REVIEW

Best Practices for Conducting Observational Research to Assess the Relation between Nutrition and Bone: An International Working Group Summary

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ABSTRACT

Diet is a modifiable factor that can affect bone strength and integrity, and the risk of fractures. Currently, a hierarchy of scientific evidence contributes to our understanding of the role of diet on bone health and fracture risk. The strength of evidence is generally based on the type of study conducted, the quality of the methodology employed, the rigor and integrity of the data collected and analysis plan, and the transparency and completeness of the results. Randomized controlled trials (RCTs) are considered to be the gold standard from a clinical research paradigm, but there is a dearth of high-quality diet-related intervention trials with bone as the primary outcome, forcing the use of observational research to inform research and clinical practices. However, for observational research to be of the most utility, standardization and optimization of the study design, accurate and reliable measurement of key variables, and appropriate data analysis and data reporting are paramount. Although there have been recommendations made in relation to RCTs in the field of nutrition, no clear rubric exists for best practices in conducting observational research with regard to nutrition and bone health. Therefore, the purpose of this paper is to describe the best practices and considerations for designing, conducting, analyzing, interpreting, and reporting observational research specifically for understanding the role of nutrition in bone health, amassed by a global panel of scientific experts with strengths in bone, nutrition epidemiology, physical activity, public health, clinical and translational trials, and observational study methods. The global panel of scientific experts represents the leadership and selected participants from the 10th annual International Symposium for the Nutritional Aspects of Osteoporosis. The topics selected and best practices presented reflect expert opinion and areas of scientific expertise of the authors rather than a systematic or comprehensive literature review or professional reporting guidelines. *Adv Nutr* 2019;0:1–19.

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Introduction

Bone is a dynamic tissue in which a range of modifiable lifestyle factors, including diet, can affect strength and integrity, and therefore, the risk of bone fractures (1–3). Currently, a hierarchy of scientific evidence contributes to our understanding of the role of diet on bone health and fracture risk. The strength of evidence is generally based on the type of study conducted, the quality of the methodology employed, rigor and integrity of the data collected, appropriate analysis plan, and transparency and completeness of the results presented in the scientific literature.

Randomized controlled trials (RCTs) are considered to be the gold standard from a clinical research paradigm (4), but there is a dearth of high-quality diet-related intervention trials with bone as the primary outcome (1, 5). There are a number of factors that make RCTs of dietary interventions with health-related outcomes (including bone) challenging to conduct and interpret, including the cost, the time commitment and difficulties with maintaining adherence to a given dietary protocol, health problems or medication changes, and ethical issues associated with assigning people to a nonintervention control comparison group (6). For trials with bone as an outcome, a long-term follow-up is typically required because the entire bone remodeling cycle typically takes around 6–9 mo. Furthermore, many trials suffer from limited external validity given that they generally focus on

homogeneous groups, which is, however, purposeful to gain a greater understanding of the mechanisms and biological events or outcomes. Another major challenge with dietary interventions is that it is often difficult to use a true placebo-controlled design, because the absence of nutrition is not a practical study arm and blinding of participants is difficult or impossible to achieve. When RCT data are not available, data from observational studies are often used to inform research and clinical practice, but observational data suffer from a lack of causal inference. Nevertheless, observational research is suitable for nutrition and health research because individuals can be followed for longer periods, permitting researchers to observe usual dietary practices and health exposures, but have varying degrees of limitations in their design, methods, and interpretability of data.

Because age-related changes in bone typically occur slowly over time, the nature of observational studies are well suited for evaluating the role of lifestyle effects on bone (7). Well-designed observational studies have provided a tremendous amount of information to tailor public health messaging to reduce bone loss and reduce fracture risk. However, for this research to be of most utility, standardization and optimization of the research is paramount. Although there have been research recommendations made in relation to RCTs in the field of nutrition (8) and for reporting of studies in nutritional epidemiology (9, 10), none are specific to bone as a health outcome. Therefore, the purpose of this paper is to describe the best practices and considerations for designing, conducting, analyzing, interpreting, and reporting observational research specifically for understanding the role of nutrition in bone health amassed by a global panel of scientific experts with strengths in bone, nutrition epidemiology, physical activity, public health, clinical and translational trials, and observational study methods.

Methods

Observational studies for the purpose of this review included prospective and retrospective cohorts, cross-sectional, and case-control studies. Clinical trials were used as a reference, but were not specifically addressed. The global panel of scientific experts represented the leadership and selected participants from the 10th annual International Symposium for the Nutritional Aspects of Osteoporosis (ISNAO) meeting in Hong Kong in November of 2017. ISNAO is a meeting that represents the scientific work from a group of physician, clinician, academic, and government researchers who specialize in the role of nutrition and bone health. The topics selected and best practices presented in this work reflect expert opinion and areas of scientific expertise of the authors rather than a systematic or comprehensive literature review or professional reporting guidelines. The panel of experts was selected by the organizers of the ISNAO meeting: Drs. Weaver, Daly, and Bischoff-Ferrari. Initial meetings to describe the scope of the project and delineate writing roles were held via conference call and culminated in a writing meeting held in Hong Kong. It should be noted that authoritative recommendations for observational research reporting should also be considered and applied, including but not limited to the Strengthening the Reporting of Observational Studies in Epidemiology (11) and the Strengthening the Reporting of Observational Studies in Epidemiology for use in nutrition epidemiology (10).

Pertinent Factors for Consideration in the Research Design Phase

The first step in any study is to clearly specify the research question and then to design a study or utilize an existing data source to address the research question. In establishing or utilizing an existing data source, it is important to understand and document how the sample or cohort was derived. Having a clear plan in place before data are collected or analyzed is always recommended. It is also recommended that all observational studies are registered with an established registry (e.g., clinicaltrials.gov) a priori, despite not having an intervention per se.

Selecting the study population and parameters

Clearly specifying and matching the study population to the research question is critical, including having predefined inclusion and exclusion criteria. These eligibility criteria should relate to key factors appropriate to the bone and nutrition research questions such as age, disease state, sex, or physical activity (12). In observational research, groups compared based on nutritional exposures should be as similar as possible with regard to other important baseline and/or demographic or geographic parameters to address the primary objective of the study. It should be noted that certain subpopulations are more prone to changes in bone and require specific considerations in recruitment and analysis. Some examples include adolescents, perimenopausal women, older adults, bariatric surgery patients, and those with compromised kidney function. The large changes in bone in these subgroups can mask more subtle effects of the diet. Larger sample sizes are needed to assess differential rates of bone accrual in puberty or bone loss as occurs with menopause, or a more narrowly defined sample can be selected to create a more homogenous group.

Nutritional exposures across the life course have the potential to influence bone health. However, the risk of osteoporosis and low trauma fragility fractures in adults increases exponentially with age, especially among women

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Abbreviations used: 25(OH)D, serum 25-hydroxy-vitamin D; aBMD, areal bone mineral density; BMC, bone mineral content; BMD, bone mineral density; BTM, bone turnover markers; CTX, C-terminal crosslinking telopeptides; FRAX, fracture risk assessment tool; HAL, hip axis length; ISCD, The International Society for Clinical Densitometry; ISNAO, International Symposium for the Nutritional Aspects of Osteoporosis; P1CP, 1 C-terminal propeptide; P1NP, procollagen 1 N-terminal propeptide; pQCT, peripheral quantitative computed tomography; QUS, quantitative ultrasonometry; RCT, randomized controlled trial; vBMD, volumetric BMD.

(13). Thus, carefully matching the age of the participants to the research question is critical. In addition, baseline or underlying nutrition status of the cohort or sample is also critical in any type of research study in the field of nutrition as this may influence the magnitude of response or the strength of any association (8). Similarly, UV exposure patterns differ substantially based on geographic location and must be considered. Provision of nutrients to an already adequate diet is unlikely to produce measurable health benefit—we have seen this with regard to a number of health outcomes but information is limited for bone. One example comes from the Women's Health Initiative that studied the effect of calcium and vitamin D supplementation on hip fracture. When individuals who were using their own calcium and vitamin D supplements (i.e., essentially receiving recommended intakes of calcium and vitamin D) were included in the analysis, there was no significant reduction in hip fracture (14). However, in re-analysis of the same data with the exclusion of those taking personal supplements and those who were noncompliant with the study supplements, there was a significant reduction (30%) in hip fracture among those randomly assigned to receive the study supplements (15).

Selecting appropriately validated endpoints or surrogate markers of disease risk or bone health also needs careful consideration to ensure that a study is able to accurately answer the question included (16). Endpoints should be quantitative or adjudicated [e.g., bone mineral density (BMD) or fracture], if possible, and biomarker measurements should be compared to a reference standard, if available. Additionally, nutritional biomarkers, such as serum 25-hydroxy-vitamin D (25(OH)D), can also be used to measure nutritional status. The National Institute of Standards and Technology has reference methods and materials for use in research studies for some nutritional biomarkers. Careful planning of the frequency and types of nutrition assessments to be made is recommended. This is especially true for 25(OH)D, which is known to change appreciably with UV exposure; so in some locations, seasonality should be considered (17).

The effect size of any 1 nutritional exposure on bone may be small, even in RCTs, but that is not to negate its importance (18). Furthermore, in the context of nutrition and bone, measurement error in dietary assessment tends to attenuate nutrition and health relations, so null findings may be observed even if a true relation exists. With changes in bone, the intervention effect may be small but compared to a control group that exhibits traditional bone loss, the difference may be larger. For example, daily calcium supplementation for 18 mo in French women was associated with a small change in BMD (19), but a substantially lower risk of bone fracture (19, 20). Also, small or short-term changes can have a cumulative impact (21). Therefore, it is necessary to examine the totality of the evidence, respecting that even null and small effect sizes taken together are important when considering the level or strength of the evidence. Finally, nonpublication of null studies could lead to publication bias.

Reducing confounding and bias

All studies are susceptible to different levels and types of biases. The goal of the investigators is to minimize bias during the development and execution of the study and to report and consider bias while interpreting the study results. Bias in evaluating the effects of diet on the risk of any disease may relate to imperfect sampling, data collection and handling, and statistical analysis and confounding effects of risk factors that are correlated with the aspect of diet under study (22). Choice of the right comparison groups can dictate the extent of bias in a study; for example, in cohort studies, differential loss to follow-up tends to be 1 of the main sources of bias (23). A number of nutrition and bone studies tend to be retrospective cohort studies, where selection of exposed and unexposed groups should be done without knowing the outcome. Strategies for reducing confounding and bias vary depending on the study design. In observational research, confounding can be reduced in the study design phase by restricting the sample on an important dimension (such as sex or age) or by matching of participants, or in the analysis phase through use of stratification and adjustment as discussed later in "Pertinent factors for consideration in statistical analysis." Failure to account for and document all potentially confounding factors and assumptions limits our ability to synthesize the research literature with meta-analyses (24). This results in high variability and dampens effect sizes for relations between diet and health.

Sample size estimation

Sample size is a vital aspect of any study and the statistical power of a study should be estimated at the design phase. The sample size not only dictates the cost of collecting data, but also is critical to ensure that true or clinically meaningful associations or effects can be detected (25, 26). When reporting, sample size calculations should consider the primary outcome, the test statistic, the null hypothesis and the alternate hypothesis, the probability of erroneously rejecting the null hypothesis (the type I error), and the probability of erroneously failing to reject the null hypothesis (the type II error) (12). For nutrition research, effect size and expected variability of a nutrient (27), along with the duration of the study to observe any effect of a given nutrition intervention and any statistical interactions that will be tested should also be considered.

Statistical analysis

Ideally, a researcher will decide on the statistical methods to employ either before the data are collected or when designing a research study of existing data (12). The analytical sample of participants in the primary analyses should be documented in any research dissemination efforts (12). Because all studies are susceptible to bias, the goal of the investigators is to minimize bias during the development and execution of the study and to take bias into account while interpreting the study results. Bias in evaluating the effects of diet on the risk of any disease can stem from imperfect sampling, imperfect

information gathering, and the effects of risk factors that are correlated with the aspect of diet under study (22).

Overview of Methods to Assess Bone

This section provides a brief overview of the measures currently available to estimate different parameters of bone health and fracture risk. This information can be useful to inform selection of appropriate outcomes in the design of studies as well as to help researchers select outcome measures for secondary analysis of previously collected data. All measures of bone health or fracture risk outlined below have strengths and limitations that need to be included when interpreting dietary studies. The selection of the bone outcome, and therefore, the measure to assess it are largely driven by the research question, sample size, length of follow-up, the skeletal sites of interest, the outcome of interest, safety and risk considerations, and the availability and costs associated with measurement.

Bone mass or density, turnover, structure, and strength can be assessed (or estimated) with use of a number of different methods including direct measurement of the bone or through the use of surrogate endpoints or biomarkers. Bone quality is an umbrella term often used to encompass 3 key facets of bone health: the composition of the bone, the geometry of the bone, and the microarchitecture of the bone (28); however, because bone is in a constant state of flux, understanding the rate of turnover is also an important dimension not captured directly by bone quality. Developments in imaging technology have allowed assessment of bone properties in humans noninvasively. Different approaches are required for each of these properties. The best approach may vary according to the life-stage and methods available, and may be more limited in children than in adults (29). To date, only bone density or mass and bone geometry are used extensively to evaluate changes in bone prospectively. Bone fracture is an ideal outcome to study in observational research rather than in clinical trials because of the large sample sizes needed with this as the primary outcome. However, skeletal and nonskeletal factors are associated with fractures, so careful consideration is needed when interpreting nutritional exposures. A comparison of methods for assessing bone mass, BMD, bone strength, and bone turnover is outlined in **Table 1**. If possible, it is recommended that different methods of assessment are examined because of limitations in measurement, and comparing different methods with multiple bone sites may yield similar or dissimilar results (30).

Measurements of bone composition, microarchitecture, and geometry

DXA is the most common bone densitometry tool used to assess bone (and body composition) that relies on the use of 2 X-ray beams of different energy to determine the bone mineral content (BMC, in g) and areal BMD (aBMD, in g/cm^2). Most prospective studies since the late 1980s have used DXA to measure these skeletal traits at the whole body level or at specific sites such as the lumbar spine, proximal femur, and radius. The strengths of this technique are the short scan times, low radiation dose, and wide availability. However, a key limitation is that it only provides a 2-dimensional assessment of bone and accounts for only 60–70% of variation in bone strength (31, 32).

Currently DXA represents the gold standard for the diagnosis and monitoring of osteoporosis in adults because it is linearly related to fracture risk (32). For diagnosis of osteoporosis, WHO recommends that aBMD be expressed in terms of SD and compared to a reference range derived from a population of young healthy adults (33). Specifically, osteoporosis is defined as an aBMD value > -2.5 SDs below the young adult reference group, whereas low bone mass (osteopenia) is defined as an aBMD value between -1.0 and -2.5 SDs below the young adult reference group mean.

DXA is a complicated measure in obese individuals because of weight limitations and scan area restrictions (i.e., width of bed for a total body scan). Some reports suggest that DXA BMD measurements are falsely elevated with increasing body fat, which leads to an overestimation of BMD in obese individuals. Other reports have shown that the precision of DXA BMD declines with increasing BMI (20, 34), and suggest that serial measurements in obese subjects should be treated with caution and may require a longer time interval between scans to detect changes in BMD because of the higher least significant change. One of the recommendations from the International Society for Clinical Densitometry (ISCD) is to perform a forearm BMD scan for very obese patients who are over the weight limit of the DXA tables.

Bone strength is only partly described by DXA. A hip structural analysis algorithm can be used in adults together with BMD to estimate structural strength from subperiosteal width, cross-sectional area, and section modulus in the proximal femur (35, 36). The hip axis length (HAL) is a measure of the distance from the base of the greater trochanter to the inner pelvic rim (37). The ISCD released a position paper on measures of hip geometry in 2015, including specific recomendations on hip structural analysis and HAL; currently, HAL is the only endorsed measure to assess hip fracture risk in women (38).

In children, BMC is preferred over BMD as the DXA measure to evaluate/report, together with weight and height, to capture the changes with growth over time (39, 40). In a study of 6,213 children at mean age of 9.9 y followed prospectively for 2 y, total body (less head) aBMD showed a weak inverse relation with subsequent fracture risk (OR per SD decrease of 1.12; 95% CI: 1.02, 1.25), but BMC, adjusted for bone area, height, and weight, showed a stronger relation (OR 1.89; 95% CI: 1.18, 3.04) (41). An important application of prospective DXA measures through the pubertal growth spurt is the determination of timing and magnitude of BMC gains as well as sex differences (42–44). The National Osteoporosis Foundation position paper on development of peak bone mass discusses various ways to adjust BMC to account for size in children (1).

MRI provides a volumetric measure of bone geometry and bone microarchitecture without radiation exposure. It has the

TABLE 1 Comparison of methods for determining density or mass, strength, and turnover in humans[1]

Outcomes	DXA	pQCT	HRpQCT	MRI	QUS	Dynamic histomorphometry	Biochemical markers of bone turnover
BMC or BMD	Yes	Yes	Yes	No	No	Yes	No
Bone balance	Weak	No	No	No	No	No	No
Bone turnover rates	No	No	No	No	No	Bone formation rate	Yes
Specificity	✓✓✓	✓✓✓	✓✓✓✓	✓✓✓✓	✓	✓	✓
	aBMD/BMC/bone Ca content, whole body and individual sites	vBMD, individual sites trabecular and cortical	trabecular and cortical	trabecular and cortical			
Ability to detect short-term changes	✓	✓	✓	✓	✓	✓✓	✓✓✓
Safety	✓✓	✓	✓✓	✓✓✓	✓✓✓✓	✓	✓✓✓✓
Cost	✓✓	✓✓	✓	✓	✓✓✓✓	✓	✓✓✓
Availability of method	✓✓✓✓	✓✓	✓	✓	✓✓✓✓	✓✓	✓✓✓✓
Overall assessment	FDA approved but large sample size required	Get measure of bone strength	Measures microarchitecture	Not well developed	Less understood	Invasive	Bone formation and bone resorption can be determined separately but higher variability

[1] More checks indicates higher impact. aBMD, areal bone mineral density; BMC, bone mineral contents; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; HRpQCT, high resolution peripheral quantitative computed tomography; MRI, magnetic resonance imaging; pQCT, peripheral quantitative computed tomography; QUS, quantitative ultrasonometry; vBMD, volumetric bone mineral density.

advantage of being able to measure the axial or peripheral skeleton, but does not provide a measure of BMC or BMD. While application of the method is still in development (29), given the high cost and limited availability and the fact that assessment of MRI scans is technically challenging, it is not ideal for use in observational research with large sample sizes. More research is needed to determine whether use of MRI scans will offer more information about the effects of nutrition on fracture risk beyond DXA.

Quantitative ultrasonometry (QUS) is measured on an inexpensive, portable device, is easy to use, involves no radiation, and thus may be particularly suitable for use in children or in settings where DXA is not available (29). QUS operates through transmission of sound waves through the bone to quantify the speed and/or the degree of attenuation of the sound wave (i.e., broadband ultrasonic attenuation) (45). Calcaneal QUS is the only site that is endorsed by the ISCD for QUS use because other sites have not been extensively or rigorously studied for use. However, there is much uncertainty about what the variables reflect, thus limiting its use (29), particularly its use to assess relations between diet and bone. Although it has been suggested as useful for screening for osteoporosis (45); at this time, QUS is not recommended as a single measure for assessing nutritional influences.

Peripheral quantitative computed tomography (pQCT) came into use later than DXA to more directly measure 3-dimensional or volumetric BMD (vBMD) and geometry of bone at appendicular skeletal sites, with minimal to low doses of radiation (46). Importantly, this technique allows trabecular and cortical bone vBMD to be measured separately and can provide an assessment of total and cortical bone area, cortical thickness, and estimates of bone strength. Measures are taken at the distal and/or mid-shaft of the tibia or forearm. However, relatively few studies have used this technique to quantify the association between nutrition and the various bone traits. A limitation of this technique is that it typically provides a single slice at each of the skeletal sites, which may not reflect skeletal changes at all bone regions. The use of pQCT to assess fracture risk is less preferred than DXA (47). pQCT has the advantage of being able to assess vBMD and bone geometry at the clinically relevant hip and spine sites. Using more advanced imaging software, it is also possible to reconstruct the 3-dimensional image/shape of bone and performed finite element analysis to stimulate the mechanical behavior of bone. However, because of the relatively high radiation dose it is not routinely used and not appropriate for use in children (47). Although this tool has been used to assess response to pharmacological treatments, much less is known with regard to nutrition (46).

High resolution pQCT is a newer instrument (available since the mid-2000s) that assesses microarchitecture and vBMD in cortical and trabecular bone of the distal radius and distal tibia. These instruments are becoming more widely available, and offer the opportunity to evaluate bone mechanical competence (48). The resolution is sufficient to build finite element models of whole bone failure load, a direct measure of fracture resistance (49). Cortical porosity of trabecular plate and rod microstructure can also be measured. This superior resolution has allowed characterization of sex differences in bone microarchitecture and vBMD (46).

Measurements of bone turnover markers

A number of biomarkers exist to measure bone turnover in the blood and urine; thus providing a noninvasive and relatively cost-effective tool. Bone turnover markers (BTMs) are an attractive tool because they allow determination of changes in bone formation rates versus bone resorption rates and may help to identify patients at high risk of fracture and to monitor the efficacy of medications or other treatments, such as nutritional therapy or modifications. Moreover, changes in bone are assessed in real time and therefore provide a shorter period than a serial collection of BMD or BMC.

A complete review of the utility and function of BTMs is published elsewhere (50, 51). Briefly, BTMs are divided into those that measure bone resorption and those that measure bone formation. Osteocalcin, a noncollagenous protein abundant in bone matrix, measures both dimensions of the bone remodeling cycle and can be used as an overall indicator of bone turnover because it is secreted during bone formation, and can be monitored in fasting serum or urine. However, osteocalcin has other actions not specific to bone formation including bone resorption, described below (16). Ideally, having a combination of both types of measures gives a better overall picture of turnover rates and choosing only 1 BTM in isolation is limited in interpretability.

BTMs are subject to large variability, so their use requires large sample sizes and does not give quantitative measures of changes in bone, thus limiting their fit for purpose, especially for monitoring (52). Many BTMs are specific to bone collagen, not changes in mineralization. Overall, BTMs have varying analytical and predictive quality and have some limitations (see Table 1) (16). As with any analytical specimen, protocols for collection and storage of BTMs are recommended. Shetty et al. (53) recommend that BTM samples be collected after an overnight fast, and that subsequent serial measurements be done at the same time of day and during the same season to reduce random variation as much as possible.

Markers of bone formation.

These biomarkers reflect the activity of the osteoblast, the bone building cells that support the formation of the bone or procollagen metabolism (51). Because type I collagen represents 90% of the organic bone matrix, measurement of its peptides has been employed to assess bone formation primarily through procollagen 1 N-terminal propeptide (P1NP), but to a lesser extent procollagen 1 C-terminal propeptide (P1CP). P1NP and P1CP concentrations in the serum are highly specific for bone formation (i.e., type I collagen is synthesized in other tissues like skin), and most serum P1NP originates from bone, but is influenced substantially by the assay technique used and by medications (e.g., glucocorticosteroids and bisphosphonates). P1NP is considered the most precise of the bone formation markers and is recommended as a reference marker for the prediction of osteoporosis risk and treatment-induced changes by the International Osteoporosis Foundation and International Federation of Clinical Chemistry (16). Bone alkaline phosphatase is another widely used bone formation marker and has been identified as an independent risk factor for osteoporosis (54).

Markers of bone resorption.

These biomarkers reflect the activity of the osteoclast, or provide a marker for collagen degradation. BTMs for bone resorption are more variable than those for formation. Most bone resorption markers are degradation products of type I collagen; pyridinoline and deoxypyridinoline are released during bone resorption and excreted in urine in a free form and in a peptide-bound form, which are C-terminal crosslinking telopeptides (CTX) and N-terminal crosslinking telopeptides. CTX is the most precise bone resorption marker (55) and is recommended by International Osteoporosis Foundation and International Federation of Clinical Chemistry (16). Both serum and urine CTX are fairly specific to bone, but influenced by many sources, including renal function as CTX is degraded in kidney (56). Tartrate-resistant acid phosphatase (TRAP) 5b, which reflects a noncollagenous protein, has also been used as a resorption marker.

Various bone turnover markers have been used in the observational literature of nutrition and bone health, usually in combination of some bone formation and resorption markers (57–62). For example, in a cohort of perimenopausal Scottish women, higher dietary anthocyanidin intake was associated with lower markers of bone resorption markers (pyridinoline and deoxypyridinoline) as well as higher BMD (59).

Assessment of fracture risk

Bone fracture can result from skeletal and nonskeletal causes; this is an important distinction in understanding how nutrition can influence fracture risk. Fractures have been self-reported, confirmed by a medical professional or a medical record, or self-reported and then adjudicated. The latter 2 offer the best classification of risk. Incident bone fracture is a complicated endpoint. Fracture ascertainment has also been assessed using bone fracture registries. Previous studies have demonstrated the utility of fracture registries to successfully examine the relation between nutrient intake and bone fracture outcomes (63). However, the association of a nutrient with fracture risk and incidence can vary by site (e.g., in 1 study protein was associated with decreased hip fracture, but not spine fracture) (34); thus, multiple sites should be examined as differential effects of nutrients can be exhibited by type of bone and skeletal site.

Fracture risk assessment tool (FRAX) is a country-specific algorithm that integrates important clinical risk factors for fracture and mortality risk, with or without information on BMD to compute the 10-y probability of hip fracture or major osteoporotic fracture (64). It was developed with the WHO Collaborating Centre for Metabolic and Bone Diseases at Sheffield, UK, and to date is the most widely used tool to generate fracture probabilities (65). FRAX is not useful for

monitoring effectiveness of treatments and is limited to hip BMD and therefore does not reflect the spine. Reference data may not reflect certain populations and are limited to the ends of the age range.

Pertinent Factors for Consideration in Dietary Assessment

Nutrition is an important and modifiable factor with regard to many diseases of public health concern (66). However, how a researcher operationalizes "nutrition" is key to examining relations with bone as well as to select the appropriate bone health biomarker or outcome. Nutritional status can be assessed by reported or observed dietary intakes and practices, biomarkers of nutritional status, anthropometry measures, clinical observation, and functional testing. Diet can be further broken down into nutrient or bioactive intake, dietary patterns, or intakes of selected food groups. How diet and/or nutritional status is operationalized should be clearly defined when designing and analyzing data.

Dietary assessment methods

Multiple methods exist for assessing dietary intake and are reviewed elsewhere in great detail (67). Briefly, several methods exist to collect dietary intakes including methods for short-recall periods ranging from a day to several weeks (24-h diet recalls, diet records, and diet diaries) and methods to estimate usual diet over an extended period of time, such as FFQ and screening tools. No method is perfect and assessment of self-reported diet has several limitations, including recall bias and measurement error.

Food records and diaries.

A food diary or record is a summary of all foods and beverages consumed within a specified period of time. Accuracy of records is enhanced when participants/people are trained to weigh or measure the foods and beverages being consumed. In general, recording multiple nonconsecutive days of intake puts large burden on a participant and correlated days of intakes can occur (68). Food diaries are subject to reactivity, that is, when a person changes their intake because they are recording it.

24-h dietary recalls.

A 24-h recall is a mean to assess an individual's intake over the previous 24 h and can be assessed in person, over the phone, or through web-based platforms and mobile applications. Multiple 24-h recalls are recommended based on day-to-day variability in intake. Day of the week, season, mode of interview (telephone or in-person), and the sequence of the 24-h recall are known to influence reported energy intakes. Reported intake of macronutrients from 24-h recalls, which are consumed in large amounts every day, is generally more stable than those for micronutrients (69). The use of probing questions aids the ease of responses and has been shown to enhance data accuracy (70).

Frequency-based methods.

An FFQ is a longer-term instrument and is often used in large cohort or case-control studies. FFQs assess dietary intake over a specified period of time and query how often a person consumed multiple food items that are aggregated into groups with similar nutrient profiles. FFQs can be quantitative, semiquantitative, or qualitative (71). FFQs offer a more cost-effective alternative to the 24-h recall; however, they limit the scope of foods that can be queried. The FFQ may create participant burden, and may be difficult or confusing to complete. Most importantly, the accuracy of nutrient profiles determined by FFQs has been questioned. The 24-h recall has been shown to be a less biased estimator of energy and protein from foods in adults than a frequency-based instrument (72), but little is known about other nutrients for which recovery biomarkers (reflect dietary intake in a specific time period and, therefore, can be used to estimate absolute intakes) do not exist.

Screening tools.

Various dietary screeners are available to rapidly assess different dimensions of the diet such as nutrient intakes (73, 74), food group intakes (75), dietary patterns (76), nutrition risk (77), food-related behaviors (67), and for identifying malnutrition (78, 79). Short screening tools may be advantageous when focusing on narrow research questions. For example, Yang et al. (74) developed a calcium screening tool that was validated against BMC in adolescent girls; such tools provide bone researchers opportunities to leverage comprehensive assessment of bone and combine it with rapid and reliable measurement of nutritional exposures.

Measuring dietary supplement use.

Given that dietary supplement use can be pervasive (80–82), knowledge of nutrients and bioactives are critical to determine nutritional status. In adults, but not in children, those who use dietary supplements tend to have significantly higher intakes of vitamins and minerals from food sources alone, so it is always important to include assessment of dietary supplements (83). Dietary data without the inclusion of dietary supplements overestimate the prevalence of inadequacy and underestimate the prevalence of potentially excessive intakes (83–85).

Selection of methods

Many factors should be considered when choosing the method to assess or characterize dietary exposures. The National Cancer Institute Dietary Assessment Primer is a valuable resource to guide researchers in making such decisions; **Table 2** comes from the Primer and compares the common dietary assessment methods (67). An important issue with nutrition studies is to assess and characterize "baseline" or underlying nutrition status that can greatly influence the relations observed between diet and bone. Utilizing biomarkers in addition to self-reported diet is recommended, when available and appropriately validated, including recovery biomarkers, predictive biomarkers, and

TABLE 2 Comparing dietary assessment instruments[1]

		24-h recall	Food record	FFQ	Screener
Study design	Cross-sectional	✓	✓	✓	✓
	Retrospective	—	—	✓	✓
	Prospective	✓	✓	✓	✓
	Intervention	✓	—	✓	✓
Scope of interest	Total diet	✓	✓	✓	—
	One or a few components	—	—	✓	✓
Captures contextual details regarding food preparation, timing of meals, location of meals, etc.	Yes	✓	✓	—	—
	No	—	—	✓	✓
Time frame of interest	Short term	✓	✓	—	—
	Long term	—	—	✓	✓
Can be used to query diet in distant past	Yes	—	—	✓	✓
	No	✓	✓	—	—
Allows cross-cultural comparisons	Yes	✓	✓	—	—
	No	—	—	✓	✓
Major type of measurement error	Random	✓	✓	—	—
	Systematic	—	—	✓	✓
Potential for reactivity	High	—	✓	—	—
	Low	✓	—	✓	✓
Time required to complete	<15 min	—	—	—	✓
	>20 min	✓	✓	✓	—
Memory requirements	Specific	✓	—	—	—
	Generic	—	—	✓	✓
	Does not rely on memory	—	✓	—	—
Cognitive difficulty	High	—	—	✓	✓
	Low	✓	✓	—	—

[1] Reproduced from the NCI Diet Assessment Primer (67).

concentration biomarkers (see "Biomarkers of nutrition status"). More details on how regression calibration can be used to combine dietary and biomarker information for predicting health outcomes are given by Freedman et al. (86); but, as yet, this has not been applied to the context of nutrition and bone.

Participant characteristics.

Energy and nutrient consumption and dietary supplement use varies with age, sex, and race/ethnicity, particularly for bone outcomes. Therefore, valid nutrition assessment tools should be selected based on the population group assessed (87, 88). Valid dietary questionnaires should detail foods typically consumed by the population of interest, including appropriate portion size and preparation, and should use an adequate food composition database for nutrient calculation (88). Regarding FFQs, several culturally appropriate FFQs have been developed and validated for use in different populations around the world (89, 90). For example, among Puerto Rican adults, the use of a general FFQ for the US population significantly underestimated energy and nutrient intake relative to a 24-h dietary recall. Regarding food preparation, the FFQ excluded cooking oil in rice, a highly consumed food in this population, thus underestimating both energy and fat intake (89). Population-specific dietary assessment tools should be considered to aid researchers looking at bone.

Time/duration of study.

For some research questions, usual intakes may be more informative but other questions may require a more temporal assessment. For bone, dietary exposures may need to be examined as a cumulative function, rather than a snapshot (91). For cohort studies, measurement of current diet and repeated assessments over the follow-up period are necessary to evaluate diet at baseline and changes over time. The use of repeated measurements of dietary intake provides analytic opportunities to reduce the effect of measurement error and to evaluate a variety of temporal relations between diet and disease (i.e., long latency, short latency, cumulative exposure) (88). In cohorts followed for longer duration, multiple dietary measures must be collected frequently because the diet may change over time.

Nutrients, foods, and dietary patterns.

Researchers may be interested in specific nutrients, groups of nutrients (i.e., calcium and vitamin or B vitamins), foods, food groups, or eating/dietary patterns in relation to bone (2, 3, 91, 92). However, the National Osteoporosis Foundation position paper on development of peak bone mass concluded in a series of systematic reviews that only the evidence for calcium intake and development of peak bone mass was grade A evidence given the lack of available data on the other nutritional factors examined (1).

Foods can be categorized into similar groups on the basis of their content of nutrients and other bioactives. Food groupings also serve as the basis for deriving dietary patterns and evaluating how diets conform to reference standards for intakes such as the Dietary Guidelines. Dietary patterns are defined as "the quantities, proportions, variety or combination of different foods, drinks, and nutrients in diet, and the frequency with which they are habitually consumed" (93). The dietary pattern approach has advanced nutrition research by capturing overall food consumption behaviors and its quality in relation to health and may better predict disease risk and provide more meaningful food-based public health recommendations (94), and dietary patterns have been successfully applied to study bone outcomes (95).

Limitations with dietary assessment

All dietary assessment methods are subject to error (72, 96, 97). It is now recognized that the error in self-reported dietary assessment instruments must be considered in analysis and interpretation of findings because measurement error in dietary assessment can lead to spurious associations (98). Underreporting has long been demonstrated in nutrition research and in diverse populations to be associated with age, BMI, and education among other factors (99, 100). Other work suggests that 24-h recalls are much less biased for energy intakes and intakes of protein, sodium, and potassium than FFQs (96). Although measurement error is unavoidable in any biologic or physical measurement, it is important to estimate the magnitude of the error and if the effect of measurement error is substantial, statistical corrections should be considered (88). Furthermore, all dietary data, including dietary supplements, are also limited by the accuracy and currency of the databases that are employed to estimate intakes. Therefore, it is important to have a standardized survey protocol and dietary assessment method based on the past research experience of diverse researchers carefully matching the method to the study population. Furthermore, to calculate highly reliable nutrient intake from dietary assessment methods, it is important to secure a researcher with sufficient expertise in using databases to convert foods and beverages as reported by study participants to the component nutrients, food groups, and/or dietary patterns of interest.

Biomarkers of nutrition status

Nutritional biomarkers are reliable and accurate biochemical or other measurements that can be objectively measured and evaluated and used as indicators of dietary intake, biological processes, nutritional status, pathological processes, pharmacological responses to an intervention, or health outcomes (101, 102). The National Institute of Health defines biomarkers as any biological measurements that indicate "normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention" (101). There are a wide range of biomarkers available for use, but their utility depends on the purpose (103).

Nutritional biomarkers are biochemical, functional, or clinical indices of nutrient intake, status, or their functional effects that can reveal information about biological or physiological responses to dietary behavior or pathological processes, as well as monitor responses to therapeutic interventions and provide information on inter-individual differences in response to diet and nutrition (104). Nutritional biomarkers can be obtained from blood, urine, bone, saliva, skin, adipose tissue, and finger- and toenails. Nutritional biomarkers are loosely broken down into those that reflect nutritional exposures (i.e., intake), nutritional status, and those that can be used to predict health outcomes, often referred to as surrogate endpoints (105).

A complete review article of the available nutritional biomarkers with their fit for purpose and limitations can be found elsewhere (106). Recovery biomarkers reflect dietary intake in a specific time period and, therefore, can be used to estimate absolute intakes (e.g., energy intake estimated by doubly labeled water technique). Concentration biomarkers refer to those that are correlated with dietary intakes and can be used to estimate the ranking of an individual's intakes [e.g., 25(OH)D] (107). Urinary sucrose plus fructose reflects dietary sucrose intakes with unusually high correlations, but is labeled as a predictive biomarker because only a small proportion of sucrose intake is recovered in urine. Biochemical markers of bone turnover and BMD are the most commonly used biomarkers of fracture risk as discussed earlier. Some biomarkers, such as folate, are represented by dietary intakes (108), whereas estimates of vitamin D from the diet differ from serum 25(OH) D (109, 110). A newer, rapid, but elaborate method for precisely evaluating bone calcium retention makes use of the long-lived isotope ^{41}Ca to label bone and follow the appearance into the urine to quantitate whole body net bone loss in response to an intervention (111). Use of natural stable calcium isotope ratios has potential as a future biomarker of bone turnover (112).

To be of utility in research, nutritional biomarkers must be analytically valid. There are many biological and methodologic issues that must be considered (105) and not all nutrients have a biomarker to estimate status or intake. For some nutritional biomarkers, factors such as hydration status, inflammation, and diurnal variation all influence measurements. Furthermore, the cost can be high for assessing nutrition status through biomarkers, which is often why dietary data are used. The use of cutpoints to determine nutritional status is difficult (113).

Pertinent Factors for Consideration in Statistical Analysis

Before formal analyses, data exploration and visualization techniques should be used to calculate descriptive statistics and generate plots using means and SD for continuous measures, with medians and interquartile ranges for measures that deviate from a normal distribution (27). Any outlier value should be further explored and appropriate approaches can be used to limit the effect of outliers on the data.

These approaches include i) keep the outlier and treat it like any other data point; or ii) winsorize it (i.e., assign it lesser weight or modify its value so it is closer to the other sample values); or iii) eliminate it (114). To fulfill the model assumptions, data can usually be transformed to a more normal distribution with the use of the natural logarithm or other appropriate transformation. However, such transformation can result in challenges related to data interpretation.

Data exploration and examination of the scale of a nutrient will further shed light on the question of examining a nutrient on a continuous scale versus in categories and utilization of parametric methods versus nonparametric methods. These 2 types of analyses can yield different relations. The most powerful treatment of a variable is to retain a continuous scale of measurement; however, outliers and multimodal distributions can create problems and exert undue influence. However, categorization is common using arbitrarily defined quantiles, standard round-numbered cutpoints or points determined a priori such as the DRI; similar issues exist with the use of cutpoints for nutritional biomarkers (113). The primary analysis should be based on cutpoints determined a priori, whereas secondary analysis may use alternative cutpoints that best describe the biological relation, or finer divisions of extreme categories to extend the examination of the dose-response relation (88). Categorical measures should be described using frequencies and percentages.

Missing data are unavoidable in research studies for various reasons and can dramatically shrink the sample size. As a result, the precision of confidence intervals is harmed, statistical power weakens and the parameter estimates may be biased (115). Missing data can therefore lead to substantial bias; however, this issue is often inadequately handled in statistical analyses (116). During study execution, attempts should be made to determine the full set of exposure and outcome values for each subject at each examination. At the analytic phase, several approaches can be used to account for missing data, including maximum likelihood methods and the multiple imputation methods described by Little and Rubin (117). Overall, authors should explicitly state the assumptions underlying the handling of the missing outcomes and justify them through data descriptions and sensitivity analyses (116).

Consideration of covariates and confounders

While analyzing a single nutrient it is key to adjust for important or clinically relevant confounders or covariates related to bone measures such as age, sex, weight, height, current smoking, physical activity, menopausal status and current estrogen replacement (in women alone) (118). In addition to these covariates, careful consideration should be given to dietary confounders, such as intakes of total energy, calcium, vitamin D (including from UV exposures), alcohol, and caffeine intake (3, 119, 120). Furthermore, substitution analyses can be used to answer the research questions related to the effect of substituting a specified percentage of energy from 1 nutrient (e.g., protein) for the same percentage of energy from another nutrient (e.g., carbohydrates) (107). While analyzing type of nutrients, nutrient types should be adjusted for each other, provided there is no collinearity between nutrient types. For example, animal and plant protein should be adjusted for each other in the same model when analyzing the association of protein type with bone measure (121). Imperfectly measured or unmeasured confounders cannot be ruled out in observational studies and this can result in residual confounding. Directed acyclic graphs (122) and change-in-estimate procedures (123) can be used for confounder identification and selection during data analysis.

Addressing interactions in nutrition studies

Nutrition analyses are often complex because of issues related to interactions between nutrients. For example, in the context of nutrition and osteoporosis studies, interaction is commonly reported between dietary protein and calcium intakes. Investigators should review the nutrition literature to plan the analyses related to interactions a priori. Similarly, in osteoporosis research, sex is an important effect modifier and analyses are often conducted stratified by sex and sometimes by age (young compared with old). Any significant interactions should be further analyzed in stratified analyses, which could be key to isolating the individual effect of a nutrient. Most statistical procedures have an underlying assumption that the data are independent (12), which may or may not be the case in nutrition and bone. There is often collinearity of both foods and nutrients. Similarly, BMC from 1 skeletal site is likely to be related to BMC of another site or total body BMC. Therefore, before formal analyses, data exploration techniques should be used to assess correlations and collinearity between nutrients of interest. Appropriate modeling strategies should be used if 2 nutrients are collinear.

Issue of multiple comparison

In nutrition and bone studies, there are often many variables under consideration, with the potential for a large number of statistical comparisons to be performed. While the issue of *P* value adjustment for multiple comparisons in observational epidemiology has been a source of discussion for years among epidemiologists (124), it is generally accepted that the *P* value is not the sole criterion for assessing relations in observational studies (e.g., 95% CI are an informative approach to presenting the data) (125). Therefore, conclusions should be based on the preponderance of scientific evidence related to the hypothesis, considering the point estimates and confidence intervals rather than a single statistical result. However, emerging research is utilizing genomic, proteomic, and metabolomic approaches as endpoints in nutrition studies. Such studies often have multiple endpoints and no prior hypotheses, which raises statistical issues (126). In such studies establishing a threshold for statistical significance (i.e., a *P* value) to address multiple comparisons becomes even more important (124).

Special Considerations for Nutrition and Bone Relations

In addition to various nonmodifiable factors (e.g., genetics, sex, maturation), a range of modifiable lifestyle and physiological factors are known to influence bone, including physical activity, sedentary behaviors, body weight or obesity, muscle mass and strength, as well as certain medications (**Table 3**). Given that various nutritional factors can influence or interact with some of these factors and directly or indirectly impact on bone, it is important to consider and plan analyses for any potential interactions with these factors a priori.

Body weight

Excessive body weight (i.e., overweight and obesity) and higher BMI has been linked to an increased bone density, which has been attributed largely to the additional strain placed on bone from increased load (1, 2, 127, 128). In terms of fracture risk, there are mixed findings regarding the association with BMI or the level of obesity. A meta-analysis of prospective studies (6 studies with 105,129 adults) reported no overall relation between BMI and the risk of vertebral fracture; however, when stratified by sex, there was a significant inverse association between BMI and fracture risk only in men (129). Interestingly, BMI was associated with an increase in fracture risk in women when models controlled for BMD (129). In part support of these findings, a meta-analysis of prospective cohorts from more than 25 countries (398,610 women, average age 63 y) reported that a high BMI (35 kg/m^2) was associated with a reduced risk of osteoporotic fracture, but when adjusting for BMD the risk of fracture was increased (130). However, adding to the complexity, this study also showed that the association between BMI and fracture risk differed by skeletal sites.

Previous research has also demonstrated that caloric restriction and weight loss are associated with increased bone loss (131, 132), but this can be counteracted by the inclusion of exercise in a weight loss program (133, 134). Although the mechanism(s) for bone loss during weight loss is likely to be multifactorial, reduced mechanical loading associated with weight loss is likely to be an important contributor. However, it is also important to acknowledge that the relation between weight (fat) and bone is influenced by a number of hormonal and inflammatory cytokines or adipokines (cytokines secreted by adipose tissue). For instance, adipose tissue acts as an active endocrine organ that can secrete a large number of proinflammatory adipokines as well as attenuate the release of anti-inflammatory markers (e.g., adiponectin), all of which have been implicated in bone and muscle loss, osteoporosis, and an increased risk of fracture. Although further research is needed, it is important that weight or BMI (or the degree of change in weight) be included in any models when assessing the association between nutrition and bone or fracture risk.

Muscle (sarcopenia)

Muscle and bone are inextricably linked by a shared loading environment (and common genes regulating body size) (135). This biomechanical link between muscle and bone is supported by the concept of the functional muscle-bone unit, which predicts that changes in muscle mass, size, or strength should influence the mass, structure, and/or strength of bone predictably and correspondingly (136). Indeed, previous research has reported that both muscle mass and strength (and changes in these measures) are associated with BMD (and bone gains or losses) in both children and older adults (137–140). More recently, there has been interest in the interrelation between sarcopenia, which is defined as low muscle mass, strength, and/or impaired function, with osteoporosis and fractures (141). In 2009, the term "sarco-osteopenia" was coined to emphasize that low BMD or osteoporosis and weak muscles may contribute to fractures in elderly adults (142); data suggest that those with low BMD and sarcopenia are at a higher risk of fracture than those with low BMD or sarcopenia alone (143). Because poor nutrition is a risk factor for both these conditions (144), it is important to consider indices of muscle or sarcopenia in the studies of nutrition and bone health.

Physical activity and sedentary time

There is consistent and compelling evidence that physical activity or exercise training, particularly weightbearing impact sports or activities and muscle-strengthening exercises, can improve peak bone mass during growth and maintain or slow bone loss throughout life (145–147). Central to understanding how bones adapt to exercise (loading) is Frost's mechanostat theory, which postulates that loads (strains) imparted on bone must exceed a given threshold or setpoint to turn on bone modeling or remodeling to elicit an osteogenic response. Conversely, when strains on bone are reduced such as during disuse or bedrest, there is rapid bone loss. As a result, not all forms of physical activity or exercise training have a positive effect on bone; regular walking, swimming, and cycling have been shown to have little or no effect on bone. Recent research has also shown that prolonged periods of sedentary behavior during daily life, such as sitting, are associated with low BMD and sarcopenia (148–150). It is therefore important that all nutrition and bone studies include physical activity or exercise as a confounding factor and/or test for any potential exercise-nutrition interaction. For instance, there are some reports that additional calcium may enhance the effects of exercise on bone in children and older adults, particularly those in a state of nutritional calcium insufficiency (151–153). Others have reported that the provision of additional protein can lead to greater resistance training–induced gains in muscle mass and/or strength compared to resistance training alone in older adults (154), particularly in those with inadequate basal protein intakes or those who are institutionalized or sarcopenic (155, 156).

TABLE 3 Potential confounders to the relation between nutrition and bone, and their methods of assessment and strengths and limitations[1]

	Method of assessment			
	Direct measure	Self-report	Advantages	Limitations
Anthropometry				
Weight and height	✓	✓	■ Simple, quick and noninvasive	■ If self-reported, errors in underreporting weight and overestimating height
BMI	—	—	■ Simple, inexpensive and noninvasive	■ Does not distinguish fat and lean mass
Body composition				
DXA	✓	—	■ Easy to use ■ Short scan time ■ Low radiation ■ Good precision ■ Assess total body and regional fat and lean mass	■ Expensive ■ Not suitable for very obese individuals
CT (including peripheral CT)	✓	—	■ High accuracy and reproducibility ■ Excellent soft tissue differentiation	■ Expensive ■ Limited access ■ High radiation exposure (CT) ■ pQCT has lower radiation dose but is limited to appendicular sites
MRI	✓	—	■ High accuracy and reproducibility ■ Excellent soft tissue differentiation ■ No radiation	■ Expensive ■ Limited access ■ Claustrophobia
Bioelectrical impedance	✓	—	■ Simple, quick, noninvasive, and portable ■ Accurate measurements	■ Can be affected by hydration status
Physical activity/sedentary time				
Recalls and questionnaires	✓	✓	■ Simple and easy to administer ■ Able to measure a large number of participants at low cost ■ A variety of physical activities can be assessed ■ Able to compare results from different locations when using the same questionnaire	■ Issues related to reliability and validity ■ Recall challenges from some populations ■ May lack sensitivity for detecting modest changes
Pedometers	✓	—	■ Small, lightweight, noninvasive, and inexpensive ■ Easy to administer to large groups ■ Objective measure of most common activity (walking)	■ Does not measure frequency, intensity, or duration of activity ■ Cannot be worn during aquatic events ■ No differentiation of activity type
Accelerometers/ inclinometers	✓	—	■ Small, lightweight, and noninvasive ■ Low participant burden ■ Real-time monitoring ■ Provide information on sedentary time and intensity, frequency and duration of activity (inclinometers can also distinguish sitting and standing)	■ Expensive ■ Cannot be worn during aquatic events ■ No differentiation of activity type ■ Underestimates activity during certain activities (e.g., cycling and upper body activities)

[1] CT, computed tomography; pQCT, peripheral quantitative computed tomography.

One of the challenges when examining the association between bone and physical activity or exercise is that it is difficult to quantify activity characteristics which are relevant to musculoskeletal responses. Physical activity is a multidimensional behavior and factors such as the type, frequency, intensity, and duration need to be considered given that not all forms of activity have a beneficial effect on bone. For example, the Timed Up and Go test performance and speed of walking is related to BMD and risk of nonvertebral fracture (157), as measures such as grip strength (158) have been associated with BMD and risk of osteoporosis (159). Traditional measures of intensity that focus on cardiovascular load, such as heart rate or estimates of total energy expenditure, are not particularly useful in studies with bone outcomes because they fail to capture key bone-loading characteristics (e.g., type, magnitude, or rate of loading) known to elicit an osteogenic response (see Table 3). A review of measurement tests and tools to assess physical performance and their strengths and limitations is published elsewhere (160).

Physical activity questionnaires are often used because they are easier to administer and can record current or

past participation in a broad range of different sporting or leisure activities, which can be classified according to weightbearing or nonweightbearing or whether they are muscle-strengthening activities (161, 162). Future observational studies with bone as an outcome should consider using bone-specific physical activity questionnaires that can take into account load characteristics (e.g., vertical ground reaction forces) (162–164). However, as all self-reported questionnaires they are subject to recall bias.

An alternative approach is to objectively measure physical activity using devices such as pedometers and accelerometers. Although there are some reports that higher pedometer-determined activity (step counts) are associated with greater BMD (165, 166), a limitation of these devices is that they do not distinguish between activities of different load magnitude (e.g., walking compared with jumping). In contrast, accelerometers which can be worn at the hip or wrist and measure acceleration [e.g., the change in speed with respect to time (m/s) or multiplies of the acceleration of gravity ($g = 9.81$ m/s)] offer a unique approach to provide an estimate of the intensity of mechanical loading of bone (167). Indeed, previous research has shown that accelerometers can be used to quantify the number of daily vertical impacts at different magnitudes and loading rates (167, 168), with higher acceleration levels (> 3.9 g) found to be associated with increased hip BMD (169, 170). Given the importance of moderate to high magnitude loading to bone, observational and prospective studies should consider using accelerometers when studying the relations between nutrition, exercise, and bone outcomes.

Medication and supplement use

Many pharmacological agents are available for treatment or prevention of bone-related disorders and should be assessed in research studies. Medications can confound diet and bone relations. The most common medications used currently are bisphosphonates (171). After menopause, estrogen loss induces bone loss; evaluation of any sex steroid medications, including raloxifene, should be examined in both women and men. Calcium and vitamin D, as well as other micronutrient supplements, are often prescribed or recommended to individuals at risk of poor bone health, so, as previously mentioned, assessment of dietary supplement use is an important parameter to include in data collection and analysis.

With regards to medications, it is important to consider drugs that may affect both BMD and fracture risk, and that may also be correlated with nutritional status. For example, selective serotonin reuptake inhibitors have been associated with bone mass loss and increased risk of fractures. In a recent meta-analysis, use of selective serotonin reuptake inhibitors was significantly associated with lumbar spine BMD reduction (154). In a recent study from NHANES, selective serotonin reuptake inhibitor use and inadequate daily intake of zinc was associated with low BMD (172). Another example is antihypertensive drugs such as thiazides, which have been associated with less annualized loss of BMD (156) and fractures (157). Thiazides have been associated with hypercalcemia even when used in combination with calcium and vitamin D among seniors with compromised renal function or hyperparathyroidism (158).

Other issues to consider

There are many special considerations when examining diet and bone. First, not all nutrient effects are linear; a "saturation" effect can occur with nutrients, meaning that beyond a certain point we would not expect to see any more change occurring in the endpoint; this has been demonstrated with calcium and BMD (173). Similarly, U-shaped and J-shaped risk curves may occur between the exposure and the outcome; this has been previously described for vitamin D and mortality (174) and for iron and osteoblast activity (175). Thus, the expected shape of the dose-response curve can differ with nutrients and this requires careful modeling of the data. Second, it is well known that nutrients have a very narrow range of effects (18), so it is of utmost importance to take care to reduce any measurement error in exposure and outcome so as not to attenuate what small relations may exist. Third, genetic backgrounds of individuals make studying specific effects of nutrition and bone particularly challenging. Genetic data have been useful in our understanding of nutrition and bone (144, 176). For assessment of gene-diet interactions, the nutritional assessment method must represent long-term intake. As these studies generally require a large sample size to accommodate the comparison of subgroups, FFQ might be the most cost-effective method to identify relations with genes and bone (177). There is much potential for genomewide association studies to help advance the field, especially in interpreting diet and bone, as reviewed elsewhere (176). Fourth, comorbidity can substantially influence diet and bone relations. Kidney function and renal disease modulate bone independent of diet, and those with renal issues also tend to be on restrictive diets. Thus, compromised kidney function is likely to be an exclusion factor in research on nutrition and bone. Diabetes and antidiabetic medications can alter BMD and negatively affect bone health (178).

Discussion and Conclusions

Observational research is prime for looking at the relation between nutrition and bone. Insights garnered from this type of work can be used to generate hypotheses for other types of studies, including RCTs. However, given all of the limitations described above in measuring dietary intakes and biomarkers, special attention to research design and analysis is paramount to making the most strides in observational research (**Box 1**). Observational research can never demonstrate a cause and effect relation (179), but with careful consideration of the causal criteria, it can help address research questions for which RCT data are lacking or questions that RCTs are not suitable to address

(180). For example, prospective cohort or cross-sectional data analysis can stratify based on key factors, including nutritional status, and can be used to identify nutrition risk. Prospective and longitudinal studies are important to study changes in bone trajectory over time and are critical to tailor dietary recommendations based on age and life-stage. In research on the relation between health and nutrition, it is important to consider association among human indicators (e.g. health, body function, bone strength and integrity, nutritional status, and behavior) and nutritional indicators (nutrients such as calcium and vitamin D, food, and diet patterns). Those indicators have a hierarchical structure from the subordinate to the upper indicators. It is necessary to set contexts including relation of lower indices (such as food, diet patterns, and eating behaviors) to higher indicators (nutrients). Therefore, selection of factors used in observational research is an important dimension of research in and of itself, but also for its potential to guide the design of RCTs to help elucidate the causal role of nutritional exposures on bone health in various life-stages. Study design of observational research will influence the design of RCTs, further highlighting the importance of observational research design and methodology. Considerable research to date has suggested that multiple nutrients, foods, and dietary patterns are associated with bone health so this is an exciting field and offers much promise for the future when genomic and metabolomics data will offer new insights into diet-microbiome-bone relations.

Box 1.
Key recommendations

- Build a multidisciplinary research team with strengths in nutrition, bone, and research design and statistics.
- Clearly specify the research question(s), including primary and secondary outcomes.
- Register observational studies and research questions at clinicaltrials.gov or a similar entity before data collection.
- Select exposure and outcome assessment methods that address the research question.
- Select the appropriate study population and appropriately validated endpoints or surrogate markers.
- Determine how diet and/or nutritional status will be operationalized. Clearly define reported or observed dietary intake, biomarkers, anthropometry measures, nutrients, dietary patterns, or intakes of selected food groups.
- Population-specific dietary assessment tools should be considered as a tool to aid researchers looking at bone.
- Measure all factors related to the research using validated methods.
- Consider multiple outcome measures to provide verification for triangulation of assessment (i.e., accurately measuring all domains).
- Use biomarkers in addition to self-reported diet when available, and validate methods with recovery markers, predictive biomarkers, and concentration biomarkers, as appropriate.
- Detail reporting of all the biases possible and measures taken to reduce the bias both at the stage of data collection and during analysis.
- Account for and document all potentially confounding factors and assumptions, as failure to do this limits our ability to synthesize the research literature with meta-analyses. This results in high variability and dampens effect sizes for relations between diet and health.
- Examine data carefully, examine outliers, use purposeful data classification, control for multiple testing, apply appropriate adjustment for confounders and effect modifiers, employ a statistician with nutrition experience.
- Design and implement a data-sharing plan and/or identify a repository that other research teams can access.

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