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Best Practices for Food-Based Clinical Trials

Guidance for Planning, Conducting and Reporting on Human Studies to Support Health Claims

May 2013

Canada

Best Practices for Food-Based Clinical Trials
Guidance for Planning, Conducting and Reporting on Human Studies to Support Health Claims

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Pratiques exemplaires pour les essais cliniques sur les aliments
Lignes directrices pour la planification, la réalisation et la communication de données issues d'études chez l'humain, pour le soutien d'allégations santé

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List of Abbreviations and Acronyms

AAFC – Agriculture and Agri-Food Canada

ANOVA – Analysis of variance

CFIA – Canadian Food Inspection Agency

CRF – Case report form

CONSORT – Consolidated Standards of Reporting Trials

CORE - Course on Research Ethics

CTD - Common Technical Document

DASH – Dietary Approach to Stop Hypertension

DELTA – Dietary Effects on Lipoproteins and Thrombogenic Activity

FDA – *Food and Drugs Act*

FDR – Food and Drug Regulations

FFQ – Food frequency questionnaire

GCP – Good Clinical Practice

ICH – International Conference on Harmonisation

LDL – Low-density lipoprotein

MRCT – Multicentre randomized clinical trial

NHP – Natural health product

OECD – Organisation for Economic Co-operation and Development

RCT – Randomized controlled trial

REB – Research Ethics Board

SOP – Standard Operating Procedure

TCPS – Tri-Council Policy Statement

1.0 Introduction

1.1 Background, rationale and importance of optimizing the design of human clinical studies in support of food health claim petitions to Health Canada

Food health claims are statements regulated by Health Canada that translate a science-based food–health relationship into clear public health messages. The regulations support informed consumer choice and guard against misleading the public with unsubstantiated health claims [1].

Supporting data for food health claims must be based on high-quality, non-biased human clinical studies and be assessed in a systematic, comprehensive and transparent manner. One of the main aspects of substantiating a food health claim submission is the evaluation of the supporting evidence from studies to assess causality, safety, population generalizability and strength of the association [2]. Optimizing the design, conduct and methods for reporting food-based human clinical trials is therefore essential in carrying out a successful clinical trial aimed at providing convincing evidence for the substantiation of a food health claim. Studies that are not robustly designed to address the evidence required by Health Canada or that display dubious reporting will directly weaken the justification of the health claim based on the totality of evidence and reduce the prospect for submission approval.

Randomized controlled trials (RCTs) are the "gold standard" of clinical trials, valued for their precision and robustness in accurately assessing the impact of a bioactive ingredient or food product on a particular physiological condition or response. RCTs are ideal tools for investigating the cause and effect relationship between an intervention and an outcome, and in establishing sound scientific evidence for this relationship, provided the RCT is well conducted. Therefore, guidelines that outline best practices for these clinical trials are required. These best practices guidelines will enable the data collected from well-controlled clinical trials to be used in support of food health claims and to verify bioactive efficacy.

Health Canada has developed policies, regulations and standards regarding health claims for food and for natural health products (NHPs). For health claims on food, the Food Directorate at Health Canada has developed policies and guidance documents for petitioners, and the Canadian Food Inspection Agency (CFIA) is responsible for the enforcement of these policies under the *Food and Drugs Act* (FDA). The use of health claims on NHPs is regulated under the Natural Health Products Regulations [3], and the Natural Health Products Directorate at Health Canada is in charge of policies, regulations and applications for NHPs.

Information about the food–NHP transition, including transition and classification criteria and an updated list of foods transitioned, can be consulted at www.hc-sc.gc.ca/fn-an/prodnatur/auth-food-aliment-eng.php.

Health Canada has transitioned certain products at the food–NHP interface to the food regulatory framework based on criteria outlined in the classification guidance document [4].

1.2 About this document

This document focuses on best practices for clinical trials using foods. It provides guidance for planning, conducting and reporting on data from human nutrition research required to substantiate a food health claim in Canada. This practical manual fills a need for food and nutrition researchers to have guidance that addresses the criteria used by Health Canada to evaluate a food health claim submission.

This manual was commissioned by the Food Regulatory Issues Division of Agriculture and Agri-Food Canada and prepared by food and nutrition research experts with extensive experience in clinical trials. The methods used to create a broad, yet comprehensive guidance document include a systematic review of the literature, extensive interviews with food and nutrition researchers, and experience-based clinical practice knowledge. Although the document covers a wide range of topics, it is not intended to be a definitive manual on food-based clinical trials. Readers are encouraged to use this manual as a guide and to refer to the cited references for more information.

This report is divided into five sections. The Introduction outlines the scope of the project. Section 2 covers food health claims and their substantiation based on Health Canada requirements Section 3 focuses on pre-clinical trial activities, and includes details on generating a research question and hypothesis, choosing a study design and population, preparing test products and selecting control foods. Section 4 covers clinical trial implementation, including recruitment of participants, managing research diets, and record keeping. Section 5 details post-clinical trial activities such as sample analysis, data analysis and publishing. Each of these sections begins with an overall Summary and includes synopses of Best Practices Keypoints. The resource also includes a brief conclusion, appendices, a list of references and a short glossary.

2.0 Health Claims and Their Substantiation

SUMMARY

- Food health claims are classified by categories depending on how they are regulated and evaluated
  (See Section 2.1 Categorization of food health claims in Canada)
- Food health claims must comply with applicable legislation and regulations. The substantiation of most food health claims should follow Health Canada's guidance documents for preparing a submission for food health claims
  (See Section 2.2 Health Canada's guidance for substantiating function, therapeutic and disease risk reduction claims)

A health claim is "any representation in labelling or advertising that states, suggests, or implies that a relationship exists between the consumption of a food or a constituent in the food and a person's health" [5]. Food health claims may be classified by categories depending on the nature of the claim: function claims (including nutrient function claims), disease risk reduction claims, therapeutic claims and general health claims (claims about dietary guidance). The use of health claims on food is voluntary. However, when a health claim is made, it must comply with applicable legislation and regulations. Health claims are regulated under sections 3 and 5 of the FDA [6–8] and several sections of the Food and Drug Regulations (FDR) (B.01.311, B.01.600-B.01.603, D.01.006, D.02.004) [9, 10].

For information on the claims that have been accepted for food sold in Canada and their conditions of use, see Function Claims on CFIA's website at www.inspection.gc.ca/english/fssa/labeti/instfunce.shtml and Health Claim Assessments on Health Canada's website at www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/assess-evalu/index-eng.php.

All health claims must be truthful and not misleading (Section 5 of the FDA). This means that manufacturers must have scientific evidence to validate the health claim before they use it in food labeling and advertising [11]. Also, certain food health claims require pre-market authorization, which involves an obligation to prepare and submit an application to Health Canada. Consultation with Health Canada's Food Directorate is encouraged when manufacturers are uncertain about the status of the claim they are planning to use.

2.1 Categorization of food health claims in Canada

Food health claims may be classified by categories, depending on how they are regulated and evaluated.

Function Claims relate to the specific beneficial effect of a food or a constituent in a food on the normal functions or biological activities of the body. These claims refer to a positive contribution to health, or physical or mental performance. They must not refer directly or indirectly to the treatment, mitigation or prevention of any disease, disorder or abnormal physical state, or of their symptoms. These claims are

based on the role that a food or food constituent may have on human physiological or psychological function when consumed at levels consistent with normal dietary patterns.

Three function claims have been reviewed and found to be acceptable by Health Canada.[1] They link coarse wheat bran with regularity; green tea unfermented and/or bud from *Camellia sinensis* with protection from oxidation of blood lipids; and psyllium with regularity. It is expected that companies wanting to make function claims have scientific evidence to validate the claim prior to its use on food labels or in advertisements. This evidence may be used by the CFIA, in collaboration with Health Canada, to evaluate product compliance with the FDA and the FDR. In order to ensure the health claim is properly substantiated it is recommended that the Health Canada *Guidance Document for Preparing a Submission for Food Health Claims* [2] be used. Submissions for function claims are voluntary but recommended; manufacturers and importers can contact the Food Directorate of Health Canada for advice regarding the acceptability of new function claims prior to their use.

Acceptable nutrient function claims can be found in CFIA's *Guide to Food Labelling and Advertisement*, Section 8.6.4 (available at: www.inspection.gc.ca/english/fssa/labeti/guide/ch8e.shtml#a8_6_4). For information on documenting the supporting evidence for new nutrient function claims, see the CFIA website at www.inspection.gc.ca/english/fssa/labeti/guide/ch8e.shtml#a8_6_5.

Nutrient Function Claims are a subcategory of function claims. These claims describe the well-established roles of energy or known nutrients that are generally recognized as an aid in maintaining the functions of the body necessary to the maintenance of good health and normal growth and development.

Disease Risk Reduction Claims establish a link between a diet, a food or a food constituent and the reduced risk of developing a diet-related disease or condition, in the context of the total diet. The following disease risk reduction claims are acceptable for use in Canada:[1]

- a diet low in sodium and high in potassium, and the reduction of risk of hypertension;
- a diet adequate in calcium and vitamin D, and the reduction of risk of osteoporosis;
- a diet rich in vegetables and fruits, and the reduction of risk of some types of cancer;
- a diet low in saturated fat and trans fat, and the reduction of risk of heart disease;
- gum, hard candy or breath-freshening products with low fermentable carbohydrates, and the reduction of dental caries.

Therapeutic Claims describe a link between the characteristics of a diet, a food or a food constituent and the treatment or mitigation of a disease or health-related condition, or about restoring, correcting or modifying body functions. Five therapeutic claims have been accepted by Health Canada:[1]

- plant sterols in foods and blood cholesterol lowering (May 2010);
- oat products and blood cholesterol lowering (November 2010);
- food products containing psyllium and blood cholesterol lowering (December 2011);

[1] Note: the lists of claims in this section that have been reviewed and found to be acceptable by Health Canada are current as of May 15, 2013.

- replacement of saturated fat with mono- and polyunsaturated fat and blood cholesterol lowering (February 2012);
- barley products and blood cholesterol lowering (July 2012).

For using general health claims that involve content from Canada's Food Guide, consult the principles published by Health Canada [12]. CFIA's *Guide to Food Labelling and Advertising* also provides guidelines on the use of third-party endorsements, logos and seals of approval (available at www.inspection.gc.ca/english/fssa/labeti/guide/ch8ae.shtml#a8_13).

General Health Claims do not refer to a specific health effect, disease or health condition. These statements promote health through healthy eating or provide dietary guidance.

2.2 Health Canada's guidance for substantiating function, therapeutic and disease risk reduction claims

Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims* [2] helps to ensure that function, therapeutic, and disease risk reduction claims are substantiated in a systematic, comprehensive and transparent manner.

Important considerations for substantiation of a health claim include the demonstration of causality (the relationship between a food and a health effect), the generalization of results to the target population (general population or subgroup of the population), and the demonstration of the feasibility of achieving an adequate food exposure, as part of a healthy and balanced diet, to obtain the claimed health benefit.

This guidance document for food health claim submissions applies to food considered as safe. If the health claim is intended for use with novel foods—"foods that have been produced through new processes, that do not have a history of safe use as a food, or that have been modified by genetic manipulation"—a novel food application must be submitted to Health Canada before or at the same time as a food health claim submission [6, 7].

Detailed information on the substantiation of new health claims can be found in Health Canada's guidance documents for preparing a submission for food health claims (available at www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/guidance-submissions-eng.php). These documents apply to all food health claims, except for nutrient function claims and general health claims.

Petitioners can use an existing systematic review of the scientific literature to help demonstrate that a proposed health claim is substantiated. The *Guidance Document for Preparing a Submission for Food Health Claims Using an Existing Systematic Review* [13] was developed by Health Canada to guide petitioners wishing to use an existing systematic review to demonstrate causality between the food and the health effect.

Of the scientific evidence recognized within the assessment of causality, only human studies—both intervention and prospective observational studies—are allowed for the substantiation of the claim. Animal or *in vitro* studies can be used only in support of the main argument and cannot be used to demonstrate causality, mainly because extrapolation of animal data for humans is subject to significant uncertainties. Studies must be performed on free-living, generally healthy adults to permit the generalization of the effect. Also, in the case of multi-component intervention studies, the effect of the food should be isolable from the effect of other factors to avoid confounding. The *Guidance Document for Preparing a Submission for Food Health Claims* also provides criteria to assess the quality (i.e., to discriminate between studies that have a high or low internal validity and risk of bias) of the studies,

including inclusion/exclusion criteria, participant allocation, blinding, attrition, characteristics of the exposure/intervention, characteristics of the health effect, and potential confounding.

The submission management process for health claims involves many steps. A **pre-submission stage** is encouraged. Applicants should consult the Nutrition Premarket Assessment Division of Health Canada at healthclaims-allegationssante@hc-sc.gc.ca to obtain assistance before submission and possibly expedite their application.

The formal submission begins with an **administration stage**, when the application is received by Health Canada's Submission Management and Information Unit (smiu-ugdi@hc-sc.gc.ca). Within 7 calendar days, the petitioners will receive an acknowledgement letter with the file number of the submission. The **preliminary review** permits the Nutrition Premarket Assessment Division to identify if additional information or clarification is required. The **full scientific review stage** evaluates the submission to determine if the scientific evidence substantiates the health claim of the food. During the **approval stage**, revisions may be required before final approval.

BEST PRACTICES KEYPOINTS

Health claims and their substantiation

- Petitioners should follow the Health Canada *Guidance Document for Preparing a Submission for Food Health Claims* to ensure that function, disease risk reduction and therapeutic claims are substantiated in a systematic, comprehensive and transparent manner.
- Studies, either intervention or prospective observational, which are used in support of a proposed health claim must be of high quality with a focus on:
  - human studies
  - the ability to effectively demonstrate causality
  - generalizability to the target population.

3.0 Pre-Clinical Trial Activities

SUMMARY

- Generating a well-defined research question and hypothesis is the initial step in designing a clinical trial. It should be based on a comprehensive review of the existing evidence and clearly state how the food intervention will influence a health effect. From this step should emerge the specific objectives that will be undertaken to answer the research question. (See Section 3.1 Generating a research question and hypothesis)
- Key elements to consider when choosing the optimal study design are: (See Section 3.2 Choosing the optimal study design)
  - Appropriate type of study for research question (Section 3.2.1)
  - Appropriate method of randomization (Section 3.2.2)
  - Level of dietary control (Section 3.2.6)
  - Type of statistical methods to be used for data analysis (Section 3.2.8)
- Outcome measures are indicators measured in a participant or a biological sample that assess the efficacy or safety of an intervention (See Section 3.3 Identification of outcome measures)
- Three types of outcome measures are important in a clinical trial:
  - Primary outcomes (Section 3.3.1)
  - Compliance outcomes (Section 3.3.2)
  - Adverse event outcomes (Section 3.3.3)
- When selecting a target study population for clinical trials, the sample size must be large enough to detect an intervention effect. It must have been populated using a definitive list of eligibility criteria and be generalizable to the Canadian population, especially for data that will be used in support of food health claim petitions (See Section 3.4 Selection of study population)
- Development of a test product for a food clinical trial should account for: (See Section 3.5 Test product preparation and suitable control selection)
  - Intended use of the food product being investigated (Section 3.5.1)
  - Appropriate dose required to achieve a physiological effect (Section 3.5.2)
  - Safe food handling and storage (Section 3.5.3)
  - Batch variability (Section 3.5.4)
  - Appropriate control treatment for comparison (Section 3.5.5)

Randomized control trials (RCTs) are experimental interventions that are widely used to evaluate the efficacy of a dietary component. RCTs are considered the “gold standard” for testing interventions as they allow investigation of the temporal relationship, where they demonstrate that the independent variable of

interest is the cause of the outcome of interest [14]. In RCTs, the independent variable (i.e., the treatment, the exposure, the dietary component of interest) is manipulated and the dependent variable (i.e., outcome measure or “health” effect) is measured under controlled conditions. Hence, any difference in the outcome measure can be attributed to the tested component with a high degree of certainty. The many stages involved in preparing an RCT include generating a research question and hypothesis, planning the study design, selecting outcomes of interest, estimating required sample size, specifying participant characteristics, and deciding which treatment and which control to use and how to prepare them.

3.1 Generating a research question and hypothesis

Initial planning of any research study begins with a research question related to a particular element, behaviour or process that is currently unknown, with the intention to answer that question. Developing the research question is the first methodological step an investigator must take in designing a study—the question often stems from voids in science that have been identified in systematic literature reviews.

The research question must be clear and precise. This essential step determines many aspects of a research project, such as the objectives, study design, test product and outcome measures, and thus determines the quality and success of the study. In cases where a clinical trial is designed with the objective of producing data for a food health claim petition, the desired health claim could aid in generating the research question and focus the hypothesis on the proposed food and health association. In such cases, collaboration with industry partners or funding sponsors is needed.

Trying to answer several questions in one clinical trial is not good practice. For example, an RCT aimed at documenting the impact of a food or nutrient on blood cholesterol will trigger different considerations than an RCT aimed at investigating the impact of the same food on inflammation. To be successful, an RCT must have a clear, focused primary objective, according to which all other aspects of the RCT will be defined. If an RCT has secondary or tertiary objectives, the study design and sampling strategy need to be properly constructed to respond to these additional objectives.

Following the generation of a definite research question, a well-defined hypothesis must be clearly stated. A scientific hypothesis is a proposed explanation that may answer the research question. This hypothesis should be based on a comprehensive review of the existing evidence. Systematic testing of the hypothesis will infer a probable solution to the research question. However, there is a tendency in research publications to provide an outline of the proposed research aims and objectives instead of a statement of the hypothesis. A specific hypothesis cannot always be assumed. Best practice is to clearly state the hypothesis that will be tested in the clinical research project. A good hypothesis is one that contains a single clear statement.

Data from clinical studies that may be used to support food health claims should have hypotheses that estimate the impact of the food or ingredient on the primary outcome very precisely. For food-based human clinical trials, the hypothesis should explicitly state how the food component would impact the primary outcome measure (health effect). Examples of well-stated hypotheses for food-based human clinical trials are shown in the box **Examples of well-defined hypotheses** [15, 16].

Examples of well-defined hypotheses

Hypothesis example #1

"Daily consumption of a baked food product containing whole soy for 6 weeks will significantly reduce serum LDL-cholesterol in individuals with hypercholesterolemia." [15]

Hypothesis example #2

"We hypothesized that consuming 1.5 g high-molecular weight oat β-glucan incorporated into a ready-to-eat cereal twice daily would reduce serum low density lipoprotein (LDL) cholesterol compared with a control wheat-bran cereal." [16]

3.2 Choosing the optimal study design

An optimal study design reduces all sources of bias as much as possible. Choosing the optimal study design for a research question is important for the validity and reliability of results that will be generated.

Aspects of design that should be carefully considered include:

- type of design
- method of randomization and allocation concealment
- blinding
- selecting the intervention and control foods
- level of dietary control and monitoring of compliance
- length of the study
- type of data analysis
- conducting pilot studies
- the number of investigating sites to be involved

These design aspects are discussed in detail in the following sections.

3.2.1 Types of randomized clinical trials

The major types of randomized controlled trials are:

- **Parallel-groups or parallel-arms design:** Participants are randomly assigned to receive only one treatment. Therefore, there are as many groups in a parallel-arm trial as there are treatments, including the control. Each participant remains assigned to the same randomized group until the end of follow-up of the assigned treatment. Parallel studies allow for between-group comparison and are also useful for assessing dose-response relationships of a given food component. An example of this type of study design is shown in Diagram 1.

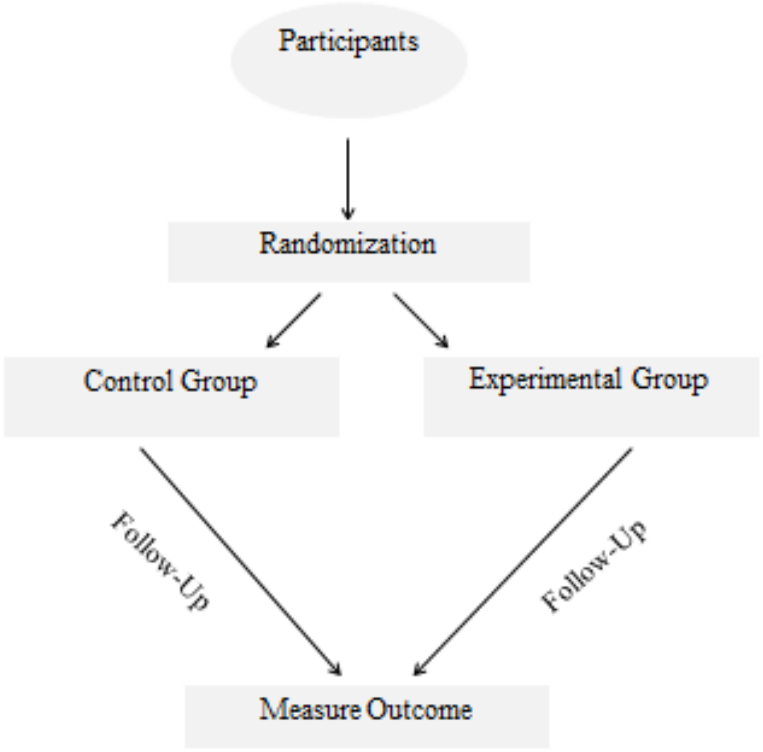


Diagram 1. Parallel-arm two-group design

- **Crossover design:** Participants are randomly assigned to a sequence of the different treatments and control and hence will receive all treatments. Each experimental phase is ideally separated by a washout period in which participants revert to consuming habitual diets. Crossover studies allow for between- and within-treatment comparisons. An example of this type of study design is shown in Diagram 2.

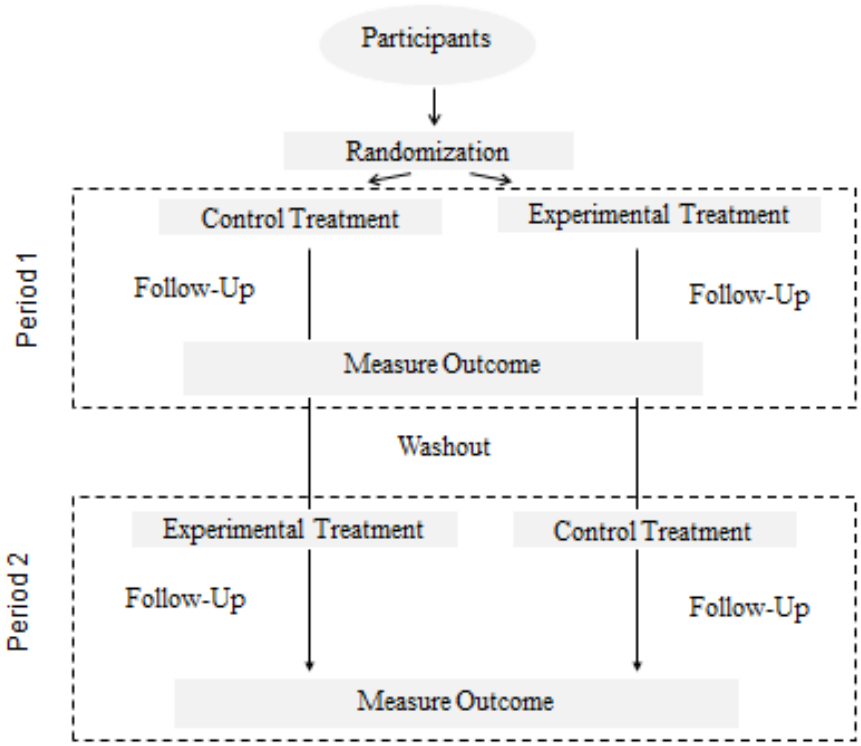


Diagram 2. Two-sequence, two-period crossover design

The selection of one design over the other depends on the type of research. For example, a crossover design can only be used with reversible treatments of chronic stable conditions [17], such as mild to moderate hypertension and hyperlipidemia. Crossover studies may also be favoured for short-term studies, such as those examining post-prandial glycemic response or satiety [18]. The advantages and disadvantages of using a crossover design instead of a parallel design are substantial and should be carefully considered by investigators.

A crossover design has two main advantages over a parallel design. Because each participant serves as his or her own control in crossover design, this:

- minimizes the effect of confounding variables and increases the precision of the study [19]; and
- reduces the required sample size. A crossover trial needs only half the sample size of that used in a parallel trial if there is no correlation among repeated measurements of the primary outcome. However, this rarely occurs. More commonly, in cases where the correlation coefficient between repeated measures over time is 50% or greater, a crossover trial needs only a quarter of the sample size of a parallel design [20].

A crossover study design also has disadvantages [20] as it:

- increases the duration of the study—the higher the number of test treatments, the longer the study duration will be;
- is significantly more inconvenient for participants due to increased study length, and increases the chance of participant withdrawal during the study (dropouts); and
- is susceptible to carryover effect, which is the confounding effect of a treatment from the previous period on the response of the outcome measure to the subsequent treatment.

When choosing a crossover design over a parallel design, investigators should consider:

- introducing a washout period (i.e., untreated period between treatment periods of enough duration to allow the outcome of interest to return to its baseline values); and
- the need to account for the effect of sequence, period, tests for carryover effects and the repeated nature of the data in the statistical analyses.

Features of crossover designs appear to be preferred over parallel-arm designs when the number of study participants is limited and when a washout period can sufficiently reduce the carryover effect [21]. However, a parallel-arm design may be more suitable if the condition is unstable and the outcomes are subjective. Parallel studies may also be necessary in cases where it may be unethical to return intentionally to baseline (e.g., if body weight or bone mineral density may be affected) [18]. Conversely, it is known that less variability is found within a participant (intra-individual variation) than between different participants (inter-individual variation) in response to diet. Thus, crossover designs may be more suitable for dietary intervention trials, providing that the aforementioned factors are considered.

Examples of crossover and parallel trials and their key features are shown in Appendix 1.

3.2.2 Randomization

Randomization is done after the participants have been screened and selected. In some cases, randomization may also be done after a run-in period [21]. See Section 3.2.7 Length of intervention and overall study.

Once a participant has been found to satisfy all eligibility criteria (see Section 3.4.2 Eligibility criteria), he or she can be allocated to the experimental treatment or control by chance. This randomization is a critical step in RCTs and should be conducted appropriately to ensure well-balanced characteristics between treatments. The investigator must randomly allocate the participants to two or more groups in a parallel-arm study or sequences of treatments in a crossover study. A group that is allocated to receive the dietary component is called the treatment group. A group that is not allocated the dietary component of interest is called the control or comparison group. The control group may receive no treatment at all, an inactive treatment such as a placebo, or a standard accepted treatment (see also Section 3.2.5 Defining the intervention and control treatments).

The advantages of adequate randomization are that it [22]:

- ensures that the comparison between the different treatments is free from selection biases and confounding from other variables so the conclusion derived from the sample can be drawn with a high degree of confidence;
- allows the use of probability theory to examine whether the differences between the control and experimental groups are due to chance or not; and
- facilitates blinding.

Improper methods of randomization which should not be used include practices such as allocation according to coin-flips, date of birth, medical record number, days of week or alternate assignment [23, 24]. Appropriate randomization techniques should be based on chance alone.

Selection of a randomization technique depends on the study design:

- **Simple randomization** is appropriate for simple randomized clinical studies with parallel arms. It is performed by first establishing an algorithm that specifies random numbers to the different study groups (i.e., odd and even numbers will represent treatments A and B, respectively), which generates a list of random numbers using a computer or a random number table. The numbers are assigned in the order that the participants enter the study [14, 21].

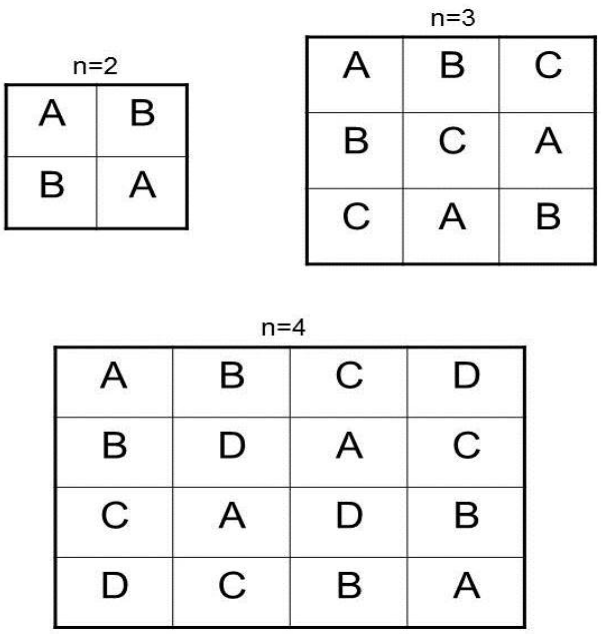
  Example: Assume that an investigator needs to carry out randomization for a study with two arms, treatments A and B, and 16 participants. Ideally, a third party is used to complete this process to allow for double blinding for investigator, research team and participant. First, numbered envelopes are prepared from 1 to 16. A list of random numbers can be generated using a random number table or computer-generated list, where odd and even numbers are predetermined to stand for treatments A and B, respectively. A pencil is blindly placed on a number in the random number table and moved according to the predetermined direction (up or down the columns for example). If the first number is even, this correlates to “treatment A,” which is written on a slip and placed in envelope number one and so on until all of the envelopes are assigned. The first participant to enter the trial is assigned to the group indicated in the first envelope. Alternatively, the investigator can use a computer program to generate a list of random numbers [25]. Simple randomization is easy to carry out but unpredictable as it may produce an uneven number of participants in each group especially if the sample size is limited.

- **Blocked randomization** (also called restricted randomization) is used to ensure that the number of participants is the same in the different study groups [25]. First, an investigator chooses the block size and the number of blocks needed to cover the number of participants in the study. The size of each allocation block must be an integer multiple of the number of treatment groups. A list is then prepared with all the possible combinations of treatments in a block, producing a randomization code for the order in which to select each block [26].

Example: Assume that an investigator needs to carry out randomization for a study with two arms, treatments A and B, and 24 participants. The investigator can choose a block size of 4 and so will need 6 blocks. The possible combinations of treatments A and B in a block size of 4 are AABB, ABAB, BBAA, BABA, ABBA, and BAAB. Finally, these different blocks are assigned random numbers from 1 to 6. A disadvantage of blocked randomization is that the assignment of treatments becomes predictable toward the end of the sequence in a block. This is a problem when treatments are not masked.

Appendix 2 shows an example of a complete block randomization list.

For crossover trials, blocked randomization provides the means of obtaining balanced designs in which each treatment is preceded and followed equally by the other treatments. These blocks are often called Latin squares or Williams design. First, the investigator should construct, at random, a proper Latin square or block, and then randomly allocate the appropriate sequences of the treatments to the participants in equal numbers. The number of participants should be a multiple of possible treatment sequences. Examples of appropriate blocks with different sizes are shown in Diagram 3 [27].



Note: Rows represent different participants or sequences, and columns represent different time periods.

Diagram 3. Examples of appropriate blocks with different number of treatments and periods

Again, equal numbers of participants should be allocated to each treatment sequence [25, 28].

Appendix 3 presents an example of a computer-generated list of sequences of treatments in an RCT with a crossover design and three treatments.

- **Stratified blocked randomization** is used when investigators want to ensure that important baseline characteristics that could affect the outcome of interest are distributed equally between the treatment groups [21]. Stratified blocked randomization ensures that the number of participants in the different groups is balanced, and that predetermined characteristics that may affect the outcome of interest are evenly distributed between the different groups. The investigator should first determine the baseline characteristic that may influence the primary outcome of interest. Second, the investigator must determine the number of strata for that characteristic, and finally produce a randomization list for each stratum using simple or blocked randomization techniques [26]. Common stratification parameters include gender, age and baseline values of outcome of interest. For example, Hendriks et al. randomly allocated participants to treatments using serum cholesterol concentrations, gender and age as stratification parameters [29].

An example of stratified blocked randomization is shown in Appendix 4.

Example: Assume that an investigator needs to carry out a stratified blocked randomization for a study with two arms, treatments A and B, and 192 participants. The investigator first determines that baseline serum cholesterol concentrations, gender and age are factors that determine a participant's response to a dietary intervention. So the investigator determines the total number of strata that depend on the level of each factor, which in this example are:

- baseline serum cholesterol concentrations: two strata, optimal and high
- gender: two strata, males and females
- age group: two strata, < 50 years and ≥ 50 years

The total number of strata is the product of the number of levels of each factor, which in this case is 2*2*2= 8. Then the investigator generates a blocked randomization for each stratum and combines all the factors. A large number of participants is needed when several strata of randomization are considered simultaneously.

3.2.3 Allocation concealment

Allocation concealment is the method used to ensure that participants and investigators who are in direct contact with participants remain fully blinded to treatment before and until allocation. In other words, allocation concealment ensures no one involved in the project is aware of the upcoming assignment, thereby limiting influence on the randomization sequence and hence on selection bias.

Allocation concealment can be achieved by [21, 24, 30, 31]:

- Central randomization, which is carried out by another office or centre that is unaware of participant characteristics. The clinical coordinator contacts this external organization (by telephone, fax, or email) to provide the name and the study number of new participants. In turn, the organization randomly assigns the new participant to a treatment group or sequence.

- Use of opaque, sealed and sequentially pre-numbered envelopes, which are administered serially to participants as they enter the study. It should be noted that in a case of stratified random design more than one set of envelopes is required.
- Use of an on-site computer program that can perform random allocation and is tamper proof.

Approaches to allocation concealment that are clearly inadequate include alternation, the use of record numbers, dates of birth or day of the week, use of an open list of random numbers or any transparent method.

3.2.4 Blinding

Blinding seeks to prevent ascertainment bias by protecting the allocation sequence of treatment groups. Whenever possible, participants, clinical coordinators and investigators who are in direct contact with participants or who will analyze study data should not be aware of the study group assignments.

Blinding can be carried out at different levels, each of which provides potential benefits to the study design [21, 23]:

- Participants blinded:
  - improves participant compliance
- Investigators and study personnel blinded:
  - evens out attitudes toward participants, such as their own perception of treatments, administration of co-intervention, and encouragement or discouragement for continuation of a trial
- Outcome assessors blinded:
  - prevents biased assessment of outcome of interest

To achieve successful blinding, it should be difficult to distinguish between the different treatments in terms of physical appearance and the placebo being used [19]. Placebo is a treatment that is identical to the test intervention or experimental treatment, but without the active ingredient. Food products that have either the active ingredient or the placebo should be packaged in a similar manner and carry a label with a generic description and a treatment code.

However, in studies involving dietary intervention, preparing a placebo and blinding participants and/or clinical coordinators can be difficult. Challenges unique to trials with food or food ingredients arise from their colour, texture, aroma and taste characteristics. Dietary components that could easily be blinded are phytosterols as they have no smell or strong taste, while it is challenging to blind fish oil, cocoa, and soy isoflavones due to their smell and taste characteristics [32]. Whole yellow-pea flour has been shown to have unfavourable sensory characteristics that limit its incorporation into functional foods [33]. It is useful to assess whether blinding is successful among the participants, the person doing the intervention, the outcome assessors, and the data analysts by asking them to guess participants' treatments and then comparing the answers with the actual treatments [34].

Other examples of blinded dietary intervention clinical trials are illustrated in Appendix 5.

In situations where it is impractical or impossible to blind the interventions, the investigator should limit other potential treatments as much as possible and instruct the participants to follow a standardized protocol [21]. The investigator should also maintain blinding with outcome assessors and statisticians. For example, a study on the impact of dairy consumption on health outcomes such as blood pressure or cholesterol cannot be double-blinded. However, sample and data analysis performed by researchers blinded to the treatment assignments will strengthen study integrity [35]. Such a study would be considered single-blinded.

Occasionally, there are unforeseen circumstances, such as an acute adverse reaction to a treatment, where emergency unblinding must occur. In such cases, blinding of an individual participant is broken to determine which treatment caused the acute side effects. Only limited research personnel (clinical coordinator or principal investigator) should be authorized to break blinding to maintain confidentiality and study integrity.

3.2.5 Defining the intervention and control treatments

Factors to be considered when designing the experimental intervention are safety, the possibility of blinding, use of single intervention versus multiple interventions, and generalizability of use in practice [21]. In addition, whether the dietary intervention will be delivered via a food category (such as fruits and vegetables), a food containing an inherent bioactive substance or a food containing an added bioactive substance must be considered [32, 36].

The following factors must also be considered when determining the type of food carrier to be used for delivering the bioactive ingredient:

- Source of the bioactive ingredient and its bioavailability. For example, phytosterols extracted from shea nut and rice bran are not bioavailable [37].
- Are the physical/chemical characteristics of the food carrier suitable to deliver the bioactive ingredient of interest? For example, phytosterols that are lipid soluble should either be added to a fatty carrier, or properly formulated by methods such as esterification to fatty acids [38].
- Food processing and storage conditions. For example, the chemical structure of soy isoflavones is altered during storage and processing, which results in lower bioactivity [39].
- The presence of other compounds that may affect the efficacy of the bioactive ingredient in humans. For example, the presence of prebiotics and milk components can improve probiotic survival in foods [40].
- Amount of bioactive component and/or the food to deliver it can be feasibly consumed as part of a healthy balanced diet.
- Will the incorporation of a bioactive component or food demand a change in the usual dietary pattern by eliminating or substituting another food?
- Level of dietary control: See Section 3.2.6 Dietary control and compliance.

Generally, use of a placebo provides the best comparison or control group/phase for food-based human clinical trials. This type of control ensures that any differences in outcome between study groups are attributed to the biological effect of the active ingredient or dietary intervention and not to other non-

specific factors [35]. The use of a placebo also facilitates participant and investigator blinding and thus reduces bias [35]. Placebo-controlled studies are capable of distinguishing adverse effects caused by the component of interest from those resulting from an underlying disease or condition [35].

Nevertheless, the study question or hypothesis, as well as the nature of the intervention, may necessitate the use of other kinds of control or comparison groups, including:

- **Standard or usual treatment:** Participants randomized to this comparison group will receive an active treatment, which can be a standard or usual treatment. Such trials are conducted to either show the efficacy of a new treatment or to compare two treatments. Using this type of control may require increasing the sample size in order to detect smaller differences than in placebo-controlled trials [21].
- **No-treatment:** Participants randomized to this comparison group will receive no treatment at all. A disadvantage of this comparison is that participants in this group may seek other interventions. Differences in outcome between treatments can be attributed to co-intervention and not only to the intervention of interest.

To demonstrate absolute efficacy of a bioactive dietary component, it should be concealed in a food product so that a control product can be used, whenever possible. When designing a placebo treatment for a dietary intervention, the placebo should be comparable to the active treatment in terms of energy, macronutrients and micronutrients, presence of other bioactive ingredients, and texture, colour, taste and smell. See also Section 3.5 Test product development and selecting a suitable control.

Example of treatments comparable in dietary composition in studies by Regand et al. and Korpela et al. are shown in Appendix 6. In the study by Regand et al., the oat and the wheat products were formulated to have an energy and nutrient composition as similar as possible [41]. In the study by Korpela et al., the control product was identical to the experimental product but without the added food ingredient of interest, in this case plant sterols [42].

3.2.6 Dietary control and compliance

Many levels of dietary monitoring exist to ensure that participants are compliant with the dietary intervention. Each level has specific characteristics (Table 1) as well as certain planning and expense considerations [43, 44]. These levels are:

- **Metabolic ward:** Participants live or stay for different periods of time in a metabolic unit where all food is provided and eaten in the unit.
- **Free-living:** There are two sublevels of free-living dietary interventions, both allowing participants to continue their normal lifestyle with the provision that either:
  - food is fully provided; the treatment foods and all or some meals are consumed in the research facility (free-living, eat-in/controlled feeding); or
  - food is to be freely selected with the exception of the treatment foods and the placebo.

Table 1. Features of degrees of dietary control

	Recruitment	Compliance	Method for determining/ estimating diet composition	Study duration	Cost	Generalizability
Metabolic ward	Hardest	Supervised	Determined	Short	Very costly	Low
Free-living -food provided and eat-in	Hard	Partially supervised	Determined and Estimated	Possibly long	Costly	Wide
Free-living -selection of food	Easier	Not supervised	Estimated via 3-day food record or FFQ	Long	Less expensive	Wider

FFQ = food frequency questionnaire

A metabolic ward setting is suitable for studies requiring frequent measurements that will be conducted over short periods of time [43]. Free-living settings provide wide generalizability and are more suitable to providing data for a food health claim submission. To increase the degree of compliance in free-living studies, the investigator can provide food and require participants to eat a portion of meals in the research unit 5 to 7 days a week. Overall, free-living, eat-in settings provide a high level of dietary intake control, which is important for clinical trials in which the data will be used for food health claim petitions. Nevertheless, studies on free-living populations where participants select foods freely can generate valuable data specifically on the effectiveness of a dietary component.

In a controlled feeding study, investigators should ensure that the following additional facilities are in place:

- Metabolic research kitchen with [43, 45]:
  - cooking facilities
  - kitchen weighing scales
  - workstation for packaging and preparing foods
  - insulated coolers and ice packs
  - enough storage space for refrigerated, frozen and dry food items
  - dishwashing and laundry facilities
- A separate dining room to seat several persons at one time
- Office space for clinical coordinator and/or dietitian equipped with computer, phone, fax and printer
- Other specific considerations for meal planning are:
  - to give variety, plan a rotating cycle menu of at least 3 days
  - select palatable and usual foods
  - use food recipes that are easy to prepare: re-heat and serve
  - select foods that are available throughout the study duration

See Appendix 7 for an example of a 3-day cycle menu.

See also Section 3.3.2 Compliance outcome measures, Section 4.4.4 Food distribution and compliance and Section 4.5 Compliance assurance and monitoring.

3.2.7 Length of intervention and overall study

The length of food-based human clinical trials can vary significantly depending on the study design, ranging from a few days or weeks to several months. Overall study length is determined by the duration of the enrolment period, the length of the feeding periods, sample size required to achieve the required study power, the number of treatments and whether the design involves run-in and/or washout periods.

During the enrolment period, participants are identified, screened and accepted into the trial. This process may take one month or more depending on the condition or disease of interest, the required sample size, and type and number of screening tests. The length of the feeding trial and the application of different study treatments should be long enough to demonstrate the intended effect on the outcome of interest. The duration of the dietary intervention should be sufficient to achieve a stable steady state of both the dietary component and the outcome [46].

See Appendix 8 for the estimated time required to demonstrate the effect of dietary intervention on selected outcomes.

A run-in period is a pre-randomization period during which all participants are placed on placebo or given a standardized diet for a specified time. The advantages and disadvantages of including a run-in period should be considered during the design stage.

- Advantages of including a run-in period:
  - Participants experience the study protocol and so non-adherent participants and early dropouts can be excluded from the study before randomization; consequently, the power of the study is increased [47].
  - All participants begin the trial by consuming the same placebo or control diet, and the effects of any dietary style are minimized before feeding the experimental diets [43].
  - Run-in periods are useful to estimate energy needs of participants and to make adjustments to maintain initial body weight if it needs to be stable [48].
  - Run-in periods are also useful to increase the intake of certain dietary components, such as beans and fibres, in a gradual fashion to minimize any side effects that could appear from giving large doses at baseline.
- Disadvantages of including a run-in period:
  - Run-in periods increase the study duration and costs.
  - They also compromise generalizability and applicability as some participants with certain characteristics may be excluded from the trial [49].

For crossover studies, a washout period can be incorporated whereby no treatment or the placebo treatment is consumed for a specified period of time between treatments. This minimizes any carryover effect induced by a previous treatment to the next treatment [20]. The length of the washout period should be sufficient to allow measured outcomes to return to baseline values or become re-stabilized. Generally, the duration of the washout period is the same as the feeding period. Introducing a washout period increases the overall study time [43].

Sample size also has a considerable impact on overall study duration. When a feeding trial includes large numbers of participants, the investigator must divide participants into groups, each processed in a staggered order, to efficiently use the available facilities. An alternative option would be to conduct a multicentre trial [50]. See also Section 3.2.10 Multicentre controlled feeding trials.

3.2.8 Defining the statistical approach

Investigators must decide in the design stage whether the results of the clinical trial will be analyzed on an intention-to-treat or per-protocol basis. An intention-to-treat analysis compares outcomes between study groups with every participant analyzed according to his or her original randomized assignment group, including the dropouts. Although this analysis includes participants who did not comply with the study protocol, it minimizes bias caused by loss of participants. A per-protocol analysis includes only participants who were fully compliant with the study protocol. This type of analysis is able to measure the maximum efficacy of an intervention [21, 51].

According to the quality appraisal tool for intervention studies in Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims*, an intention-to-treat analysis is preferred over per-protocol analysis [2]. However, if the study reported no participant attrition, it is appropriate to conduct a per-protocol analysis as an intention-to-treat analysis is not applicable [21, 51]. See also Section 3.4.1 Sample size calculation and Section 5.2 Data manipulation and statistical analysis.

3.2.9 Pilot studies

Pilot studies are small studies used to pretest the actual study methods and to provide training and experience for investigators and clinical coordinators. Pilot studies are conducted on a small number of participants and cannot be used to test a hypothesis and to evaluate efficacy of an intervention. Aspects of a clinical trial that can be pre-evaluated by a pilot study include feasibility of recruitment, randomization, assessment procedures, new methods, and introduction of a new intervention [52].

In controlled-feeding trials, pilot studies are also useful for assessing different aspects of a feeding regime, including recipe development, verifying the composition of menus, assessing the feasibility of blinding, and testing the acceptability of new foods [43]. Conducting a pilot study will increase the cost and overall duration of a trial. However, a pilot study can improve compliance, as well as the overall success and quality of a clinical trial, by discovering any impractical issues or challenges ahead of time. See also Section 3.4.1 Sample size calculation, Section 3.5.2 Appropriate and effective dose and Section 3.5.6 Test product development pilot studies.

3.2.10 Multicentre controlled feeding trials

Multicentre randomized clinical trials (MRCTs) use one standardized protocol at multiple sites. This type of clinical trial ensures an adequate sample size to detect significant treatment differences and widens the pool of participant characteristics. Consequently, it will yield an accurate and precise effect size and results will be more generalizable [53, 54]. When designing MRCTs, one must take into consideration the diversity among regions regarding medical practice, disease epidemiology, race or ethnicity, social status, culture and food

Appendix 9 lists three examples of multicentre feeding trials—the Dietary Approach to Stop Hypertension (DASH) trial, the Dietary Effects on Lipoproteins and Thrombogenic Activity (DELTA) trial, and the Dietary Portfolio trial.

preference as they may differ substantially and possibly affect study outcomes [55]. This will improve the representativeness of the study sample and allow for better extrapolation of the study findings to the general population.

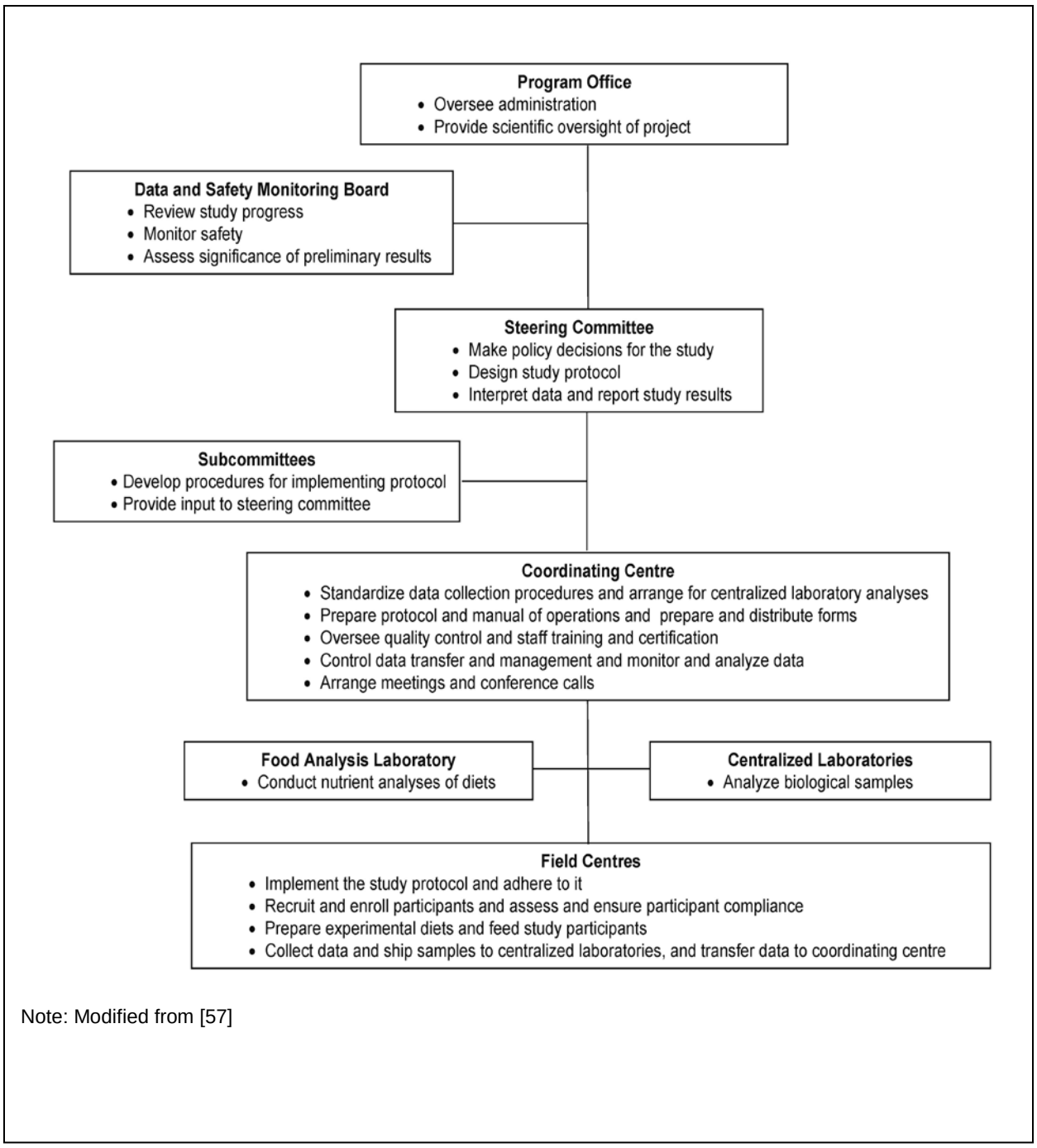
Two key groups involved in multicentre trials include [53]:

- **Steering committee**, which is responsible for making policy decisions, designing study protocol, and interpreting and reporting study results; and
- **Coordinating centre**, which is responsible for preparing the protocol and manual of operations, training staff, standardizing data collection procedures, ensuring quality control, and arranging for centralized laboratory analyses.

Other units required for conducting a multicentre feeding trial include a program office, a data and safety monitoring board, subcommittees, a food analysis laboratory and centralized laboratories for specific or all outcomes, and field centres. (See Diagram 4.)

Essential steps involved in conducting a multicentre controlled feeding trial are similar to single feeding trials, but the following critical organizational issues in MRCTs require attention and are recommended [56, 57]:

- Ensure that each centre has an on-site research kitchen, specific to feeding trials, or has access to an alternate facility kitchen and sufficient food storage space. Alternatively, foods/treatments can be prepared centrally and shipped to various sites.
- Carefully determine the number of staff required and their period of employment for each centre.
- Implement similar menus and foods in each centre, accounting for cultural food preferences and regional food availability.
- Standardize food preparation and cooking procedures.
- Conduct taste tests for selected food items at each centre in order to replace or modify any unacceptable foods in specific centres.
- Arrange for central procurement with distribution to the different centres for foods that are highly variable in a critical nutrient or dietary component.
- Verify chemical composition of diets during menu development and throughout the study by chemical analysis of all samples collected from each centre performed in the same laboratory.
- Ensure that all centres operate according to the same procedure (guidelines for all participants, feeding, other foods allowed, time of visits, and compliance assessment).
- Communicate regularly.



Note: Modified from [57]

Diagram 4. Organizational structures of the Dietary Approach to Stop Hypertension (DASH) study and the function of each unit

BEST PRACTICES KEYPOINTS

Choosing the optimal study design

- Key elements of study design for intervention studies that should be carefully considered so the data can be used in support of a food health claim are:
  - appropriate type of study (i.e., parallel-arm vs. crossover) for research question
  - appropriate method of randomization
  - allocation concealment and method of blinding
  - level of dietary control (i.e., full-feeding vs. free-living)
  - type of statistical analysis method (i.e., intention-to-treat vs. per-protocol).
- Pilot studies may be useful to pre-evaluate many aspects of study feasibility and thus improve the overall quality and success of the trial.
- Multicentre randomized clinical trials can detect treatment efficacy over a large population and increase the generalizability of a health effect, which is ideal for food health claim substantiation.

3.3 Identification of outcome measures

Outcome measures are indicators measured in a participant or a biological sample that assess the efficacy or safety of an intervention. Selection of the outcome to be measured can influence the reliability and interpretability of clinical trials [58]. Generally, three different types of outcome measures are important: primary outcomes, compliance outcomes and adverse event outcomes.

3.3.1 Primary outcome measures

The primary outcome is used to answer the principal research question and to calculate sample size. The primary outcome should be well defined and reliable for assessing important aspects of health, sensitive to the effect of the intervention, and measurable and interpretable [58]. The most powerful outcomes of a trial are clinical endpoints such as death, hospital admission, and myocardial infarction in coronary heart disease [58]. However, measuring such outcomes will increase study duration, sample size and cost, and are largely dependent on the research question and intervention being investigated [59].

Alternatively, biomarkers, surrogate endpoints (biomarkers that are intended to substitute for a clinical endpoint) or indirect measures that predict disease risk can be used. The chosen surrogate endpoint for a treatment must be valid by having a proven relationship with the clinical outcome of the disease of interest, which is demonstrated through:

- biological plausibility as established through basic research and mechanistic trials in humans [60];
- statistical proof from epidemiological data that the surrogate is a risk factor of the disease and that alteration in the level of the surrogate reduces mortality or major morbidity [61];

- estimation of anticipated clinical benefit, predictable from a change in that surrogate biomarker [60];
- validation of its analytical method so that the biomarker is well defined, convenient, easily quantifiable and reproducible [60]; and
- confirmation that the presence or absence of unanticipated outcomes in response to the treatment is unrelated to the surrogate biomarker, such as toxicity [62].

To evaluate a new surrogate endpoint based on the above-mentioned criteria, investigators can use various statistical approaches such as:

- **Bayesian analyses**, which combine information across studies when it is reasonable to make assumptions about distributions of the parameters that characterize the relationships under study [63]; and
- **Biomarker-Surrogacy Evaluation Schema**, which evaluates and ranks the surrogacy status of biomarkers and surrogate endpoints using defined levels of evidence [64].

  (Note: The Institute of Medicine has also recently proposed an evaluation process for biomarkers [65].)

When selecting biomarkers for studying a food or dietary components for a potential claim, Health Canada requires the use of validated surrogate biomarker(s), whenever it is impossible to measure a true clinical outcome. Several documents have recently been published on appropriate surrogate outcomes for certain diseases and conditions [36, 66].

Table 2 outlines some of the variables that have been proposed as surrogate biomarkers for certain diseases and conditions.

Table 2. Validated surrogate biomarkers in certain diseases and conditions

Disease	Biomarker	Reference
Cardiovascular disease	LDL cholesterol Total cholesterol Blood pressure	Aggett et al., 2005 [36] FDA, 2009 [66]
Osteoporosis	Bone mineral density	Aggett et al., 2005 [36] FDA, 2009 [66]
Cancer	Pre-cancerous lesions	Aggett et al., 2005 [36]
Colon cancer	Adenomatous colon polyps	FDA, 2009 [66]
Type 2 diabetes mellitus	Elevated blood sugar concentrations Insulin resistance	FDA, 2009 [66]

Trials may have additional outcomes to measure different aspects of the intervention effect, known as secondary or tertiary outcomes. However, *a priori* sample size is generally calculated to determine power for primary outcomes; therefore, if the study includes secondary and tertiary outcomes, it is important to ensure the sample size can adequately investigate the impact of additional outcomes.

Outcomes can be measured at the beginning of a trial and at the end of the follow-up period to observe an effect in response to time. Outcomes can also be measured many times during the follow-up period to allow detection of any changes over time, such as when an intervention treatment effect begins to plateau.

3.3.2 Compliance outcome measures

In addition to measuring outcomes related to treatment efficacy, several other outcomes can be measured to assess different aspects of a feeding trial, such as compliance and adverse effects [21]. In free-living trials, monitoring of participants' compliance with the intake of food or dietary component under study is essential to validate study results. Biomarkers of intakes or food exposure can be blood, tissue, urinary, or fecal levels of the nutrient or dietary component under study, or its metabolite.

Table 3 outlines validated biomarker measures of dietary intakes or food exposure. In addition, a marker can be added to the food that can be detected in blood, urine or breath such as para-aminobenzoic acid, which is excreted in urine. Other measures that can be used as biomarkers are urine osmolality and urinary sodium levels [32]. See also Section 3.2.6 Dietary control and compliance, Section 4.4.4 Food distribution and compliance and Section 4.5 Compliance assurance and monitoring.

Table 3. Validated biomarker measures of dietary intakes or food exposure [32]

Component	Biomarker
Energy	Carbon dioxide production using doubly labelled water
Protein	Urinary nitrogen levels
Fatty acids	Plasma and erythrocyte fatty acid composition
Fibre	Stool hemicellulose levels
Retinol	Retinol plasma levels
Vitamin E	Vitamin E plasma, adipose levels
Vitamin D	25-OH D plasma levels
Vitamin C*	Vitamin C plasma levels
Vitamin B6	Vitamin B6 plasma levels
Folic acid	Folic acid serum, erythrocyte levels
Selenium	Selenium serum, toenails
Iron	Iron serum levels
Sodium	Sodium urinary levels (24 h)
Calcium	Calcium urinary levels (24 h)
Potassium	Potassium urinary levels (24 h)
Magnesium	Magnesium urinary levels (24 h)
Polyphenols*	Post-prandial polyphenol plasma and urinary levels
Lycopene*	Lycopene plasma levels
Alpha-carotene*	*Alpha*-carotene plasma levels
Beta-cryptoxanthin*	*Beta*-cryptoxanthin plasma levels
Lutein/zeaxanthin*	Lutein/zeaxanthin plasma levels
Beta-carotene*	*Beta*-carotene plasma, adipose levels

*Can also serve as a biomarker for fruit and vegetable intake

3.3.3 Adverse event outcome measures

Investigators should also measure outcomes that can detect any adverse effects related to intake of the food or dietary component under study. Adverse effects may range from minor symptoms to serious complications [21]. Outcome measures that can be used to track adverse effects include simple measures of clinical biochemical indices that are routinely done (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, lactate dehydrogenase, total protein, albumin, total bilirubin, creatinine, urea, glucose, iron, sodium, potassium and chloride) or hematological parameters (hemoglobin, hematocrit, leukocytes, erythrocytes, mean corpuscular volume,

mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, prothrombin time and platelets).

Other possible outcomes include subjective measures of participant well-being evaluated by direct questionnaires that contain broad, open-ended questions about all types of potential adverse effects [21]. For example, the clinical coordinator can ask the participants questions such as “Have you had any adverse events since your last visit?”

BEST PRACTICES KEYPOINTS

Identification of outcome measures

- Choosing appropriate outcome measures involves selecting surrogate biomarkers that will demonstrate a relationship between the effect of the test treatment and the disease or health condition of interest.
- Outcome measures should demonstrate:
  - biological plausibility
  - statistical proof
  - method validation
  - absence of any alternative effect.
- Outcome measures other than those used to determine treatment efficacy may be tested to measure compliance and/or to detect adverse effects.

3.4 Selection of study population

Several factors should be considered when selecting a study population for a food-based human clinical trial. One critical factor is ensuring that the participant cohort provides a good representation of the population of interest and a suitable scientific platform to test various food components for physiological health effects.

For clinical studies to support a food health claim, the study population must be representative of the population for which the food health claim will be applied, in this case for the Canadian population. In Canada, the population demographic is diverse geographically, economically and ethnically, so it is important to ensure that a study population is not biased toward a particular demographic and remains applicable to all citizens.

The study population must also be relevant and generally representative of the overall health status of the Canadian population. As an example, the barley health claim accepted by Health Canada was substantiated with studies using healthy normocholesterolemic and hypercholesterolemic participants. This was considered to be generalizable to the Canadian population as a high proportion of the Canadian population (about 40% of Canadian adults aged 20 to 79) has unhealthy total cholesterol levels (>5.2 mmol/L) and is at higher risk for developing heart disease. Conversely, if a study uses a diseased

population, such as people with diabetes, the claim wording should be specific about the target population. Study findings based on a diseased population are usually not applicable to the general Canadian population.

3.4.1 Sample size calculation

Once the study population has been selected, a power analysis is necessary to determine the optimum number of participants that will allow conclusions to be drawn with the highest degree of confidence; it allows for the detection of the hypothesized intervention effect of a food-based human clinical trial within a set level of confidence, when such an effect exists. It is recommended that a power analysis be done at the design stage of the study (*a priori*). It is done to determine sample size using the background variation from pilot studies or previously conducted studies of similar context, the desired level of power, detectable effect size and significance level. Estimating sample size for an RCT is directly linked with the study design and the planned analysis. It can be complex when repeated measures are correlated over time. Sample size estimation also must not be based solely on the number of participants who have been included in previous studies in the same area.

The power of a study is the probability of rejecting the null hypothesis when the alternative hypothesis is true (power = 1-β = P (reject H_0 | H_1 true); where β is Type II error; H_0 is the null hypothesis; H_1 is the alternate hypothesis). A null hypothesis is a proposition that implies no effect or no relationship is observed between a treatment and proposed outcome. A null hypothesis can be tested and found to be false, which implies that an effect or relationship is present between treatment and health effect.

An alternative hypothesis states the opposite of the null hypothesis and implies that a relationship exists between treatment and outcome. When a null hypothesis is tested and found to be false, it implies that the alternative hypothesis is true. Type I error is the incorrect rejection of a null hypothesis when the null hypothesis is true, and Type II error is the failure to reject a false null hypothesis, implying that the null hypothesis is true whereas the alternative is actually true.

The power of a study can be affected by the significance level (**α**), the effect to be detected (**δ**), the variation in the outcome ($\boldsymbol{\sigma^2}$), the sample size (**n**), and whether it is a one-tailed or two-tailed test [67]. Generally, the power of an experiment increases when the sample size, significance level and effect to be detected are high, when there is little variation, and when the test is a one-tailed rather than a comparable two-tailed test.

Here is a simple example of how power and sample size calculations can be performed [67]:

$$Power = (1 - \beta) \propto \frac{\delta\alpha\sqrt{n}}{\sigma}$$

And can be rearranged to solve for n, or sample size,

$$n \propto \frac{\sigma^2(1 - \beta)}{\alpha\delta}$$

Given this equation, it can be implied that sample size is proportional to the variation in the outcome, scaled by the power, partitioned by the significance level and effect to be detected. These factors are required to determine sample size.

A worked example of an *a priori* power analysis test used to determine the optimum number of participants required for a food-based human clinical trial to detect an intervention effect at a desired power level of 0.95 is shown in the box **Example of an *a priori* power analysis test**. (Note: the example shown below is for a one-tailed t-test. A statistician should be consulted for more complicated power analyses.)

Example of an *a priori* power analysis test

Effect of plant sterol consumption versus control on lowering LDL-C concentrations in humans

Pilot study:

- **Variance of LDL-C (σ^2) = 0.5 mmol/L**
- **Mean of LDL-C (μ) = 5.0 mmol/L**

Aims:

- to detect 15% decrease in LDL cholesterol due to plant sterol supplementation (i.e., LDL cholesterol decreases from 5.2 mmol/L to 4.5 mmol/L and improving lipid profile to reduce cardiovascular risk), therefore detectable effect size is 15%, or **δ = 0.15**
- to ensure that the effect of decreasing LDL cholesterol by the desired effect size is measurable with a 95% confidence, therefore power = 0.95 or (1- 0.05); type 2 error or **β = 0.05** (Note: the level of confidence can vary from 80 to 95%;the value for β will vary accordingly.)
- type 1 error is defined at 5%, P<0.05; **α=0.05**

How many replicates, or participants, would be needed in each treatment to achieve these parameters?

$$n \propto \frac{\sigma^2(1-\beta)}{\alpha\delta}$$

$$n \propto \frac{0.5(1-0.05)}{0.05 * 0.15}$$

$$n \propto \frac{0.475}{0.0075}$$

$$\mathbf{n \propto 63}$$

63 is the minimum total number of participants required to achieve a 15% detectable effect with a power of 0.95. (An adjustment for expected attrition should be factored into the number of participants required for the trial.) (See also Section 4.3.2 Estimating dropout rate and Section 4.8 Management of dropouts and missing data.)

3.4.2 Eligibility criteria

After determining sample size, the next step is to develop a comprehensive and concise list of inclusion and exclusion criteria. This step is crucial for compiling a participant cohort that will be an ideal target population to test the study hypothesis, treatment and outcome measures of the research project.

Inclusion and exclusion criteria are factors based on physiological, anthropometrical, functional, demographic and lifestyle characteristics to define a study population. Eligibility criteria for food-based human clinical trials usually include variables such as:

- age;
- gender;
- health status (including presence or absence of specific diseases);
- medication and/or NHP use;
- anthropometric and biochemical values (which would typically imply blood screening and measurements to obtain these values); and
- dietary and lifestyle habits such as smoking, alcohol consumption, physical activity, food preferences and allergies.

Although the development of concise eligibility criteria for clinical trials is common practice, inclusion and exclusion criteria are sometimes not reported in scientific publications, especially the quantifiable ranges of clinical and anthropometric values used.

It is recommended as good practice to clearly define all eligibility criteria, including acceptable clinical ranges for biomarkers (i.e., LDL cholesterol ≤5.2 mmol/L; fasting blood glucose ≤6.1 mmol/L). Depending on the study design and research question, the use of certain medications and NHPs is an important eligibility criterion as these substances can potentially be study confounders. For example, if the objective of the study is to investigate a food product's ability to lower cholesterol, the use of lipid-lowering medications could dramatically confound results, either deeming the food product ineffective or unsafe, depending on the research objectives.

It is also common practice for food-based human clinical trials to include the term "healthy" or "in good health" as an inclusion criterion. This is highly subjective as "healthy" can range from specific biomarker values to simply the absence of disease related to the outcome. Best practice is to provide a definition for "healthy" in the context of the study. A clear rationale for the chosen eligibility criteria should always be established and stated to allow full understanding of the study target population. Although restricting the eligibility criteria in order to collect a uniform cohort will precisely test the hypothesized effect of treatment, it may ultimately lead to significantly limiting the target population. This can complicate recruitment and reduce the applicability to the population as a whole [68].

Examples of best practices in selecting and reporting a study population are outlined in Appendix 10.

BEST PRACTICES KEYPOINTS

Selection of study population

There are three essential components for selecting a study population for a food-based human clinical trial:

- Determine the number of participants required to provide a level of power with which the study can detect meaningful changes in study outcomes via power analysis.
- Develop a set of robust, comprehensive eligibility criteria to assemble a participant cohort that will precisely test the study hypothesis.
- Select a target population that is representative of the demographics of the target population, so that the results of the food-based human clinical trial intended to support a potential food health claim can be generalizable to the Canadian population at large, or a subpopulation as appropriate.

3.5 Test product preparation and suitable control selection

Formulation of the research test product, or treatment, for a food-based human clinical trial requires extensive research, planning and preparation to successfully develop a treatment that will effectively assess the health properties of the test food or food constituent. Some developmental steps must be systematically followed during formulation of the test treatment as they are key components of a food-based human clinical trial. These include dosage and biological effect, food safety, storage practices, participant acceptability and reduction of batch-to-batch variability. Test food research and development should be performed in facilities intended for food preparation and production which are Hazard Analysis and Critical Control Point-accredited.

3.5.1 Intention for use

The intended use of the food product being investigated must be established and will usually determine how the test food product will be administered during the research study. For example, if the test product is a cooking oil, such as olive oil or canola oil, the test product can be incorporated into a part of the research diet that will be consumed as naturally intended, such as a salad dressing or consumed in a mixed meal, rather than in an unusual preparation like an extract. Conversely, when the test product must be double-blinded but blinding is a challenge, such as for soy or cow's milk, it may be necessary to incorporate the test product into a formulation that may not be typical, such as a pudding or shake. If a particular test product would generally be consumed once a day (at breakfast, for example), this pattern should be reflected in the study design and followed through the feeding stage. The way in which the test product will be used should also be discussed with the sponsor or industry partner.

3.5.2 Appropriate and effective dose

The appropriate dose required to achieve a physiological effect must be determined based on data from previous research studies, if available. The bioavailability and any other physiological effects related to dosage must be taken into account. Additional factors to assess when determining an effective dose are whether the amount to be consumed will be palatable and whether the dose administered would be feasible for normal consumption. Once the test product dosage has been determined, it is best practice for food-based human clinical trials to report the rationale and appropriate evidence for this treatment dosage [18]. If there are no available data to determine the effective dosage, pilot studies for that purpose are strongly recommended. See also Section 3.2.9 Pilot studies, Section 3.4.1 Sample size calculation and Section 3.5.6 Test product development pilot studies.

Specifically for feeding trials, it is important that the test product dose be incorporated into a food where the serving size is appropriate, if incorporation is the means of administration. For example, if plant sterols were to be incorporated into a yogurt product, it would be appropriate to provide this treatment in a 100 g serving such as yogurt snacks that are readily available in the marketplace.

Significant consideration must be given to the appropriate vehicle in which a test product will be incorporated, its potential interaction with other dietary ingredients, and the desired nutritional profile. For instance, if the composition of the test product were a lipid, choosing a vehicle that will allow the lipid to disperse or emulsify to retain the original structure, texture and palatability for blinding and acceptability purposes would be the most suitable. The food matrix chosen to house the test product should not drastically alter the desired macronutrient profile of the study diet, especially if body weight and composition are endpoint outcome measures or potential confounders of the outcome measure.

The macronutrient composition of the test product should be incorporated into the overall desired daily macronutrient and caloric intake so as to maintain body weight and related metabolic processes. For instance, if the test and control product contains 30 g of fat per day, this amount must be integrated into the total daily fat intake of the predetermined diet composition (i.e., 20–35% of total daily calories) or body weights and lipid metabolism may be skewed in a manner that may confound results. See also Section 3.2.5 Defining the intervention and control treatments.

The serving size must also be able to accommodate the determined dose of the test product without disturbing the matrix of the treatment food vehicle as a whole. For example, adding 30 g of oil into a 100 g serving of yogurt will produce an oily, unpalatable product, possibly affecting compliance. Therefore, careful attention must be given to the treatment matrix as well as the serving size. If the study design has determined that the food bioactive will be administered several times throughout the day, it is recommended that several different incorporations of the food bioactive—such as a yogurt, shake, bread or cookie—be developed to provide variety.

3.5.3 Safe food-handling and storage practices

Considerable attention must be given to food safety when developing a test treatment intended for human consumption. Specific factors to assess for treatment development would be assurance of food safety and elimination of food toxins (e.g., salmonella, pathogens, mycotoxins), the stability of the test product and how to preserve the test product safely by freezing or other storage practices [69].

To ensure the test and control treatments are safe for consumption, food microbiological tests should be done on the final test product, including tests for contamination with *Salmonella enterica*, *Escherichia coli*, yeasts and moulds, deoxynivalenol and other vomitoxins and total bacterial aerobic plate count. These tests can be outsourced to a reputable food microbiology-testing laboratory or performed in-house by trained laboratory technicians specializing in food microbiology assays. To avoid contamination of the test and control treatments during development and administration, proper food-handling and food storage practices must be followed to prevent cross-contamination with foodborne pathogens, specifically *Salmonella* and *E. coli*, and to prevent growth of mycotoxins due to improper storage humidity and temperature [70].

In addition to ensuring food safety, the method of storing and preserving the test and control treatment is also important for food stability and retaining the intended food matrix for the intervention trial. Choosing the correct food storage method must be considered in a food-specific manner as some methods may differentially alter certain food properties, such as texture, viscosity and other rheological parameters, and usually affect consumer acceptability and compliance in the long run. For example, low moisture test products such as chips or crackers would benefit from dry cool storage like freezing, whereas yogurt, margarine and other dairy treatments should be refrigerated, and oil treatments should be stored away from heat and light to protect them from spoilage and bioactive degradation.

Test and control products suited for prepackaged and frozen storage can be administered as needed during the trial, significantly increasing efficiency of food production and distribution. Proper food storage to maintain food stability is especially important during long-term feeding trials, and when test and control products are produced on a large scale in big batches where the remainder is stored for future consumption [71].

Most importantly, conducting bioactive viability tests on the test product at various times during storage is recommended to determine whether the bioactive has been compromised due to lengthy storage times. For example, if a test product is to be frozen at -20°C for 3 months after processing, either for the feeding trial or commercial storage, it should be known whether the bioactive in the test food is still viable; therefore, it should be tested before freezing and again after being thawed. Further analysis is also recommended to assess the viability and stability of macronutrients of the test food in relation to storage length and method. It is recommended that samples of the control and intervention foods be stored in archive in the event that further analyses are required during the reporting phase of the study.

3.5.4 Batch variability

Minimizing variability between test product batches is a common challenge for long-term feeding trials, especially when transitioning from the small-scale development processes to production of large batches of the test treatment in preparation for administration. Food-specific procedures that will help to reduce variability should be followed; these include quality control testing between batches, appropriate sizing of batches and standardized procedures for preparation and administration of test and control treatment [72]. High variability between test or control treatment batches weakens the study integrity, especially if batch variability alters treatment dosage or serving size, possibly leading to inconsistent physiological effects. Thus, strategies must be enforced to minimize variability for each test and control treatment. These may include tracking macronutrient levels and performing proximate analysis whenever new batches of ingredients are purchased. For example, in a study exploring the role of dietary fibre it would

be important to ensure that there is minimal batch-to-batch variation in the various starch fractions of the foods being administered.

3.5.5 Selecting a suitable placebo or control

Selecting a suitable placebo or control for food-based human clinical trials is crucial at the design stage as the appropriate control treatment will determine how effective the test product is when outcome measures are analyzed. The control treatment must be completely void of all bioactive components that the trial is investigating but hedonically resemble the test product as closely as possible (e.g., texture, taste, smell) to maintain blinding among participants and investigators.

Maintaining blinding may become difficult if the test product is a whole food rather than a food constituent, but test and control treatments can be packaged and coded identically to conceal their identity. The control treatment should not be able to alter outcome measures. While the test and control treatments should have equal energy densities and analogous sensory characteristics, the control treatment should not have any components that may have physiological effects of their own.

Table 4 outlines basic examples of test foods and their appropriate control foods for a feeding trial. However, depending on the outcome of interest, the control food may be modifiable.

Table 4. Examples of test foods and suitable control foods

Test food	Control food	Rationale for choosing the control food
Pulses; peas, beans	Potatoes	• Lacks bioactive • Similar intended use • Similar food matrix, texture
Oat bran	Wheat bran	• Identical fraction • Lacks bioactive
Oatmeal	Glucose solution	• Ideal reference for effect on blood glucose
Soy protein	Milk protein isolate	• Identical fraction • Different food origin • Similar intended use
Soy milk	1% Cow's milk	• Similar use • Different food origin
Fish oil	Corn oil	• Similar use • Different food origin • Lacks bioactive
Probiotic yogurt	Probiotic-free yogurt	• Identical use • Lacks bioactive
Omega-3 eggs	Regular eggs	• Identical use • Lacks bioactive

3.5.6 Test product development pilot studies

Once test and control treatments have been designed, produced and tested for safe consumption, a small pilot study should be conducted before the official food-based human clinical trial. Pilot studies are able to identify any issues or challenges related to the production and quality of developing test and control treatments as well as issues with participant acceptability. This process allows for the modification of aspects of the study design found to be problematic during the pilot study, before the human trial is started [73]. For example, if a test product is assessed via a pilot study to satisfy palatability but it is found to be difficult or costly to produce, this element can be changed. See also Section 3.2.9 Pilot studies, Section 3.4.1 Sample size calculation and Section 3.5.2 Appropriate and effective dose.

BEST PRACTICES KEYPOINTS

Test product preparation and suitable control selection

- Establish intended use of the food product being investigated.
- Determine an appropriate dose required to achieve a physiological effect and ensure bioavailability, while still being an appropriate serving size typical for normal consumption.
- Use various methods for enforcing safe food-handling and storage practices and to minimize batch variability.
- Determine appropriate control treatment to effectively compare differences to test the food product.
- Implement test product development pilot studies to test feasibility of various elements of the food-based human clinical trial, focusing on quality control, production and participant acceptability.

4.0 Clinical Trial Implementation

SUMMARY

- For all clinical trials, researchers should dutifully employ the guidelines and policies defined in the Tri-Council Policy Statement regarding the ethical conduct of research involving humans, including:
  (See Section 4.1 Application and approval of research ethics/regulations)
  - Obtain informed consent for participation (Section 4.1.1)
  - Maintain confidentiality of participant privacy and data (Section 4.1.5)
  - Outline appropriate compensation for participation (Section 4.1.6)
- For all clinical trials, researchers must obtain approval from their institutional Research Ethics Board (Section 4.1.3)
- All clinical trials must be registered in a publicly accessible, recognized clinical trial database (Section 4.1.4)
- Appropriate staffing requirements must be considered for the successful planning and execution of a food-based human clinical trial
  (See Section 4.2 Administration and human resources for clinical trials)
- Appropriate recruitment strategies are needed to acquire adequate participation (Section 4.3.1)
- Screening of prospective participants is mandatory for determining whether eligibility criteria have been satisfied (Section 4.3.3)
- Allowance for dropouts must be configured into the number of participants recruited to ensure that sample size and power of experiment are not jeopardized (Section 4.3.2)
- It is important to consider the energy requirement for each participant when designing, producing and delivering research diets (Section 4.4.1)
- Food preparation must be implemented in a standardized manner and distribution of meals must be done according to participant identifier code (Section 4.4.3)
- Compliance of consumption can be monitored by inspecting the contents of the returned food containers and daily food records (Section 4.4.4)
- Methods for monitoring, promoting and assessing compliance should be implemented throughout the clinical trial to minimize protocol deviation by participants
  (See Section 4.5 Compliance assurance and monitoring)
- Implementation of a formal data management strategy is recommended to maintain current and organized study records while protecting the privacy of participants and maintaining confidentiality of data during and after trial completion
  (See Section 4.6 Record keeping and database management)

- Sample collection must follow a standardized protocol, using confidential identifier codes to ensure correct sampling from participants followed by appropriate storage practices to ensure sample stability
  (See Section 4.7 Collection, labelling and storage of samples)
- It is important to implement strategies to manage dropouts and prevent further attrition
  (See Section 4.8 Management of dropouts and missing data)
- Robust statistical analyses should be used to assess outcome measures in trials with attrition to prevent selection bias and maintain data integrity
  (See Section 4.8 Management of dropouts and missing data)
- Adverse events should be recorded immediately and a report should be filed with the concerned ethics committee, concerned authorities and funding sponsor within 7 days if the adverse event is fatal or life-threatening or within 15 days if it is not
  (See Section 4.9 Reporting of adverse events)
- Post-experiment communication with participants is useful and needed for informing volunteers of study results, thanking them for their participation, obtaining informed consent for future participation, supplementary analyses and retrospective studies
  (See Section 4.10 Post-experiment communication with participants)

4.1 Application and approval of research ethics/regulations

4.1.1 Tri-Council Policy Statement

Research involving humans brings with it the immense responsibility of maintaining high scientific and ethical standards to safeguard the respect for human dignity. This responsibility forms an important underlying value of the *Tri-Council Policy Statement (TCPS): Ethical Conduct for Research Involving Humans* [74]. The principle of respecting human dignity in the TCPS is reflected in its three core values:

- Respect for Persons
- Concern for Welfare
- Justice

These values are to be applied in all cases. The extent to which each is applied will depend on the type and invasiveness of the research.

The first core value, Respect for Persons, acknowledges the respect and courtesy that is duly warranted for all people. This value focuses on the moral responsibility to respect and maintain an individual's autonomy and to protect those whose autonomy is developing, weakened or declining, and is facilitated by obtaining participant informed consent. The informed consent to participate in a research trial must be based on a full understanding in lay terms of the type of research, its purpose and what it entails for the participant, specifically outlining all benefits and risks associated with the proposed research.

Obtaining informed consent from participants for a research trial ensures that they understand the conditions of the trial and have chosen to participate voluntarily and as such have exercised their right of autonomy.

For examples of typical required elements of an informed consent form and template, see www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/consent/index-eng.php and www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/applic-demande/_form_docs/e-eng.php, respectively.
If the study design requires genetic testing, a separate informed consent form must be completed; for an example see www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/consent/h-eng.php.

The second value, Concern for Welfare, describes the responsibility of the researcher and the Research Ethics Board (REB) to respect and protect the welfare of the participant—mentally, physically and emotionally—during the research trial. REBs are institutional review boards that oversee, enforce and regulate good ethical practices in academic, industrial and medical research. REBs determine ethics approval in all research trials. Most institutions (e.g., hospitals, public health and government departments, and universities) will have an internal review board but independent institutional review boards also exist. In the design phase of the research trial, the principal investigator and the research team must try to minimize any possible risks and outline the associated benefits the participant may experience. The REB will not approve a research trial with an unfavourable balance of risks to benefits.

The final value, Justice, emphasizes the responsibility of the principal investigator and the research team to treat people fairly and equitably throughout the research process. This value stresses the equal distribution of burden and benefits among participants during the research trial, along with treating each participant with equal respect and consideration. This value is tested in the recruitment process, as inclusion and exclusion of participants must be based on the research question. Any arbitrary exclusivity not based on the previously determined criteria demonstrates inequality toward prospective participants.

An online tutorial, Course on Research Ethics (CORE), which provides an applied approach to the guidance provided in the TCPS can be found at www.pre.ethics.gc.ca/eng/education/tutorial-didacticiel/.

These three core principles must be practised and reinforced throughout the research process to ensure the respect for human dignity and safeguard the ethical conduct of research. These elements form the foundation of the TCPS and every well-designed feeding trial [74].

4.1.2 Assessment of invasiveness

During the design stage of a clinical trial, the level of invasiveness required to test the research hypothesis needs to be assessed. Generally, for food-based human clinical trials, the extent of invasiveness is limited to blood draws; however, it can occasionally include stable isotope administration, dual energy X-ray absorptiometry, magnetic resonance imaging or the application of intravenous lines. If multiple time points and phases are included in the study design, these procedures can quickly heighten the invasiveness to a point that may threaten recruitment or retention of study participants. Researchers must be fastidious in determining the minimum level of invasiveness needed for sufficient sample collection, as REBs carefully review this subject and any unnecessary invasiveness identified will need to be revised.

4.1.3 Research ethics approval

Before starting any clinical trial, the study protocol must be approved by the institutional REB where the trial will be conducted (e.g., university, hospital, clinic). This process is initiated when the REB application outlining the research proposal is submitted in detail. In addition to the study proposal, a REB application should also include the informed consent form, all data collection tools to which the participant will be exposed, including questionnaires, and any advertising materials.

See an example of a REB application at www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/applic-demande/_form_docs/a-eng.php.

Depending on the type of study, the researchers must identify the appropriate review board(s) and/or organizations from which approval must be obtained:

- For food and nutrition clinical intervention trials, institutional REB approval is mandatory and is usually the only regulatory approval required.
- For drugs, medical devices, NHPs or homeopathic drugs, approval must be obtained from Health Canada. REB approval is also required.
- For food clinical trials that use novel foods, a Letter of No Objection must be obtained from the Food Directorate of Health Canada before their incorporation into a clinical trial. REB approval is also required.
- For multicentre studies, each participating institution must obtain approval from its local REB and a copy of each centre's approved REB submission should be submitted to the main centre to include in the master data file.

Once research ethics approval has been obtained from the institutional REB and recruitment has begun, supporting ethics documents such as informed consent forms and medical questionnaires must be completed and signed by the participants.

4.1.4 Clinical trial registration

The World Medical Association's Declaration of Helsinki has introduced an initiative for the registration of all clinical trials through a publicly accessible database before recruitment, called *a priori* registration [75]. Clinical trial registration is now considered to be an ethical, moral and scientific responsibility of researchers.

As of July 1, 2005, the International Committee of Medical Journal Editors no longer considers non-registered or non-*a priori* registered trials for publication [76, 77]. The importance of clinical trial registration is intended to reinforce research validity by reducing the occurrence of selective reporting, non-publication of negative studies and intention-to-treat analyses, indirectly protecting the integrity of clinical trials and emphasizing transparent and ethical research [18, 76].

General information required to register a clinical trial includes:

- study type
- study design

- study title
- study objective
- primary and secondary outcome measures
- length of study
- number of study sites
- number of participants recruited
- treatment arms and assigned interventions
- type of treatment
- eligibility criteria
- dates of study commencement and completion

Information supplied to the clinical trial registry must be updated regularly after completing major steps such as recruitment, study phases and study completion. Table 5 shows recognized Canadian clinical trial registry databases.

Table 5. List of recognized clinical trial registry databases for Canadian clinical trials

Clinical trial registry databases	National/International	ID scheme	Website
Current Controlled Trials	International	International Standard Randomised Controlled Trial Number	www.controlled-trials.com
ClinicalTrials.gov	International; National Institute of Health initiative	ClinicalTrials.gov identifier (e.g., NCT00632788)	www.clinicaltrials.gov

4.1.5 Confidentiality

Maintaining confidentiality of participant data and biological samples is required throughout the research process. It begins with the development of confidential identification systems, strategies for confidential storage of data and samples, and appropriate conduct of authorized staff to protect privacy of participants.

To maximize confidentiality of participant data, identifier codes should be created for each participant. All corresponding biological samples and data will receive this identifier code to maintain anonymity during analysis. Identifier codes for participants can be random alphanumeric or numeric codes, or a combination of both. The use of initials followed by a number is not recommended as this method may compromise confidentiality and participant privacy. Biological samples and data files should be kept securely locked.

Access to data files, participant information and samples should be limited to authorized staff who have secure passwords for electronic data and who abide by the regulations specified in the *Personal Health*

Information Act. As universities and hospitals are considered trustees of this act, the onus is on the trustee to enforce and safeguard personal health information in any manner necessary to maintain privacy. Research staff should conduct themselves in an ethical and professional manner during and after the clinical trial to protect the confidentiality of participants and their property and to maintain ethical integrity of the clinical trial.

4.1.6 Compensation

Providing suitable financial compensation for participation is an important ethical issue in clinical trial design—unsubstantiated compensation can raise numerous ethical issues that must be reviewed. For instance, excessive remuneration may inappropriately influence various ethical aspects, such as participation, recruitment and compliance. On the other hand, inadequate compensation for the personal inconvenience and invasiveness involved in participating in a trial violates core TCPS values.

Determining appropriate financial compensation for participation should be based on two main factors: participation inconvenience and protocol procedures. First, a major cost to participants is transportation—gas, parking, and public transit. Foregone wages should also be incorporated into costs. Second, procedures performed on the participant to obtain biological samples must be factored into the remuneration, taking into account the number of procedures and their invasiveness [78]. Compensation rationale based on these two important ethical elements will provide clear justification for remuneration.

A fair schedule for compensation is also an important ethical issue in clinical trial design. Large payments for participation, which usually result from lengthy feeding trials, are generally pro-rated and partially paid out at the sample collection point and at the end of a study phase. Other pro-rating options include small payments throughout the trial and one large payment after successful study completion. However, this option may be unacceptable to the REB as it may represent unethical influence over study adherence and continuance. Furthermore, prompt payment is occasionally an issue in research trials; thus, proactive communication with institutional departments will ensure that payment is punctual and help maintain a positive participant–research relationship.

All aspects of compensation and ethical conduct of a clinical trial must be fully disclosed to the participant and the REB. This disclosure ensures a study with high scientific and ethical merit. It also maintains research integrity and transparency while reinforcing the core TCPS values.

BEST PRACTICES KEYPOINTS

Application and approval of research ethics/regulations

- For all clinical trials, researchers should dutifully employ the values defined in the TCPS for the ethical conduct of research involving humans, including:
  - Respect for Persons
    - obtain informed consent for participation
    - enforce confidential research practices
  - Concern for Welfare
    - assess invasiveness
    - declare associated risks and benefits
  - Justice
    - use appropriate inclusion and exclusion criteria
    - provide appropriate compensation for participation.
- Researchers must obtain approval for all clinical trials from their institutional REB and register their clinical trials in a publicly accessible, recognized clinical trial database.

4.2 Administration and human resources for clinical trials

A skilled and knowledgeable research team is essential for the successful planning and execution of a food-based human clinical trial. The research unit must include the key components, qualified staff and essential equipment required to execute fundamental aspects of a controlled feeding trial.

The central functions of the research team include:

- scientific, ethical and financial management and supervision
- coordination and recruitment of participants
- preparation and administration of research foods and/or diets
- sample and data collection and analysis

The principal investigator is accountable for all skilled personnel in a research team which typically consists of collaborators, clinical coordinators, food production staff, medical technicians (e.g., phlebotomist, nurse), a physician and laboratory technicians. A qualified biostatistician is also often part of the research team and reviews the study protocol, helps with the study design and sample size analysis, and addresses more complex statistical analysis of the study data. Typical duties required in clinical trials, the associated personnel with the skills to conduct these duties, and the skills or training required by personnel are outlined in Table 6.

Table 6. Research personnel and their respective duties and related training

Research personnel	Typical duties	Education, skills or training required
Principal investigator	• Organize, design, manage and supervise all aspects of trial • Initiate research program • Obtain and secure funding for research project • Keep sponsor informed of decisions and progress of the study	Doctorate; extensive experience working in clinical trials
Research manager	• Organize and manage clinical trial and clinical staff • Organize clinical data among clinical centres • Order and purchase supplies	Bachelor of Science or Registered Nurse/Bachelor of Nursing; on-site training; clinical research certification; clinical experience
Clinical coordinators	• Complete and submit research ethics application • Register clinical study • Recruit and screen participants • Inform, discuss and collect completed informed consent forms from eligible participants • Organize and prepare test and control treatments and enforce allocation concealment • Organize and distribute research diets • Prepare a production sheet for each meal from each menu for each participant • Engage in in-house communication with participants • Assess and monitor compliance • Coordinate and organize anthropometric and biological sampling schedule • Order all clinical consumables • Create and implement sample coding system for proper sample storage organization • Enforce correct storage of biological samples (e.g., aliquot blood fractions) • Maintain current and correct participant records	Bachelor of Science or Registered Nurse/Bachelor of Nursing; on-site training; clinical research certification; clinical experience
Food production manager	• Hire food production staff • Order all diet consumables • Enforce safe food-handling techniques • Train kitchen staff on proper food preparation techniques • Prepare menus • Prepare a production sheet for each meal from each menu for each participant • Organize participant-specific research diets and direct staff for proper handling and preparation	Dietitian, technician in dietetics; on-site training; food-handling certification

Research personnel	Typical duties	Education, skills or training required
Food production staff	• Prepare research diets according to food product manager or clinical coordinator instructions	Dietitian, technician in dietetics; on-site training; food-handling certification
Medical technician	• Administer blood sampling and transfer to clinical coordinators for proper storage	Nurse, Phlebotomist certification; clinical experience
Medical doctor	• Assess overall health and counsel on any health issues related to trial	Doctor of Medicine; clinical experience
Laboratory staff	• Conduct all laboratory analyses on collected biological samples	Bachelor of Science; Diploma in Medical Laboratory Science; on-site training; extensive laboratory experience
Biostatistician	• Perform and analyze necessary statistical analyses on datasets	Doctorate of Statistics; clinical trial experience

Staffing requirements of a food-based human clinical trial must be guided by the level of effort required as determined by the elements of the study design, including intervention type, study length, number of participants, complexity of research diet, menu cycle, research setting and complexity of endpoint outcome measures [79]. It is also important to assess the tasks to be done for the respective clinical trial and the estimated time required for each task. This step involves performing a functional time analysis estimate, which will take into account the type of research diet used and all tasks generally required for that type of trial. Staffing requirements may be considerably higher when conducting multiple concurrent studies as the burden on dietary, clinical and laboratory staff is generally heavier.

Assembling a clinical research team comprising skilled and knowledgeable staff who can execute core functions of a clinical trial protocol and who have excellent communication, organizational and planning skills is essential for conducting a well-controlled food-based human clinical trial [79]. The extra effort and time in assembling a research team with the skills to ensure the well-being of the participant during the study is important as experience shows that this investment has a huge impact in limiting dropout rates during a long intervention study.

BEST PRACTICES KEYPOINTS

Administration and human resources for clinical trials

- Compiling a research team of skilled personnel to conduct all scientific and administrative duties is essential for a successful clinical trial.
- Assessing the staffing requirements for a research trial is dependent on the following aspects of the study design:
  - intervention type
  - study length
  - sample size
  - complexity of research diet
  - research setting
  - complexity of endpoint outcome measures
  - tasks required and estimated completion time.

4.3 Recruitment of participants

4.3.1 Recruitment strategies

Recruitment of participants for a clinical trial has two goals: to select a sample that reflects the eligibility criteria and to enroll the required sample size [80]. A variety of generally acceptable recruitment strategies can be used, including posting on a clinical trials registry, as well as [81]:

- newspaper advertising
- radio and television advertising
- media release and interviews with project principal investigator
- mass mailing and electronic communications (e.g., social media, emails, website)
- physician referrals
- flyers and poster advertisement
- personal contacts with potential participants or with previous research participants who have signed an informed consent form for future contact (see Section 4.10 Post-experiment communication with participants)
- recruitment meetings and information sessions

Clinical trials usually use a combination of these strategies to recruit participants. For example, the Dietary Approach to Stop Hypertension (DASH) trial [82] recruited participants using a mass distribution of brochures to licensed drivers and registered voters delivered in coupon packs by commercial mailing companies. The DASH brochures were also distributed by hand to people living near the participating

research centres. The participating research centres used mass media including newspaper, radio and television advertising. Other recruitment strategies used in the DASH trial included blood pressure measurements at health and community fairs, presentations at churches and community centres, and word-of-mouth. The most successful recruitment strategies were mass mailing of brochures and word-of-mouth.

An effective advertisement contains general information about the clinical trial and contact information. It should also generate interest and discourage ineligible persons [81]. All advertisements used for recruitment need to be included in the REB application and be approved by the REB before undertaking the study.

An example of advertisement used for recruitment is found in Appendix 11.

When recruiting participants to a clinical trial, investigators should ensure that the interests of participants are not jeopardized. A conflict of interest may appear when physician-investigators enroll their own patients in their trials. Financial and non-financial incentives to physicians for referral of participants are not ethical and should not be practised by investigators [83]. Maintaining confidentiality during recruiting, particularly for excluded volunteers, is important. All participants' personal details should be kept confidential and participants should be identified only by code numbers.

4.3.2 Estimating dropout rate

In planning a food-based human clinical trial, recruitment goals should account for attrition (dropouts). Thus, a higher number of participants (10% to 30%) should be recruited and screened than the number of participants determined by *a priori* power analysis (see also Section 3.4.1 Sample size calculation). A slightly higher dropout rate should be assumed for long-term trials or ones that require more from participants, in terms of invasiveness and lifestyle inconvenience. Statistical tools for data analysis also exist to reduce the impact of attrition. See also Section 4.8 Management of dropouts and missing data.

4.3.3 Prospective participant screening

Screening identifies participants who meet the eligibility criteria. Early in the screening process participants must be made aware that they are not permitted to participate in concurrent food intervention trials in order to prevent confounding results. The time and complexity of the screening process depends on the eligibility criteria [81]. Usually, the screening starts by a telephone interview to complete a simple questionnaire, which may include questions about general dietary habits and specific dietary habits that may affect the study.

See an example of a questionnaire form in Appendix 12, which was designed to screen for a feeding trial to test the efficacy of a cholesterol-lowering agent.

Screening may only require a questionnaire, but in many cases, other assessment parameters may be necessary, including laboratory, dietary and/or anthropometric measures and, for some studies, a full medical examination. The entire process may require participants to undergo a series of screening visits.

If recruitment is a challenge, it may be necessary to redefine the eligibility criteria. However, this should be done with caution as changing the eligibility criteria may invalidate the study outcomes. This decision should also be discussed with the sponsor.

BEST PRACTICES KEYPOINTS

Recruitment of participants

- Recruitment strategies necessary to acquire adequate participation include:
  - mass distribution of written advertisements (i.e., brochures, newspaper advertising)
  - mass media (i.e., radio, television and internet advertising)
  - word-of-mouth
- Recruitment strategies must:
  - include general information about the clinical trial
  - generate interest in the research
  - discourage ineligible persons
  - be accepted by the REB.
- Screening of prospective participants is required to identify those who meet the eligibility criteria.
- Sufficient participants must be recruited to allow for dropouts and ensure sample size and power of experiment are not jeopardized.

4.4 Designing, producing and delivering research diets

Research diets should be designed to meet the recommended intake of nutrients set by the Dietary Reference Intakes committees [84] or should be based on current Canadian intake, unless the research question necessitates other modifications. The energy requirement for each participant must be considered where body weight must remain stable, as this factor may affect the provision of food and the cost of the feeding trial [44].

4.4.1 Energy requirement determination

The most accurate method of measuring resting energy requirement is indirect calorimetry; however, the cost and availability of required equipment may limit its use in controlled feeding trials. Thus, it is more common to estimate energy requirement using predictive equations. Equations are available to estimate basal energy expenditure or resting energy expenditure. These estimates are multiplied by an activity factor to determine total energy needs. Table 7 outlines predictive equations to calculate estimated energy requirements for study participants. These equations should not be used for obese participants, children, and pregnant or lactating women; other appropriate formulas should be used when studying these population groups. Other methods for assessing total energy intake are 24-h recalls, 3- to 7-day food journals and food frequency questionnaires (FFQs). However, under- and over-reporting of food intake is common with these methods and can compromise baseline results and thus result in selection bias.

Table 7. Equations for calculating estimated energy requirement in kilocalories per day

Predictive equation	Formula
Harris-Benedict	Men: (66.5 + 13.8*W + 5.0*H – 6.8*A) * AF Women: (65.5 + 9.6*W + 1.8*H – 4.7*A) * AF
Mifflin-St. Jeor	Men: (10*W + 6.25*H – 5*A + 5) * AF Women: (10*W + 6.25*H – 5*A – 161) * AF
World Health Organization	Men: Age 18–30 y: (15.3*W + 679) * AF Age >30–60 y: (11.6*W + 879) * AF Age >60 y: (13.6*W + 487) * AF Women: Age 18–30 y: (14.7*W + 496) * AF Age >30–60 y: (8.7*W + 829) * AF Age >60 y: (10.5*W + 596) * AF

W = weight in kilograms; H = height in centimetres; A = age in years; AF = activity factor: 1.5 can be used initially and adjusted if necessary

The predictive equation is first used to estimate energy needs, but it is important to monitor weight frequently during an intervention. Maintaining stable weight throughout the trial may be required, as weight changes may significantly affect the trial outcomes such as blood cholesterol concentration and blood pressure. Energy intake in feeding trials can be adjusted when body weight changes from baseline values by 2% [85]. In free-living studies, it is more common for body weight to fluctuate as energy intake is not controlled; however, instructions can be given to participants to maintain normal eating and activity patterns as much as possible to prevent these fluctuations. Investigators should always consider variables that may affect daily weights before making changes in a participant's caloric level, including inconsistency in standard weighing procedure, illness, change in fluid status, or an increase in physical activity [86].

In a feeding trial, a basic diet of 2000 kcal/day is set; then the food quantity, expressed in gram weights, is modified to supply the energy requirement for each participant. Alternatively, menus can be designed to provide four to five different calorie levels and each participant would be given the calorie level closest to their needs. Extra unit foods can be used to modify the participant's caloric intake in order to meet that person's individual caloric requirements [87]. In free-living clinical trials where only the test food is provided, food intake can be assessed at several time points to monitor dietary energy intake.

4.4.2 Menu design

As discussed in Section 3.2.6 Dietary control and compliance, a rotating cycle menu of at least 3 days should be used for variety. Menus should be designed to encourage participant compliance with the trial diets and efficiently use the research kitchen facilities [56]. Participant compliance with the study regimen may be higher if the diet is more diversified (5- to 7-day vs. 3-day cycling menu) [88].

An example of a 3-day cycle menu is shown in Appendix 7.

The following criteria should be considered in menu design [56]:

- **Target nutrient:**
  - nutrient ranges
  - food sources
  - nutrient absorption inhibitors/enhancers
  - nutrient variability
  - spices
  - discretionary food, beverages and alcohol
- **Menu type:**
  - number of energy levels
  - number of menu cycles
  - weekday vs. weekend menus
  - emergency meals/menus
  - food choices
  - food units
- **Participant considerations:**
  - age
  - lifestyle
  - religion (holidays and/or special celebrations)
  - culture
  - meal storage
  - food preparation and packaging
  - variety of foods in menu cycle
  - distribution of foods and drinks
  - serving sizes
  - allergies/intolerances
  - nutritional adequacy
- **Kitchen and production planning:**
  - food similarity across diet arms
  - food weighed in raw vs. cooked state
  - commercially prepared food items, regional and seasonal food availability, canned/frozen/fresh produce
  - batch vs. individually prepared recipes; menu complexity
- **Standardization:**
  - nutrient database
  - chemical nutrient analysis
  - food specification
  - food preparation procedures and recipes

Menus can be created by:

- manually using available food exchange lists
- using computerized and online systems (however, the use of online systems does not allow entering new recipes) such as:
  - Food Processor SQL
  - My Food Guide from Health Canada (www.hc-sc.gc.ca/fn-an/food-guide-aliment/myguide-monguide/index-eng.php)
  - Daily Food Plan available from the United States Food and Drug Administration (www.choosemyplate.gov/supertracker-tools/daily-food-plans.html)

The menus can be kept for other future trials and modified accordingly. After a cycle menu has been created, its nutrient content should be evaluated with respect to the study nutrient goals. Nutrient composition can be determined by chemical analysis; however, this will increase the duration of preparing menus and the cost of the trial. Alternatively, nutrient composition can be verified using dietary analysis systems available as software packages or online. An advantage of using computerized systems is that the nutrient content can be checked while building the menu and adjustments can be made immediately.

Factors to consider when selecting a computerized dietary analysis system are discussed in detail by Lee and Nieman [89]. The selected computerized dietary software should:

- contain more than 15,000 food items in its database
- contain a significant number of brand name items
- analyze for at least the basic components (energy, water, cholesterol, lipids, dietary fibre, vitamins and minerals)
- allow addition of new food items to the database
- provide updates every 1 to 2 years
- have the ability to enter any variation of the food name
- be able to adapt different measures of portion size
- make it easy to convert errors during data entry
- allow the view of nutrients for a food item during data entry
- allow comparison of dietary intake with dietary reference intakes
- provide nutrient information in the form of tables
- give an indication of the number of missing values when calculating nutrient intake

The following computerized dietary analysis systems meet most of the criteria specified by Lee and Nieman [89]:

- Food Processor SQL
- NutriBase Clinical
- Nutrition Data System for Research (NDSR)
- FoodWorks
- Nutritionist Pro

4.4.3 Food preparation, safety and coding

Food items should be prepared according to a standardized protocol to ensure consistency of nutrient composition throughout the trial. Standardized protocol should follow these guidelines [90]:

- Adequately describe all food items. For example, milk can be whole, low fat or fat free.
- Identify food brands to be used throughout the study, including description of the food items, size and type of packaging.
- Standardize recipes, including cooking technique, time and temperature.
- Weigh food items using electronic analytical balances that are accurate to at least 0.1 g. Accuracy of the balances should be checked weekly.

Dealing with the same food distributor over time can also help ensure consistency [90].

The amount of individual food items to be purchased is based on edible portions. For cooked items, appropriate calculations should be made to convert to raw weight after considering losses or gains during preparation and cooking. Usually, the estimated amount needed for different food items is increased by 25% to ensure enough is purchased [90]. However, when deciding on the amount of foods to be purchased, the storage space and facilities should also be considered. The shelf life for each food item must be noted as well [90].

The food production process should be tracked by coding and all prepared food items should be clearly labelled [90]. During food production and storage, a label should carry a full description of the food item. The label on foods served to the participant should show a generic description and a code to preserve the masking of foods in a blinded study. These food labels can be computer generated using labels that can tolerate cold storage temperatures.

4.4.4 Food distribution and compliance

Some clinical feeding trials may require participants to consume all or some of their meals at the research site. With such trials, the research facility should have a dining area that is comfortable and clean. The dietitian, clinical coordinator or food production manager should prepare a production sheet for each meal from each menu for each participant. The production sheet shows the food items and their weights according to the caloric needs of each participant. The food production staff assembles food trays according to the production sheet. The dietitian then checks the trays to ensure that each food item appropriate to the dietary treatment is present. Returned trays must also be inspected to ensure that participants have consumed all required food items, especially the food(s) associated with the dietary treatment.

An example of a food production sheet is shown in Appendix 13.

Participants should be given clear instructions to [91]:

- eat all the provided food items
- not to share food items
- report if any food items were lost due to spillage

If participants consume all or some of the meals outside the research unit, they should be provided with packaged meals—this will increase the amount of work required by the clinical and food production staff [91] as it requires additional considerations:

- Food must be packed in containers that do not leak or spill.
- Each participant should be provided with a food cooler and ice packs.
- Food storage and safety issues should be discussed with the participants.
- Participants should be instructed to consume only the food provided.
- Compliance should be monitored by inspecting returned food containers for uneaten items, and weighing and reporting uneaten items in a daily food record.

See also Section 3.2.6 Dietary control and compliance, Section 3.3.2 Compliance outcome measures and Section 4.5 Compliance assurance and monitoring.

BEST PRACTICES KEYPOINTS

Designing, producing and delivering research diets

- Participant energy requirements need to be determined beforehand to effectively monitor changes in anthropometric measures.
- Cycle menus need to be developed that encourage subject compliance, considering:
  - target nutrient
  - menu type
  - individual participant considerations
  - kitchen burden
  - standardization.
- Food preparation must be implemented in a standardized manner to ensure consistency of nutrient composition throughout the trial.
- Meals must be distributed according to participant identifier code.
- Compliance with consumption requirements can be monitored by inspecting the contents of the returned food containers and daily food records.

4.5 Compliance assurance and monitoring

Compliance assurance is one of the principal challenges for food-based human clinical trials. There are various methods of assessing compliance, but no single approach—except for a full metabolic ward approach—is guaranteed to prevent participants from deviating from protocol. Poor compliance can negatively affect the robustness of the study by reducing the ability to detect effects, causing false reporting of findings and, most importantly, causing reporting of erroneous health effects of a food component to the nutrition and health community. To maintain study integrity and minimize non-compliance, a system of complete and inclusive methods for assessing and monitoring compliance, and dealing with non-compliance should be used throughout the trial.

The methods for monitoring compliance commonly used in food-based human clinical trials depend on the study design, level of invasiveness, study length, and feeding type. Compromised compliance can become a study limitation. The monitoring methods themselves can also become a study limitation as they can be difficult to control. Thus, extra attention is required in selecting the most suitable methods of compliance assessment and enforcement.

For qualitative and epidemiological nutrition dietary studies, it is common to use dietary records, including FFQs and 24-h food records, to obtain actual intake data. However, these methods are notorious for under-reporting of unhealthy foods and over-reporting of healthy foods. Manipulation of such records in an attempt to misrepresent actual consumption of particular foods by participants can significantly confound findings and produce false associative reports. If FFQs are to be used, standardized and

quality-tested resources such as the National Health and Nutrition Examination Survey food frequency questionnaire are recommended [92].

Compliance with study treatments in food-based human clinical trials can be maximized by:

- having the study treatment consumed under the supervision of study personnel;
- having the study treatment supplied to participants for take-home consumption while determining intake through the return of emptied study treatment containers; or
- monitoring biomarkers of intake or food exposure (see Section 3.3.2 Compliance outcome measures).

The second method of determining compliance is less robust as empty treatment containers do not guarantee consumption. This approach may be used when there is a lack of resources (i.e., shortage of study personnel, statutory holidays, weekends, lack of research venue, or impracticality of consuming the test food at the research facility). A checklist can also be used to monitor compliance, but again this is less robust than physical verification by study coordinators at the time of consumption.

Participant compliance in full-feeding food intervention trials is usually monitored by direct observation, where one meal and the study treatment are consumed under the supervision of study personnel to confirm compliance with treatment intake. The main challenge with compliance in full-feeding trials is adherence to consuming the background diet provided. Full-feeding trials require that participants consume only the food components provided by the research kitchen and no additional outside food.

In such cases, the provided background diet is custom designed to tightly control individual energy and macronutrient intake to maintain various anthropometric or metabolic factors set in the study design, and to prevent misrepresenting any of the findings as bioactive effects. Participant adherence to full-feeding trials is generally thought to be good, with more than 90% compliance. The compliance rate is high as study volunteers are generally enthusiastic in participating as they know that they may possibly improve their health [93].

Factors that have been found to affect compliance in full-feeding intervention trials include self-selected weekend meals as well as the allowance of alcohol. One would think these freedoms in an otherwise restrictive setting would aid in compliance, but they actually exacerbate the extent of deviation from the dietary protocol [93].

Conversely, factors that should improve adherence and participant contentment such as increased menu variety, less repetition of foods and short study length, which are welcomed by participants, have been shown not to improve compliance but aid in participant satisfaction [93]. Consuming all foods provided daily has shown to be more difficult for participants than refraining from prohibited foods outlined by the study design. Thus, reformulation of menu items may be required to improve the level of compliance [93].

Another important factor to improve study compliance is regular contact with the same key study staff members. Regular contact with participants by research coordinators provides repeated reminders of proper compliance (i.e., consume only foods and treatments that are being provided). During a clinical trial, this creates participant–coordinator relationships. These bonds strengthen compliance by developing loyalty, as well as feelings of accomplishment or guilt. Compliance is reinforced when staff turnover is low.

This results in a sense of stability in the workplace and indirectly displays feelings of professional quality care and attention to participants [94]. Relationship building also encourages volunteers to reapply for subsequent study opportunities, creating a pool of dependable research participants for multiple study protocols.

Although compliance is generally good in short-term food-based human clinical trials, deviation does occur even with the use of the above-mentioned methods. If non-compliance is detected, the research coordinator must impose appropriate consequences depending on the severity of the deviation. In general, respectful reminders are sufficient. However, if blatant non-compliance occurs, participant dismissal may be warranted, especially if individual biological or anthropometric values are skewed enough to jeopardize overall study findings. The methods for monitoring, promoting and assessing compliance discussed here will help to minimize deviation. See also Section 3.2.6 Dietary control and compliance, Section 3.3.2 Compliance outcome measures and Section 4.4.4 Food distribution and compliance.

BEST PRACTICES KEYPOINTS

Compliance assurance and monitoring

- FFQs and 24-h food recalls can be used for compliance assurance for qualitative and epidemiological nutrition trials.
- Supervised meal consumption and inspection of take-home meal containers can assess compliance for food-based human clinical trials.
- Regular contact between participants and research staff is recommended to improve study adherence.
- Participant deviation can be addressed by enforcing appropriate consequences. In general, respectful reminders are sufficient. Blatant non-compliance can warrant study termination.

4.6 Record keeping and database management

Good record-keeping practices are important during a clinical trial. Document accumulation is extensive and continuous, requiring high-quality database management. Records largely consist of participant data files, case report forms (CRFs) and study procedures, such as study protocol, menus and laboratory standard operating procedures (SOPs). These records form the master permanent data file that will be used for final reports and publications.

The Common Technical Document (CTD) format, described in guidance document “M4” of the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, provides a common format to organize records related to product quality, safety, and efficacy. Since food and nutrition clinical trials do not require prior authorization by regulatory authorities, the CTD has not been commonly used by researchers in this field. However, this consistent format for information on products associated with health claims has been adopted by Health

Canada, the European Union, and Japan, and recommended by the U.S.A., for a variety of submission types, including drug clinical trials, pharmaceutical and biological drug licence applications, master file submissions, periodic safety update reports, risk management plans, and pre-submission meeting information. Acceptance of the electronic version (e-CTD) is also being implemented for these various purposes. While not required by Health Canada at this time for submissions related to food health claims, there will be clear advantages for food and nutrition clinical researchers to document their data in this format. According to ICH, the international agreement by clinical researchers to assemble all the quality, safety and efficacy information in the CTD format has revolutionized regulatory review processes and led to a harmonized electronic submission that, in turn, enabled implementation of good review practices. For industries, it has eliminated the need to reformat the information for submission to different regulatory authorities.

More information on the CTD format can be found on the ICH website at www.ich.org/products/ctd.html and Health Canada's website at www.hc-sc.gc.ca/dhp-mps/prodpharma/applic-demande/guide-ld/ctd/index-eng.php

Clinical research data that are relevant to the roles and responsibilities of the institutional REB are to be retained for 25 years. All other research data collected should be retained as long as is necessary to fulfill the initial research objectives for verification or auditing of research results required by collaborators, regulators or publishers. The retention period is at least 3 years post-completion as per Good Clinical Practice (GCP) guidelines [95, 96]. Good data management practices are essential for secure data storage. A formal data management strategy needs to be approved by the REB as part of the process leading to the approval of the consent form and the study as a whole. See also Section 5.4 Archiving of data.

4.6.1 Data management approaches

All participant and study records should be regularly updated in a clear and organized manner to maintain a current database throughout the study. Regular updating also prevents any reporting oversight due to data entry delay. Instructing participants to keep a study log or journal to record treatment intake, deviations or any issues arising from the study such as illness, over-the-counter medication use, and treatment contraindications can also be useful for researchers from both an objective and compliance standpoint. This study journal can also contain the study summary, responsibilities of each participant and a schedule for clinic visits. A study journal emphasizes the notion of compliance by giving participants a constant reminder that their role within the study is to comply. Providing a report to the clinical research team instills a feeling of teamwork and collaboration with researchers.

4.6.2 Data archiving and storage

Records should be kept in a secure storage place with restricted access to protect the confidentiality of both the participant and the research. Initially, paper records such as signed documents including informed consent are kept; these should be later scanned to generate an electronic copy or manually entered into an electronic spreadsheet database. Data should be double entered and cross-referenced to find entry errors [97].

Hard copies of data records should be secured in a locked storage area. Electronic data should be password-protected and backed up regularly to protect from invasion of property, power failures and data loss. A secure electronic database, online or shared network (useful for multicentre trials) is

recommended and can be used to upload electronic data and be shared with authorized users. This system is usually securely managed by professional information technology staff in the faculty or academic institution [98]. A secure database lets users share data with authorized staff, permits remote access, and automatically executes system backups and performance checks. Most importantly, the method ensures optimum security measures are in place to guard against data loss and unauthorized use [99].

4.6.3 Confidentiality of data and records

Participants' privacy and consent to use personal health information are key considerations. All records containing participant information should be classified using the confidential identifier code given to the participant at the beginning of the study. The careful use of identifier codes for participant data protects the privacy of the participant, eases the data analysis process, and fulfills the researcher's obligation to maintain participant confidentiality, as stated in the informed consent [100].

When it comes to data manipulations or analysis, raw, unprocessed datasets may be shared with other scientists or might be needed for publications [101]. A dataset is the collection of participant information, including socio-demographic and clinical information, to summarize the findings, and is presented in the main report, published or not [101]. Records that could identify participants must be protected to respect the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s) [100].

Practical guidance has been developed proposing a minimum standard for de-identifying datasets for publication or data sharing [101]. De-identification and anonymization are strategies to remove patient identifiers in electronic health record data. De-identification of medical record data refers to the removal or replacement of personal identifiers so that it would be difficult to re-establish a link between the individual and his or her data [102]. Anonymization is the irreversible removal of the link between the individual and his or her medical record data to the degree that it would be virtually impossible to re-establish the link [102].

4.6.4 Administrative reporting

The annual REB status report and final REB study report form the permanent study data file and are useful for determining successful recruitment and trial operation. These required reports contain important information such as number of initial participants screened, initial eligible participants, number of dropouts, final participant study size and participant deviations. These reports are reviewed by the REB, but also provide the researcher with a view of completion success and help evaluate whether strategies for promoting recruitment and minimizing dropouts are required.

See an example of an annual study status report at www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/applic-demande/_form_docs/k-eng.php and an example of a final study status report at www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/applic-demande/_form_docs/l-eng.php.

BEST PRACTICES KEYPOINTS

Record keeping and database management

- Implementation of a formal data management strategy is recommended to maintain current and organized study records during and after trial completion.
- Study records should be maintained in a secure storage area with restricted access to protect the confidentiality of the participants.
  - Hard copies of data should be safely secured in a locked place.
  - Electronic data should be password-protected and backed up regularly to maintain data integrity.
- To protect the privacy of the participant, confidential identifier codes should be given to the participant and used for all participant data during and after trial completion.

4.7 Collection, labelling and storage of samples

The collection of biological samples from participants during a clinical trial is a procedure that requires systematic planning, organization and meticulous care to ensure accuracy and integrity of study results. A seamless process requires scheduling of technical staff and participants for the sampling and enforcement of SOPs for quality assurance and proper storage of samples. See also Section 5.5 Sample preservation, coding and storage.

4.7.1 Sample collection organization

Biological sample type and quantity required from participants depend on the study design and the outcome measures to be analyzed. In turn, these requirements define the organizational requirements of staff and participants. For instance, common procedures such as blood sampling require the correct type of blood collection tube suited for the particular analysis. (See box **Common blood sampling procedures**.)

Common blood sampling procedures

- For all serum chemistries, tubes coated with a clot activator and serum separator additive should be used.
- For plasma determinations, a heparinized tube or an ethylenediaminetetraacetic acid (EDTA) tube should be used to separate plasma.
- For stat serum determinations, a thrombin-based clot activator tube should be used.
- For trace-element, toxicology and nutritional-chemistry determinations, a tube coated with clot activator should be used.

For blood specimens, the type of anticoagulant may alter the integrity of some biomarkers [103]. Different specimen types may reflect different exposure periods; for example, concentrations of folate and selenium measured in plasma represent recent dietary intake, while concentrations measured in red blood cells reflect longer-term body stores [104].

The treatment of blood with enzyme inhibitors is occasionally needed as some blood proteins are quickly degraded by various enzymes. For example, dipeptidyl peptidase-4 inhibitors can be used to keep glucagon-like peptide-1 from degrading. Thus, depending on the analysis determined, careful attention must be paid to the appropriate blood collection container.

In addition, labelling of blood collection tubes, aliquot tubes and other sample collection containers must be meticulous to ensure that proper collection containers are being used. These containers must also be labelled using the confidential identifier code assigned to the respective participant to facilitate organized blood collection for the phlebotomist or research nurse, and for proper storage and future data analysis. Other useful labels are date of draw, study phase and day within phase (e.g., ID code: ABC; Date of draw; 01/01/12; Study phase and day within phase: P1D1).

The use of a sample log and sample index is recommended to organize biological samples. When the sample is received, the appropriate identifier code, type of sample and number of aliquots should be recorded in a logbook and then transferred to an electronic index for easy reference. Discarded aliquots should be recorded in the sample log as depleted and the sample index should be updated to represent the current aliquot inventory.

4.7.2 Scheduling of sample collection

When scheduling participants for sample collection, they must be informed of any advance preparation required (e.g., fasted, post-prandial, no alcohol or heavy exercise for 24 h). If fasting is required, morning appointments are preferred. If saliva, urine or fecal sample collection is required, sampling containers should be discretely distributed and collected by research staff to minimize embarrassment. If participants are to collect urine and faces samples at home, they should be instructed to promptly and safely store the samples in their freezer (away from food) until delivery to the research facility. All samples must be clearly labelled according to the confidential identifier code assigned to the participant. This identifier code must be carried through all phases of the study to ensure confidentiality and accurate identification [100].

4.7.3 Biological sample storage

Appropriate methods for sample storage must be used to ensure their security and stability before analysis. Urine and feces should be frozen immediately. Blood samples should be processed and stored at the appropriate temperature for the analyte of interest. Sample storage can influence specimen integrity, and different storage methods can influence assay variability; for example, beta-carotene concentrations degrade after long-term storage at -20°C to -40°C, but not at -70°C. Generally, storage at colder temperatures is desirable [103]. If fatty acid or sterol analysis is part of the study, a scavenger or antioxidant such as butylated hydroxytoluene should be added immediately to the sample to preserve fatty acid status.

It is important for samples to be collected and processed in a standardized manner for all participants, or across all centres in the case of a multicentre study [103]. Biological samples are usually frozen for a

minimum of 2 years to enable analysis of the research project; however, it is important to consider the stability of the analytes of interest. In prospective cohort studies, however, long-term storage (>10 years) may be required.

The storage time of biological samples must be declared in the REB submission and informed consent must be obtained so that biological samples can be retained for the length of time requested. It is considered good practice to duplicate each sample into different freezers so that if a major power failure occurs for one system, investigators are still able to access usable study samples.

4.7.4 Handling and safe disposal of biohazardous materials

Due to the biohazardous nature of sample collection, SOPs that outline safe collection, analysis and storage procedures are critical to protect research technicians and sample stability. Maintenance of safe and attentive laboratory etiquette, reputable standardized laboratory training and supervision of all research staff and students will help reduce errors and minimize biohazardous contamination.

To prevent contamination of other spaces, biohazardous materials should be handled with laboratory protective attire, including lab coats, nitrile gloves and safety glasses, in an assigned biohazard workspace. Biohazard waste, including blood tubes, used pipets, and tips, should be disposed of in a designated biohazard waste container. Yellow containers are used for human blood and body fluid waste. Red containers lined with an autoclave-safe bag are used for human anatomical waste; when full, the biohazard bag and its contents must be steam autoclaved at the recommended settings for proper sterilization before disposal [105].

Adherence to these recommendations and regulations is good clinical practice and significantly contributes to the integrity and merit of a food-based human clinical trial.

BEST PRACTICES KEYPOINTS

Collection, labelling and storage of samples

- Biological sample type and quantity are predetermined by study design and outcome measures.
- Sample collection must follow a standardized protocol, and appropriate sample collection containers must be organized and labelled using the confidential identifier code to ensure correct sampling from participants.
- Proper instruction is necessary so that participants come prepared for the biological sampling procedure (e.g., fasted, post-prandial, no alcohol or heavy exercise for 24 h).
- Appropriate storage methods must be used for sample collection to ensure sample stability before analysis.
- Biohazardous materials must be handled and disposed of appropriately.

4.8 Management of dropouts and missing data

Attrition is common in all food-based human clinical trials and can seriously compromise the power of a study if the dropout rate becomes excessive. Participants may drop out of a study for many reasons. Many are personal, including relocation, inconvenience, and family issues. While problems related to study conduct or design are not common, participants may find long-term feeding trials monotonous, which can increase attrition rates.

In a survey of RCTs it has been reported that more than 10% of primary outcome data are missing from approximately 25% of controlled trials [106]. Strategies to promote participant satisfaction should be developed to maintain study integrity and to prevent tardiness, absenteeism and dropouts. Examples of such strategies would be the implementation of monthly incentives, such as recreational outings or event tickets. If a participant chooses to drop out of a trial, it is wise to respectfully inquire why. This information can help prevent further attrition throughout the trial. This information should be recorded by following the Consolidated Standard of Reporting Trials (CONSORT) guidelines. See Section 5.3 Publishing of results.

Analyzing data from food-based human clinical trials with high attrition rates can be challenging statistically, as attrition may introduce selection bias due to imbalances in baseline values [107]. Attrition becomes an issue when the sample size is small or when data are not missing at random. For instance, it has been suggested that an attrition rate of 5% or lower is unlikely to threaten trial validity; however, a dropout rate of 20% or higher can cause bias and becomes more of an issue when the attrition is non-random [108].

Non-random attrition results in data that are missing for a specific reason, such as participants deliberately skipping a blood draw. Non-random attrition may affect multiple baseline parameters as opposed to random attrition and should not be treated equally over treatment arms, as the rationale for attrition may not be the same between treatment and control. To accurately report findings without creating baseline bias, it is important to clearly report baseline differences of participants across treatment arms for the analyzed parameter. Robust statistical analyses should be used to assess outcome measures and to reduce impact of attrition in an RCT [107]. The statistical tools, particularly with mixed models, have improved significantly over the last decade and are better able to deal with missing data in an RCT. See also Section 5.2 Data manipulation and statistical analysis.

BEST PRACTICES KEYPOINTS

Management of dropouts and missing data

- Implementation of strategies to manage dropouts and prevent further attrition is important.
- Robust statistical analyses should be used to assess outcome measures in trials that experience attrition to prevent selection bias and maintain data integrity.

4.9 Reporting of adverse events

Adverse events are rare in clinical food trials. However, they require full disclosure when they do happen. Definitions of an adverse event, adverse reaction, serious adverse reaction and serious unexpected adverse reaction that occur during clinical trials are set out in the FDR (C.05.001) for biological drugs, pharmaceutical drugs, and radiopharmaceuticals [9] and in the Natural Health Products Regulations (sections 1 and 63) for NHPs [3]. Researchers involved in food-based human clinical trials must follow the directions for adverse event reporting that are outlined by the institutional REB. While there are no specific definitions for food-based human clinical trials, those applicable to drug trials are broad enough to be applied to food adverse events and reactions.

- An “adverse event” means any adverse occurrence in the health of a clinical trial subject who is administered a drug (or NHP or food), that may or may not be caused by the administration of that product, and includes an adverse reaction.
- An “adverse reaction” means any noxious and unintended response to the product that is caused by the administration of any dose of the product.
- A “serious adverse reaction” means an adverse reaction that requires in-patient hospitalization or prolongation of existing hospitalization, that causes congenital malformation, that results in persistent or significant disability or incapacity, that is life threatening, or that results in death.
- A “serious unexpected adverse reaction” means a serious adverse reaction that is not identified in nature, severity or frequency in the risk information set out in the investigator’s brochure or on the label of the product.

Based on research or clinical experience, surrogate outcomes can be used to identify whether the intervention being tested results in an adverse effect. A change could be seen in a surrogate outcome long before an adverse event occurs [109]. For example, an increased level of alanine aminotransferase can predict liver damage. Simple laboratory measures and/or a direct questionnaire can be used to track adverse events. See also Section 3.3.3 Adverse event outcome measures. An abnormal laboratory measure can be considered an adverse event, as it may indicate disease or organ toxicity. Important anticipated adverse events can be discovered by specific queries [21]. For example, as stomach problems, flatulence, bloating or abdominal pain can be a side effect of increasing fibre intake, the signs and symptoms of abdominal discomfort can be queried in trials feeding high amount of fibres.

Moreover, general physical examination and laboratory measures at the screening visit should be used to record any pre-existing clinically significant abnormality. At the end of each treatment phase, general physical examinations and laboratory measures should be repeated to check if the frequency or intensity of a pre-existing abnormality is exacerbated, and to check for any new abnormalities.

Each clinical trial should have a qualified investigator who is, according to the FDR (C.05.001), a medical doctor or a dentist who is entitled to provide health care under the laws of the province where the clinical trial site is located. The qualified investigator is responsible for the safety of the participants and supervising medical care and medical decisions during the clinical trial.

Details of all adverse events reported by participants or observed by clinical coordinators or the investigators must be recorded in an adverse event form retained in the participant’s file. In addition,

all adverse events should be reported to the sponsor or funding agency and to the REB. As outlined in the FDR (C.05.014), a clinical trial sponsor is legally required to report serious unexpected adverse events to Health Canada. Reports must be submitted within 7 days of becoming aware of the information if fatal or life-threatening or within 15 days for any other unexpected problems [9]. Investigators must also report adverse events to their institutional REB following the same timelines. Each institutional REB will have designated forms for reporting these events. Adverse reactions dealing with marketed food products should also be reported to CFIA [110].

Within 8 days after submitting a report, the sponsor must complete another report that includes an assessment of the importance and implication of any findings. All adverse events must be recorded separately and kept for 25 years (FDR C.05.012). All adverse events should be followed up by the investigator. In some cases, it may not be possible to follow up if the whereabouts of the participant is unknown. In other situations, it may be determined that the adverse effect was not caused by the trial.

If a serious adverse event occurs, the investigator should break the treatment code for that participant while retaining the blinding for those who will be responsible for outcome measures and data analysis and interpretation [111]. Interim monitoring in a clinical trial carried out by an investigator or an independent committee is important for participant safety. If there are a large number of serious adverse events, the trial may be stopped or suspended until the protocol is modified [112].

To reduce the occurrence of adverse events, participants who are less likely to have adverse effects such as young people and those who are not on multiple co-therapies could be selected for the trial [112]. However, this selective process may affect the generalizability of efficacy data.

BEST PRACTICES KEYPOINTS

Reporting of adverse events

- Each clinical trial should have a qualified medical doctor who is responsible for the safety of the participants and is capable of supervising medical care and medical decisions regarding the clinical trial.
- Adverse events should be recorded immediately and a report should be filed with the concerned ethics committee, concerned authorities and funding sponsor within 7 days if the adverse event is fatal or life-threatening, or within 15 days if it is not.

4.10 Post-experiment communication with participants

Post-experiment communication with participants is generally conducted for three main reasons:

- to provide individual volunteers with their study results, thank them for their participation and maintain contact to generate interest in future research participation;

- to obtain informed consent to perform supplementary analyses or subsequent research after ethics approval has been already been granted from the REB or the feeding trial has been completed; and
- to obtain informed consent to perform retrospective studies.

Post-experiment communication with participants to obtain informed consent for supplementary measures is uncommon, as a well-designed clinical trial would have these measures incorporated into the original REB submission. However, if additional analysis would improve the robustness of the study, a REB amendment request must be submitted and informed consent must be obtained from participants.

An informed consent form may be presented to participants to obtain permission to be contacted for future research participation. This step eliminates the issue of when it is appropriate to make contact and would considerably help recruitment for future studies.

See an informed consent form template for contacting participants about future research at www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/consent/consent_futur-eng.php.

See an example of a request for amendment to a previously approved study at www.hc-sc.gc.ca/sr-sr/advice-avis/reb-cer/applic-demande/_form_docs/d-eng.php.

If these supplementary measures were not included in an original REB submission, the principal investigator and the clinical research team should draft a new or revised consent form that incorporates all revisions to be featured in the REB amendment or new REB submission and be sent by courier to the participant.

As with the original REB approval, the revised informed consent form must include a clear and honest explanation of all additional analyses to be conducted, what the subsequent experiment would entail, what would be expected of the participant, and what would be done with the participant's biological samples. Information provided in the consent form must be stated in plain language so that the participant completely understands the study details and how confidentiality will be ensured. This is especially important for retrospective genetic analyses, which can be sensitive in nature.

Following full communication of study objectives and participant requirements, the decision to participate should be entirely voluntary and occur without obligation or influence. Upon successful REB approval of the request for study amendment, signed informed consent can be obtained from all participants taking part in the subsequent research.

Residual clinical samples that are anonymous or become anonymized with the removal of participant identifiers may be used for future analyses without informed consent with REB guidance [113].

BEST PRACTICES KEYPOINTS

Post-experiment communication with participants

- Post-experiment communication is useful and is needed to:
  - inform volunteers of study results
  - thank them for their participation
  - maintain contact to generate interest in future participation
  - obtain informed consent to perform supplementary analyses
  - obtain informed consent to perform retrospective studies.
- In the event of an amendment to the approved protocol, informed consent for the supplementary procedures may be required from the participants depending on the outcome of the REB review.

5.0 Post-Clinical Trial Activities

SUMMARY

- All sample analyses should follow rigorously standardized and validated operating procedures to ensure high-quality assurance, and low intra-batch and low inter-assay variability
  (See Section 5.1 Sample analysis)
- Data analysis involves three major steps:
  - data preparation
  - descriptive statistics
  - inferential statistics

  (See Section 5.2 Data manipulation and statistical analysis)
- Quality control of data capture should be enforced through random audits, double entry of data and use of electronic data capture systems (Section 5.2.3)
- Dissemination of results for a scientific audience should follow the Consolidated Standards of Reporting Trials (CONSORT) guidelines to ensure a standardized method for reporting clinical trial results in a comprehensive and transparent manner
  (See Section 5.3 Publishing of results)
- Study archives should consist of all key documents collected during the design, conduct and analysis of a clinical trial for future access and interpretation
  (See Section 5.4 Archiving of data)
- Effective biological sample preservation requires a comprehensive strategy for the collection, transport and long-term protection of samples
  (See Section 5.5 Sample preservation, coding and storage)

5.1 Sample analysis

Clinical trial samples refer to any biological sample collected from a participant of a clinical trial as required by the protocol. Samples may include blood, plasma, serum, saliva, urine, feces, tissues, DNA, RNA and cells. These samples are used to quantify biomarkers reflecting a step in the process between exposure to an ingredient or a food, for example, and disease or health condition [114].

Three common sources of error are introduced when using biomarkers:

- issues related to biological specimen collection, processing and storage [115];
- analytical imprecision of laboratory measurements associated with high within-batch (intra-assay) and between-batch (inter-assay) variation and lack of adequate quality control; and
- within-person (intra-individual) variability over time [116].

5.1.1 Sample processing and quality assurance

Either external or in-house laboratories can be used to perform analyses of biological samples depending on the availability of established and validated analytical methods. Regular communication with the laboratory conducting the assay is critical during all phases of a study [103]. If analysis or evaluation of clinical trial samples is subcontracted to another laboratory, the ability of the subcontractor to perform the work must first be assessed [117]. Using a specialized laboratory that is certified or accredited by an external proficiency testing program for the main outcome measure is strongly recommended to enhance the overall quality of the study. However, for newer methods, such as blood polyphenol profile or LDL particle size, analysis may have to be done by in-house laboratories that do not subscribe to an external policy program. In such cases, training can be specifically tailored to the needs of the in-house research facility staff, but must be tied to an internal quality control checks that will allow for an assessment of analytical variation of the method.

In MRCTs, the use of a centralized laboratory (whether in-house or external) is a useful solution for the interpretation of results as it ensures that the same method and reagents for assays will be applied for all participants and that the results will comply with the same system of quality assurance [118]. In the absence of a centralized laboratory, methods across centres should be standardized.

All samples received by the laboratory should be assessed on arrival to check for physical integrity and proper labelling. The sponsor (or representative) or the investigator should be notified promptly when samples have been compromised in transit. Samples should not be analyzed until their identity is confirmed. The sample labelling method has to be well organized and structured [117].

5.1.2 Batch analysis

As most intervention studies accumulate several biological samples over time, assessment for specific outcome measures often needs to be done in multiple batches [119]. However, separating the analyses into different batches can introduce additional variability beyond the intrinsic, within-batch (intra-assay) variation [120–124]. For intervention studies primarily interested in assessing changes in biomarkers over time, biomarkers at all time points during the study should be analyzed concurrently. Baseline samples should not be assayed alone so baseline cross-sectional associations can be assessed more quickly. This concept may also be extended to other study designs. (See Table 1 of [103] for more details on sample allocation issues for different study designs.) This concept does not apply to samples assessed at screening, before randomization of participants.

In some cases, it is not possible to analyze study outcomes in batches because some biomarkers are unstable over time and have to be measured immediately. For example, markers of oxidative stress often undergo further modification over time even after the serum/plasma has been frozen. In such cases, the analytical method of unstable biomarkers has to be extremely robust to fluctuations. This is an advantage of working with a certified laboratory when possible.

5.1.3 Variability in assay results over time

A large inter-assay variability can potentially bias the effect size estimates, even when samples are properly stored and managed [103]. In long-term studies, years can separate the measurements of study outcomes between participants. Substantial laboratory assay drift may occur, causing different mean

levels between assay batches. Contracting sample analysis to a certified laboratory will greatly minimize this risk.

Some other solutions include:

- creating batch-specific quantile cutpoints, allowing the relative relationship (e.g., high vs. low levels) between biomarker and disease or exposure to be assessed [103];
- including a subset of quality control samples, also called drift samples, assayed in all batches to allow recalibration of results from batch to batch to achieve comparable distributions; and
- developing a regression model to compare sample values from the new and reference batches [103].

BEST PRACTICES KEYPOINTS

Sample analysis

- All sample analyses should follow rigorously standardized and validated operating procedures to ensure high-quality assurance, and low intra-batch and low inter-assay variability.
- In the absence of external quality assurance, perform repeat analysis of aliquots of a designated in-house quality control sample along with routine sample analysis
- Contracting the analysis of the main study outcomes to accredited laboratories is generally recommended, when possible.

5.2 Data manipulation and statistical analysis

Data analysis is an intricate phase that involves more than just analyzing the results from a statistical standpoint. Analysis involves three major steps [125]:

- cleaning and organizing the data for analysis (data preparation);
- describing the data (descriptive statistics); and
- testing hypotheses and models (inferential statistics).

Data preparation involves checking for accuracy and running frequency analyses to make sure that the output matches the expected values for each variable [125, 126]. This has to be done even if data entry uses verification methods such as double entry of data [126]. See also Section 5.2.3 Validity and quality control of data capture. Errors, implausible values and outlier values need to be checked and any errors need to be corrected [126]. According to best practices in clinical research, all changes in the dataset at this point need to be carefully justified, monitored and documented for future reference.

Descriptive statistics outline the basic features of the study data and help to create a description of the study population [125, 126]. They provide simple summaries about the study sample and the

outcome measures [125]. More precisely, they describe the distribution, the central tendency and the dispersion [125].

An important aspect of the description of a variable is the shape of its distribution, which indicates the frequency of values from different ranges. Researchers are interested in how well the distribution can be approximated by the normal distribution. Visual examination of the data using a histogram and performance of a test of normality are used to determine the probability that the sample came from a normally distributed population of observations [127]. This analytical step is fundamental [126] as many statistical tests are based on assumption of normality. For example, statistical tests that compare means, such as two-sample *t*-tests and analysis of variance (ANOVA), are called parametric tests and assume that the data from the samples being compared are normally distributed [126].

When data are not normally distributed, the data can be transformed to normality successfully by using log or trigonometric transformations [126]. For instance, dietary and nutrient data are often skewed. To proceed with parametric statistical inference, the data must be transformed [126]. If the data are not normally distributed and cannot be transformed toward normal distribution, non-parametric tests must be used [126]. A good example of this would be data presented as prevalence or proportion.

It is beyond the scope of this document to present an in-depth view of how to conduct statistical testing of the hypotheses being investigated in a clinical trial. Statistics is a complex area and support from an expert in biostatistics is highly recommended. Nevertheless, the main issues to consider when performing statistical tests in the context of an RCT are briefly outlined here.

Inferential statistics are performed according to the statistical plan defined *a priori* at the design stage. Tests are devised specifically to provide a level of confidence to accept or reject the working hypothesis [126]. See also Section 3.4.1 Sample size calculation. An observed effect that is considered large enough so that it cannot be ascribed to chance is considered statistically significant. To draw a conclusion about a result being statistically significant, the appropriate statistical test needs to be completed [126]. Statistical tests are conducted considering a level of confidence of 95% or more, or a margin of error of 5% or less.

The simplest inferential test used to compare the difference in means between two groups is the two-sample *t*-test, also known as independent samples *t*-test [126]. This test is used when analysis involves a dichotomous categorical variable (e.g., two groups, or two treatments) and a quantitative variable, which is the outcome measure [126]. For a crossover study with two treatments, a paired *t*-test would be used as it defines an analysis where the participant is its own control. However, a simple paired *t*-test cannot account for period and carryover effects. The *t*-test is also limited to comparing the means of two groups. When more than two groups or treatments are involved, use of repeated *t*-tests between the various pairs of groups is not recommended as this increases the risk of observing significant differences between groups/treatments due to chance.

Using ANOVA is more powerful and more appropriate than using multiple two-group comparisons by *t*-tests [127]. ANOVA is a series of statistical tests that can be used to compare the means of two or more independent groups or treatments and identify factors that explain the variation in the outcome measure [128]. In a typical food-based human clinical trial, many potentially confounding factors need to be taken into account. For instance, factors such as gender, age and obesity may be related to the outcome measure and therefore need to be accounted for when assessing the response to dietary changes. Using

the ANOVA approach, these factors can be integrated into the modelling of the treatment effect, providing a more refined appreciation of the "true" treatment effect.

When the treatment effect takes into consideration the impact of other co-variables, it can be stated that the treatment effect is "adjusted" for, or independent from, the potential confounding effects of variables known to be related to the study outcome. The capacity to adjust the main treatment effect for the contribution of potential confounders is another strength of the ANOVA compared with simple *t*-tests and is very important in clinical studies [127].

An additional advantage of ANOVA over simple *t*-tests relates to its capacity to test interactions among variables in determining the outcomes of the study. This allows the testing of more complex hypotheses [127]. An interaction is present when the main treatment effect on the outcome measure varies as a function of one or several characteristics of the study sample [128]. In other words, the interaction reflects how one characteristic of the study participants can modify their response to a nutritional change. For example, studies have shown that age and gender may alter the impact of a dietary manipulation on cardiovascular risk factors (e.g., blood cholesterol). Potential interaction effects need to be well thought out and planned *a priori*. The study has to be designed so it is able to investigate the variability of the response according to any given characteristic [128].

Most studies involve multiple measurements of a variable under different conditions or at different times on the same participant [128]. ANOVA for repeated measures is used when a group of participants has the same variable measured several times over a specified period of time or after exposure to two or more conditions [128]. It is relevant when the investigator wants to look at the trends in a biomarker level over time and the sustainability of the effects after an intervention [128]. The test determines whether there are statistically significant differences in biomarker levels over time when there are more than two repeated measurements [128].

Three classes of models are used in the ANOVA:

- fixed-effects models
- random-effects models
- mixed-effects models

Fixed-effects models of ANOVA are used when one or more treatments are applied to the participants to see if the response variable changes. Random-effects models are used when the treatments are not fixed; that is, when various factor levels are sampled from a larger population. Mixed-effects models include both fixed- and random-effects experimental factors. Mixed-effects models are more flexible for repeated measure or longitudinal data than univariate or multivariate approaches. Mixed models are powerful methods for handling non-independence of data over time (data in any given individual at various time points during an experiment are correlated), while allowing missing datasets and not requiring measurements that occurred at the same time points.

The possible presence of a carryover effect (also referred to as residual effect) in crossover studies complicates design and analysis. Mixed model analysis can take into account the limitation inherent to residual effects. The sequence of treatments for each individual becomes one of the independent variables predicting the outcome measure in the model, along with time period and the treatment. A

significant carryover effect implies that the treatment effect varies according to study periods, as emphasized by a significant treatment by period interaction. When the treatment by period interaction is significant, the usual practice is to compare the various treatments within each sequence. However, such post hoc data analyses have to be interpreted with caution, particularly if they were not part of the original study design.

5.2.1 Blinding

In cases where clinical trials are blinded, maintaining the integrity of the blinding process is an essential part of conducting a clinical trial. Laboratories that perform the analysis or evaluation of clinical trial samples must exercise due diligence to ensure they do not inadvertently compromise the blinding process. The trial protocol should include directions for breaking the blind (generally after the statistical analysis is completed), including the conditions under which the code is allowed to be broken and by whom [117]. Any intentional or unintentional breaking of the blind should be reported and explained at the end of the trial, irrespective of the reason for its occurrence. The procedure and timing for revealing the treatment assignments should be documented [129].

5.2.2 Expression of results

There are several acceptable ways to report data. For example, a report may be prepared that contains data, interpretation of results and conclusions, or the results of clinical analysis may simply be supplied as electronic source data or printouts from the analytical equipment used to perform the testing. Regardless of how data are reported, the format used must be accurate, complete and reflect all analyses that were performed [100].

5.2.3 Validity and quality control of data capture

Any analysis is only as convincing as the quality of the underlying data [130]. For paper-based clinical trials, data quality is traditionally assessed through audits that compare database listings against data recorded on paper CRFs [131]. Research organizations are now moving from paper-based data collection to electronic data capture systems. Some research centres have developed their own tool but there are also commercially available e-CRFs (e.g., Biogenix, Itec Services, Entrypoint Plys, Ofni systems). If novel technologies are to be successfully integrated into clinical trials, their data quality is assessed differently [131].

Some authors have attempted to provide scientists with practical recommendations on how to ensure efficiency in the task of data entry, which is the backbone of any credible analysis [130]. It is recommended that pilot testing of the system be done before data entry at a more systematic level [130]. See also Section 5.2 Data manipulation and statistical analysis. While not an absolute regulatory requirement, double entry of data is considered the definitive gold standard of good clinical practice.

BEST PRACTICES KEYPOINTS

Data manipulation and statistical analysis

- Data analysis involves three major steps:
  - Data preparation
    - checks for data accuracy and verification
  - Descriptive statistics
    - describes and summarizes data focusing on the normality of the data distribution
  - Inferential statistics
    - tests hypotheses and models outlined *a priori* during study design
    - simple *t*-tests or ANOVA tests using various classes of models (i.e., fixed effects, random effects and mixed effects).
- Blinding should be maintained during data analysis.
- Quality control of data capture should be enforced through random audits, double entry of data and use of electronic data capture systems.

5.3 Publishing of results

Any food-based human clinical trial will mean little if the results are not disseminated and used in clinical practice [132]. Results of a trial can be made widely available using a variety of media, such as articles in medical journals, online journals, trial registers, systematic reviews and conference presentations [132]. Reporting in a scientific journal involves not only choosing the appropriate journal depending on sponsor needs and target audience but also deciding whether to publish in open access journals. For some authors, publication quality is more important than free public availability and increased exposure when choosing publication venues [133]. On the contrary, industries might prefer publications that are freely available for dissemination as part of their marketing strategies.

The CONSORT Guidelines [134, 135] provide a 25-item checklist of essential items for reporting clinical trials, standardizing the quality and transparency of trial reporting [132]. (See box **The CONSORT 2010 checklist**.)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results and conclusions (for specific guidance see CONSORT for abstracts)	
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	
	2b	Specific objectives or hypotheses	
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	
Blinding	11a	If done, who was blinded after assignment to interventions (e.g., participants, care providers, those assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	

Section/Topic	Item No	Checklist item	Reported on page No
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

* We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist, see www.consort-statement.org.

The aim of the CONSORT guidelines is to improve the quality of published reports [136]. Some of the details of reporting of clinical interventions involving plant materials, recommended in the CONSORT Extension for Herbal Medicinal Interventions [137], may be particularly applicable to clinical studies of foods. Currently, some journals have adopted CONSORT and suggest or require its use in submitted manuscripts [136]. Trial results should be published whatever the outcome of the trial, and it is regarded as scientific misconduct not to publish [137, 138].

There has been significant public debate about the susceptibility of research to biases of various kinds. The overwhelming focus of the discussion to date has been on industry-funded science. Given the critical role that industry has played and will continue to play in the research process, the International Life Sciences Institute North America Working Group on Guiding Principles has proposed conflict-of-interest guidelines regarding industry funding, for protecting the integrity and credibility of the scientific record, particularly with respect to health, nutrition and food-safety science [139]. (See box **Eight principles for industry-sponsored research**.)

Eight principles for industry-sponsored research [139]

1. Conduct or sponsor research that is factual, transparent and designed objectively; according to accepted principles of scientific inquiry, the research design will generate an appropriately phrased hypothesis and the research will answer the appropriate questions, rather than favour a particular outcome.
2. Require control of both study design and the research itself to remain with scientific investigators.
3. Not offer or accept remuneration geared to the outcome of a research project.
4. Before the study begins, ensure that there is a written agreement that the investigative team has the freedom and obligation to attempt to publish the findings within some specified time frame.
5. Require, in publications and conference presentations, fully signed disclosure of all financial interests.
6. Not participate in undisclosed paid authorship arrangements in industry-sponsored publications or presentations.
7. Guarantee accessibility to all data and control of statistical analysis by investigators and appropriate auditors/reviewers.
8. Require that academic researchers, when they work in contract research organizations or act as contract researchers, make clear statements of their affiliation; require that such researchers publish only under the auspices of the contract research organizations.

These guidelines should be considered only as a first step in creating a firewall against bias in research. That is, each organization wishing to adopt these guidelines needs to develop its own quality control mechanism to ensure integrity of scientific records, conformity with guidelines and transparency [139].

Publishing a paper in a scientific journal is not a quick process. The general steps include manuscript preparation in accordance with the chosen journal guidelines (e.g., style, format) and its submission. The journal editorial board will decide if the research topic and quality of the study in very broad terms meet

the journal's standards. If it does, the paper is sent to reviewers for critical review. The turnaround of the review process varies widely between journals and can range from a few days to months. Investigators may be given the opportunity to respond to comments and concerns raised by the reviewers before the paper can be accepted or rejected. If rejected, the paper can be submitted to another journal. Of course, data cannot be submitted concurrently to two or more journals for publication. When finally accepted, the manuscript has to be edited and proofread before publication. Even if a manuscript requires only minor revisions and is finally accepted, the whole process may take several months. Overall, the various stages described above can consume a period of well over a year, particularly if the paper is initially rejected.

Other dissemination activities include presenting the results to the study participants (see also Section 4.10 Post-experiment communication with participants), presenting the results at scientific meetings, sharing the results with interested stakeholders, regulators and policy makers, as well as disseminating the results to health professionals that may find clinical applications with their own practice. However, in many instances, dissemination of newly emerged data is often too rapid, generating media hype that may not be justified. Indeed, one study is often not sufficient to draw a firm conclusion on a particular health effect related to a particular food.

BEST PRACTICES KEYPOINTS

Publishing of results

- Preparation of results for dissemination to a scientific audience should follow the CONSORT guidelines to ensure a standardized method for reporting clinical trial results in a comprehensive and transparent manner.
- To prevent susceptibility to publishing bias, declarations of conflict of interest and industry funding must be stated to protect the integrity and credibility of the research presented.
- Manuscripts must be formatted and organized according to the specified journal guidelines.
- Several other methods can be used for disseminating results depending on whether the results are intended to be reviewed by research participants, research scientists, stakeholders, policy makers, or others.

5.4 Archiving of data

The archiving of a dossier of essential documents of an entire trial, including the database, is a requirement of GCP [100]. Archiving ensures that data are generated in an adequate format and properly documented so that scientists or professionals in the future may use the data and knowledge to answer additional questions or explore additional secondary hypotheses. For instance, other scientists might request further information on a study (perhaps when performing a meta-analysis) or on topics that have not been published. Indeed, not all information and results of a trial can be put in a publication due to length limits. Archiving remains the only option, ensuring that everything related to the study will be preserved and accessible. See also Section 4.6 Record keeping and database management.

5.4.1 Archiving of study data

Management of data derived from a food-based human clinical trial can be tedious. Data management and archiving need to be planned carefully. Both the data associated with the study itself (i.e., description of study, raw data, analyzed data, reports and publications) and the processes used to manage the study have to be archived. Backing up electronic data is also an imperative part of this process.

The study data that need to be archived for each study will depend on the type of study and the degree of standardization in the field. The archived study should include everything needed for another scientist to be able to interpret and use the data. Each study should be stored in a separate dossier that includes only information relevant to the study. Each study dossier should include a one-page summary of the study providing key information.

The study dossier should also include:

- a background folder with a description of general considerations and preliminary thoughts on methodology;
- a methods folder that provides a detailed description of the various methods used in the study—final versions of questionnaires, informed consent forms, and other forms that were used should be segregated from preliminary versions;
- a raw data folder as well as an analyzed data folder containing the outcome of the analysis, and if relevant the statistical scripts used; and
- a reports and publications folder with any published or submitted manuscripts.

The relevant study management information to be archived includes contract records, grant proposals and signed addenda. Also, work plans and budgets for each year and changes that occurred need to be formally documented. Progress reports, minutes of management (and other) meetings, menus, as well as administrative, financial and procurement information are also useful to keep.

5.4.2 How and where to archive

Facilities should be designed to accommodate the types of material that will be archived. Archive design and environmental conditions should protect contents from untimely deterioration and should safeguard the confidentiality of any trial participants [100].

Archiving may take several different forms, including a building or room specifically designated for the retention of trial materials, a fireproof safe or a lockable cabinet. Some research facilities may not be equipped with adequate archiving rooms. In such instances, researchers can contract archiving services from specialized companies. All archive facilities should be secure to prevent unauthorized access to the retained materials. Access to the archive should be restricted to designated staff members. Procedures for the removal of material from the archive and its subsequent return should be documented [100].

Requirements for the archiving of electronic records are the same as those for other record types. Some additional issues that should be considered include [100, 140]:

- long-term access to, and readability of, electronic information (data format);

- the shelf-life of the storage medium where appropriate (e.g., CD-ROM, DVD, server); and
- quality control checks following data migration to a secure server or other storage medium.

5.4.3 Archiving duration

Information from a clinical study—in both paper and electronic form—is a valuable asset that should be archived to preserve both the records and access to the information [141]. The FDR require that the sponsor maintain all pertinent REB records for a period of 25 years [9]; all other essential documents not unique to the REB should be retained for at least 3 years as per GCP guidelines [95, 96].

BEST PRACTICES KEYPOINTS

Archiving of data

- Each study archive should include these key folder articles for future access and interpretation:
  - summary of key information
  - background general information regarding study considerations
  - description of methods used
  - final copies of all study forms (e.g., questionnaires, informed consent forms)
  - raw and analyzed data, final reports and published or working manuscripts.
- Administrative records of studies should also be archived, including:
  - contract records, grant proposals, addenda
  - work plans, budgets, progress reports, menus, meeting minutes.

5.5 Sample preservation, coding and storage

Preservation of biological samples requires a comprehensive strategy for collection, transport and protection. The clinical trial protocol should specify a designated person in charge of sample management as well as the duration of storage. Tracking of these temperature-sensitive materials specified by the clinical trial protocol should be included in the work instruction or associated documentation. Information about the sample should comprise its source (e.g., study participant), its characteristics (e.g., skin tissue, blood, serum), and its post-collection processing and storage (e.g., placement in freezer 10 minutes after collection). Laboratory staff should monitor storage conditions to provide evidence that the samples have been stored in a way that ensures they remain fit for the intended purpose [100].

Storage in a biorepository (or biobank) necessitates following specific regulations and a management framework supervised by the research institution. For instance, in October 2009, the Organisation for Economic Co-operation and Development (OECD) Council adopted a Recommendation on Human Biobanks and Genetic Research Databases. OECD is an international economic organization of 34 countries, including Canada, founded in 1961 to stimulate economic progress and world trade. The recommendation provides guidelines for the establishment, management, governance, operation, access,

use and discontinuation of human biobanks and genetic research databases. It recognizes that one of the fundamental objectives of such databases is to foster scientific research. Overall, it seeks to facilitate wide access to data and materials for biomedical advances while ensuring that research is conducted in a manner respectful of participants, and upholds human dignity, fundamental freedoms and human rights.

6.0 Conclusion

Food health claims that are authorized and accepted by Health Canada are the culmination of extensive evidence-based research compiled from numerous human studies that have been robustly appraised based on their validity and quality. Food health claim petitions based on supporting studies that are appraised to be of high internal validity and low risk of bias are more likely to support claim substantiation as they provide clear information on the validity of the food–health relationship and its relevance for the Canadian population.

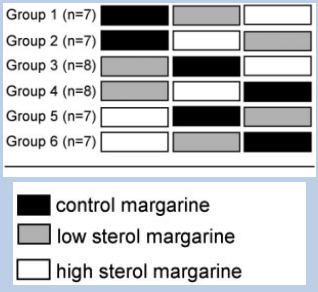
This document fulfills a need for Canadian researchers to have a broad and comprehensive resource that describes each step of a food-based human clinical trial from inception to publication from a food health claim perspective. Such a document is required to improve the validity of claim petitions for the benefit and health of the Canadian population.

This practical manual is intended to be used by academic and industry researchers, with a focus on food manufacturers and agricultural sectors so the final food health claim can be applied to foods and food constituents consumed by Canadians. This document, used in tandem with Health Canada's *Guidance Document for Preparing a Submission for Food Health Claims* [2], should provide the guidance needed to design, conduct, analyze and report quality food intervention trials and incorporate the evidence in support of a food health claim petition to Health Canada.

7.0 Appendices

Appendix 1. Key features of dietary intervention trials with crossover design

Crossover design

	Vanstone et al., 2002 [142]	Veenstra et al., 2009 [143]	Naumann et al., 2003 [144]
Design	4 factors x 4 levels	4 factors x 4 levels	3 factors x 3 levels
Objective	• To examine the effect of supplementation with unesterified plant sterols and stanols on plasma lipid and phytosterol concentrations and cholesterol absorption, synthesis, and turnover	• To determine the influence of regular consumption of a moderate amount of chickpeas, lentils and green peas on perceived gastrointestinal function in a sample of healthy adult males	• To examine if plant sterols and stanols influence serum plant sterol, stanol, lipid and lipoprotein concentrations
Outcomes	• Plasma lipid • Plasma phytosterol concentrations • Cholesterol absorption, synthesis, and turnover	• Perceived flatulence • Abdominal comfort • Bowel movements • Overall gastrointestinal function	• Serum plant sterol and stanol concentrations • Serum lipid and lipoprotein concentrations
Sample size	15	21	44
Treatment	• Plant sterols (NS) • Plant stanols (SS) • 50:50 mixture of sterols and stanols (NSS) • Cornstarch (control)	• 100 g dry weight Kabuli chickpeas • Green Laird lentils • Green peas • Potato control	• 1.5 g/d of plant sterols plus 0.5 g of plant stanols (high sterol margarine) • 1 g of each (low sterol margarine) • Control margarine
Sequences	To reduce the error term associated with diet sequencing, subjects were randomly assigned to 1 of 4 predetermined Latin squares, each of which possessed 4 sequenced phases and 4 subjects. In this manner, we ensured that the crossover design was balanced.	Each participant was randomly assigned to consume the four study treatments in a different order from a possible 24 order groups.	
Treatment duration	3 weeks	4 weeks	3 weeks

	Vanstone et al., 2002 [142]	Veenstra et al., 2009 [143]	Naumann et al., 2003 [144]
Washout period	4 weeks	28 days	None
Statistical analysis	Means ± SEMs; ANOVA; carryover term	Repeated measures ANOVA; ANCOVA; Tukey's test	ANOVA with Tukey's test

Parallel design

	Maki et al., 2010 [145]	Polagruto et al., 2006 [146]
Design	2 factors	2 factors
Objective	• To evaluate whether a whole-grain ready-to-eat (RTE) cereal lowers LDL cholesterol	• To evaluate whether a flavanol-rich cocoa snack food with phytosterols lowers LDL cholesterol and affects serum fat-soluble vitamins in hypercholesterolemic individuals
Outcomes	• Serum lipoprotein levels • Waist circumference • Triceps skinfold thickness • Body weight	• Serum lipid levels • Serum carotenoids and fat-soluble vitamins
Sample size	204	67
Treatment	• Whole-grain RTE oat cereal (3g/d oat beta-glucan) • Energy-matched low-fibre food	• Cocoa flavanol-enriched snack bar with 1.5 g phytosterols • Control product with no phytosterols
Sequences	N/A	N/A
Treatment duration	12 weeks	6 weeks
Washout period	N/A	N/A
Statistical analysis	Repeated measures ANOVA	Mean ± SEM; 2-way repeated measures ANOVA

Appendix 2. Example of block randomization

Random code of block	Participant	Treatment
5	1	A
	2	B
	3	B
	4	A
2	5	A
	6	B
	7	A
	8	B
4	9	B
	10	A
	11	B
	12	A
3	13	B
	14	B
	15	A
	16	A
1	17	A
	18	A
	19	B
	20	B
6	21	B
	22	A
	23	A
	24	B

Appendix 3. Randomization schedule for a crossover trial

Participant	Period 1	Period 2	Period 3
1	A	B	C
2	C	B	A
3	B	C	A
4	C	A	B
5	B	A	C
6	A	C	B
7	C	A	B
8	B	A	C
9	A	C	B
10	B	C	A
11	A	B	C
12	C	B	A
13	B	A	C
14	A	C	B
15	C	B	A
16	C	A	B
17	B	C	A
18	A	B	C

A, B, C = treatments

Appendix 4. Example of stratified blocked randomization

Stratum	Baseline serum cholesterol	Gender	Age	Randomization
1	Optimal	Male	< 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
2	Optimal	Male	≥ 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
3	Optimal	Male	< 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
4	Optimal	Male	≥ 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
5	High	Female	< 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
6	High	Female	≥ 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
7	High	Female	< 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB
8	High	Female	≥ 50 years	AABB, ABAB, BBAA, BABA, ABBA, and BAAB

Appendix 5. Examples of relevant blinding approaches

	Vanstone et al., 2002 [142]	Bloedon et al., 2008 [147]	Bïorklund et al., 2005 [148]
Dietary component	Phytosterols	Ground flaxseed	ß-glucan as powder
Control	Corn starch	Wheat bran	Rice starch
Incorporation method and similarity of the treatment characteristics	"the phytosterol and phytostanol mixtures and the cornstarch control were blended into the butter component of the diet at a dosage of 1.8 g/d; the butter was warmed to 37°C and administered equally across the 3 daily meals."	"Twenty g of ground flaxseed or wheat bran was baked into 1 of 3 breads or muffins designed and pretested to have similar appearance, taste and texture."	Beverages flavoured with a blackcurrant fruit juice concentrate. "Three times during the study the subjects filled in a questionnaire for sensory evaluation concerning taste, smell, consistency, appearance and total impression of the beverages, using a nine-graded scale that ranged from 'dislike very much' (grade 1) to 'like very much' (grade 9)."
Who was blinded?	Double-blind; participant and researcher	Double-blind; participant and researcher	Single blind; participant

Appendix 6. Examples of treatments and their dietary compositions

Table 2. Energy (kcal/serving) and nutrient composition (g/serving) of oat and wheat food products

	oat crisp bread	wheat crisp bread	oat porridge	oat granola	wheat muffin	oat pasta	wheat pasta
Calories	365	343	317	318	333	312	325
serving size (g)	115	107	270	97	128	366	329
fat (g)	6	4	9	9	10	8	9
carbohydrates (g)	85	85	60	62	58	71	66
fiber	22	20	17	18	12	29	22
β-glucan	4	1	4	4	0.2	4	0.4
glucose	2	1	7	6	8	3	2
starch	62	64	36	38	38	39	42
available carbohydrates[a]	64	65	43	44	46	42	44
protein (g)	15	11	16	16	15	18	18
ash (g)	3	2	2	1	2	2	3
moisture (mL)	6	4	184	9	43	267	233

[a]Calculated as starch (g) + glucose (g)

Regand et al. (2009) [41]

Excerpted from Table 2 Composition of plant sterol-containing yoghurt, low-fat plant sterol-containing hard cheese and fresh cheese and control (standard) food items

Nutrients	*Yoghurt*	
	Sterol (150 g)	*Control (150 g)*
Energy (kJ)	536	540
Protein (g)	5.25	5.25
Carbohydrates (g)	19.5	19.5
Total fat (g)	3.0	3.0
Fatty acids		
Saturated (g)	1.95	1.95
Monounsaturated (g)	0.6	0.6
Polyunsaturated (g)	0	0
Cholesterol (mg)	7.8	7.8
Plant sterols (g)	1.65	0
β-Sitosterol (g)[a]	1.24	
β-Sitostanol (g)[a]	0.16	
Campesterol (g)[a]	0.16	
Campestanol (g)[a]	0.03	
Other plant sterols (g)[a]	0.03	
Milk fat (g)	0.75	3.0
Vegetable fat (g)	0.6	0

Values are per daily dose of the product.

[a]Plant sterol amounts are calculated from the manufacturer's information of plant sterol powder (75% β-sitosterol, 10% β-sitostanol, 10% campesterol, 2% campestanol, 2% others)

Korpela et al. (2006) [42]

Appendix 7. Example of a 3-day cycle menu based on 2000 kcal

	Day 1	Day 2	Day 3
Breakfast	255 g Orange Juice – Unsweetened 220 g Milk – 2% 80 g Cereal with Fruit and Fibre 12 g Whole Egg, 135 g Egg White 65 g Tomato – Red 30 g Bell Pepper – Green 5 g Butter, 27 g Margarine 58 g Bagel – Plain 13 g Jam – Strawberry	152 g Orange Juice – Unsweetened 185 g Yoghurt – Non-Fat Fruit 31 g Maple Syrup 109 g Bread – Multigrain 211 g French Toast Mixture 3.7 g Olive Oil 3.3 g Butter, 25.5 g Margarine 21 g Dried apricots 21 g Raisins	320 g Orange Juice – Unsweetened 66 g Cottage Cheese – 2% 66 g Oranges – Fresh Peeled Quartered 66 g Blueberries – Fresh or Frozen 66 g Bananas – Fresh Sliced 112 g Whole Wheat English Muffin 21 g Margarine 12 Whole Egg, 66 g Egg White 31 g Sausage – Pork Cooked 26 g Jam – Strawberry
Lunch	255 g Apple Juice – Unsweetened 110 g Pita Bread Whole Wheat 65 g Pizza Sauce 33 g Chicken Breast – Cooked 45 g Mushrooms – Sliced Raw 37 g Bell Pepper – Green Chopped 15 g Back Bacon – Cooked 30 g Mozzarella Cheese 1% shredded 40 g Zucchini – Sliced Raw 25 g Onions – Chopped Raw 12 g Margarine, 2 g Butter 100 g Tomatoes – Red Chopped Raw 100 g Cucumber – Chopped Raw 4 g Vinegar – White, 3 g Olive oil 35 g Carrot Cake, 12 g Cake Icing – White 25 g Seedless Grapes	220 g Orange Kiwi Passion Fruit Juice 80 g Bread – Multigrain 12 g Margarine 5 g Butter 70 g Ham – Lean 5% Fat Sliced 20 g Swiss Cheese 25 g Lettuce – Iceberg Raw 5 g Alfalfa Sprouts – Raw 20 g Bell Pepper – Green Sliced Raw 25 g Tomatoes – Red Sliced 330 g Soup – Cream of Broccoli 35 g Grapes – Red Slip Skin Seedless 65 g Yoghurt Cake 60 g Fruit Sauce	210 g Apple Juice – Unsweetened 220 g Turkey Loaf 85 g Tomato Sauce 146 g Potatoes – Peeled Chopped Boiled 70 g Carrots – Sticks Raw 90 g Beans – Green Snap/String Cooked 22 g Margarine 90 g Fruit Salad – Canned Light Syrup 60 g Date Square
Supper	240 g Apple Juice – Unsweetened 81 g Chicken Breast – Roasted 50 g Cranberry Sauce – Canned 2 g Butter 100 g Potatoes – Peeled Chopped Boiled 45 g Carrots – Stick Raw 50 g Peas – Green Frozen Boiled 23 g Margarine 26 g Chocolate Fudge Syrup 100 g Frozen Yoghurt – Vanilla 40 g Stoned Wheat Crackers	175 g Orange Banana Strawberry Juice 51 g Celery – Sticks Raw 45 g Cottage Cheese Dip 142 g Spaghetti Pasta – Cooked 102 g Egg Noodles – Cooked 238 g Spaghetti Sauce 16 g Parmesan – Cheese Grated 16 g Margarine 36 g Whole Wheat Bread 3 g Butter 57 g Sherbet – Orange	185 g Orange Peach Mango Juice 290 g Soup –Tomato Lentil 27 g Whole Wheat Bread 6 g Butter 12.5 g Margarine 200 g Beef Stir-Fry 175 g Rice – Long Grain Cooked 100 g Apple Sauce – Unsweetened 37 g Ginger Snaps

Appendix 8. Estimated time required to measure the effect of selected dietary intervention on selected outcomes

Study outcome measure	Minimum intervention duration	Reference
Lipoprotein	2–4 weeks	Sandstrom, 1995 [46]; Kris-Etherton and Dietschy, 1997 [149]
Blood pressure	4–8 weeks	Appel et al., 1997 [150]; EFSA [151]
Glycosylated hemoglobin	3 months	EFSA [152]
Weight loss	6 weeks	Franz et al., 2007 [153]
Glucose tolerance	4 weeks	Marinangeli and Jones, 2011 [33]

Appendix 9. Examples of multicentre feeding trials

Trial	Number and location of centres	Participants	Treatments	Outcomes	Reference
DASH	4	"456 healthy men and women, aged 22 years or older, with systolic blood pressure less than 160 mm Hg and diastolic blood pressure 80 to 95 mm Hg"	A control dietary pattern that is relatively low in potassium, magnesium, calcium and fibre, and has a fat and protein profile mirroring current consumption. The first experimental diet, arguably termed "ideal," is high in fruits, vegetables, whole cereal products, low-fat dairy products, fish, chicken, and lean meats designed to be low in saturated fat and cholesterol; moderately high in protein; and high in minerals and fibre. The second experimental diet tests the effect of fruits and vegetables alone.	Blood pressure	Appel et al., 1997 [150]
DELTA	4	"One hundred three individuals completed the protocol: 55% were women and 45% men. The mean age of the entire group was 37.9 years, with a range from 22 to 67 years. Thirty percent of the women and 20% of the men were black. Thirty-two percent of the women were postmenopausal; 35% of the men were 40 years old. Body mass index ranged from 17.3 to 32.1, with a mean of 24.4 for the women and 24.7 for the men."	"Three diets were designed; diets were to provide 37% of calories from fat with 16% SFA, 14% MUFA, and 7% PUFA; a Step 1 diet with 30% of calories from fat and 9% SFA, 14% MUFA, and 7% PUFA; and a low-fat diet with 26% of calories from fat and 5% SFA, 14% MUFA and 7% PUFA fats."	Plasma lipids, lipoproteins, and thrombogenic factors	Ginsberg et al., 1998 [154]

Trial	Number and location of centres	Participants	Treatments	Outcomes	Reference
Dietary Portfolio	4	"included men and postmenopausal women in the low (0%-10%) and intermediate (10%-19%) Framingham 10-year risk categories who had LDL-C values ranging from 135 to 205 mg/dL and 116 to 178 mg/dL, respectively (to convert to millimoles per liter, multiply by 0.0259). Exclusion criteria included a history of cardiovascular disease, cancer or a strong family history of cancer, untreated hypertension (blood pressure >140/90 mm Hg), diabetes, renal or liver disease, and currently taking lipid-lowering medications."	"Participants received dietary advice for 6 months on either a low-saturated fat therapeutic diet (control) or a dietary portfolio, for which counseling was delivered at different frequencies, that emphasized dietary incorporation of plant sterols, soy protein, viscous fibers, and nuts. Routine dietary portfolio involved 2 clinic visits over 6 months and intensive dietary portfolio involved 7 clinic visits over 6 months."	Percentage change in serum LDL-C	Jenkins et al., 2011 [155]

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

Appendix 10. Examples of best practices in selecting and reporting a study population

Reference	Example
Judd et al., 1994 [156]	Selection of subjects Male and female subjects 25-65 y of age were recruited by advertisement in the area of the Beltsville Agricultural Research Center, Beltsville, MD. Subjects were recruited without regard to race or smoking habits. From 1250 people who responded, 72 were deemed eligible for the study on the basis of the criteria described below, and 64 of these chose to enter the study. Participants were required to be within 85-120% of desirable body mass index (BMI) when compared with the life insurance reference tables, and to be in basic good health with no history of cancer, heart disease, hypertension, hyperlipidemia, diabetes, peripheral vascular disease, gout, liver or kidney disease, or endocrine disorders. Those taking lipid-lowering drugs were not included in the study. Volunteers who reported regular use of dietary supplements, or eating patterns incompatible with the study protocol (e.g., vegetarian or low-fat diets), or who were unwilling to forgo consumption of alcoholic beverages for the duration of the study were not included. Although exercise was not controlled, subjects were asked to maintain their usual exercise pattern throughout the study and to report on a daily questionnaire any deviation from usual behavior. Women taking hormones for birth control or as hormone-replacement therapy were accepted into the study with the provision that they maintain the same type, amount, and timing of medication throughout the study, and that they report such medication on the daily questionnaire. From volunteers meeting other screening criteria, those with plasma cholesterol concentrations between the 50th and 75th percentiles of those screened for blood lipids were included in the final selection (Table 1). HDL cholesterol was required to be > 0.91 mmol/L for men and 1.03 mmol/L for women. Plasma triglyceride concentrations < 3.39 mmol/L were required. Subjects eligible for the study were required to read and sign a written consent form before entry into the study. All procedures were approved by the Institutional Review Board, Georgetown University School of Medicine.
Wolever et al., 2010 [16]	Subjects We conducted a double-blind, randomized, multicenter, parallel design, controlled clinical trial at 2 contract research organizations and 3 university nutrition research centers. Males and nonpregnant females aged 35–70 y with a body mass index (BMI; in kg/m2) >18.5 and <40.0, fasting serum total cholesterol >5.0 and <8.0 mmol/L, and fasting serum LDL cholesterol >3.0 and <5.0 mmol/L were invited to participate. Subjects were excluded for any of the following: fasting serum triglycerides >4.0 mmol/L, serum aspartate transaminase (AST) >1.5 times the upper limit of normal (ULN), serum urea and creatinine >1.8 times the ULN, unstable body weight or intention to lose or gain weight, presence of diabetes mellitus (fasting plasma glucose >7.0 mmol/L or use of insulin or any hypoglycemic or antihyperglycemic medication), presence of any prescription or nonprescription drug, herbal or nutritional supplement known to affect blood lipids (except for stable doses of thyroxine, oral contraceptive agents, hormone replacement therapy, and medications for controlling blood pressure), recent major surgical or medical events, presence of a gastrointestinal disorder or medication that alters the digestion and absorption of nutrients, consumption of a diet containing >15% of energy from saturated fat, allergy to wheat or oats, or consumption of >5 servings of oatmeal, oat bran, or psyllium-containing cereals weekly.

Appendix 11. Example of participation advertisement used for recruitment in RCTs

Want to lower your cholesterol?

We are conducting a study to investigate the effects of yogurt on blood lipid levels.

The study is open to men and postmenopausal women who meet the following criteria:
- Aged 19-60 yr
- Slightly overweight
- Moderately elevated LDL-cholesterol
- Not taking medication to lower blood lipids

Volunteers will be compensated for their participation

Dinner is provided daily upon participation

If interested, please call (680) 555-1234

Appendix 12. Example of a study screening information form

General Subject Screening Form

Circle appropriate YES/NO responses

Name:			
Date of Birth:	Month	Day	Year
Sex:	Male:	Female: Postmenopausal: YES NO	

Contact Information	
Street Address	
Postal Code	
City	
Home Phone:	
Cell Phone:	
Email:	

Medical History			
Diabetes mellitus	YES	NO	If Yes to Other, please specify:
Thyroid disease	YES	NO	
Kidney disease	YES	NO	
Liver disease	YES	NO	
Heart disease	YES	NO	
Hypertension	YES	NO	
Other	YES	NO	

Cholesterol lowering medication? *(in the last 3 months)*	YES	NO	
Other medications	YES	NO	If Yes, specify: Are the doses of these medications stable? YES NO
Vitamin, Mineral supplement	YES	NO	If Yes, specify:
Herbal, food supplement	YES	NO	If Yes, specify:

Laxatives	YES	NO	
Fiber	YES	NO	
Allergies (food such as corn)	YES	NO	If Yes, specify:
Vegetarian	YES	NO	
Any metallic bone components	YES	NO	

Lifestyle		
Smoker?	YES	NO
If Yes, how many per day?		
Drink Alcohol?	YES	NO
If Yes, how many drinks/week		

To be filled out by a study coordinator:

Screening Information		
Weight	lbs:	kg:
Height	‘ “	m:
BMI (kg/m^2)		
Waist circumference (inches)		
Hip circumference (inches)		
Blood pressure	Systolic	Diastolic
Screening code (initials:mm:day:year; eg TR:07:22:10)		

Is subject fasted for blood sampling? YES NO

Appendix 13. Example of a study participant food production sheet

(PETGE302)
Breakfast 1
BEVERAGE
197.7 g Orange Juice – unsweetened
152.1 g Milk – 2%
CEREAL
79.1 g Cereal - Fruit and Fibre
SCRAMBLED EGG DISH
12.2 g Whole Egg
136.9 g Egg White
65.9 g Tomato – Red
30.4 g Bell Pepper – Green
28.4 g Margarine
YOGURT DRINK
1 Container
BAGEL
58.8 g Bagel – Plain
13.2 g Jam – Strawberry
5.1 g Butter

8.0 References

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9.0 Glossary

Alternative hypothesis (H_1) – States the opposite of the null hypothesis and implies that a relationship exists between treatment and outcome

A priori – Before the event (e.g., *a priori* power analysis—power analysis done before data collection; *a priori* clinical trial registration—clinical trial registration to a publically accessible database before participant recruitment

Attrition – The loss of participants during a human clinical trial

Baseline value – Measurement taken on a study participant at the beginning of a study

Bioactive – A substance having an effect on, or causing a reaction in, living tissue exposed to it (e.g., probiotic strains could be a bioactive found in yogurt, omega-3 fatty acids could be the bioactive in omega-3 eggs)

Biomarker – An objectively measured variable, often a laboratory measurement, that is a risk factor or an indicator of a clinical condition or of a response to treatment

Blinding – A research method in which the treatment a participant receives is unknown to either the investigator, the participants or both in order to avoid bias (See *double blinding*)

Carryover effect – The confounding effect of a treatment from the previous period on the response of the outcome measure to the subsequent treatment

Control group – A control group is a group that has not received the exposure of interest and is being compared to the treatment or intervention group in the randomized trial

Crossover study design – A study in which each participant is assigned to a sequence of the different treatments and control and hence will receive all treatments

Double blinding – A research method in which the investigator and the participants do not know which treatment the participant receives

Eligibility criteria – Factors that are based on physiological, anthropometrical, functional, demographical and lifestyle characteristics that are used to define a study population (see *inclusion criteria* and *exclusion criteria*)

Endpoint value – Represents a final value of a measurable quantity following the administration of a treatment and represents the change in the value in response to the treatment

Exclusion criteria – Eligibility criteria that define which participants should be excluded from the study population

Food frequency questionnaire – Research instrument designed to obtain information about long-term patterns of food consumption; consists of a list of foods and frequency-of-use response categories

Food health claim – Any representation in labelling or advertising that states, suggests, or implies that a relationship exists between the consumption of a food or a constituent in the food and a person's health

Free-living trial – A study in which participants are allowed to maintain their normal diets and lifestyles

Good Clinical Practice (GCP) – An international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analyses, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of trial subjects are protected., developed by the International Conference on Harmonisation (ICH).

Hypothesis – A proposition tentatively assumed in order to draw out its logical or empirical consequences and test its consistency with facts that are known or may be determined; a proposed explanation for a research question

Inclusion criteria – Eligibility criteria that define which participants can be included in the study population

Intention-to-treat analysis – A strategy for analyzing data in which all participants are included in the group to which they were assigned, regardless of whether they completed the intervention given to the group. This analysis prevents bias caused by loss of participants which may disrupt the baseline equivalence established by random assignment and may reflect non-adherence to the protocol.

Latin square – A statistical research method that is used when the researcher desires to control two sources of variation in an experiment

Null hypothesis (H_0) – A proposition that implies no effect or no relationship is observed between a treatment and proposed outcome

Outcome measures – Indicators measured in a participant or biological sample that assess the efficacy or safety of an intervention

Parallel-arm study – A study in which a participant is assigned to receive only one treatment. Therefore, there are as many groups in a parallel-arm trial as there are treatments, including the control. Each participant remains assigned to the same treatment group until the end of follow-up of the assigned treatment.

Per-protocol analysis – A strategy for analyzing the set of data generated by the subset of participants who complied with the protocol sufficiently to ensure that the data would be likely to exhibit the effects of the treatment according to the underlying scientific model. Compliance covers such considerations as exposure to treatment, availability of measurement and the absence of major protocol violations.

Placebo – Treatment that is intended to hedonically resemble the test treatment but is void of the bioactive

Primary outcome – An endpoint measure that is used mainly to answer the principal research question

Randomized controlled trial – Quantitative, comparative, controlled experiments in which investigators study two or more interventions in a series of individuals who receive them in random order; considered the "gold standard" of clinical trials; can provide convincing evidence of a cause-and-effect relationship between an intervention and an outcome

Research Ethics Board – A review board established by an institution to review the ethical acceptability of all research involving humans conducted within the institution's mandate

Research question – A query focused on something unknown that outlines a research project

Retrospective studies – A study performed post hoc where the data are collected from past records

Run-in period – A period of time before the commencement of a clinical trial where all participants are given a placebo or standardized diet to ensure baseline observation results

Selection bias – Bias caused by the fact that the types of participants who take part in studies are not a random sample of the population from which they are drawn

Significance level – The minimum level at which the null hypothesis can be rejected

Study population – The entire group of people who could potentially be recruited to a study and, therefore, the population to which any findings and conclusions should apply

Surrogate endpoint – A biomarker that is intended to substitute for a clinical endpoint

Test food – Food that is to be tested as a treatment as part of a food-based human clinical trial; usually contains a bioactive ingredient

Tri-Council Policy Statement (TCPS) – A policy implemented by Canada's three federal research agencies—the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Social Sciences and Humanities Research Council of Canada (SSHRC)—outlining the essential ethical issues to ensure the ethical conduct for research involving humans

Washout period – A period where no treatment or the placebo treatment is consumed for a specified period of time between treatments; incorporation of a washout period can minimize any carryover effect induced by a previous treatment to the next treatment