CHAPTER 14

PLANNING AND PRODUCING FORMULA DIETS

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DECIDING TO USE A FORMULA DIET

Formula diets are well suited for use in strictly controlled studies. Of all the types of research diets, they provide the most accurate and constant intake of calories, macronutrients, vitamins, and minerals. These diets can comprise liquids (the most common option), solid foods, or both. Because formula diets are based on a limited number of ingredients, variability in nutrient content is minimal, and each ingredient can be analyzed for nutrient content to ensure that the composition of the diet is known with high accuracy. Formulas are convenient to use and can be adapted for many study designs. The dietary treatments are easily masked (or ‘‘blinded’’) and the recipes can be adapted to deliver numerous combinations of nutrients at markedly different dose levels.

Another advantage of formula diets is that they are economical. They maximize the number of participants and studies that can be accommodated with a minimum of work area and staff, because labor, kitchen space, and food-handling costs are low. In fact, once the recipe is calculated, the formula diet can be prepared in advance in bulk, frozen, and served as needed; some formulas can be stored at –15°C for years without adversely affecting nutrient content (1). This means that storage space for the ingredients and the prepared formula is minimal. For example, a 40-L batch of liquid formula, which can be prepared in one hour by two foodservice workers, can supply 50,000 kcal. Costs associated with food composition analysis also are relatively low, because only a few component ingredients and the finished formula must be analyzed, as opposed to daily menus of different foods.

The main disadvantage of liquid formula diets is that most people prefer to eat solid foods, and the participants typically find that the regimen becomes increasingly monotonous over time. The lack of variety, lack of chewing, and the change in taste and texture from usual foods may

Source: "Well-Controlled Diet Studies in Humans, A Practical Guide to Design and Management", American Dietetic Association, © 1999.

contribute to participant dissatisfaction and lack of compliance. Formula diets also lack natural fiber, and tend to be low in starch and high in sugars. As a result, consumption of formula diets may change bowel habits, causing diarrhea, constipation, or other types of gastrointestinal discomfort such as flatulence, belching, nausea, and distention. These problems may resolve after a few days but often make it difficult to recruit and retain participants. Investigators considering use of formula diets must also decide whether these gastrointestinal side effects, or treatments for them, may ultimately influence study endpoints and thus confound interpretations of data. Concerns have been expressed that liquid-formula diets may differ from solid-food diets with respect to absorption and metabolism of nutrients (2, 3); more research is needed to clarify this poorly understood phenomenon.

Liquid formulas can be used to provide nutrients for protocols as short as 1 day (for example, postprandial fat load studies). The typical time span tolerated by participants is 3 to 6 months, but some participants have been maintained on liquid formula diets for several years (4).

WORKING WITH PARTICIPANTS

Screening

The success of a study starts with careful screening of potential participants. Highly motivated individuals are needed to undertake the rigors of formula diet protocols. For outpatient studies, motivation and support from family and friends should be evaluated and reinforced at the time of screening. It is helpful to have potential participants consume the entire volume of a single serving of a formula diet prior to committing to the study. This allows individuals to judge their tolerance of the formula and to evaluate their ability to adhere to the requirements of the study. If individuals are aware of the disadvantages of formula diets prior to the study, they are less likely to encounter problems later on.

A key step in the screening process is to review the formula ingredients with potential participants in order to eliminate individuals with allergies or intolerances to common ingredients, such as milk or egg protein.

Instructing the Participant

Water Intake

Participants on a liquid formula diet might be tempted to restrict their water intake, because they tend to have increased urinary output; they may wish to avoid carrying extra urine collection bottles. Nevertheless, participants should be advised to drink adequate amounts of water, particularly in hot weather, and to maintain adequate fluid balance when exercising. Calorie-free beverages may be allowed if the ingredients do not interfere with the study. It is best to provide water of known mineral composition (or deionized or distilled water) for studies where mineral intake is of interest. (See Sources of Nutrients; also see Chapter 15, ‘‘Meeting Requirements for Fluids.’’)

Free Foods

If they can be allowed, noncaloric items such as sugar-free chewing gum, bouillon, and herbal tea add variety and enhance participant compliance. Gum provides the chewing action that the participants may miss and also helps rid the mouth of aftertastes. Bouillon can be used as a source of sodium and can provide some warmth and saltiness to counter the monotony of the formula diet. Herbal teas offer yet more variety in flavor and may have a soothing and relaxing effect. The ingredients of any such teas must be reviewed to make sure they do not contain active ingredients that might interfere with study outcomes.

Eating Techniques

To ensure accurate intake of formula diets, participants are instructed on correct eating techniques. Participants should drink slowly without gulping to eliminate gagging and swallowing air, which may cause burping, nausea, and vomiting. Glasses or containers should be rinsed with water (distilled water for micronutrient studies), which also is drunk. Drinking the rinse ensures complete consumption of nutrients that otherwise might adhere to container surfaces. No visible formula residue should be left in the container.

Presenting the Diet

Volume and Number of Daily Meals

The volume of individual liquid formula meals depends on the tolerance of the study participants. As a general guideline, no more that 400 mL to 450 mL per serving is given to elderly participants and no more than 600 mL to 700 mL to young healthy adults. Individualized guidelines should be established for pediatric and other specialized populations.

Multiple isocaloric meals are recommended. The number depends on the total volume of the diet; the age and other characteristics of the study population; and the aims of the study. A regimen of four to five meals per day is acceptable to most participants. However, a schedule of three meals per day can be used if necessary by supplying part of the daily total calories as formulated solid food. (The use of solid food formulations is discussed later.)

Tray and Table Set-up

As with any meal, the appeal of formula diets can be enhanced through effective presentation. Colorful napkins or place mats, attractive glassware with unusual shapes, and appropriate garnishes (eg, paper flowers, twist of lemon rind if allowed) can add flair and make a positive impression on participants.

Compliance

As mentioned earlier, subjects prefer conventional food diets and this is a major factor affecting compliance with formula diets. Subjects consuming liquid formulas on an outpatient basis are more likely to have compliance problems, because they are routinely exposed to the temptations of more desirable foods. Even within the confines of a metabolic ward, participants have been known to drop out from a formula protocol when they smelled the aroma of food being prepared or served nearby for another study. Objective measures of compliance can be obtained by measuring urine osmolarity or by examining urine recovery of paraaminobenzoic acid added to the formula (5, 6). (Also see Chapter 24, ''Biological Sample Collection and Biological Markers of Dietary Compliance.'')

Designing Formula Diets

Planning the Production Mode

Liquid and Solid Forms

Liquid-formula diets are sometimes used in conjunction with specially formulated solid foods such as puddings (Exhibit 14-1), protein-free cookies (Exhibit 14-2), and unit muffins (see Chapter 18, ''Documentation, Record Keeping, and Recipes''). These items offer a variety of textures, ease boredom, and allow for mastication of food while maintaining constant levels of nutrients. Solid foods are particularly useful in incorporating ingredients such as saturated fats, fiber, and certain complex carbohydrates, which are difficult to keep in homogeneous solutions.

Site-produced and Commercial Formulas

Formula diets usually are made in the research kitchen. The major advantages of on-site preparation of formulas are: (1) complete control of ingredients and (2) precise tailoring of the formula to the specifications of the study design. The needs of many studies, however, can be met by using commercial preparations. Among the numerous advantages of using commercial formulas, the first is financial: labor costs are minimal. Other advantages of using commercially prepared formulas include: rigorous sanitation standards at the manufacturing plant, manufacturer-solved issues of palatability and acceptability, and consistent texture and taste.

Storage requirements also may be simplified; already mixed dry powdered formulas that only need reconstitution with water or milk will require less storage space than their constituent dry basic ingredients. Liquid commercial formulas, of course, already contain the water component, a feature that adds both bulk and weight.

Purchased formulas can be considered if they provide a reliable nutrient composition in close accord with study goals. It is important in such cases to purchase single lots large enough for all study needs; to verify ingredient specifications with the manufacturer; to check that no recent processing changes in either content or handling have occurred; and to verify nutrient content by chemical analysis because it may vary somewhat from the labeling information. If the commercial formula is used as a base, rather than as a complete diet, pilot tests should be done to make sure that any added ingredients are fully compatible with the formulation. For example, a change in the type of fiber might alter the solubility of added mineral salts.

Meeting Nutrient Requirements and Study Goals

Macronutrients

There are two ways to tailor formula diets to meet macronutrient requirements: a single-formula method and an individualized-formula method.

Single-formula Method

When a constant nutrient composition is to be provided to all participants for the entire length of the study, one formula calculation can be used for all participants. Energy requirements are met by varying the amount of formula given. This method is commonly used to maintain energy homeostasis and to conduct baseline studies. The diet is first calculated to meet the protein goals of the diet protocol; protein is factored in first because many protein sources contain small amounts of fat (Table 14-1). The remainder of the calories is derived from carbohydrate and fat in amounts required to fulfill the study requirements.

Example:

- Liquid Formula B contains 15% protein, 40% fat, and 45% carbohydrate. Each 100 g of Formula B provides 4.7 g of protein, 5.6 g of fat, 14.1 g of carbohydrate, and 125 kcal (1.25 kcal/g). (See Exhibit 14-3.)
- Participant W, who requires 3,000 kcal daily, will receive 2,400 g of Formula B (3,000 kcal ÷ 1.25 kcal per g); Participant X, who needs 2,800 kcal a day, will receive 2,240 g of Formula B. (See Exhibit 14-3.)

Individualized-formula Method

When a specific level of a macronutrient is provided per kilogram of body weight, an individual formula calculation and recipe is needed for each participant. Other macronutrients are then distributed as a percent of the remaining calories.

Example:

- Liquid Formula C will be used for a dietary protocol requiring an isocaloric diet that provides 1.5 g protein per kilogram of body weight, 40% nonnitrogenous calories from fat, and 60% nonnitrogenous calories from carbohydrate. (See Exhibit 14-4.)
- Individually tailored recipe calculations are needed to make up Liquid Formula C for each participant. (See Exhibit 14-4.)

EXHIBIT 14-1

Solid Formulations: Puddings

BACKGROUND

- Puddings can be useful vehicles for providing complex carbohydrates or saturated fats that would not otherwise suspend in a liquid formula.
- Puddings should be used within three days of preparation. The mixtures do not freeze well and the texture is lost upon thawing.
- There are two ways to add saturated or hydrogenated fats to the pudding mix: (1) melt the fat and blend it into the pudding before the mix is brought to a boil; (2) finish cooking the pudding, remove it from the range, and blend in the solid fat; the fat will then be melted by the heat of the pudding.
- The Basic Pudding recipe is meant to provide a general approach to formulation. The ratio of starch, sugar, protein, and fat varies with each research diet protocol, so it may be necessary to experiment with the recipe to identify the best amount of liquid and the best order in which to add the ingredients.
- The protein-free puddings are useful for amino acid studies (such as described in Exhibit 14-5).

RECIPES

Basic Pudding

Ingredients	Quantities
Liquid (water or milk)	2,000 mL
Sugar	200 g–250 g
Protein source (optional)[1]	150 g–200 g
Starch	100 g–150 g
Fat	100 g–150 g
Flavoring extract	20 mL–30 mL

[1]The protein source can be egg (whole, whites, or yolks), milk powder, soy protein, etc.

Preparation Procedure

1. Mix liquid and half of the sugar to make a syrup. Bring to a boil, then lower the heat.
2. Beat eggs (or other moist protein source) until smooth. Add slowly to the hot sugar syrup, beating thoroughly after each addition. (It is helpful to first stir a small amount of the hot sugar syrup into the egg mixture. This will raise the temperature of the eggs and will prevent coagulation when they are added back into the syrup.)
3. Mix the remaining sugar (and any dry protein source) with the starch. Sift slowly into the liquid, mixing well.
4. Mix until smooth. Remove from heat.
5. Blend in fat and flavoring.
6. Pour appropriate quantity into serving dishes and cool.

Protein-free Wheat Starch Pudding (Lemon Flavor)

Ingredients	Quantities
Water	2,240 g
White sugar	1,054 g
Lemon juice	660 g
Wheat starch	312.4 g
Yellow food coloring	14 drops

Preparation Procedure

1. Combine all ingredients.
2. Cook over medium heat to a slow boil, stirring constantly.
3. Weigh out into serving dishes. Cool, cover, and refrigerate.
4. Yield: 27 servings, each weighing 152 g.

(*continued*)

EXHIBIT 14-1

Continued

Nutrient Content[1]	Amount
Protein	0.1 g
Fat	3.2 g
Carbohydrate	46.0 g
Energy	207 kcal

[1]For one 152-g serving.

Protein-free Cornstarch Pudding (Fruit Flavor)

Ingredients	Quantities
Water	2,240 g
Corn oil	240 g
White sugar	680 g
Cornstarch	140 g
Koolaid powder, unsweetened	24 g
Salt	14 g

Preparation Procedure

1. Weigh water and corn oil into separate bowls.
2. Weigh dry ingredients into large mixing bowl and transfer to a large (2-qt) saucepan.
3. Rinse the mixing bowl with some of the weighed water. Add to the saucepan, together with the rest of the water. Mix thoroughly and add the oil.
4. Cook over medium-high heat until thickened, stirring constantly.
5. Remove the mixture from the heat when it starts to bubble. Let it set for 5 minutes and stir again. (This step will help to keep the oil in suspension.)
6. Weigh out into serving dishes, stirring occasionally. Cool, cover, and refrigerate.
7. Yield: 19 servings, each weighing 160 g.

Nutrient Content[1]	Amount
Protein	0.1 g
Fat	12.2 g
Carbohydrate	40.1 g
Energy	266 kcal

[1]For one 160-g serving.

Vitamins and Minerals

The purified ingredients that are used to make formulas generally have a low vitamin and mineral content. The quantities of vitamins and minerals that are needed should first be calculated; the content of the formula can then be assayed directly or compared with predetermined nutritional standards, and the micronutrients supplied in the necessary quantities to make up any differences. There are two main ways to do this: (1) adding the vitamins and/or minerals directly to the formula or (2) giving the participant a pill or other form of supplement. Some pharmaceutical companies can produce customized vitamin and mineral preparations, such as a niacin-free vitamin. Research pharmacies at some facilities also may accommodate these special requests.

Micronutrients for formula diets should have high nutrient bioavailability. For example, calcium citrate might be chosen over calcium carbonate, which is not as well absorbed. It is also important to check the stability and solubility of any supplement. Some mineral salts may not dissolve readily; they may settle out if the formula is not consumed promptly after preparation. To avoid this problem, the formula should be stirred or shaken before serving; any residue adhering to stirring rods or spatulas should be consumed.

Fiber

Most formula diets are low in fiber; therefore, they have the potential to cause gastrointestinal discomfort. Fiber supple-

EXHIBIT 14-2

Solid and Liquid Formulations for an Egg-protein Diet: Protein-free Cookies and Liquid Formula A

BACKGROUND

- This diet uses egg protein as the main source of nitrogen.
- Part of the energy requirement is supplied by formulated solid food (protein-free cookies).
- The study protocol specifies the protein level as 1 g/kg body weight and the nonnitrogenous calorie ratio as 40% fat, 60% carbohydrate.
- The reference participant weighs 70 kg and has an estimated energy requirement of 3,150 kcal.
- The daily diet consists of three isocaloric meals, each containing protein-free cookies (2 cookies, uncooked total weight 90 g) and liquid formula A (599 g).

CALCULATIONS FOR DAILY DIET

Ingredient/Food	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Protein-free cookies (n = 6)	270[1]	1,195	1.1	60.8	164.7
Liquid Formula A[2]					
Egg white solids	72	273	60.0	0.0	3.2
Whole egg solids	20	118	10.0	8.2	0.8
Beet sugar	210	808	0.0	0.0	209.0
Orange sherbet[3]	195	273	2.1	3.9	59.3
Safflower oil	55	484	0.0	54.8	0.0
Koolaid® beverage, unsweetened (reconstituted)	1,245	0	0.0	0.0	0.0
Subtotal	1,797	1,956	72.1	66.9	272.3
Daily total (Cookies + Liquid Formula A)	2,067	3,151	73.2	127.7	437.0

[1]This refers to weight of unbaked (raw) cookie dough.
[2]The preparation procedure for Liquid Formula A is the same as for Liquid Formula C in Exhibit 14-4.
[3]Orange sherbet can be used to improve the flavor of liquid formulas. The small amount of protein in the sherbet usually will not affect study outcomes.

RECIPE

Protein-free Cookies

Ingredients	Quantities (g/batch)
Wheat starch	1,000
Beet sugar	300
Butter	454
Safflower oil	55
Baking powder	5.5
Salt	6
Water	80
Vanilla flavoring	10

Nutrient Content	Amount[1]
Energy	199.1 kcal
Protein	0.2 g
Fat	10.1 g
Carbohydrate	27.4 g

[1]Dough weight = 45 g raw, 41 g cooked.

Preparation Procedure

1. Mix sugar, butter, and oil together until smooth.
2. Add all dry ingredients and mix well.
3. Weigh raw dough for each cookie at 45 g.
4. Bake at 400°F for 15 minutes.
5. The cooked weight of each cookie averages 41 g.

TABLE 14-1

Selected Whey Protein Products

Product	Trade Name	Supplier	Nutritional Characteristics: Energy (kcal/100 g)	Protein (g/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)
Whey protein, concentrated	Pro Mod	Ross Laboratories Columbus, Ohio	424	75	9.1	10.2
Whey protein, caseinate	Propac	Sherwood Medical St Louis, Mo	400	76.8	8.0	5.2
Whey protein, ultra-filtered	Promix	Corpak, Inc Wheeling, Ill	400	75–78	6–8	8–10
Lactalbumin	Alatal 812	New Zealand Milk Products Petaluma, Calif	397	90.5	3.5	0.8

EXHIBIT 14-3

Single-formula Diet Using Liquid Formula B: Recipe and Preparation Procedures

BACKGROUND

- This diet provides all the participants with the same proportional distribution of energy from macronutrients: 15% protein, 40% fat, and 45% carbohydrate.
- Liquid formula B provides the entire daily diet.
- The quantity of formula is adjusted to meet the calorie requirements of individual participants.

CALCULATIONS FOR DAILY DIET

Subject	Energy Requirement (kcal/day)	Formula Portion[1,2] (g/day)	Macronutrient Distribution (% Energy)			Nutrient Intake (g/day)		
			Protein	Fat	Carbohydrate	Protein	Fat	Carbohydrate
Participant W	3,000	2,400	15	40	45	112.8	134.4	338.4
Participant X	2,800	2,240	15	40	45	105.3	125.4	315.8

[1]Formula Portion (g/day) = Energy Requirement (kcal/day) ÷ Energy density of formula (1.25 kcal/g).
[2]Energy density of Liquid Formula B: 1.25 kcal/g.

RECIPE

Liquid Formula B

Ingredients	Quantities[1]
Promix[2]	2.5
Corn oil	2.1
Polycose[3]	5.8
Water	29.6

[1]Weight in kg/batch.
[2]Promix (whey protein) (Corpac, Wheeling, Ill).
[3]Polycose (hydrolyzed cornstarch) (Ross, Columbus, Ohio).

Nutrient Content[1]	Amount
Energy	125 kcal
Protein	4.7 g
Fat	5.6 g
Carbohydrates	14.1 g

[1]Content per 100 g.

(*continued*)

EXHIBIT 14-3

Continued

Preparation Procedure (Batch Production)

1. Employee complies with hygiene measures (uses hairnet, washes hands, wears gloves).
2. Zero scale with bowls for dry ingredients and fat.
3. Weigh Polycose and Promix into one bowl.
4. Weigh corn oil into other bowl.
5. Remove these ingredients from scale and zero scale with a large bowl.
6. Weigh water into the large bowl.
7. Stir the dry ingredients with slotted spoon to blend Polycose and Promix. (Milk protein alone becomes lumpy when added to water.)
8. Pour water into the homogenizer; turn it on to obtain vortex in water.
9. Gradually blend dry ingredients into water.
10. Add corn oil. Turn off homogenizer. Rinse bowl three times with formula from the homogenizer to incorporate any residual oil into the formula.
11. Stir formula often with wire whip during mixing phase, after homogenization and before putting into quart-size storage containers.
12. Rapidly decant formula into quart containers (Conocups Tucker, Harrison, NJ), leaving a 2-in space on the top for expansion upon freezing. If producing more than one batch, refrigerate one batch while making the second.
13. To speed the cooling of formula to a safe temperature of 40°F or below, do not close the container. Place open containers on the coldest part of the freezer (where the circulating cold air originates) in order to enhance the cooling process.
14. When the formula temperature reaches 40°F (5°C), close the containers, clip them, and label with the number of the formula, the batch number, and the date made.
15. Freeze immediately.

ments can be added to a formula diet to promote more frequent bowel movements and to soften stools. Bulking agents should be carefully evaluated, however, for their potential effects on the gastrointestinal tract. This is especially critical for studies of nutrient absorption and mineral bioavailability.

Microcrystalline cellulose powder can be used as a source of fiber. Because of its thickening effect, it should be added to the formula at the time of service; it also can be offered as an additional supplement at meal time. Most participants find 20 g per day to be acceptable, administered either as a single 20-g dose or as two 10-g doses. *Alpha-cellulose* can also be given in amounts of 8 g to 10 g per day to increase stool bulk. *Methyl cellulose,* which is used less often, is more potent; it should not be used unless other sources have been evaluated and rejected. Typical doses of methyl cellulose are comparatively small (4 g per day).

Solute Load

Fluid and electrolyte homeostasis in living systems is maintained by the movement of water toward physiological compartments having higher concentrations of dissolved particles. Under free-living conditions with self-selected diets, healthy individuals will regulate their water balance through a variety of behavioral mechanisms that include the sensation of thirst, the drinking of water and other fluids, and the partially subconscious preference for salted or unsalted foods.

In a formula diet study, it is particularly important to evaluate and monitor the solute load because the protocol may constrain these mechanisms in several ways:

- The protocol may specify that all fluid be provided through the formula, rather than through *ad libitum* water consumption. This requirement deprives the participant of one of the most important physiological mechanisms for self-regulation of fluid balance.
- Formula diets often are made from highly purified ingredients, which may have a very low content of electrolytes in comparison with conventional foods.
- Unlike participants who are enrolled in many research protocols that use conventional foods, subjects who are enrolled in formula studies may not be permitted to add salt to their food, or they may consider it unappealing to add salt to a relatively sweet milkshake-like formula.

The solute content of a formula diet thus should be evaluated from several vantage points: first, the *enteric solute load,* which will influence gastrointestinal symptoms upon ingestion; second, the *renal solute load;* and finally, the *electrolyte content* of the entire diet.

The enteric solute load of a prepared formula is measured by its osmolality. *Osmolality* is a property of solutions that represents the number of dissolved particles per unit mass of solvent (ie, per kg water). One Osmol is defined as the number of dissolved particles required to depress the

EXHIBIT 14-4

Individualized Formula Diets Using Liquid Formula C, Recipe and Preparation Procedures

DIET DESIGNS FOR TWO REFERENCE PARTICIPANTS Y AND Z

BACKGROUND

- This formula diet uses egg protein as the main source of nitrogen.
- The study protocol defines the protein level as 1.5 g/kg body weight and the nonnitrogenous calorie ratio as 40% fat, 60% carbohydrate.

Subject	Body Weight (BW) (kg)	Energy Requirement (ER)[1] (kcal/day)	Macronutrient Requirements (g/day)			Energy Distribution (% kcal)		
			Protein	Fat	Carbohydrate	Protein	Fat	Carbohydrate
Participant Y	60	2,400	90	90.7	306	15	34	51
Participant Z	70	3,150	105	121	410	13	35	52

[1]Each participant's energy requirement is estimated using standard algorithms. (See Chapter 17, ''Energy Needs and Weight Maintenance.'').
[2]Protein requirement (g/day) = 1.5 g/kg BW × BW (kg).
[3]Fat requirement (g/day) = (40% of nonnitrogenous calories)* ÷ 9 kcal/g fat.
 *Nonnitrogenous calories (kcal/day) = ER (kcal/day) − (4 kcal/g protein × protein required (g/day)).
[4]Carbohydrate requirement (g/day) = (60% of nonnitrogenous calories) ÷ 4 kcal/g carbohydrate.

CALCULATIONS OF INDIVIDUALIZED FORMULA FOR PARTICIPANT Z

Ingredient/Food	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Egg white solids	105	398	87.5	0	4.7
Whole egg solids	30	176	15	12.4	1.2
Beet sugar	306	1,173	0	0	303.5
Safflower oil	102	900	0	101.8	0
Orange sherbet[1]	361	505	3.9	7.3	109.8
Koolaid beverage, unsweetened (reconstituted)	1,400	0	0	0	0
TOTAL	2,304	3,152	106.4	121.5	419.1

[1]Orange sherbet can be used to improve the flavor of liquid formulas. The small amount of protein in the sherbet will not affect study outcomes.
[2]The daily diet consists of four isocaloric meals, each containing 576 g of Liquid Formula C.

RECIPE

Liquid Formula C

Ingredients	Quantities (g/batch)[1]
Egg white solids	115.5
Whole egg solids	33
Beet sugar	336.6
Orange sherbet	397
Safflower oil	112
Unsweetened Koolaid® (reconstituted)	1,540

[1]Quantities are 110% by weight of each ingredient from the formula calculation in order to allow for losses such as those during transfer from blender to serving containers. Weight is in grams.

Preparation Procedure

1. Make a dry mix of the first three ingredients in a bowl.
2. Combine the remaining items in a blender with 1-gallon capacity. Mix for 3 minutes.
3. Add dry ingredients to the blender cup and mix for another 3 minutes.
4. Scrape down the lid, sides, and blades of the blender. Mix again for several minutes to ensure homogeneity of the formula.
5. Portion four servings of formula, each weighing 576 g, into four labeled serving containers. Cover containers with lids and refrigerate until serving time.

freezing point of one liter of water by 1.86°C (7). Osmolality (mOsmoles per kg water) is a *mass basis* expression of concentration, whereas *osmolarity* (mOsmoles per L solution) is a *volume basis* expression of concentration (6). Most dilute solutions have similar values for osmolality and osmolarity.

The macronutrient energy sources that suspend rather than dissolve in the formula (ie, lipids, complex carbohydrates, and proteins) have little effect on the solute load. Rather the most important contributors to the osmolality of the formula are the simple sugars, amino acids, and electrolyte salts (primarily sodium, potassium, chloride, and to a lesser extent magnesium, calcium, phosphate, and sulphate). The osmolality of formula diets can be estimated from algorithms that account for the formula recipe and the measured osmolality of each component ingredient (8, 9). Another approach, often used in the food industry, is to analyze a sample of the formula (and/or the component ingredients) in the laboratory with a vapor pressure or freezing point osmometer, making appropriate adjustments for viscosity, turbidity, and other types of interference from suspended solids (V Mustad, personal communication).

The osmolality of the formula diet should always fall within a certain range, or there will be major implications for safety and acceptability. (The constancy of the osmolality measurements over time can also serve as a quality control measure for formula diet production.) Formulas are considered *iso-osmolar* if they have a solute concentration similar to that of plasma and other physiological fluids (ie, approximately 275 mOsm/kg to 300 mOsm/kg).

Another simple and useful parameter for evaluating the concentration of the formula is the *energy density,* which reflects energy intake per unit of fluid or water. The usual daily total water allowance for mixed diets is approximately 1.5 mL/kcal (10). This value corresponds to an energy density of approximately 1.25 kcal/g formula (under the assumption that water comprises three-fourths of the weight of most formulas); additional fluids may be needed if this level is exceeded. The formulas used for enteral nutrition support, which resemble the formulas used for research diets, have a typical energy density of 0.5 kcal/mL to 2 kcal/mL and range in osmolality from a low of 120 mOsm/kg for polymeric mixtures (with intact protein, starch, and triglycerides) to a high of 650 for monomeric mixtures (with hydrolyzed starch, amino acids, and peptides) (11).

Dehydration and unpleasant gastrointestinal symptoms are the most serious consequences of imbalanced enteral solute loads. The ingestion of a hyperosmolar formula will draw water into the lumen of the gastrointestinal tract; this can cause nausea, rapid transit time, and diarrhea (11). Longer-term ingestion of such a formula may cause dehydration by forcing the kidneys to dilute the solutes in additional volumes of urine. Formulas that have a high content of salt, simple sugars, or amino acids often are hyperosmolar. To avoid this problem, the formula can be diluted or reformulated (for example, by using starch instead of sugar as a carbohydrate source); if the protocol allows, participants should be encouraged to drink water or other noncaloric beverages. (See Chapter 15, ''Meeting Requirements for Fluids.'')

On the other hand, the research diet must provide electrolyte minerals (notably sodium, potassium, and chloride) in sufficient quantities to prevent deficiency (10), unless this is a planned aspect of the protocol. Long-term intake of a hypo-osmolar diet may result in excessive urine output, reduced plasma electrolytes, and other electrolyte imbalances (12). It may be necessary to supplement the diet with electrolyte salts in the form of pills or powder blends.

The renal solute load of the diet can be predicted from the electrolyte content and from nitrogen derived from all dietary sources, whether ingested as intact protein (such as casein) or as individual amino acids; carbohydrates do not enter into this calculation. It also is relatively simple to measure urinary osmolality as a way of checking renal solute load and risk of dehydration. Some researchers consider that a comparison between predicted and observed urinary osmolality can provide an objective measure of dietary compliance (6). (Also see Chapter 24, ''Biological Sample Collection and Biological Markers of Dietary Compliance.'')

Carbon-13 (^{13}C) Content

Stable isotopes are often used to trace the transformation and transport of biological compounds during the various steps of metabolic processing. (See Chapter 16, ''Compartmental Modeling and Balance Studies.'') Carbon is commonly chosen as the tracer element because of its preeminent role in the chemistry of organic molecules. About 99% of naturally occurring carbon comprises carbon-12 (^{12}C); other isotopes, including the stable isotope carbon-13 (^{13}C), are generally rare. Certain plants such as sugarcane and corn, however, tend to concentrate ^{13}C, and food products made from them are thus also enriched in ^{13}C. These include cane sugar, molasses, and all corn products (eg, cornstarch, corn syrup, and corn oil). Diets providing such foods can confound the results of studies using ^{13}C-labeled tracers because of alterations in the isotopic enrichment of metabolic products (such as expired CO_2 during fasting and feeding) (13). The research diet can be rendered ^{13}C-neutral by avoiding the use of any corn and sugarcane products.

Verifying Composition

Nutrient data for individual formula ingredients are readily available, and this facilitates calculating the formula prescription. However, for more accurate formulation of diets, laboratory analysis of selected ingredients should be performed prior to recipe calculation. For example, the fatty acid composition of some oils varies substantially, so in studies of fatty acids, it may be important to assess the profile of each fat and oil prior to use. Chemical verification after formulation is also recommended. (See Chapter 22, ''Validating Diet Composition by Chemical Analysis.'')

Sources of Nutrients

This section describes sources of *macronutrients* (water, protein, carbohydrates, and lipids). Sources of *micronutrients* are discussed in Meeting Nutrient Requirements and Study Goals. The food source for each nutrient is selected in accordance with the research question, the extent of control desired, and the physical and nutritional characteristics required. For example, the nutrient content of some food products varies with season (vitamin A in butter) or region (trace mineral content of wheat flour because of the wheat's growing area). Such foods will not provide consistency throughout the study and are not appropriate for certain protocols.

The supplier or manufacturer of each food product also must be carefully considered, because even purified sources of particular nutrients (such as protein) will often contain small amounts of other nutrients as well (such as fat); the levels of such "extraneous" nutrients will vary with the raw ingredients and processing method. Comprehensive listings are available that can simplify the task of choosing specialty food suppliers whose products meet the needs of the protocol (14).

Water

The decision to use tap or distilled water in liquid formula diets depends on study goals and outcome variables to be measured. Some investigators, particularly those who study minerals, use deionized water in formula and all other beverages. In other studies the source of the water is not particularly important, but investigators may prefer to keep it consistent with the source used in their prior studies to facilitate comparisons. (Also see Chapter 15, "Meeting Requirements for Fluids.")

Protein

Because of their high biological value and palatability, egg and milk are the most frequently used sources of protein in formula diets. Soy protein products and pure amino acids are less palatable and therefore are not as suitable for general-purpose formulas. Soy products and pure amino acids are more commonly chosen for protocols that evaluate their specialized properties or for situations in which egg or milk cannot be used.

Egg Protein

Egg white solids, which are usually used in the form of sprayed dried egg white, provide high-quality protein with a low mineral content. The amount of fat and carbohydrate in egg white solids is also small compared to other protein sources. Using egg white as the only source of protein, however, can have drawbacks: the formula tends to have an undesirable sulfur taste and a very foamy texture. To prevent the formation of lumps, the powder should be mixed first with other dry ingredients before water is added.

Whole egg solids supply a high-quality protein but also contain fats, cholesterol, vitamins, minerals, and trace amounts of carbohydrates. Unless strict control of lipids and vitamins is specified, a combination of egg white and whole egg solids is advised as the protein source for most formulas. Formulas that use whole egg as the sole protein source may have unacceptably high cholesterol content; reduction in the total cholesterol content can be achieved by replacing part of the whole egg with egg whites. Combining whole egg solids with egg white solids also can improve the taste and texture of the formula over those containing egg white alone (see Liquid Formulas A and C in Exhibits 14-2 and 14-4). The lecithin in the egg yolk serves as an emulsifier and promotes stability of the formula.

Egg white solids require no refrigeration and can be kept indefinitely at room temperature. Whole egg solids need no refrigeration for periods of up to 4 weeks if they are kept at room temperature (for example, on a cool dry shelf). If held for longer, storage below 50°F is recommended. Egg products suitable for formula diets may be obtained from Henningsen Foods Inc, White Plains, NY; Oskaloosa Food Products Corporation, Oskaloosa, Iowa; and Siegel Egg Products, Boston, Mass (14).

Milk Protein

Milk protein is available as processed caseins, whey proteins, and milk solids.

Caseins are the main proteins of milk. They are used principally as casein salts, of which sodium and calcium caseinates are the most common. In addition to providing a source of protein, caseinates function as emulsifiers and water binders (15). Because casein contains only trace amounts of water-soluble vitamins, it is preferable to egg solids in diets that are designed to be devoid of water-soluble vitamins. Caseinates contain fat and carbohydrate (lactose); amounts of these components must be evaluated in the context of specific studies. These proteins are hygroscopic and can absorb odors; temperatures below 25°C, relative humidities below 65%, and an odor-free environment are thus all essential to extend storage life. Casein products may be obtained from New Zealand Milk Products, Inc, Santa Rosa, Calif (14).

Whey proteins are obtained from skim milk after separation of casein during cheese production. Whey proteins have low water-binding capacities, permitting high protein concentrations without excessive viscosity (16). They also exhibit good emulsifying properties. The two main whey proteins are β-lactoglobulin and α-lactalbumin. Lactalbumins are water insoluble; therefore, they are difficult to use in liquid formulas. Lactalbumins are also hygroscopic and can absorb odors. Storage temperatures should be maintained below 25°C and relative humidities below 65%. Stocks should be used preferably within 6 months.

Some whey protein preparations contain substantial amounts of lactose; for certain protocols it may be necessary to obtain manufacturer's information about the lactose content and choose the supplier accordingly. Table 14-1 shows selected commercially prepared whey proteins and their energy values and macronutrient compositions.

Milk solids are produced by spray- or roller-drying processes, which remove the water fraction of whole milk. Dried milk offers convenience of transportation, utility, stability, and resistance to microbial growth. Another advantage is ease with which milk powder can be incorporated into many formulations. However, whole milk solids must be used with caution because the butterfat content makes the formula prone to develop rancid flavors during storage (16). Dried skim milk has excellent flavor, high nutritional value, and good functional properties such as water binding and emulsification. It is the most readily available form of nonfat milk solid and is easily obtained through retail grocery stores, wholesale food suppliers, and specialty suppliers such as Clofine Dairy and Food Products, Linwood, NJ (14).

Soy Protein

Soy protein contains nearly optimal proportions of all the essential amino acids for humans, including lysine and tryptophan, but has insufficient (limiting) amounts of cystine, methionine, and valine. Its digestibility is high, being equivalent to milk and superior to whole egg (17). The use of processed soy proteins in the formula diet has the additional benefit of being hypoallergenic for certain groups of individuals who are sensitive to milk or other proteins such as gluten.

Soy protein must be used cautiously, because it may contain biologically active constituents, such as estrogen-like compounds, which could confound the results of some studies. In addition, the high phytic acid content of many soy preparations renders them unsuitable for mineral absorption studies.

Isolated soy protein (ISP) can be used when a vegetable protein source is required in a formula diet. The isolate is prepared from soybean flour by extracting the protein and precipitating it to yield a product that is approximately 90% protein on a dry weight basis. It is virtually free of carbohydrate and fat. ISP is a bland, soluble powder with low viscosity and gelling properties. It is highly digestible, forms a stable suspension, and provides smooth mouth feel. Grains Processing Corporation (Muscatine, Iowa) and Protein Technologies International (St Louis, Mo) offer a wide range of ISP products (14).

Soy protein concentrate (SPC) is prepared by removing most of the water-soluble nonprotein constituents from defatted soy flakes. This yields a product that is approximately 75% protein on a dry weight basis. SPC is available from Protein Technologies, International (St Louis, Mo) (14).

Amino Acid Powders

Purified crystalline amino acids can be mixed in different ratios for studies of amino acid turnover or requirements. Crystalline amino acids are water-insoluble; therefore, they are not mixed directly into liquids. Instead, a liquid formula is used to provide nonprotein nutrients and calories, and amino acid mixtures are weighed and served as a solid item. (See Exhibit 14-5.) In order to mask their metallic and bitter taste, a similar amount (by weight) of beet sugar and a small amount of flavoring is typically added to each portion of the amino acid mixture. In some cases, tolerance and acceptance can be improved by making a gelled product, which is made by mixing the amino acid blend with water and a trace amount of carrageenan (Gelcarin SA 812, FMC Corporation, Rockland, Maine). Purified food-grade amino acids may be obtained from the Ajinomoto Company, Tokyo, Japan.

Carbohydrates

Carbohydrates typically provide the main source of calories in formula diets.

Simple Carbohydrates

Simple carbohydrates dissolve easily in liquid formulas. Dextrose and sucrose, in particular, are often used as a major source of calories and as a way of sweetening the formula. The type of simple carbohydrate selected depends on the degree of the sweetness desired; combinations of different sugars can be used to obtain the desired taste (Tables 14-2 and 14-3). The major sources are sucrose, glucose, dextrose, fructose, maltose, and lactose. Several of these (glucose, mannitol, lactose, sorbitol, and maltose) are chemically refined and are costly, so it is best to minimize their use. Sorbitol and mannitol are sugar alcohols that can have adverse gastrointestinal side effects because they are not completely absorbed. They usually are chosen as sweetening agents rather than as major sources of energy.

The relative sweetness of various sugars and syrups is sometimes expressed as a ‘‘dextrose equivalent’’ value. During the acid hydrolysis conversion of cornstarch to corn syrup, an array of simple sugars and small polysaccharides is formed (such as dextrin, dextrose, maltose, maltotriose, and maltotetrose). As the conversion proceeds, increasingly higher proportions of dextrose are formed. Stopping the process at various defined points yields a corn syrup having a particular dextrose content (ie, dextrose equivalent, or DE, value). Pure dextrose is assigned a DE value of 100. For corn syrup products, the DE value thus indicates the achieved degree of polysaccharide hydrolysis; a higher DE value indicates more complete hydrolysis, higher dextrose content, and greater sweetness (18).

Dextrose (D-glucose) is a dry corn sweetener that is made from cornstarch by the action of heat, acid, or enzymes, resulting in the complete hydrolysis of the cornstarch to its component D-glucose molecules. Purified, crystallized D-glucose is commonly available from chemical supply companies and specialty food suppliers as dextrose monohydrate (9% water by weight) or anhydrous dextrose (less than 0.5% water) (14).

Sucrose, a disaccharide composed of one molecule of glucose and one molecule of fructose, is extracted from sug-

EXHIBIT 14-5

Solid and Liquid Formulations for an Amino Acid Diet:
Amino Acid Blend, Protein-free Cookies, and Liquid Formula D

BACKGROUND

- This metabolic balance diet uses a specifically patterned amino acid blend as the main source of nitrogen.
- The study protocol defines the protein level as 1 g/kg body weight and the non-nitrogenous calorie ratio as 40% fat, 60% carbohydrate.
- The reference participant weighs 70 kg and has an estimated energy requirement of 3,150 kcal.

CALCULATIONS FOR DAILY DIET

Ingredient/Food	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Amino Acid Blend					
Amino acids	70.0	279.6	69.9	0.0	0.0
Beet sugar	80.0	308.0	0.0	0.0	79.6
Subtotal	150.0	587.6	69.9	0.0	79.6
Protein-free cookies (n = 6)[1]	270.0	1,194.8	1.1	60.8	164.7
Liquid formula D[2]					
Orange sherbet[3]	230.2	322.1	2.5	4.7	70.0
Safflower oil	64.8	575.7	0.0	64.8	0.0
Beet sugar	122.8	470.6	0.0	0.0	121.7
Koolaid®, unsweetened (reconstituted)	833.0	0.0	0.0	0.0	0.0
Subtotal	1,250.8	1,368.4	2.5	69.5	191.7
Daily total (Amino Acid Blend + Cookies + Liquid Formula D)[4]	1,670.8	3,150.8	73.5	130.3	436.0

[1]The recipe for protein-free cookies is shown in Exhibit 14–2.
[2]The preparation procedure for Liquid Formulas is shown in Exhibit 14–4.
[3]Orange sherbet can be used to improve the flavor of liquid formulas. The small amount of protein in the sherbet usually will not alter the outcome of the study.
[4]The daily diet consists of 3 isocaloric meals, each containing Amino Acid Blend (23.3 g amino acids and 26.7 g beet sugar), Protein-free cookies (2 cookies, uncooked total weight 90 g), Liquid Formula D (433.6 g).

arcane or sugar beets. The general composition and appearance of sucrose from these two plant sources are identical, but beet sugar contains less natural enrichment in ^{13}C and is therefore more appropriate for stable isotope studies. Sucrose is available in powdered, fine-grain, and coarse-grain forms, all of which have different dissolving properties. Brown sugars are less refined, retaining greater (dark) or lesser (light) amounts of molasses, which increases the water and mineral content and adds a distinct, strong flavor. Cane sugar is widely available from retail and wholesale grocers; pure beet sugar may be obtained from specialty suppliers (such as Western Sugar Company, Bayard, Nebr; Holly Sugar Corporation, Hereford, Tex; and Great Lakes Sugar Company, Fremont, Ohio) (14).

TABLE 14-2

Properties of Selected Carbohydrates[1]

Product	Solubility[2]	Sweetness[2]	Viscosity[2]	Dextrose Equivalents[3]
Starch	4	4	1	0
Maltodextrin	3	3	2	6–20
Corn syrup solids	2	2	3	20–58
Dextrose	1	1	4	100

[1]Adapted from Igoe RS (15).
[2]Range of values: 1 = greatest; 4 = least.
[3]For a discussion of dextrose equivalents, see Carbohydrates in this chapter.

TABLE 14-3

Relative Sweetness of Natural and Artificial Sweeteners[1]

Sweetener	Type	Sweetness Level (%)[2]
Saccharin	Artificial	300–400
Aspartame	Artificial	200
Fructose	Natural	150
Sucrose	Natural	100
Dextrose (glucose)	Natural	70
Sorbitol	Natural	55
Mannitol	Natural	50
Lactose	Natural	20

[1]Adapted from Igoe RS (15).
[2]Values are expressed in comparison with sucrose. Sucrose = 100%.

Complex Carbohydrates

At times the protocol requires use of a complex carbohydrate like cornstarch or wheat starch. For liquid formulas, this causes several types of problems. The first problem is that complex carbohydrates do not dissolve or suspend well in a cold liquid medium, so that a homogeneous mixture is hard to achieve. The second is that most liquid formulas made with complex carbohydrates have a thick, unpalatable texture because the uncooked starch granules swell but do not gelatinize. This problem can be alleviated by incorporating the cornstarch or wheat starch with other formula ingredients into cooked solid food forms, such as cookies and puddings.

Cornstarch is derived from the endosperm of corn and contains amylose and amylopectin starch molecules. Cornstarch is generally considered to be a good choice as a source of complex carbohydrate for a formula, provided its relatively high ^{13}C content does not pose a problem. (Also see the earlier discussion of ^{13}C.) Compared with other complex carbohydrate sources, cornstarch mixes more readily with water and other ingredients. Formulas made with cornstarch are not stable for freezing and thawing. Although cornstarch imparts a chalky taste to the formula, the taste can be masked with artificial flavorings. Highly purified cornstarch products are readily obtained from retail grocery stores as well as general and specialty wholesale suppliers (such as CPC International, Englewood Cliffs, NJ; Corn Products, Summit Argo, Ill; and American Maize Products, Decatur, Ill) (14).

Wheat starch produces lower viscosity and more tender gels than does cornstarch (15). Wheat starch imparts a pasty, chalky taste to formulas and thickens the formulas to an undesirable degree when not consumed immediately after preparation. Although not ideal for use in liquid formulas, wheat starch is preferred in solid baked formulations, such as cookies.

Maltodextrin is a spray-dried product. It is made from a purified, concentrated aqueous solution of nutritive saccharides obtained by hydrolysis of cornstarch. It usually has a DE of 20 or less. Maltodextrin is slightly sweet with no undesirable taste, and it disperses rapidly in cold water (Table 14-2). Specially processed maltodextrin (Amino Products, Philadelphia, Pa) can provide an effective avenue for emulsifying certain foods, nutrients, and ingredients. A wide range of maltodextrin products with differing DE values is available from Grain Processing Corp (Muscatine, Iowa). Several commercially available maltodextrin products are listed in Table 14-4.

Fats and Oils

Fats and oils play multiple functional roles in formula diets. They provide concentrated sources of calories and specific combinations of particular fatty acids, and are vehicles for flavoring agents and biologically active compounds such as antioxidants and fat-soluble vitamins. Oils are the most frequently used lipid sources in formula diets because they are liquid at room temperature and are readily incorporated into suspension (18). Because they usually have a more saturated fatty acid profile than oils, fats tend to be solid at room temperature. As a result, fats are not preferred as lipid sources for chilled liquid formula diets: their tendency to solidify at cool temperatures means that they will stick to the sides of preparation and serving containers, causing quantitative losses, and that they will leave an unpleasant coating in the mouth. For the same reasons, hydrogenated shortenings, which are commonly used in baked goods, are seldom used in liquid formulas.

There are many naturally occurring food fats and oils that can be chosen to obtain the desired combination of characteristics. Custom-made blends can also be produced by specialty manufacturers. (Information about the customized production of specialty fats and oils may be obtained from the Institute of Shortening and Edible Oils, Inc, 1750 New York Avenue, Washington, DC 20006.) The principal fatty acids of selected fats and oils are shown in Table 14-5. Several commercially available specialty oils are listed in Table 14-6.

Despite the technical problems already mentioned, some liquid formula protocols nevertheless require the use of saturated fats. The usual choices include butter, lard, and

TABLE 14-4

Selected Maltodextrin Products

Product	Trade Name	Supplier	Nutritional Characteristics: Energy (kcal/100 g)	Carbohydrate (g/100 g)
Maltodextrin	Sumacal	Sherwood Medical St Louis, Mo	380	100
	Moducal	Mead Johnson Evansville, Ind	380	100
	Maltodextrin 180	Grain Processing Corp Muscatine, Iowa	376	94
Cornstarch, hydrolyzed	Polycose	Ross Laboratories Columbus, Ohio	380	94
Corn syrup solids, deionized	Nutrisource Carbohydrates	Sandoz Minneapolis, Minn	320	100

margarine. Another alternative is anhydrous butterfat (also called *anhydrous milk fat* or *ghee*), which is the clarified fat portion of milk, cream, or butter. Anhydrous milk fat is produced from cream that has been spray-dried, then immediately centrifuged to remove traces of water (19). It is remarkably pure (ie, free of protein and carbohydrate) and can be kept for years at room temperature if stored and packaged appropriately. Anhydrous milk fat may be obtained from Clofine Dairy and Food Products, Linwood, NJ (14).

There are several ways of incorporating solid fats into formula diets. One way is to bring the fat into suspension by using an emulsifier such as lecithin (Lucas Meyer, Inc, Decatur, Ill). Another approach is to first melt the fat in a double boiler and then mix it manually into the formula using a food whip. The entire mixture is then homogenized under pressure (see the discussion under Equipment). The formula will remain stable at room temperature for many hours, but the emulsion is broken upon heating, freezing, and thawing; restoring the emulsion likely will require an additional blending process. Finally, as with the complex carbohydrates, using a solid food form such as a pudding or cookie can be an effective way of presenting a solid fat in a palatable preparation.

PRODUCING FORMULA DIETS

Choosing Suitable Ingredients

A starting point in the development of a formula diet is to identify available ingredients and to compile a reference list of potential formula components and their characteristics. Ideal ingredients (1) are easy to weigh, transfer, mix, and form into a homogeneous product; (2) have a well-defined nutrient composition that is constant among lots and shipments; and (3) come with manufacturer's product specifications that are detailed and accurate. Other characteristics of ingredients that should be considered include the shelf-life of the product, any special requirements for storage, and the likelihood of spoilage during storage or preparation. For example, deterioration of whole egg powder and whole milk powder has caused investigators to seek alternative sources of protein.

Some food ingredients are available in different forms for various uses; the investigator must identify the product that best suits the needs of the study. For example, egg white solids are available as nonfoaming and foaming types. Nonfoaming egg whites are preferable because foaming incorporates air during mixing, which can complicate measuring and handling. Casein is also available in various forms (eg, sodium caseinate and calcium caseinate).

Solutions, Suspensions, and Emulsifiers

Certain products in solid or powder form do not dissolve well in liquid (eg, amino acid powders or lard). The miscibility of the ingredients is crucial for production of a homogenized formula that provides an even distribution of nutrients. Ingredients that are not well dissolved will produce a gritty texture, and there is a great likelihood that the first portions decanted will differ in composition from the last.

Difficulties can occur in keeping certain ingredients in suspension. Starch and solid fats such as butter, margarine, and lard are difficult to incorporate into a liquid formula with an appealing taste and texture. The alternative is to combine the fat, the starch, and some of the sugar into formulated solid products like cookies or puddings. The remaining ingredients are served as a liquid formula that can maintain a stable suspension.

Some fat-containing food ingredients serve as emulsifiers in formula diets (eg, egg yolk solids, whole egg solids, and lecithin). When food ingredients do not provide adequate emulsification, an emulsifying agent such as polygly-

TABLE 14-5

Principal Fatty Acids of Selected Fats and Oils[1]

	Weight % of Each Fatty Acid													
Oil or Fat[2]	**4:0**	**6:0**	**8:0**	**10:0**	**12:0**	**14:0**	**16:0**	**18:0**	**20:0**	**16:1**	**18:1**	**20:1**	**18:2**	**18:3**
Soybean oil							11	4			24		54	7
Corn oil							11	2			28		58	1
Cottonseed oil						1	22	3		1	19		54	1
Palm oil						1	45	4			40		10	
Peanut oil[3]							11	2	1		48	2	32	
Olive oil							13	3	1	1	71		10	1
Low erucic acid rapeseed oil (canola)							4	2			62		22	10
Safflower oil							7	2			13		78	
Sunflower oil							7	5			19		68	1
Coconut oil		1	8	6	47	18	9	3			6		2	
Palm kernel oil			3	4	48	16	8	3			15		2	
Cocoa butter							26	34	1		34		3	
Butterfat[4]	4	2	1	3	3	11	27	12		2	29		2	1
Lard						2	26	14		3	44	1	10	
Beef tallow[5]						3	24	19		4	43		3	1
Menhaden oil[6]						9	19	4		13	16	2	2	1

[1]Table adapted with permission from: *Food Fats, and Oils.* 7th ed. Washington, DC: Institute of Shortening and Edible Oils; 1994. Fatty acid composition data determined by gas-liquid chromatography and provided by member companies of the Institute of Shortening and Edible Oils, Inc. Fatty acids (designated as number of carbon atoms:number of double bonds) occurring in trace amounts are excluded. Component fatty acids may not add to 100% because of rounding.
[2]Common names: C 4:0 = butyric; C 6:0 = caproic; C 8:0 = caprylic; C 10:0 = capric; C 12:0 = lauric; C 14:0 = myristic; C 16:0 = palmitic; C 18:0 = stearic; C 20:0 = arachidic; C 16:1 = palmitoleic; C 18:1 = oleic; C 20:1 = gadoleic; C 18:2 = linoleic; C 18:3 = linolenic.
[3]Peanut oil typically contains C 22:0 plus C 24:0 at 4%–5% of total fatty acids.
[4]Butter fat typically contains C 15:0 plus C 17:0 at about 3% of total fatty acids.
[5]Beef tallow typically contains C 15:0 plus C 17:0 at about 2% and C 14:1 plus C 17:1 at about 2% of total fatty acids.
[6]Data on menhaden oil from Bimbo AP. *J Am Oil Chem Soc.* 1987;64:706. Menhaden oil typically contains the omega-3 fatty acids C 20:5 (EPA) and C 22:6 (DHA) at about 13% and 8% of total fatty acids, respectively. In addition, the minor fatty acids C 22:1, C 18:4, C 20:4, and C 22:5 typically compose about 6% of total fatty acids.

TABLE 14-6

Selected Specialty Oils

Product	Source Oil	Trade Name	Supplier
Microparticulated lipid	Safflower oil	Microlipid	Sherwood Medical St Louis, Mo
Medium chain triglycerides	Fractionated coconut oil	MCT oil	Mead Johnson Evansville, Ind
	Fractionated coconut oil	Nutrisource Lipid-MCT	Sandoz Minneapolis, Minn
Long-chain triglycerides	Soybean oil	Nutrisource Lipid-LCT	Sandoz Minneapolis, Minn

cerol ester (Drew Chemical Corp, Boonton, NJ) or carrageenan (FMC, Inc, Philadelphia, PA) may be added to the formula to ensure a well-homogenized suspension (1).

Organoleptic Aspects

Mouth-feel and Taste-testing

The tactile oral sensation of a food is sometimes called its ''mouth-feel.'' Liquid formula diets preferably should have a smooth texture that does not leave a sensation of ''coating'' in the mouth. Ingredients that may conflict with this goal are highly saturated fat sources, poorly soluble carbohydrates, fibers, and minerals.

Before settling on the final recipe for the product, the dietary staff and investigators or a panel of independent testers should consume a full serving of the product to assess its odor, texture, flavor, and its overall acceptability. The formula served at taste-testing should also be presented at the same temperature at which it will be served to the subjects. A final step in taste-testing is to present the formula to volunteers who have demographic and other characteristics similar to those of the anticipated study participants.

Flavor

Many participants prefer formulas that have a sweet, rather bland flavor, especially for a long-term study. Other participants may wish to have a choice of flavors to help relieve monotony. Variety in flavor can be provided by using flavor packs or extracts at the time of serving (Table 14-7). Slices of lemon can also be offered to cut the sweetness of a formula if the fruit does not interfere with the nutrients under study. Before any flavorings are added to a controlled formula diet, their chemical composition and any potential metabolic activity should be evaluated.

Flavor extracts, such as vanilla, lemon, almond, and orange, are available commercially. Local ice cream manufacturers can also provide a variety of flavorings. Flavor extracts may contain alcohol and may not be suitable for use in certain protocols.

Instant coffee powder (regular or decaffeinated) is another well-liked flavoring agent.

Vari-Flavor® Flavor Pacs (Ross Laboratories, Columbus, Ohio) provide five flavor options (cherry, lemon, orange, pecan, strawberry). Each 1-g packet contributes 4 kcal and trace amounts of minerals; contains dextrose, artificial flavor, and artificial color.

Koolaid® (Kraft General Foods, Inc, White Plains, NY) is a powdered soft drink mix. It contains citric acid, salt, calcium phosphate, and vitamin C. Koolaid powder is available in a variety of fruit flavors. There is a choice of naturally (sugar) sweetened, artificially sweetened, and unsweetened forms.

Vivonex flavor packs (Sandoz, Minneapolis, Minn) have five flavor options (cherry vanilla, raspberry, lemon-lime, orange-pineapple, and vanilla). Each packet contains 0.111 g (raspberry flavor) to 0.169 g (vanilla flavor) of aspartame, trace amounts of citric acid, dextrose, artificial colors, and flavors. Because aspartame contains phenylalanine, this product is not recommended for use in studies restricting phenylalanine. It is also not recommended for use in formulas that will receive heat treatment because aspartame decomposes at cooking temperatures. Aspartame decomposition can also occur in acidic mixtures that undergo prolonged storage.

Color

In formulas the appearance of the finished product should be consistent with standard expectations for colors that are associated with particular flavors or ingredients. For example, milk- and egg-based formulas are best developed to be off-white or in light tones of yellow or orange, which are typically associated with milk- and egg-based foods. Fruit flavors are sometimes associated with yellow, orange, red, and purple. Brown tones would be expected for coffee and chocolate flavors; green for mint flavor. Several of the flavoring blends listed in Table 14-7 also include compatible food colors.

Temperature

Formula feedings are typically served at 35°F to 40°F, or common refrigeration temperature. Not only are these temperatures in keeping with food safety standards, but the palatability of formulas is best in this range. Exceptions must be made for formulas that need to be warmed before serving because they contain certain ingredients such as lard and other saturated fats that may not remain in solution at this temperature. An alternative approach is to freeze the formula and serve it as a milkshake-like slush. As mentioned earlier, formulas that have been frozen and thawed may lose their homogeneous texture; the broken emulsions sometimes can be restored through vigorous shaking.

Recipes and Preparation Techniques

Once the formula ingredients have been determined, the recipes can be calculated and standardized preparation steps can be specified. The same ingredients are always used for any one formula, preferably all from the same lot, to attain consistency of nutrient composition throughout the protocol. The standardized format for recipes and preparation procedures should specify:

- Names and quantities of ingredients
- Expected yield; number of servings
- Nutrient composition for each serving
- Amount of water needed to achieve an appropriate concentration
- Type and amount of flavoring
- Weighing method
- Mixing procedures
- Types of storage and serving containers
- Type of serving utensils

TABLE 14-7

Extract Blends for Flavoring Formula Diets[1, 2]

Flavor	Ingredient	Quantity[3]
Butternut	Banana extract	12 drops
	Coconut extract	12 drops
	Butter pecan extract	15 drops
	Brown food color	1 drop
Butternut spice	Banana extract	12 drops
	Coconut extract	12 drops
	Butter pecan extract	15 drops
	Cinnamon powder	2 shakes
	Brown food color	1 drop
Cinnamon nog	Cinnamon powder	1/16 Tsp
Cinnamon nut	Butter pecan	30 drops (¼ tsp)
	Coconut extract	12 drops
	Cinnamon powder	2 shakes
Cream de toffee	Rum toffee extract	12 drops
	Banana extract	6 drops
	Cinnamon powder	Pinch
	Brown food color	1 drop
Hawaiian basic	Banana extract	12 drops
	Coconut extract	9 drops
	Yellow food color	1 drop
Hawaiian comfort	Lemon extract	12 drops
	Coconut extract	6 drops
	Banana extract	6 drops
Hawaiian mint	Coconut extract	12 drops
	Peppermint extract	3 drops
	Banana extract	9 drops
	Yellow food color	1 drop
Mint julep	Wintergreen extract	12 drops
	Banana extract	12 drops
	Coconut extract	4 drops
	Green food color	1 drop
Montage	Black walnut extract	12 drops
	Rum toffee extract	6 drops
	Maple extract	9 drops
	Coconut extract	6 drops
	Peppermint extract	3 drops
Tutti frutti	Strawberry extract	15 drops
	Banana extract	9 drops
	Red food color	1 drop
Yellow winter	Wintergreen extract	9 drops
	Banana extract	12 drops
	Yellow food color	1 drop

[1]Recipes provided courtesy of Phyllis Stumbo, PhD, RD, University of Iowa GCRC, Iowa City, Iowa.
[2]Flavors can be added to each serving rather than to the entire day's formula.
[3]Quantity is suitable for flavoring one 180-ml serving of formula.

- Portion size and serving temperature
- Labeling and coding
- Handling and storage conditions
- Caveats and hints for avoiding problems

A minimum number of containers should be used during formula preparation in order to avoid omission errors, to limit loss of ingredients, and to minimize contamination. In mixing a liquid formula, the preparer should first add some water into the blender cup, then add the dry ingredients, blend the mixture for a few minutes, then add the remaining liquids. This process will prevent the dry ingredients from adhering to the surface of the blender cup. To allow for some waste and spillage in preparation, it is advisable to increase the weight of each ingredient (including water) by 5% to 10%. Gram weights rather than volume measures should be used to portion each serving.

To ensure a safe and palatable product, the formula should not be prepared more than 2 days in advance, unless it will be frozen. The prepared formula should be clearly labeled with the code or name of the formula and the date that it is mixed.

Quality control of formula diets may be achieved by sampling each batch or random sampling of different batches and assaying for key nutrients. A less expensive procedure is to document the weight of a predetermined volume of formula, monitor it on a weekly basis, and compare the results with the established standard. Testing the pH value of the formula is quick and simple and can also be used for quality control.

Equipment

Equipment selection for preparation of liquid formula diets depends on the composition of the formula and quantities required for the study. Large quantities of formula composed of protein, carbohydrate, fat, and water can be successfully mixed into a smooth and palatable consistency using a homogenizer. *Homogenizers* are processing equipment for the production of high-quality emulsions. They differ from blenders in the ability to break food particles into smaller molecules to obtain better suspension. The Standard Gaulin Model 15M single-stage Labscale Homogenizer (APV Gaulin Inc, Wilmington, Mass) is a high-energy, high-pressure machine that can yield up to 40 L per hour. The IKA Ultra-Turrax T65 (IKA Works Inc, Cincinnati, Ohio) achieves its dispersing effect through a high-speed, rotor-stator principle (rotations per minute) yielding approximately 20 L per batch. If a homogenizer is not available, smaller batches can be processed in a 1-gallon blender (Waring Products, New Hartford, Conn) if the fat is unsaturated and ingredients are easily solubilized.

Homogenization using high pressure and/or higher temperatures is beneficial, for example, when a study design cannot accommodate using emulsifiers to keep saturated fats in suspension. Companies that manufacture homogenizers typically provide technical support when problems arise.

For preparation of formulas in batches greater than 20 L, the following are required: a top-loading scale with large capacity (30 kg or otherwise larger than total batch weight) and a smaller electronic balance (capacity 6 kg) for weighing ingredients, a large vessel with a spigot at the base for ease in decanting, a wire whip for premixing, wax-coated paper containers with plastic clips for sealing before storage (Con-ocup; Tucker, Harrison, NJ), freezer-stable labels and markers, long-handled spatulas, and freezers capable of maintaining low temperatures (−18°C or below) for long-term storage.

After defrosting, a formula can be aerated by mixing to a slurry in the half-gallon size Waring blender (model 36BL23) or the IKA Ultra-Turrax T65 (IKA Works, Inc, Cincinnati, Ohio).

Sanitation

Because the neutral pH and high protein content of formulas provide an excellent medium for the rapid growth of bacteria, the same rigorous standards and guidelines for food safety and sanitation must be followed for preparing formulas as for conventional foods (20). As with any other food, formula should not be held at "danger zone" temperatures of 5°C to 60°C (41°F to 140°F) for more than 4 hours during preparation, during short- or long-term cold storage, or during thawing. It thus is important to monitor the temperatures frequently while the prepared formula is cooling down or freezing.

Frozen formula also must be thawed in accord with safe food-handling practices, under refrigeration (20) with frequent shaking to maintain a uniformly cool mixture. In addition, all equipment used in preparing the formula must be kept meticulously clean. Care also must be taken to prevent contamination when staff repeatedly withdraws small quantities from bulk packages of dry ingredients (eg, boxes of powdered skim milk or dried egg white). Risk is highest when the container is opened and kept open and when the measuring utensil is inserted. Careful attention to storage conditions, holding temperatures and packaging, and advance portioning of dry ingredients into smaller clean containers can help to protect against this problem. It is prudent to carefully archive small samples of liquid formula in a freezer at −20°C to −80°C. These samples may be an important resource in the event that a participant becomes ill and the safety of the formula must be checked.

Participants should be given written instructions concerning appropriate handling and storage of their formula. Those who must consume their formula off-site should be given a good-quality, nonbreakable Thermos®; the formula will stay cold for several additional hours if the empty Thermos is first chilled in the freezer before filling. A telephone number should be provided in case the participant has a problem that needs immediate attention.

Conclusion: Research Applications of Formula Diets

In comparison with whole-food diets, formula diets are relatively easy to produce and deliver, and the composition can be controlled to a remarkable degree with modest cost. Provided that the nutrients of interest are available in purified or semipurified forms, formula diets can be considered when the complex background matrix of whole foods interferes with testing the hypothesis under investigation or when the composition of whole foods is too variable to deliver adequate dietary control. Formulas also are a means of evaluating food components (such as specific amino acids) that cannot easily be manipulated through whole foods. The tightly defined composition of formulas is useful for studies of nutrient balance and requirements, dose-effects of single nutrients, and between-nutrient interactions.

Formula diets usually are better suited to short-term applications because participants often find the monotony and limited sensory stimulation to be problematic (although some investigators have been successful in long-term feeding of formulas). Nevertheless, this time frame is highly compatible with many types of protocols, such as postprandial metabolism and absorption studies, nutrient load and tolerance tests, diagnostic test meals, and balance studies for nutrients with short equilibration time.

Considered from another perspective, there are research problems in which this lack of sensory variety may actually enhance the investigator's purposes. For example, some hypotheses are best tested under conditions that minimize variation in texture, taste, food volume, and visual perception of relative quantities and varieties of foods. Similarly, for many nutrients, the formula diet provides a convenient means of delivering a double-blinded intervention because changes in nutrient content are harder to perceive in the absence of associated food flavors.

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