recruitment of potential study participants can be accomplished in many ways, and will be guided by inclusion/exclusion criteria set forth in the study protocol. Reaching the target study population is key. For example, post-menopausal women would more likely see an advertisement in a community weekly than, obviously, in a high school newspaper. Therefore, recruitment tactics focus on the groups that need to be recruited, and are planned accordingly. The Institutional Review Board (IRB) must clear all recruiting materials before use to assure that the information is not misleading and the study is accurately represented. Detailed information about recruiting is available elsewhere (see Table 16.1), but is briefly discussed below.

Advertisements in the local media are effective recruitment tools. Newspaper advertisements can be general in nature or contain specific information about the study (see example, Figure 16.1). An advantage of a newspaper advertisement is that it reaches a large audience, while a disadvantage is that it can be expensive. The ability to target a specific audience may be somewhat limited unless the newspaper audience is narrow, such as for business reports or campus newspapers. Radio and television advertisements also can be expensive and are limited in the amount of information that can be disseminated about the study. However, it may be possible to obtain free radio and television advertising by submitting the material as a public service announcement. Local morning or early evening magazine-format programs often welcome an interview with the study investigator, who can describe the study and ask for volunteers.

Other recruiting tools include speaking about the study to different local community organizations, such as the Lions clubs or church groups. Although time consuming, it targets a specific audience. Health fairs provide a community service as well as a recruitment opportunity. For example, free blood pressure screenings will help identify potential participants for a study examining blood pressure-lowering diets. Distributing and posting flyers on bulletin boards in supermarkets, drug stores, or doctors' offices is an excellent avenue for communicating the need for study participants. Mass mailings of a brochure, postcard, or letter describing the study can be sent to a target population. This tends to

Is Eating Cocoa and Chocolate Good for You??

We are currently recruiting participants for a nutrition study aimed at determining whether cocoa and chocolate contain antioxidants that may be good for you.

The study dates are June 1–June 29 and July 12–Aug 10. You must complete both 4 week periods.

(There is a break from June 29 to July 12th)

You may qualify if you meet all of these criteria:

- You must be healthy and between the ages of 20–67 (male or pre-menopausal female);
- You must not be a smoker and cannot consume alcohol, coffee, or tea during the study;
- You must be willing to eat only foods (including cocoa and chocolate) provided for the study and come to the study center on University Park campus for breakfast and dinner 5 days/week — food for lunches and the weekend will be packed for takeout;
- You must not have diabetes mellitus or uncontrolled (> 140/95) high blood pressure or other serious health conditions;
- You must not be pregnant, nursing, or planning to get pregnant.

All food will be provided during the study along with monetary compensation. If you are interested in participating in this study, please call (814) 863-3168, give your name, mention that you are interested in the Cocoa Study, and give us a number where you can be reached.

FIGURE 16.1
Sample subject recruitment advertisement.

be an effective tool but is labor-intensive and costly for the materials, mailing list, and postage. Email also can be used for recruitment but, like mass mailings, it sometimes requires the purchase of the mailing list.

Regardless of the recruitment method used, careful records are required to track the recruitment progress, and more importantly, to document that the eligibility criteria are being met.

Screening Potential Participants

Once a person expresses interest and contacts the recruiter, the screening process begins. The initial contact should be used to exclude those who do not meet the most easily identifiable criteria (see Telephone interview form, Figure 16.2). Obviously, a smoker who calls to ask about a study that is recruiting only non-smokers would be excluded quickly during the telephone screening process. Another example would be the screening of a post-menopausal woman who is taking hormone replacement therapy (HRT) when the study protocol excludes women on HRT. These initial exclusions early in the recruiting process prevent bringing people in for the more expensive clinic visits, and thus prevent

Folate Study
TELEPHONE INTERVIEW FORM
Pennsylvania State University

Subject ID: _____
Today's Date: __/__/__
Reviewer's Initials: _____

Before asking any questions, please read the following paragraph to obtain verbal consent to conduct the telephone interview:
"We received your message that you are interested in participating in the Folate Study. I would like to ask you a series of questions about your willingness to participate in the study, your past medical history, and your current lifestyle behaviors. If you agree to answer these questions, and it is then determined that you meet the criteria for this study, we will schedule you for a screening visit. Are you willing to answer a series of questions, which will take about 15 minutes?"
1. ☐ Yes (continue with interview)
2. ☐ **No** (thank them for their time and interest)

Full Name w/middle initial _____ DOB _____
Local address _____
Home # _____ Work # _____

1. Are you a female between the ages of 19–35 years inclusive?	☐ Yes	☐ **No**
2. Do you plan to remain in the area for the duration of the study?	☐ Yes	☐ **No**
3. Are there any personal reasons (e.g., family problems, vacations, child care difficulties, etc.) that would keep you from participating in the study?	☐ **Yes**	☐ No
4. Are there any professional reasons (e.g., job-related travel, irregular work schedule, conferences, etc.) that would keep you from participating in the study?	☐ **Yes**	☐ No
5. There is a variety of foods in the Folate Study. Are there any foods you refuse to eat, are allergic to, or for whatever reason have to avoid?	☐ **Yes**	☐ No

Go to Food List

Listed are foods included on the diet for this study. I will go through the list — tell me if there is any food you cannot or would not eat.

FOODS	Ok?	FOODS	Ok?
Turkey breast		Penne pasta	
Zucchini (fresh)		Celery	
Green olives		Mayo dressing	
Potatoes		Radishes	
Dill pickles		Onion	
Tuna		Sweet relish	
Mayonnaise		Canned pears	
Canned peaches		Applesauce	
Grapes (fresh)		Watermelon (fresh)	
Canned pineapple		Blueberries	
Jello		Potato chips (regular/barbecue)	
Banana muffin		Cinnamon-apple muffin	
Blueberry muffin		Pineapple-orange muffin	
Beef		Mashed potatoes w/ gravy	
Chicken breast		Seasoned rice (thyme, oregano, parsley flakes)	

FIGURE 16.2
Telephone interview form.

Spaghetti		Meatballs & marinara sauce	
Ground turkey		Taco seasoning	
Cornbread muffin		Skim milk	
Crackers		Pretzels	

6. Do you currently smoke?	☐ **Yes**	☐ No
a. If No, have you ever smoked before?	☐ **Yes**	☐ No
1. If Yes, how long since your last cigarette? _____		
7. Are you willing to discontinue your consumption of alcohol for the entire 8 weeks of the study?	☐ **Yes**	☐ No
8. Do you have access to the following appliances at home/apartment/dorm:		
Refrigerator	☐ Yes	☐ **No**
Freezer	☐ Yes	☐ **No**
Microwave or oven or toaster oven	☐ Yes	☐ **No**
Explain that they will take Sat/Sun meals home on Friday and will need a place to store/cook their food		
9. It is important to maintain your current body weight for the duration of the study. Are you willing to maintain your current body weight?	☐ Yes	☐ **No**

Medical and Lifestyle Information

Do you have any of the following medical conditions:

1. Heart disease	☐ **Yes**	☐ No
2. Diabetes	☐ **Yes**	☐ No
3. High blood pressure (hypertension) treated with medication	☐ **Yes**	☐ No
4. Renal or kidney failure	☐ **Yes**	☐ No
5. Gastrointestinal condition such as Crohn's disease, irritable bowel syndrome, ulcer, or history of bowel surgery	☐ **Yes**	☐ No
6. History of blood clotting disorder	☐ **Yes**	☐ No
7. Liver disease such as cirrhosis	☐ **Yes**	☐ No
8. Condition that requires the use of steroid medication	☐ **Yes**	☐ No
9. Gout requiring treatment	☐ **Yes**	☐ No
10. Recent history of depression or mental condition requiring medication within the last 6 months	☐ **Yes**	☐ No
11. Anemia	☐ **Yes**	☐ No
12. Sickle cell anemia	☐ **Yes**	☐ No
13. Lung disease such as chronic bronchitis or emphysema	☐ **Yes**	☐ No
14. Cancer, active within the last 10 years	☐ **Yes**	☐ No
15. Thyroid disease or thyroid problem requiring treatment such as iodine or surgery, or taking medication for your thyroid	☐ **Yes**	☐ No
16. Do you have any other medical condition not previously mentioned?		
If Yes, specify: _____	☐ Yes	☐ **No**
a. **If Yes, is the subject eligible?**	☐ **Yes**	☐ No
17. Do you take any type of doctor-prescribed medication?	☐ **Yes**	☐ No
If Yes, specify medication and reason: _____		
_____		
a. If Yes, is the subject eligible?	☐ Yes	☐ **No**
b. If she takes birth control, explain she has to be willing to forego its use for the duration of the study (8 weeks) and has to have a 2-week wash-out period before starting. This means she has to finish her entire packet of pills and from that last day she starts the 2-week wash-out period.	☐ Yes	☐ **No**

FIGURE 16.2
Continued.

18. Do you take any type of self-prescribed medication, vitamin, mineral, or other supplement (including garlic or ginseng?)	☐ **Yes**	☐ No
If Yes, specify medication/supplement: _____		
a. If Yes, are you willing to discontinue? _____	☐ Yes	☐ **No**
19. Are you on a special diet prescribed by a doctor or self-prescribed?	☐ **Yes**	☐ No
If Yes, specify: _____		
_____	☐ Yes	☐ No
a. If Yes, is the subject eligible? _____		
20. Do you exercise more than 8 hours a week or play sports regularly?	☐ **Yes**	☐ No
If Yes, please describe: _____		
_____		
_____		
21. Are you willing to have a pregnancy test 3x during the 8-week study?	☐ Yes	☐ **No**
22. In order to assess folate, red blood cells, and homocysteine, blood will be taken at the beginning of the study and once every week after that. There will be a total of 9 blood draws, with no more than 2 tubes of blood taken each time. Are you willing to do this?	☐ Yes	☐ **No**

Note: This form has to be reviewed by the Study Coordinator		
If any of the bolded boxes are marked, the subject is ineligible.		
Is the subject eligible?	☐ Yes	☐ **No**
a. If Yes, go to Women's History Form and then schedule clinic visit.		
b. If No, thank subject for his/her time and terminate the interview.		

FIGURE 16.2
Continued.

wasting resources in terms of staff time and money. For those who pass the initial telephone interview, further screening is required. They will come to the study site for clinical laboratory tests or other measurements to assure that they are relatively healthy and meet all the study eligibility criteria.

To assist with identifying whether someone would be able to adequately follow the dietary protocol, general dietary information may be gathered in questionnaire form during the study screening visits. The questions identify participants who cannot or will not eat any foods due to religious reasons, allergies, or severe physical discomfort. A complete list of study foods or a copy of the study menus in layman's terms may be reviewed with potential participants. This information is especially important when particular foods in the menu cannot be substituted.

Other useful screening information includes whether a person can safely store and prepare foods to be consumed away from the clinical site, and environmental, family, or work situations that may make adherence to the protocol difficult (see General dietary questionnaire, Figure 16.3). For persons whose adherence may appear to be questionable, a meeting with the study dietitian may help to determine if reasonable provisions can be made within the protocol requirements, or they may be deemed ineligible. For example, participants who have lactose intolerance may be allowed to use lactose digestive aids and be eligible. Someone who does not have adequate facilities in their home to store and prepare study foods would be ineligible to participate in the study.

The following questions are related to your overall eating environment. Your answers will help the staff determine ways they can make your participation in the study more enjoyable.

	Yes	No
1. Do you foresee any problems transporting, storing, refrigerating, and warming your study foods when you are away from our center?		
2. Do you participate in activities where food is served, such as sporting events, religious gatherings, business meetings, etc.?		
3. Will any holidays, birthdays, family reunions, vacations, etc., occur during the period you are on the study?		
4. If you are responsible for preparing meals in your household, will this make it difficult for you to meet study requirements?		
5. Will anyone in your household be affected or inconvenienced by your participation in this study? If yes, who are they and how will they be affected?		
6. Will your employment (e.g., job transfer) or work hours (e.g., moving to a night shift) change during the study?		
7. Do you, or anyone in your household, work in the food service industry (cafeteria, bakery, restaurant, etc.)? If yes, do you eat any meals or snacks at work either as a requirement of your job or as a matter of convenience?		
8. If you have any concerns about the study, please write them in the space provided below (use the back of this page if you need additional space).		

Reviewed by (staff ID): _____

FIGURE 16.3
General dietary questionnaire.

Orientation Session

When taking part in a feeding study, it is important that each participant carefully follow the dietary protocol. An orientation form that lists these guidelines may be developed and reviewed immediately prior to the actual study start date during an orientation session. Helpful information for the form would be instructions to finish all foods and beverages provided, squeeze condiment packets until empty, use a rubber spatula or bread to clean the plate, and eat fruit and vegetable peels as appropriate. Reminders to complete the daily forms, check for accuracy of the meals, and not make substitutions may be listed. A guideline stating what to do if participants find that they are too full or hungry is useful. Heating directions for takeout foods could also be included. A list of contact persons with telephone numbers is imperative for when questions or problems arise. Participants appreciate a copy of the beverage and seasoning guidelines that specify the types and amounts that may be consumed. If there are restrictions on mints or gum, these should be listed. Participants must be instructed in the safe handling of foods provided to be consumed off site. For example, a simple handout may be given that reminds participants to use a cooler to transport foods for longer than one hour, to refrigerate all perishable foods as soon as possible, and to not eat suspected spoiled foods, but to notify the staff immediately to avoid a missed meal.

Assuring Participant Diet Adherence

Participant adherence to the diet protocol is collected, usually on a daily basis (see Daily checklist, Figure 16.4) by the participant, and is subject to review by study staff. For this

Fat Challenge 2 **Pennington Biomedical Research Center**

Name _____ Date _____

Please answer all questions below and place an "X" in appropriate column. Please fill in the additional information requested when necessary. Please return this form each day to the Pennington Metabolic Kitchen.

1. _____ Yes _____ No — Were there any study foods you did not eat/drink on this day? Reasons include missing, spilled, or inedible food, illness or other.

Food/Drink	Amount	Reason

2. _____ Yes _____ No — Did you eat or drink any foods that were not provided by the Metabolic Kitchen today? If yes, please give the food/drink (be very specific), the amount, and reason consumed.

Food/Drink	Amount	Reason

3. _____ Yes _____ No — Did you consume any decaffeinated sugar-free beverages on this day? Record all beverages, including those provided by the Metabolic Kitchen.

Food/Drink	Amount
	_____ oz.

4. **Did you eat any of the "unit foods" today? Y _____N _____**
   How may "unit foods" did you eat? 0 1 2 3 4 5 Other _____

5. _____ Yes _____ No — Is there anything you would like us to know in relation to your participation in this study?

FIGURE 16.4
Daily checklist.

reason, the participants must be encouraged to record honestly and must not feel that their participation is threatened in any way. The data, which document diet adherence and deviations, may be used to calculate daily energy and nutrient intakes and to compute a compliance score. The form, called a daily diary, daily log, or food and beverage intake form, may gather information for the following broad categories:

- The type and amount of study foods not eaten
- The type and amount of non-study foods eaten
- The type and amount of discretionary or "allowed" food items consumed
- The type and amount of beverages consumed, including coffee, tea, soft drinks, and alcoholic beverages
- The number of unit foods or calorie adjusters eaten
- Dietary supplements or over-the-counter medications taken
- Feedback regarding concerns or questions related to participation in the study

The forms should provide the participant's identification number and date for which diet information is obtained. It may also contain the kcalorie level for the participant, coded

ILSI
Weekly Monitoring Form
Pennsylvania State University

Subject ID: _____
Today's Date: __/__/__
Reviewer's Initials: _____

Week 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Blood Draw Date

Date of blood draw 1: ____/____/____ **Date of blood draw 2:** ____/____/____

1. In the past week has your exercise level changed? ☐ **Yes** ☐ No
   a. If Yes, was it
   ☐ More Active
   ☐ Less Active
   ☐ No Exercise

2. Have you taken any vitamin, mineral, or other supplements in the past week? ☐ **Yes** ☐ No
   a. If Yes, specify: description amount
   _____ _____
   _____ _____

3. Have you been ill in the past week? ☐ **Yes** ☐ No
   a. If Yes, describe illness: _____
   _____

4. If you were ill in the past week, did your eating change as a result? ☐ **Yes** ☐ No
   a. If Yes, describe: _____
   _____

If any of the bolded boxes are checked, please notify Study Coordinator

FIGURE 16.5
Weekly monitoring form.

treatment diet, a field for entering the participant's weight, and for females, menstruation information. The information gathered then may be coded by the study staff, either on the same form or another form, for data entry.

Another form for staff documentation of diet deviations may be used. This would provide a record of deviations observed during on-site meals or those called in to staff during times of off-site meals, such as weekends. If any study food or beverage is left on the meal tray and was unnoticed during the meal tray check, information regarding the food (i.e., type and amount) is recorded. Similarly, if a participant calls a staff member to report a deviation for an off-site meal (i.e., missing, lost, or spoiled food), the appropriate information is recorded. If discrepancies between the kitchen staff observations and information reported by the participant are found, the study dietitian should adjudicate those. In addition, the study protocol may require the participant to return uneaten portions of study-provided foods. The type and amount of food returned would be recorded on this form. Additional information may be collected regarding what was done with the food not consumed. For example, was the food given back to the participant to eat, replaced at the next meal and eaten, not replaced or not eaten? The data then may be coded for compliance measures and energy or nutrient calculations.

Usually on a weekly basis, the participant will complete another form that asks about general health-related items that might influence food intake or study outcomes (see Weekly monitoring form, Figure 16.5). For example, changes in exercise, any illnesses, and any medications or supplements taken may be reported. Queries for possible symptoms,

Diet D Menu 1						
Date: ____/____/____ Mm dd yy Day: M T W T F S S	**Staff Initials**	**1500**	**2000**	**2500**	**3000**	**3500**
Breakfast						
Orange juice		124.0	124.0	124.0	124.0	248.0
Puffed rice cereal		23.0	23.0	23.0	46.0	46.0
White bread		22.7	45.4	90.4	90.4	90.4
Butter		8.0	9.0	15.0	20.0	20.0
Sweets; jellies		0.0	10.0	10.0	10.0	10.0
Jellies, dietetic		14.2	0.0	0.0	0.0	0.0
Milk, whole		245.0	245.0	245.0	490.0	490.0
Lunch						
Sandwich package:						
*Turkey breast meat		35.0	50.0	56.7	56.7	75.0
*Mayonnaise, regular		4.3	5.0	6.0	9.0	9.0
*Iceberg lettuce		0.0	0.0	0.0	10.0	10.0
*White bread		45.4	45.4	45.4	45.4	90.8
Iceberg lettuce		24.0	24.0	24.0	40.0	40.0
Olive oil		0.0	2.0	2.0	10.0	8.0
Peaches, juice pack		127.6	127.6	127.6	127.6	127.6
Ginger Cookie		14.0	22.0	22.0	22.0	22.0

FIGURE 16.6
Food production form. Foods are indicated in grams for each energy level diet.

such as poor appetite, stuffy nose, fatigue, diarrhea, constipation, nausea, or headache could also be included as appropriate.

Food Production and Meal Assembly

Used in conjunction with standardized recipes, a food production form is followed for the preparation and portioning of all menu items (see Food production form, Figure 16.6). There will be a separate form for each diet and menu. It is usually helpful to color-code the forms according to diet treatment. An established menu sequence will determine which menu is to be served on each day of the study. For each menu, the portion sizes for the various kcalorie levels are listed. The number of participants receiving each kcalorie level for the corresponding diet is listed in the box above each kcalorie designation so that the kitchen staff knows how many servings are required. The food is prepared and portioned according to the list, and then the staff member responsible initials the item in the space provided. At times, it might be necessary to substitute a food item. Documentation of the deviation on the food production form or a separate form, to include the type of food and when it was used may be informative later when detailed diet information is required.

A tray assembly check sheet is valuable for assuring that all participants receive each menu item (see Tray assembly check sheet, Figure 16.7). Separate sheets may be needed for each kcalorie level, menu, and diet, and could be color-coded similar to the food production forms. As the tray is assembled, the item is checked off in the corresponding box for each participant and initialed by the staff to verify that all food items are provided. If the food production forms are used to assemble the foods, a tray assembly check sheet may be used as a quality assurance check. Similar procedures are followed for packed meals, checking off each item as it is placed in the takeout container.

Diet D Menu 1 1500 Kcal								
Date: ____/____/____ Mm dd yy **Day: M T W T F S S**	**Name or I.D. #**							
Breakfast								
Orange juice								
Puffed rice cereal (1 PC)*								
White bread (1 slice)								
Butter								
Dietetic jelly (1/2 oz.)								
Whole milk (1 PC)								
Staff Initials								
Lunch								
Turkey sandwich package								
Salad								
Salad dressing								
Peaches (1 PC)								
Ginger cookie (1 small)								
Staff Initials								
Snack								
Trail mix								
Staff Initials								

FIGURE 16.7
Tray assembly check sheet. * PC = portion control.

Another practical form for packed meals and/or snacks is a checklist of the menu items that participants use to verify that all items have been packed in their takeout containers (see Packed meal form, Figure 16.8). The form is attached to or placed inside the container, and the participant is instructed to contact a kitchen staff member if any item on the form is missing from the container. Menu cards also could be used in place of the form. These,

Diet D Menu 1

Name _____ **I.D.** _____ **Telephone Contact (___)___-____**

Day M T W T F Sa Su (Circle One) **Date** ___ ___ ___ **Packed by** _____ **Meal B L D S**

mm dd yy

Breakfast	Lunch	Dinner
Packed	**Packed**	**Packed**
☐ Orange juice	☐ Turkey sandwich package	☐ Sirloin tips with gravy
☐ Puffed rice cereal (1 box)	☐ Salad	☐ Corn
☐ White bread (1 slice)	☐ Salad dressing	☐ Salad
☐ Butter	☐ Peaches (1 can)	☐ Salad dressing
☐ Dietetic jelly	☐ Ginger cookie (1 small)	☐ Roll
☐ Milk (1 carton)	☐	☐ Butter
☐	☐	☐ Applesauce

Unit Foods		Snack
# of Units	**Packed**	**Packed**
_____	☐	☐ Trail mix
_____	☐	☐

FIGURE 16.8
Packed meal form.

when placed on the on-site meal trays, also provide a means for the participant to double-check the accuracy of the menu items.

Training Dietary/Kitchen Staff

It is imperative that all staff members who will be involved in the preparation and delivery of research diets understand the strict procedures necessitated by the protocol. For example, they must know the acceptable ranges for gram weights when portioning various food items, how to read the food production forms, and cooking procedures. Those who will be interacting with the participants must know the dietary guidelines, such as allowed beverages and seasonings. The development of a training manual is useful so that staff can periodically review the standard procedures and have a reference available to answer any questions that may arise during the course of a study. Actual observation and/or a written test may be utilized to assess staff competencies. In addition, routine quality control checks are essential, and kitchen staffs who do not consistently meet the rigorous standards set for delivering the experimental diets must be relieved of this responsibility.

Quality Assurance of Diets

During the regular production of the menus, duplicate meals should be prepared and collected for monitoring quality assurance and for chemical analysis of target nutrients. The food should be prepared and weighed following the standard procedures. Ideally, food preparation staff should not know that these will be used for quality checks, but this may be difficult to disguise. One way to overcome possible bias is for the staff to prepare a meal for analysis identical to one prepared for a participant, and the two may be switched prior to serving. For portion weight checks, as each food for one menu is placed into a container, the weight of that food item is recorded and compared to what should be present. Any discrepancies should be noted. The person who made a mistake when weighing the food or assembling the meal can be identified by looking for his/her initials on the production sheets. Retraining of that individual may be necessary to alleviate any further errors. Once the entire menu is in the container it can be blended, and aliquots taken or frozen for later analysis. When the analysis is completed, the actual nutrient values may be compared to the expected nutrient values (from the database), especially for those being controlled.

A spot-checking procedure may be employed to monitor the accuracy of meals distributed to participants. Randomly selected meals and/or food items are checked for completeness and accuracy. If a problem is found, it is described, a plan of action for correction of the problem is detailed, and documentation of the action plan or followup is provided. Again, the person making the mistake should be retrained.

Miscellaneous Forms

The use of a food service sanitary inspection checklist for personnel, food handling, equipment, storage practices, dishwashing, and department areas is standard. Inspection of refrigerator and freezer temperatures also should be conducted on a regular basis. In a research kitchen, regular accuracy checks of the electronic balances should be documented. Use of a form that lists acceptable ranges is a practical way to indicate whether to recalibrate a balance. If foods are donated to the study, their expected delivery, date received, and accuracy may be tracked on a form that also includes a description of the food items, company, address, contact person, and telephone number.

Exit Interview

At the end of the study, an exit interview may be planned for each individual or for a group of participants. Information offered could include laboratory data gathered during the study, health risk assessments, and appropriate educational materials. In addition, anonymous input from completing participants about their experiences and views is helpful for planning future studies. For example, questions asking about favorite foods, disliked foods, and about the menus in general that were served during the study can provide information for improving future menus. Factors that made it easy or difficult to follow the study protocol may be assessed. Additional questions include how study staff treated participants and whether the subjects would recommend participating in a study to their friends. This information can be invaluable for improving future studies by increasing menu acceptability and making study participation a more enjoyable experience.

Unique Study Challenges and Strategies for Addressing Them

Clinical nutrition studies may present challenges at every stage; for example, with menu development, recruitment of participants, and finally, preparation and delivery of the experimental diets. Obstacles must be overcome for a successful outcome. For illustration, studies will be described that have dealt with unique and challenging situations with the population studied, experimental design employed, and experimental diet(s) fed to study participants. The conduct of multiple center clinical nutrition trials present their own challenges, which are discussed elsewhere (see Table 16.1).

Recruitment and Retention

A study was designed to evaluate the effects of diets high in polyunsaturated fatty acids/n-3 fatty acids (PUFA/n-3FA, accounting for 12% of energy), derived from walnuts and walnut oil with different levels of n-6/n-3 FA ratios (9:1 vs. 4:1), on multiple risk factors for cardiovascular disease (CVD). In this crossover study, we recruited 30 males and females, ages 45 to 65, so that the results could be somewhat generalized to a middle-aged population. Various study endpoints, including serum cytokines and the release of TNFα and IL-6 by polymorphonuclear cells, are affected by the menstrual cycle, so this dictated that premenopausal or postmenopausal females on hormone replacement therapy (HRT) be excluded to minimize confounding. All participants were to be healthy, overweight or mildly obese, having moderate hypercholesterolemia, and taking no medications. These eligibility criteria made recruitment efforts challenging. First, many people within this age group were taking nutrient supplements, cholesterol-lowering drugs, or medications for hypertension, diabetes, or rheumatoid arthritis, so were not eligible for the study. Second, many have families and it is difficult for one or both parents to come to the clinical site for breakfast and dinner each weekday for three six-week dietary periods. Third, postmenopausal women without HRT account for only a small proportion of this population.

Various recruitment strategies were used such as advertising by posters, in newspapers and on television, and sending advertising fliers to churches, senior or retired communities, and to individuals between the ages of 45 to 65. We even agreed to feed a couple in order to recruit one of them who qualified to be a study participant while the other did

not. To overcome similar problems with recruiting persons in this age group, other clinical centers provide guest meal passes so that family members or friends may regularly join the study participants for meals.

Two studies that needed young females presented unique recruiting and retention situations. Participants with low iron status were required to observe the overloading effect of an iron supplement. Females, who generally have a lower iron status than males due to menstruation, were targeted. Therefore, participants were females, ages 19 to 47, with regular menstrual periods, in good general health and from all ethnic groups. Difficulties in recruiting occurred for several reasons in a college town. During the summer months the overall student population at the university diminishes greatly, presenting a problem by reducing the potential participant pool. Also, the prospect of strictly adhering to a controlled diet during the relaxing summer months provided another hurdle. The two-week break between diet periods was planned around the July 4 holiday and a summer arts festival to avoid further recruiting obstacles.

For this study and one that required females 18 to 22 years of age, weight concerns proved to be a barrier for recruiting. Many young women were trying to lose weight and did not want to maintain their current weight as required by the study protocols. In addition, women in this age group tend to be "fat phobic" and careful about their fat consumption. They were hesitant to consume diets that may have contained more fat than their usual diets. Therefore, it was important that the fats and oils were discreetly added to the study diets. This was accomplished by choosing meals such as turkey with dressing and mashed potatoes or stuffed flounder, which would readily accept the oils and fats without drawing attention.

Both recruitment and retention of participants were challenged in a study that compared whole-food diets with formula diets. For recruitment, potential participants sampled the formula diets. It was emphasized that the formula was all they would consume during two of the four diet periods. Once enrolled, participant retention became the paramount issue. One way of maintaining their participation in the study was to conduct a raffle during each diet period. Raffle items included tickets to a football game, a movie, and a Broadway show that came to campus. Another way was to prepare a portion of the daily formula as "ice cream." The "flavor of the day" was posted, and chocolate and strawberry flavors were always available. For the participants, this alleviated the boredom from having to always drink their meals. Many enjoyed telling their friends that they were on an "ice cream diet."

Providing specialized, prepackaged meals or foods to participants for daily consumption may make recruiting easier (i.e., more participants are willing to eat prepackaged foods at home, rather than having all meals served through a metabolic kitchen), yet challenges still abound as in other clinical nutrition studies. As one site of a year long, multicenter study testing the effect of a prepackaged meal plan on multiple CVD risk factors, 70 men and women with hyperlipidemia, hypertension, and/or Type 2 diabetes mellitus were recruited. Finding participants who met the entry criteria proved to be difficult in a small college town. Thus, the study was conducted simultaneously in a more urban location 90 miles away, where the university's medical school is located. Once all of the participants were recruited, problems arose with the weekly home delivery of the prepackaged, frozen meals. The food could not be left without a signature, which proved to be an obstacle. Participants sometimes received meals different than those they ordered, and they became bored with their limited food selection during the year-long study.

Food Products and Menu Planning

Cocoa powder is a rich source of antioxidants in the form of flavonoids. The Cocoa Study was conducted to evaluate the effects of a diet high in cocoa powder (22 g/day) and

dark chocolate (16 g/day) on LDL oxidative susceptibility and total antioxidant capacity of plasma. The study employed a two-period, crossover design. Using a randomized diet treatment assignment, participants were fed the cocoa powder/dark chocolate diet (CP/DC) and an Average American Diet (AAD, control) for four weeks each. The cocoa powder and dark chocolate were incorporated into only one experimental diet, making it impossible to employ a blinded experimental diet design. Planning a study of chocolate would appear to be easy, but the cocoa powder and menu development actually presented some challenges.

First, it was necessary to control for components present in the cocoa powder and dark chocolate to "isolate" the flavonoids for testing their contribution to a possible antioxidant benefit. To do this, the cocoa butter present in the dark chocolate was included in a similar amount in the AAD. Other components included caffeine, theobromine, and fiber, since cocoa powder and dark chocolate contain these in addition to the flavonoids. The caffeine was supplemented in the AAD with diet cola, and 431 mg/day of pure theobromine in a gel capsule was provided. The fiber was equilibrated with bran cereal. In addition, the diets were designed to be low in non-CP/DC flavonoids. Thus, foods that were limited or excluded included tea, coffee, wine, onions, apples, beans, soybeans, orange juice, and grape juice.

Second, there was a fair amount of cocoa powder in the experimental diet, so the participants were sometimes required to add cocoa powder (about 15 g) to their milk (or any other menu item). They also could add a non-caloric sweetener to the "chocolate milk" if they chose to do so. The resulting beverage was notably different from commercially available chocolate milk and that prepared from chocolate syrups. Nonetheless, it was a menu item that the participants found acceptable, albeit different from what they were accustomed to drinking, and they were willing to consume it throughout the study.

Third, the cocoa powder and dark chocolate were incorporated into the diets in different ways to avoid monotony. The daily allotment of cocoa powder was also baked into cookies, muffins, and brownies that would be served at meals. This assured that the cocoa powder and dark chocolate would be consumed throughout the day.

A soy study evaluated the effects of 31 g/day of an isoflavone-rich soy powder (equivalent to 25 g soy protein) on plasma lipids and lipoproteins and vascular reactivity in hypercholesterolemic but otherwise healthy male and female participants. All participants were first fed a Step-I (run-in) diet followed by either a Step-I diet plus soy protein or a Step-I diet with milk protein. Isoflavones are cleared rapidly from the plasma after ingestion, so soy-containing menu items were incorporated throughout the day in an attempt to achieve maximal effects. The barrier to overcome was incorporation of the soy or milk protein into baked products at an acceptable level without sacrificing the quality and acceptability of the product. Acknowledging the importance of this, we worked with a faculty member with expertise in food product design who prepared several great-tasting soy products for the study. With considerable product development effort it was possible to employ a blinded study design.

Similar barriers to flavor were overcome in a study that examined the effects of defatted rice bran on blood lipids and lipoproteins in moderately hypercholesterolemic men and women. Participants were randomly assigned to a reference diet with typical levels of dietary fiber (approximately 15 g/day) or one that contained defatted rice bran to increase dietary fiber to the recommended intake level (30 g/day). Foods were developed with the intention of incorporating the highest amount of defatted rice bran possible. The defatted rice bran had a nutty flavor, a somewhat grainy texture, and imparted a brown color to all the products to which it was added. After much experimentation, we found that one gram beyond a certain amount would yield an unacceptable food product. Because of the

color, food products that are expected to be brown, such as spice muffins and ginger cookies, were ideal for the addition of the defatted rice bran.

Folate fortification of foods in the U.S. created some obstacles in menu development for a study that examined the effects of a low-folate diet with milk (8 oz milk, 3 times/day) or with no milk (8 oz apple juice, 3 times/day) on folate absorption and blood homocysteine levels. For a low-folate diet, it was necessary to purchase foods from countries that did not fortify their food products. Pasta imported from Italy became a staple in these menus and was used for lunch salads and dinner items. Foods which are manufactured for individuals with celiac sprue are naturally low in folate because they are made with rice flour and corn starch rather than wheat flour. Some of these items included crackers, pretzels, and a delicious chocolate truffle brownie. The sources of these low-folate foods were obtained by searching shops that specialize in imported foods. Furthermore, information available on the Worldwide Web was immensely helpful to make these menus appealing. One limitation with the menus was that there was only a four-day rotation because of the lack of foods that are naturally low in folate. A short menu rotation may contribute to monotony and boredom, so encouraging adherence may require extra effort.

The Worldwide Web also proved to be useful in finding food items for other studies, such as one that manipulated the glycemic load of a low-fat, high-carbohydrate diet. The number of food items with a known glycemic index is relatively low, and therefore limited the food choices for the menus. Some of the foods are not routinely eaten in the U.S. For example, chana dal, a dried baby garbanzo bean, is a staple in Indian food and has a glycemic index of 8 compared to glucose at 100. Locating a source of the chana dal was made easy by searching various websites. It was used as the carbohydrate source for several meals for the low glycemic index menus.

Participant Adherence and Protocol Acceptability

High monounsaturated fatty acid (MUFA) diets have been studied extensively in the context of evaluating their effects on plasma lipids and lipoproteins. Previous studies have used mainly olive oil as the MUFA source, while other MUFA-rich food sources have not been evaluated. The peanut study was conducted to evaluate the effects of experimental diets high in peanuts and peanut products (e.g., peanut butter and peanut oil) that are rich MUFA sources, on lipid and lipoprotein risk factors for CVD. The greatest challenge with this study was its duration. Although there were five test diets, six 24-day diet periods were scheduled. This schedule necessitated that participants commit to the study for a period of approximately six months.

In a long-term study such as this, it is imperative that special efforts be made to sustain the commitment of the participants. The participants selected one diet period off to allow time for vacations, family activities, or celebrations. Despite participants' initial enthusiasm, some found it difficult to complete the study. Nonetheless, providing some scheduling flexibility did help with adherence. Incentives for the participants, such as t-shirts, coffee mugs, and movie tickets were also advantageous in maintaining long-term adherence. Interestingly, as the study progressed, it was increasingly challenging for the staff to maintain their enthusiasm as well. Theme parties without food (i.e., Halloween night, Thanksgiving celebration) helped participants and staff members maintain a positive attitude throughout the study.

Similarly, some flexibility was given in a nine-month study designed to test the hypothesis that replacement of a fat substitute for dietary fat would significantly decrease body fat relative to a 33% fat diet or a 25% reduced-fat diet. Testing days were scheduled at three-month intervals. Participants were given a three-day break from eating the study

meals for the Easter, July 4, and Labor Day holidays. In addition, they were allowed to take a total of seven "vacation" days within the six weeks immediately following the testing days. As for the previously described study, incentives were provided throughout the time period for encouragement.

A study designed to achieve weight maintenance after a weight loss phase presented interesting situations with participant adherence to and acceptability of the protocol. Participants who were moderately overweight were placed on one of two energy-restricted diets to achieve a weight loss of two pounds per week. Following a six-week weight loss period, they were fed the same diets, but with enough energy to maintain their weight. Despite using a metabolic cart to calculate energy needs, about 20% of the participants continued to lose weight during the weight maintenance phase. Because of this, periods longer than four weeks were necessary to establish a stable body weight.

In general, people enjoy participating in controlled weight loss studies, and consequently, adherence is ideal. For this study, the weight loss aspect generally was motivation enough for the participants, although weekly incentives were provided. The hardest part of the study was getting the participants to eat all of their food during the weight maintenance phase of the study. Most wanted to continue to lose weight and had to be encouraged to adhere to the study protocol.

Several people enrolled in the weight loss study had unrecognized eating disorders, making it difficult for them to comply with the protocol, where kcalories were restricted for a portion of the study and then increased for the remainder of the study. This problem pointed out the need for a screening questionnaire that would help identify people with eating disorders so that they would not be enrolled in the study.

In the following series of studies, each of which incorporated a particular food product, it is important that participants like the food, or at least tolerate it and be willing to eat large amounts or more "realistic" quantities daily. While efforts are made to incorporate the food product into a number of tasty products, it would be difficult for participants to adhere to the experiment diet if they dislike the particular food product or the foods that serve as a major delivery vehicle (i.e., milk for cocoa powder).

Studies were conducted using chocolate as a vehicle to assess the effects of stearic acid on plasma lipids, lipoproteins, and platelet function. Our approach was to first evaluate a large dose of the major fat in chocolate (i.e., cocoa butter) and subsequently assess a large dose of milk chocolate (i.e., 10 oz/day), as well as the fat mixture found in chocolate (cocoa butter and dairy butter — 4:1). Our rationale for using large doses was first to determine if there was any effect of chocolate on the study endpoints of interest. Then, more realistic intakes that reflect usual consumption practices would be examined. Initially we needed to assess whether a large dose of chocolate would cause any significant adverse side effects, such as gastrointestinal distress, headaches, or skin problems. Thus, a small pilot study evaluated six participants who were fed large amounts of chocolate (10 oz/day) for approximately one month. Having seen no adverse symptoms, two studies were conducted to evaluate the effects of cocoa butter and milk chocolate on plasma lipids, lipoproteins, and platelet function.

A subsequent study evaluated the incorporation of more realistic amounts of milk chocolate (1 candy bar/day) to a Step-I diet. Moreover, we wanted to mimic real-world chocolate consumption practices. Since a peak time for consumption of chocolate is in the mid-afternoon, participants were required to eat their candy bar at that time. They were required to return the wrapper to the study staff at the dinner meal for adherence assessment and for communicating an important message that participants needed to consume the chocolate bar as well as follow all aspects of the experimental protocol.

Conclusions

Well-controlled clinical nutrition studies have been invaluable in generating results that have advanced our understanding of diet–disease relationships and have provided information that has formed the basis for making nutrient recommendations. A number of recent publications have comprehensively described the process of how to conduct well-controlled feeding studies in humans that employ quality control standards at each step (Table 1). Inherent to conducting these studies is the associated myriad of challenges, not discussed in depth in the literature, that need to be resolved in order to conduct successful studies. This section has presented an overview of these, which relate globally to subject recruitment and compliance/adherence, and maintenance of subject and staff enthusiasm during the feeding study. In addition, we have provided a "forms library" that provides specific forms or a description for virtually every aspect involved in carrying out these studies. Specific examples from the many clinical studies that we have conducted are presented herein, and the approaches we implemented to resolve these problems are described. This information will help readers to overcome the challenges that can arise during the conduct of a well-controlled feeding study. Avoiding inherent pitfalls in feeding studies will facilitate conducting high quality clinical nutrition research efficiently and effectively, and therefore help advance the field.