SUGGESTED MEASURES OF NUTRITIONAL STATUS AND HEALTH CONDITIONS FOR THE THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

November 1985

Prepared for
CENTER FOR FOOD SAFETY AND APPLIED NUTRITION
FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES
WASHINGTON, D.C. 20204

under
Contract No. FDA 223-84-2059
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edited by
Susan A. Klausing, Ph.D.
Susan M. Pilch, Ph.D.

LIFE SCIENCES RESEARCH OFFICE
FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY
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Bethesda, Maryland 20814
FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was developed for the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA) in accordance with the provisions of Contract No. FDA 223-84-2059. It was prepared and edited by Susan A. Klasing, Ph.D., Associate Staff Scientist, LSRO, FASEB and Susan M. Pilch, Ph.D., Staff Scientist, LSRO, FASEB, under the direction of the ad hoc Expert Panel identified in Chapter XXIV.

The LSRO acknowledges the contributions of the scientists who served on the Panel as well as the numerous investigators who provided materials for and/or critically reviewed sections of the report. The listing of these individuals as Panel Consultants does not imply that they endorse the recommendations of the Panel. The Panel accepts the responsibility for the study recommendations and LSRO accepts the responsibility for the accuracy of data and information included in the report.

The Panel and LSRO appreciate the cooperation of scientific staff of the Center for Food Safety and Applied Nutrition, FDA, who provided information and interpretation of the agency's needs for data on nutritional status; collaborators from the National Center for Health Statistics who provided data and information on previous Health and Nutrition Examination Surveys (HANES); and staff members of the Centers for Disease Control who provided background information on analytical methodology employed in previous NHANES. These individuals are identified in Chapter XXIV.

The Panel met four times between March and September, 1985 to obtain background information, identify pertinent issues related to specific nutrients and health conditions, and develop drafts of the report. Members of the Panel and other consultants reviewed drafts of the report and provided additional documentation and viewpoints contained in the several chapters. In addition, members of the Panel formulated the specific conclusions and recommendations incorporated in the final report.

The final report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.
While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

11-27-85
(date)

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office
SUMMARY

This report reviews the recommendations of an ad hoc Panel concerning assessments of nutritional status and health conditions for possible inclusion in the third National Health and Nutrition Examination Survey (NHANES III) in 1988. The Panel, convened by the Life Sciences Research Office, Federation of American Societies for Experimental Biology, at the request of the Center for Food Safety and Applied Nutrition, Food and Drug Administration (FDA), examined information on the availability of indicators of status and, when applicable, excess accumulation for 21 selected nutrients and health conditions. In addition, they offered conclusions on the appropriateness of inclusion of each nutrient and health condition in NHANES III, and the indices that might be utilized for assessment, as well as the methodology that was feasible for use in a large-scale, cross-sectional survey. The Panel also identified specific target groups, and appropriate uses of the data based upon their understanding of the FDA needs for such data. Of the nutrients and conditions reviewed, the Panel recommended that the following be included in NHANES III: vitamins A, B-6, C, and D; folacin; riboflavin; thiamin; copper; iron; selenium; cancer; cholesterol and blood lipids; hypertension; and osteoporosis. The Panel also concluded that the following nutrients were not appropriate for inclusion in NHANES III because an indicator of status appropriate for a national survey was not available at this time: vitamin E, niacin, calcium, chromium, magnesium, manganese, and zinc. A synopsis of the Panel recommendations is included in the following summary table.
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<th>Target Groups</th>
<th>Comments</th>
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<td>III</td>
<td>Vitamin A</td>
<td>+/+</td>
<td>serum retinol level</td>
<td>yes</td>
<td>all subjects</td>
<td>rapid dark adaptation test</td>
<td>all subjects</td>
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<td></td>
<td></td>
<td></td>
<td>serum retinyl ester levels</td>
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<td>fasting subjects</td>
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<td>IV</td>
<td>Vitamin B-6</td>
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<td>plasma pyridoxal 5'-phosphate level</td>
<td>yes</td>
<td>all subjects</td>
<td>in one cycle</td>
<td></td>
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<tr>
<td>V</td>
<td>Vitamin C</td>
<td>+/−</td>
<td>serum/plasma ascorbic acid level</td>
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<td>all subjects</td>
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<td>VI</td>
<td>Vitamin D</td>
<td>+/+</td>
<td>serum/plasma 25-OH-D level</td>
<td>yes</td>
<td>all subjects</td>
<td>serum calcium level</td>
<td>subjects with high 25-OH-D + controls</td>
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<tr>
<td>VII</td>
<td>Vitamin E</td>
<td>+/+</td>
<td>- -</td>
<td>no</td>
<td>--</td>
<td>- -</td>
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<tr>
<td>VIII</td>
<td>Folacin</td>
<td>+/−</td>
<td>RBC folate level</td>
<td>yes</td>
<td>all subjects</td>
<td>serum folate level</td>
<td>all subjects</td>
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<td></td>
<td></td>
<td></td>
<td>neutrophil hypersegmentation</td>
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<td>subjects with low RBC folate + control sample</td>
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<tr>
<td>IX</td>
<td>Niacin</td>
<td>+</td>
<td>-</td>
<td>no</td>
<td>- -</td>
<td>- -</td>
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<tr>
<td>X</td>
<td>Riboflavin</td>
<td>+</td>
<td>-</td>
<td>yes</td>
<td>erythrocyte glutathione reductase act. coeff.</td>
<td>all subjects</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>in one cycle</td>
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<tr>
<td>XI</td>
<td>Thiamin</td>
<td>+</td>
<td>-</td>
<td>yes</td>
<td>transketolase thiamin pyrophosphate effect</td>
<td>all subjects</td>
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<tr>
<td>IX</td>
<td>Calcium</td>
<td>-</td>
<td>-</td>
<td>no</td>
<td>- -</td>
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<td>XIII</td>
<td>Chromium</td>
<td>-</td>
<td>-</td>
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<td>XIV</td>
<td>Copper</td>
<td>?</td>
<td>-</td>
<td>yes</td>
<td>RBC superoxide dismutase activity</td>
<td>subsample of adults</td>
<td>Should be measured only if SOD is validated as an assessment of copper status</td>
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<tr>
<td>XV</td>
<td>Iron</td>
<td>+</td>
<td>+/-</td>
<td>yes</td>
<td>serum ferritin level</td>
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<td></td>
<td>transferrin saturation</td>
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<td></td>
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<td></td>
<td>erythrocyte protoporphyrin</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mean corpuscular volume</td>
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<td></td>
<td>hemoglobin concentration</td>
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<tr>
<td>XVI</td>
<td>Magnesium</td>
<td>-</td>
<td>-</td>
<td>no</td>
<td>- -</td>
<td>- -</td>
<td></td>
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<tr>
<td>XVII</td>
<td>Manganese</td>
<td>-</td>
<td>-</td>
<td>no</td>
<td>- -</td>
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<tr>
<th>Chapter</th>
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<th>Indicator Availability</th>
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<tr>
<td>XVIII</td>
<td>Selenium</td>
<td>+</td>
<td>yes</td>
<td>serum/plasma selenium level</td>
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<tr>
<td>XIX</td>
<td>Zinc</td>
<td>-</td>
<td>no</td>
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<td>XX</td>
<td>Cancer</td>
<td>NA</td>
<td>yes</td>
<td>current cancer</td>
<td>all subjects</td>
<td>Should be obtained from medical history</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>selenium &amp; vitamin A &amp; C nutritional status</td>
<td>all subjects</td>
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<td></td>
<td></td>
<td>diet &amp; lifestyle questions</td>
<td>all subjects</td>
<td>Usual dietary intake, smoking, alcohol use, and occupation should be obtained</td>
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<tr>
<td>XXI</td>
<td>Cholesterol &amp; Blood Lipids</td>
<td>+</td>
<td>yes</td>
<td>plasma total cholesterol level</td>
<td>all subjects</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>plasma HDL cholesterol level</td>
<td>all subjects?</td>
<td>Sufficient blood may not be available for this analysis to be performed for preschool children</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>plasma apoproteins B &amp; A-I</td>
<td>random adults</td>
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<td>XXII</td>
<td>Hypertension</td>
<td>+</td>
<td>yes</td>
<td>brachial artery pressure</td>
<td>all subjects 6 yr +</td>
<td>Mercury sphygomanometer, with and without random zero device, should be used</td>
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<tr>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td>left ventricular hypertrophy</td>
<td>adults with high BP + controls</td>
<td>Can be used to assess evidence of organ damage due to hypertension</td>
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Table 1. Summary of Panel Recommendations for NHANES III (continued)

<table>
<thead>
<tr>
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<td>XXII</td>
<td>Hypertension (continued)</td>
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<td></td>
<td>blood ionized calcium</td>
<td>all subjects 6 yr +</td>
<td>Can be used to assess calcium/blood pressure relationships</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>urinary sodium, potassium, and calcium</td>
<td>all subjects 6 yr +</td>
<td>Casual urine sample for excretion ratios</td>
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<tr>
<td>XXIII</td>
<td>Osteoporosis +/- NA yes</td>
<td>forearm bone mineral content</td>
<td></td>
<td></td>
<td>bone mineral content of lumbar vertebrae</td>
<td>men &gt;30 yr &amp; postmenopausal women</td>
<td>Both current and past intake should be assessed if possible</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>calcium intake</td>
<td>all subjects 18 yr +</td>
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</tbody>
</table>

* See report chapters for detailed information.

+ = indicator of status or excess accumulation is available.

- = indicator of status or excess accumulation is not available.

+/- = indicator is available to provide some information.

NA = not applicable.
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I. INTRODUCTION

A. BACKGROUND

The Health and Nutrition Examination Surveys (HANES) are one series of health-related surveys conducted by the National Center for Health Statistics (NCHS) on the civilian, noninstitutionalized population of the United States. The first National Health and Nutrition Examination Survey (NHANES I) examined persons aged 1 through 74 years, and was conducted in 1971-1974. The second National Health and Nutrition Examination Survey (NHANES II), conducted during 1976-1980, examined persons aged 6 months through 74 years. In 1982-1984, the Hispanic Health and Nutrition Examination Survey (HHANES) was conducted to examine persons in the three major Hispanic groups in the United States: Mexican-Americans in the Southwest; Cuban-Americans in the Miami, Florida area; and, Puerto Ricans in the metropolitan New York City area.

Each of these surveys consisted of household interviews, medical and dietary histories, extensive physical examinations, various physiological measurements, and laboratory tests on blood and urine. Data from these surveys were intended to provide information on the prevalence of certain diseases and health conditions, to monitor health and nutritional status of the population over time, to identify public health problems, and to identify interrelationships among health and nutritional variables.

Planning is currently underway for the third National Health and Nutrition Examination Survey (NHANES III), which is scheduled to begin in 1988. As part of the planning process, the NCHS is soliciting recommendations from various federal agencies and other sources regarding the conduct of the survey and the data to be collected.

B. SCOPE OF THIS STUDY

The Food and Drug Administration (FDA) is a major user of HANES data in discharging its responsibility to monitor the nutritional adequacy and safety of the U.S. food supply. Thus, FDA has an interest in making recommendations about the assessment of deficiency and toxicity of particular nutrients, and the evaluation of diet-related health conditions in NHANES III. To assist in this endeavor, the FDA's Center for Food Safety and Applied Nutrition requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) assemble an ad hoc Panel on NHANES III Recommendations to review available information on the assessment of 21 nutrients and health conditions of interest to the FDA. Based on the depth of knowledge required by the agency, and the likelihood of measurements being suitable for inclusion in NHANES III, the FDA divided the list of nutrients and health
conditions into three categories. Topics for which specific detailed information and evaluation were desired included vitamin A, vitamin D, iron, calcium, copper, cholesterol and blood lipids, hypertension, and osteoporosis. Topics for which some information and suggestions were desired included thiamin, riboflavin, niacin, folacin, vitamin B-6, vitamin C, and zinc. Finally, for vitamin E, magnesium, manganese, selenium, chromium, and cancer, FDA sought the suggestions of the Panel on possible approaches to their inclusion in NHANES III.

For each topic, the Panel was asked to consider whether NHANES III would be an appropriate vehicle for the assessment of the given nutrient or condition and, if appropriate, to recommend the variables that should be measured in the survey. The Panel was also asked to make general recommendations on the methods to be used, suggest approaches to interpreting and evaluating the resulting data, identify target groups for each recommended assessment, and identify any unique requirements associated with obtaining the desired data.
II. GENERAL RECOMMENDATIONS ON CONDUCT OF NHANES III

The Panel on NHANES III Recommendations concentrated on evaluation of biochemical and clinical indices of nutrition and health status. In doing so, the Panel made several assumptions about the organization of the survey based on practices used in past surveys. The Panel assumes that the initial contact with subjects for NHANES III will be in their homes, and that most will subsequently make an appointment for examination at the mobile examination center trailers. The Panel also assumes that the examination will last no longer than 3 or 4 hours. Inasmuch as interpretation of data collected on nutrient status and health conditions will require additional socioeconomic, medical, and clinical information about each subject, the Panel has assumed that portions of the previous HANES protocols providing this information will be included in NHANES III. The possibility of longitudinal follow-up of NHANES III subjects is recognized in the Panel's descriptions of some of the intended uses of the data. In addition, some observations and general recommendations are noted in the remainder of this chapter.

A. POPULATION TO BE SURVEYED

In previous HANES, the population surveyed included individuals aged 6 months or 1 year to 74 years. In NHANES III, the Panel suggests that the population surveyed include infants as soon after birth as practical; that is, infants less than 6 months of age. In addition, because of increasing public health concern with older citizens, the Panel recommends inclusion of persons up to 85 years of age.

In the case of some nutrients, the Panel recommends that certain subgroups of the population be oversampled so that more accurate prevalence estimates of deficiency states can be obtained than would otherwise be possible. For example, oversampling of individuals older than 65 years and younger than 3 years has been recommended for certain status determinations. For disorders unlikely to be found, nutritional status assessments have been recommended to identify groups at risk of deficiency or excessive accumulation if a suitable method is available.

In previous national HANES, approximately 20,000 persons were examined in a 4-year period. The Panel understands that current plans for NHANES III call for three 2-year cycles with approximately 20,000 persons, selected to form a national probability sample, examined in each cycle.
B. HOUSEHOLD QUESTIONNAIRE

The Panel assumes that a household questionnaire, similar to the one administered in previous HANES, will be used to obtain socioeconomic and demographic information, including age, sex, race, family relationships, housing, income, occupation, veteran status, and use of food assistance programs such as food stamps. The Panel has not focused on the specific questions that should be included, but assumes that during development of the questionnaire due consideration will be given to posing questions amenable to statistical analysis. However, one potentially useful question, not included previously, would be the time interval since the previous meal or previous instance of eating.

C. MEDICAL HISTORY QUESTIONNAIRES

The Panel has reviewed the medical history questionnaires used in previous surveys for both children and adults. In regard to the questionnaire used for children under the age of 12 years, the Panel recognizes that a number of questions are asked that are of value to federal agencies other than FDA. However, from the perspective of assessing the nutrients and health conditions of interest to the FDA, the information collected previously that should be obtained in NHANES III is as follows: birth weight, infant feeding history including breast feeding, congenital conditions and inborn errors of metabolism, lead poisoning, pica, history of anemia, and participation in food programs. Previously, age at first menses was not asked of subjects under age 12 years. The Panel recommends that this question be asked of females aged 10 years and older.

Similarly, in the adult questionnaire, the Panel is concerned primarily with questions relevant to nutritional status and several health conditions. The Panel recommends that the questionnaire continue to include information about general medication use and hospitalization history, and the diagnosis and treatment of specific diseases, in particular, gastrointestinal disorders, diabetes, liver and gall bladder disease, hypertension, cardiovascular disease, and stroke. Questions relating to the reproductive history of female subjects should be included, particularly, age at first menses, history of pregnancies and miscarriages, oral contraceptive use, and use of noncontraceptive estrogens. The Panel recommends that questions be added about the occurrence of fractures, especially those not associated with trauma. The Panel also recommends inclusion of questions designed to be useful in quantifying the consumption of tobacco, tea, coffee, alcohol, and substances of abuse.

Information received from NCHS indicates that another group is considering development of an activity questionnaire and an exercise and physical fitness examination. Details of the questionnaire, and the extent of the physical examination are not known at this time. The Panel believes that such information and
testing should be included in NHANES III because it would be a valuable adjunct in the interpretation of nutritional status. Lacking other information, the Panel recommends that a record of habitual physical activity, including type and level should be obtained.

D. DIETARY QUESTIONNAIRES

At this stage of the planning process for NHANES III, the Panel recognizes that firm decisions have not been made with respect to approaches to collection of information on dietary intake. In previous HANES, two types of dietary intake questionnaires have been used: 24-hour dietary recall and food frequency. The Panel notes that both approaches to gathering dietary data used in previous HANES have inherent limitations. While the Panel has not focused on the use of dietary intake data to evaluate health and nutritional status, the Panel recommends that instruments to assess current intake be developed for use in NHANES III. To the Panel's knowledge, a feasible way to determine past usual intake of energy and most nutrients is not available; however, for a specific nutrient (e.g., calcium), a reasonable estimate may be possible. Need for such data is discussed in subsequent chapters where appropriate.

E. MEDICATION AND DIETARY SUPPLEMENT USE

Throughout specific discussions of nutrient status and health conditions, the Panel has identified the importance of collecting information on nutrient supplements, other dietary supplements, and medications containing components that may affect biochemical measures of nutritional status. Therefore, the Panel strongly recommends that the household and medical history questionnaires elicit detailed information related to current medication and vitamin/mineral supplement use as was done in NHANES. Perhaps the best approach is for the subject to show the interviewer in the home all drugs (prescription and over-the-counter) and supplements so that accurate information on the exact content and dose can be obtained from the label. With a computerized data recording and retrieval system, answers to the questions asked by the interviewer at the examination center can be used to confirm whether the subject is still consuming the same medications and supplements he or she described in the household interview.

F. MEDICAL EXAMINATION

The classical clinical signs of specific nutrient deficiencies have been detected infrequently in the U.S. population. For example, the physician's examination in the previous HANES revealed very few individuals with clinical manifestations of rickets, Bitot's spots, xerophthalmia,
follicular hyperkeratosis, etc. The Panel believes that emphasis on such manifestations is unlikely to be rewarding. However, the medical examination can be most helpful in disclosing evidence of diseases that may adversely affect nutritional status (e.g., hepatosplenomegaly, or pulmonary or cardiovascular abnormalities). The Panel assumes that the subjects' physician will be informed of significant clinical findings in order that there be individual follow-up as appropriate.

G. CLINICAL PROCEDURES

Anthropometric measures that were made in NHANES II should be repeated in NHANES III. It would be useful to collect data on length and stature measurements for infants and young children. Length measurements should be made on infants and children up to age 3 years. Stature should be measured, when possible on children between 2 and 3 years of age and on all children 3 years of age and older. In subsequent chapters, the Panel has recommended consideration of other clinical procedures.

H. ANALYTICAL PROCEDURES

The Panel recommends that all blood samples be taken by venipuncture. Only one attempt at venipuncture should be made in infants and children. Special training of laboratory personnel can assure a high success rate for this procedure. Because it is desirable to have fasting blood samples for several nutrients, it is recommended that at least part of the population arrive at the examination in a fasting state. Subjects should receive detailed information instructing them not to take nutritional supplements during the fasting period.

The Panel finds the procedures used for sample preparation, handling, and equipment maintenance in NHANES II and HHANES adequate; however, they should be reviewed by those scientists who will be responsible for the collection, storage, and analysis of samples.

The Panel recognizes that validation trials on methods and pilot studies of survey techniques will need to be completed prior to the scheduled initiation of NHANES III in 1988. This time constraint requires that validation trials start in late 1986. Nevertheless, the Panel has some concerns about its ability to identify the most appropriate methodology for measurement of some of the recommended indices because of the rapid pace of analytical developments. The Panel suggests that when the overall plan for NHANES III is finalized, NCHS make the protocols for the various analyses available to those agencies which have an interest in using the data to be collected. At that time (late 1986-early 1987), the analytical procedures already established, and those requiring further validation, could be identified. In addition, because the tentative plans for NHANES III include data
collection over a 6-year period in three 2-year cycles, the opportunity for incorporation of further improvements in analytical methodology should not be overlooked. However, changes in methodology may lead to comparability problems.

Another important aspect of analytical methodology is rigorous attention to quality assurance and control in regard to standardization of techniques, precision and accuracy of methods, and calibration against known standards. The NCHS and CDC are well aware of issues associated with quality assurance and quality control. Protocols for validation trials and pilot studies should make adequate provision for standardization of all aspects of sample collection, analysis, and data treatment. The Panel suggests that protocols related to quality assurance and control be established during validation trials and pilot studies, and that these results together with NHANES protocol be made available to interested agencies.

The Panel recognizes that many techniques used in nutrition surveys are labor-intensive. The Panel suggests that, as protocols for validation trials are being developed, consideration should be given to using semi-automated and automated analytical equipment as much as possible. The use of laboratory robotics should be explored. These efforts should be directed towards development of procedures with improved precision and efficiency in handling large numbers of samples.

I. OTHER CONSIDERATIONS

The Panel has noted the importance of taking blood pressure measurements at a standard time during the examination, particularly in relation to the blood drawing. If logistical and operational constraints allow, consideration should be given to standardizing the timing of this measurement in NHANES III.

A number of techniques for evaluating nutritional status of individuals are not appropriate for large-scale nutrition surveys. For example, repeated blood drawing, 24-hour urine collections, load tests, or biopsies could be useful, but cannot be considered for NHANES III. The Panel has focused its attention on assessment methods that do not involve such techniques.

J. CONSIDERATIONS OF THE PANEL IN DEVELOPING THE SPECIFIC RECOMMENDATIONS

In formulating its recommendations for indices of nutritional status to be assessed in NHANES III, the Panel has placed great emphasis on indicators that reflect body stores of nutrients. If measurements of body stores are not available, and functional assessments of nutrient depletion do not exist,
the Panel considered other approaches to assessment less useful for the FDA's purposes. However, the Panel is aware that in doing so, it is making recommendations to FDA and not to the NCHS, the agency that will conduct NHANES III.

The Panel has also considered criteria for acceptance of new methodology for use in NHANES III which may make comparisons between surveys more difficult. The Panel chose to recommend new methodology if the method was clearly superior to those used previously. When feasible, the previously used and currently proposed techniques should both be performed (e.g., blood pressure measurement with standard and random-zero mercury sphygmomanometers).

In describing the basis for interpretative criteria, the Panel has given cutoff values for some biochemical indicators. In such cases, the values cited are applicable only for the specific analytical method used to determine them. In addition, the Panel notes that such cutoff values are frequently based on normalized distribution curves, and that low values may not necessarily indicate low body stores of nutrients or impaired function.
III. VITAMIN A

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III. VITAMIN A

A. INTRODUCTION

Vitamin A deficiency is a major health problem worldwide with the occurrence of an estimated 500,000 cases of childhood xerophthalmia per year (Russell et al., 1984; Sommer, 1982). Clinical signs and biochemical measurements of vitamin A depletion in the United States population have been assessed in previous HANES, and the prevalence of frank deficiency found to be low. However, the prevalence of serum vitamin A levels considered indicative of vitamin A status which might be improved by additional dietary vitamin A was >20% for children in previous HANES (Pilch, 1985)

Recently, vitamin A and its carotenoid precursors have been implicated as possible cancer preventive agents. Wald et al. (1980) and Kark et al. (1981) found subjects who later developed certain types of cancer had serum vitamin A levels significantly lower than controls. Another study (Shekelle et al., 1981) found that carotenoid intake, but not preformed vitamin A intake, was inversely proportional to the number of subjects that developed lung cancer. Other studies have not confirmed this association (Willett et al., 1984). Currently, the National Cancer Institute is supporting several clinical trials in cancer prevention involving retinoids, carotenoids, and other dietary constituents (Sestili, 1984).

Concerns about vitamin A toxicity are justified because retinol and its analogues are sold over the counter and FDA consumer surveys show these agents are being taken in large quantities by some adults (Stewart et al., 1985). Emerging information about vitamin A and cancer risk may spur increased consumption of large doses. It is important to evaluate whether consumption of vitamin A is approaching the toxic range in some individuals.

B. AVAILABLE INDICATORS

There is no single method that quantitatively determines vitamin A status; however, several methods exist which may be used to identify the risk of vitamin A deficiency or toxicity.

1. Nutritional status

Rapid Dark Adaptation Test (RDAT). Tests of dark adaptation can be used to determine night blindness, an early manifestation of vitamin A deficiency (Russell et al., 1984). RDAT involves determination of the Purkinje shift [the time of
transition from day vision (cone-mediated) to night vision (rod-mediated) (Russell et al., 1984; Thornton, 1977). This method utilizes a table, light source, cone-matched red and blue chips, takes between 5 and 15 minutes to perform, and is suitable for field studies (Russell et al., 1984; Solomons et al., 1982). RDAT correlates with serum vitamin A and classical dark adaptation (Vinton and Russell, 1981). Several problems exist with this method and all other tests for visual function: false positives occur because of congenital night blindness, dark adaptation can be affected by other nutrient deficiencies (e.g., zinc and protein) (Dutta et al., 1981; Morrison et al., 1978), and great variability in results exists from tester to tester (Carney and Russell, 1980; Fisher et al., 1970).

Relative Dose Response (RDR). The RDR test (Amédée-Manesme et al., 1984; Flores et al., 1984; Loerch et al., 1979; Mobarhan et al., 1981) involves taking a blood sample just before and 5 hours after a small dose (450 μg) of vitamin A. This method provides an assessment of body stores of vitamin A and, thus, can be used to identify those individuals with marginal vitamin A deficiency. The RDR (%) is computed as:

\[
\text{plasma retinol at 5 h - plasma retinol at 0 h} / \text{plasma retinol at 5 h}
\]

The RDR may be low in those individuals with malabsorption, liver disease, severe protein malnutrition, or zinc deficiency, irrespective of differences in vitamin A status (Russell et al., 1983).

Serum Retinol. Vitamin A levels in the serum are not reflective of body stores of this nutrient until stores are severely depleted (Russell et al., 1984). Serum retinol, which is the primary form of circulating vitamin A during deficiency, can be determined by reversed-phase (Bieri et al., 1979; Catignani and Bieri, 1983) or normal-phase (Bankson et al., 1985) high performance liquid chromatography (HPLC). Various cutoff values for serum vitamin A have been suggested as indicative of vitamin A deficiency; age-specific recommendations have been made (Pilch, 1985). Adult cutoff values are less valid in populations with chronic parasitism and other factors which lower vitamin A status.

Serum Carotenoids. The only known nutritional role of carotenoids in vertebrates is that of conversion to vitamin A; existence of carotenoid deficiency has been established (Olson, 1984). Carotene or carotenoids do not relate to vitamin A nutritional status except as precursors of vitamin A (Olson, 1984). Assessment of serum carotene or carotene intake with respect to its possible relationship to cancer is discussed in Chapter XX.
2. Excessive accumulation

Hypervitaminosis A may be associated with increased concentrations of total serum vitamin A, but, more specifically, with markedly increased levels of serum retinyl esters (Russell et al., 1974; Smith and Goodman, 1976). Under normal circumstances, the concentration of retinyl esters is very low in fasting blood samples and has been reported to comprise less than 5% of the total vitamin A present (Smith and Goodman, 1976). Retinyl esters may not be toxic in and of themselves, but elevated levels may reflect a state of intoxication.

C. NHANES III APPROPRIATENESS

Total serum vitamin A levels in NHANES I and II, and serum retinol level in Hispanic HANES, were assessed as single biochemical indicators of vitamin A status. Although very depressed values are sufficient to identify severe vitamin A depletion, marginally low values are only suggestive of risk of inadequate vitamin A status. Additional indicators are required to distinguish among different degrees of vitamin A inadequacy as well as evidence of vitamin A toxicity. Although the RDR test would be valuable to assess vitamin A stores and to determine the significance of serum retinol levels in the marginal range, its use as a screening test in NHANES III cannot be recommended because of its logistical difficulties. Additionally, in children, where the potential for inadequate vitamin A nutriture is probably greatest, the Panel cannot justify the second blood sample required for this procedure. Alternatively, rapid dark adaptation might be tested in the entire population to identify individuals who have impaired functional status possibly associated with vitamin A depletion.

The determination of serum retinyl esters for evaluation of excessive accumulation of vitamin A is probably only suitable for a subset of the population because of the need for a fasting blood sample. This measurement is only an indicator of possible toxicity of vitamin A and will not identify those individuals with low vitamin A status.

D. RECOMMENDED APPROACHES

1. Indices

Serum retinol and retinyl ester levels should be measured for the assessment of vitamin A status. A test of rapid dark adaptation should also be performed, if validated for inter- and intra-test administrator reproducibility.
2. Methods

Methodology for rapid dark adaptation has already been discussed. Although relatively quick and inexpensive, the space requirement for this test is considerable. Additional problems with compliance may arise in children. Accuracy of the RDAT is dependent on tester motivation and should only be included in NHANES III if validation studies prove it to be reproducible.

Serum vitamin A or retinol levels have been measured by three different techniques in previous HANES, both colorimetric and chromatographic (reversed-phase HPLC). The latter method (Bieri et al., 1979) was greatly preferred due to its ease and specificity. Because earlier colorimetric measurements did not distinguish retinol from retinyl esters in the serum, the improved method was desirable. However, this change has made secular trends in serum vitamin A concentrations nearly impossible to identify.

Retinyl ester levels can be measured by either reversed-phase (Furr et al., 1984) or normal-phase HPLC (Bankson et al., 1985). New methodology (Bankson et al., 1985) allows routine quantitation of total fasting serum retinyl esters concurrently with retinol. Most HPLC techniques that separate individual retinyl esters lack the sensitivity required to detect the low levels found in fasting serum. Normal-phase HPLC combines over 94% of circulating esters and quantitates pooled total retinyl esters and retinol in one run of approximately 10 minutes. This method greatly enhances the speed at which retinoid compounds can be analyzed and obviates the requirement for two HPLC instruments or separate HPLC runs. If this method is chosen for concomitant measurement of serum retinol and retinyl esters, it should be evaluated for comparability with methods used in previous HANES.

3. Basis for interpretative criteria

Dark adaptation time has been correlated with serum vitamin A levels, determined by the method of Neeld and Pearson (1963), in adult subjects with documented liver disease, gastrointestinal disease, or chronic alcoholism in the United States (Carney and Russell, 1980). These studies, in diseased individuals, revealed that normal vitamin A-dependent retina function could only be predicted if serum vitamin A levels were greater than 40 μg/dl. Among nondiseased subjects there are few recent studies that record impaired dark adaptation at serum values above 30 μg/dl. Usually, dysfunction of the retina is not observed until blood levels are under 20 μg/dl and, even at this level, findings are inconsistent.

In several studies, primarily with children, relationships between clinical signs of vitamin A deficiency and commonly used biochemical indicators of vitamin A assessment have been examined. A population-based study of Sri Lankan children aged
6 months through 71 months included observations on signs of clinical vitamin A deficiency (Bitot's spots, determination of corneal scars, and blindness of one or both eyes) and microfluorometric measurements of serum vitamin A [by the method of Futterman et al., (1975)]. Results showed that the lowest mean serum vitamin A level (26.3 µg/100 ml) occurred in children with clinical eye findings; however, low serum values were not necessarily indicative of impaired visual status (Brink et al., 1979). Studies of circulating vitamin A were recommended as an estimate of populations considered at risk of developing clinical disease, but not as an indicator of prevalence of clinical disease.

Longitudinal studies have been conducted in Guatemalan children to evaluate changes in serum vitamin A concentration (Arroyave et al., 1981) resulting from a program to fortify the nation's sugar supply (Arroyave et al., 1979). In all children with initial serum vitamin A levels below 20 µg/dl, as determined by the method of Bessey et al. (1946), serum vitamin A levels increased after 1 year of fortification. Numbers of children with serum levels in the highest category of adequacy also increased during this time (Arroyave et al., 1981).

Although clinical signs of deficiency and serum vitamin A levels were not correlated directly in Philippine intervention trials (Solon et al., 1979), fortification of monosodium glutamate with vitamin A led to a decrease (p < 0.01) in active signs of xerophthalmia after intervention. A concomitant increase in serum vitamin A (p < 0.01), determined by the method of Neeld and Pearson (1963), was noted as well.

A recent retrospective analysis of vitamin A data collected in previous HANES has suggested the following age-specific interpretative criteria for low total serum vitamin A values in populations (Table 2) (Pilch, 1985).

Vitamin A toxicity is generally diagnosed by clinically distinct manifestations, including pseudotumor cerebri, skeletal pain, desquamating dermatitis, and hepatic inflammation (Frame et al., 1974; Josephs, 1944; Morrice et al., 1960; Muenter et al., 1971; Rubin et al., 1970; Russell et al., 1974). Smith and Goodman (1976) compared three patients with hypervitaminosis A with 14 control subjects of comparable age. In patients 1 and 2, whose plasma samples were obtained within 4 days after the last dose of supplemental vitamin A, retinyl esters made up 67 and 65% of the total vitamin A present. In patient 3, retinyl esters made up 33% of the total vitamin A in a plasma sample taken 3 weeks after the last dose of supplemental vitamin A. In contrast, retinyl esters ranged from 0.1 to 4.7% of plasma total vitamin A in control subjects. In the patients with hypervitaminosis A, plasma total vitamin A as well as the percentage of vitamin A as retinyl esters decreased as signs of toxicity diminished.
Table 2. Guidelines for Interpretation of Serum Total Vitamin A Levels in Selected Low Ranges in Populations

<table>
<thead>
<tr>
<th>Serum Vitamin A Levels</th>
<th>3-11 yr</th>
<th>12-17 yr</th>
<th>18-74 yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 µg/dl</td>
<td>Vitamin A status(^1) is very likely to improve with increased consumption of vitamin A; impairment of function(^2) is likely</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 µg/dl</td>
<td>Vitamin A status is likely to improve with increased consumption of vitamin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29 µg/dl</td>
<td>Vitamin A status of some individuals may improve with increased consumption of vitamin A; improvement is most likely in those with values 20-24 µg/dl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Vitamin A status refers to serum vitamin A levels and tissue levels of the nutrient.

2 Impairment of function may include impaired dark adaptation, night blindness, ocular lesions, and possibly impaired immune function.
4. **Target groups**

Vitamin A assessments of the entire population should be performed.

5. **Other considerations**

Precautions should be taken during extraction of vitamin A compounds to avoid their degradation after prolonged storage of frozen serum samples (Driskell et al., 1985).

Myocardial infarction, surgery, acute infection, parasites, large doses of vitamin E, oral contraceptive agents, pregnancy, alcohol, zinc deficiency, serum iron concentration, protein malnutrition, hyperlipidemia, time of blood collection, and seasonal variations can each affect serum vitamin A levels (Olson, 1984; Underwood, 1984). Efforts should be made to identify individuals who might have these confounding factors affecting vitamin A assessment.

Twenty-four hour recall data on vitamin A intake tends to be unreliable; however, 3-day intake data supplemented by reports of frequency of consumption of vitamin A-rich food may prove useful.

Currently, other assessments of vitamin A nutritional status are being investigated. For example, improved gas chromatographic/mass spectroscopic detection techniques are being developed for stable isotope dilution tests, which can assess the total body pool of vitamin A (Clifford et al., 1985). Serum retinoic acid level is also under study as an indicator of vitamin A nutritional status (Pilch, 1985). If and/or when these measurements are validated and become feasible to perform in large surveys, they should be considered for inclusion in HANES.

**E. INTENDED USES OF DATA**

Because different methods for determination of serum vitamin A have been used in the three previous HANES and because of the previously discussed difficulty in relating serum vitamin A level to clinical correlates of deficiency, the significance of differences in prevalence of hypovitaminosis A over time will be difficult to interpret using past HANES data. HPLC, recommended for use in NHANES III, was used to determine retinol in Hispanic HANES, but this population is not representative of the U.S. population as a whole. Care must be taken by investigators attempting to relate data from NHANES III with previous HANES, so that unwarranted conclusions are not drawn regarding changes in prevalence estimates of vitamin A inadequacy.
Sufficient data for establishing acceptable cutoff values to define rapid dark adaptation time as an indicator of vitamin A status are not available; however, values can be correlated with those of serum retinol. A finding of impaired dark adaptation in persons with serum retinol in the marginal range may provide a better indicator of clinically significant impairment in vitamin A status than serum retinol alone.

Data on serum retinyl ester levels can be obtained along with serum retinol data using HPLC methods. If sufficient numbers of high values occur in the population surveyed, it would allow the determination of prevalence estimates for excessive intakes of vitamin A in the U.S. population. In addition, it may be useful to compare the range and mean of retinyl ester levels in those individuals who are supplement users and nonsupplement users.

F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends inclusion of the following indices for assessment of vitamin A status in NHANES III:

- serum retinol level
- rapid dark adaptation test (providing it is reproducible)
- serum retinyl ester level in fasting subjects.
LITERATURE CITED


IV. VITAMIN B-6

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IV. VITAMIN B-6

A. INTRODUCTION

Selected subgroups of the United States population suspected to be "at risk" of developing inadequate vitamin B-6 status include pregnant women, adolescents, and the elderly (Cleary et al., 1975; Driskell, 1978; Driskell et al., 1985; Heller et al., 1973; Jacobs et al., 1968; Kirksey, et al., 1978; Roekpke and Kirksey, 1979; Schuster et al., 1984). Chronic drug and alcohol use coupled with insufficient dietary vitamin B-6 intake are examples of factors that may adversely affect vitamin B-6 status (Bailey, 1984; Bhagavan, 1985; Li and Lumeng, 1985; Lumeng and Li, 1974). National prevalence estimates of vitamin B-6 deficiency are not available.

Vitamin B-6 functions as a coenzyme in numerous enzyme systems affecting the metabolism of protein, carbohydrate, and lipid (Bailey, 1984). In controlled metabolic studies, vitamin B-6 depletion has been demonstrated by both biochemical and clinical deficiency signs. However, frank vitamin B-6 deficiency resulting in recognizable clinical manifestations is not suspected to be widespread in the general population. In view of the continuing reports of marginal vitamin B-6 status in population subgroups, the assessment of vitamin B-6 adequacy is desirable.

B. AVAILABLE INDICATORS

1. Nutritional status

Plasma Pyridoxal 5' Phosphate (PLP). Plasma PLP is a sensitive and reliable indicator of vitamin B-6 nutritional status (Leklem and Reynolds, 1980; Li and Lumeng, 1980; Lumeng et al., 1978; Sauberlich, 1981; Sauberlich et al., 1974). Pyridoxal 5' phosphate is a functional coenzyme form of the vitamin and represents a rapidly mobilizable storage pool of vitamin B-6, as well as the major transport form (Lumeng et al., 1978). Plasma PLP is derived primarily from the liver and its concentration correlates well with vitamin B-6 intake and PLP tissue levels, particularly with that of skeletal muscle, which is the largest repository of vitamin B-6 in the body (Li and Lumeng, 1980). The concentration of plasma PLP remains relatively constant over time with little fluctuation when subjects are ingesting constant amounts of vitamin B-6, whether the diet is regular (unsupplemented), restricted, or B-6-supplemented (Lumeng et al., 1974, 1980). When the dietary content of vitamin B-6 is altered, the plasma PLP concentration decreases or increases commensurate with dietary intake to reach new steady-state levels within 3-4 weeks (Brown et al., 1975). Following an
acute oral pyridoxine dose, plasma PLP peaks in 2-4 hours after ingestion; with large doses, it remains elevated for several days (Shane, 1978). Thus, plasma PLP levels reflect recent intake as well as tissue stores.

**Urinary 4-Pyridoxic Acid (4PA).** Urinary 4PA provides a measure of the major metabolic end-product of vitamin B-6 (Shultz and Leklem, 1980). Urinary 4PA excretion decreases during vitamin B-6 deficiency and increases during repletion with dietary vitamin B-6 (Baysal et al., 1966; Kelsay et al., 1968; Sauberlich, 1984), which suggests that urinary 4PA excretion generally reflects dietary intake of vitamin B-6. Shultz and Leklem (1980) established that a significant relationship exists between vitamin B-6 intake as well as B-6/protein ratios and 4PA and concluded that 4PA levels in 24-hour urine samples could be used as an index of vitamin B-6 intake. The data are inadequate to correlate urinary 4PA with plasma PLP, and, as yet, the relationship of urinary 4PA to vitamin B-6 status is unknown. Additionally, measurement of 4PA requires a 24-hour urine collection.

**Urinary Vitamin B-6.** In controlled studies with adult subjects, the urinary excretion of free vitamin B-6 correlated closely with the level of vitamin intake (Sauberlich et al., 1974). The measurement of urinary level of vitamin B-6 is useful as a reflection of the subject's recent dietary intake of vitamin B-6, but may be of limited value as an indication of the vitamin B-6 status of an individual (Sauberlich et al., 1974). Urinary excretion of vitamin B-6 decreases proportionately with a decreased intake of the nutrient to a critical point, after which further lowering of intake results in only minor and variable changes in urinary excretion (Sauberlich, 1981). Additionally, this method requires a laborious microbiological assay; HPLC methods are not presently considered an acceptable alternative due to methodological problems.

**Blood Transaminase Activities.** Transaminase activity measurements represent a biochemical functional test which may provide information regarding the state of deficiency or depletion of vitamin B-6 reserves (Sauberlich et al., 1974). Transaminase activities have been measured in erythrocytes, leukocytes and plasma. Because plasma transaminase activities are much lower in plasma than in erythrocytes and show a wide range in normal individuals, they are not considered useful in assessing vitamin B-6 status. Two transaminases measured in erythrocytes are alanine aminotransferase (AIAAT) and aspartate aminotransferase (AspAT), also referred to as glutamic pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT), respectively. The measurement of the stimulation of the activity of these two enzymes by the in vitro addition of PLP to the assay reaction is considered a better indicator of vitamin B-6 status than enzyme activity alone (Cinnamon and Beaton, 1970). The Panel notes that no established clinical correlates exist between either the specific activity or the percent activation of AspAT,
and thus the enzyme measurements serve only as a gross indicator of deficiency or adequacy (Bamji and Prema, 1981; Nguyen et al., 1983; Skala et al., 1982; Thurnham, 1981). AlaAT serves as a more sensitive indicator of vitamin B-6 status, but has low activity when compared to AspAT and is far less stable in storage (Thurnham, 1981), making it unsuitable for field survey studies.

**Tryptophan Load Test.** Determination of tryptophan metabolites in the urine after a tryptophan load provides an indirect measure of vitamin B-6 status because of the numerous enzyme steps that require PLP (Leklem and Reynolds, 1980; Leklem et al., 1975). The tryptophan load test has been used extensively to assess vitamin B-6 status in individuals and to determine vitamin B-6 requirements (Sauberlich et al., 1974). Although this method provides a measure of the functional adequacy of coenzyme levels, many factors other than vitamin B-6 status can influence urinary excretion of these metabolites including protein intake, lean body mass, exercise, the size of the amino acid loading dose, and estrogen levels. A major disadvantage of the method is that it requires the collection of 24-hour urine samples which is not practical for field survey studies.

2. **Excessive accumulation**

The toxicity of all forms of vitamin B-6 is low. Hayes and Hegsted (1973), in a review of toxicity of the vitamins, state that an intravenous dose of 200 mg of pyridoxine is nontoxic in man and daily oral doses of 100-300 mg have been administered without side effects. Oral doses up to 1.0 g/day daily in humans without adverse reactions have been reported (Haskell, 1978). Pyridoxine supplements containing 50 to 500 mg of pyridoxine are widely available and supplements have been promoted, despite inadequate testing, for a number of conditions including premenstrual syndrome, morning sickness, carpal-tunnel syndrome, schizophrenia, autistic conditions, and hyperkinesis (Schaumburg et al., 1983).

The possibility of sensory neuropathy from pyridoxine abuse is now recognized in view of recent reports of this side effect from daily megadoses (2-6 g) (Schaumburg et al., 1983). Vitamin B-6 assessment parameters were not measured in all cases; however, in one individual taking 4 g/day of pyridoxine, the plasma pyridoxine was approximately twice the highest level in the normal range. The plasma pyridoxine in this patient returned to normal following 1 month's abstinence from supplemental vitamin B-6. Supplementation with only 500 mg/day over a prolonged period (approximately 1 year) has also been shown to be neurotoxic (Berger and Schaumburg, 1984). Plasma PLP does not appear to be a discriminatory assay for evaluating excessive intakes of vitamin B-6; widely varying large doses may lead to similar
elevations of plasma PLP (Bhagavan et al., 1975; Brown et al., 1975). Thus, plasma PLP concentration cannot be used to estimate the prevalence of individuals consuming potentially harmful doses of vitamin B-6 in the U.S. population. Other measures of vitamin B-6 toxicity are not available.

C. NHANES III APPROPRIATENESS

Vitamin B-6 status has not been evaluated on a national probability sample. The Ten-State Nutrition Survey provided some data for urinary vitamin B-6 for five locations in the United States. Urinary excretion was higher in young children than adults, was higher in adult women than in men, and was lower in South Carolina than in the other four locations. Data from the USDA Nationwide Food Consumption Survey (U.S. Department of Agriculture, 1980) suggest that vitamin B-6 intake is inadequate for some individuals. The actual consumption of the vitamin in the U.S. population is unknown because food composition tables are incomplete with respect to vitamin B-6. In addition, food composition tables do not account for incomplete bioavailability from plant sources (Gregory and Ink, 1985).

Plasma PLP is available for the determination of vitamin B-6 status and is sensitive and amenable to a large-scale survey such as NHANES III.

D. RECOMMENDED APPROACHES

1. Indices

Measurement of plasma PLP concentration is recommended as the method of choice for inclusion in NHANES III.

2. Methods

Methods available for measuring PLP in plasma have been reviewed by Sauberlich (1984). Although some procedures for plasma PLP analysis appear to have advantages, direct comparison of methods have not been published to date. Lui et al. (1985) have compared the cation-exchange HPLC method of Coburn and Mahuren (1983) with their cation-exchange open column chromatographic (OCC) method and the L-tyrosine apodecarboxylase assay for measuring the different vitamin B-6 compounds in human plasma. Excellent correlations were obtained for plasma PLP levels measured by any two of the three methods of analysis (r values in the range of 0.93 to 0.98).
3. **Basis for interpretative criteria**

Recent diet and/or supplements should be considered when interpreting plasma PLP levels since levels do fluctuate with recent dietary intake and/or supplement use. The plasma PLP concentrations of apparently healthy unsupplemented adult subjects consuming "normal" diets have been reported by a number of different investigators (Chauhan and Dakshinamurti, 1981; Lumeng and Li, 1974; Lumeng et al., 1980; Shultz and Leklem, 1980).

Plasma PLP levels have been reported to be higher in male than in female adults; therefore, sex differences in PLP levels should be considered (Brown, 1972). Shultz and Leklem (1980) developed guidelines for assessing plasma PLP levels based on extrapolations from the regression of plasma PLP on the dietary vitamin B-6:protein ratio. Mean values below the range of 9.5-10.2 ng/ml and 5.5-6.2 ng/ml were considered as indicative of marginal vitamin B-6 status for adult males and females, respectively.

It may be necessary to consider age-specific norms for plasma PLP. Muscle mass increases during childhood and adolescence, remains relatively constant during early adulthood, and then declines in later years. Because the major storage site for vitamin B-6 is skeletal muscle, these changes in body composition may affect tissue and blood levels. Age-related changes have been observed in population studies (Hamfelt, 1964; Lumeng and Li, 1974; Rose et al., 1976).

Evaluation of the vitamin B-6 status of pregnant women is difficult. Lower plasma PLP levels in pregnant than in nonpregnant women may not signify a vitamin B-6 deficiency. Hemodilution during pregnancy caused by an increase in maternal plasma volume beginning at the end of the third month may be partly responsible for the decreased plasma PLP levels. However, the most rapid rate of decrease in plasma PLP occurs late in pregnancy after the greatest increase in plasma volume has already occurred (National Research Council, 1970). The greatest growth of the fetus and the placenta also occurs between the 30th week of pregnancy and term (National Research Council, 1970). In longitudinal studies, the second and third trimester as well as postpartum biochemical indices have been compared with first trimester levels (Roepke and Kirksey, 1979; Schuster et al., 1984). Reynolds and Leklem (1985) have pointed out that vitamin B-6 supplementation at levels several times the current RDA fails to prevent the third trimester depression. These workers suggest that the depressed plasma PLP levels in late pregnancy may be normal and perhaps are advantageous to either the mother or the fetus. As more information is obtained relating biochemical indices of vitamin B-6 status with clinical parameters such as condition of infant at birth, suggested norms can be determined and evaluated.
4. **Target groups**

The Panel recommends that vitamin B-6 status be determined on a representative sample of the U.S. population. Inasmuch as the protocol for NHANES III may call for three 2-year cycles of national probability samples, the Panel suggests that vitamin B-6, along with riboflavin and thiamin, be determined together on persons included in either the first or second cycle of NHANES III. Consideration should be given to possible emphasis on examining those groups considered at risk for inadequate status, namely, the elderly, adolescents, and pregnant women.

5. **Other considerations**

Alterations in vitamin B-6 nutriture have been noted in response to many factors, including physical activity (Leklem, 1985), oral contraceptive agent use and estrogen replacement therapy (Miller, 1985), specific drugs (Bailey, 1984; Bhagavan, 1985), and alcohol (Li and Lumeng, 1985). Individuals who may be affected by these factors should be identified to aid in the interpretation of vitamin B-6 status.

E. **INTENDED USES OF DATA**

The data collected will provide the first national probability evaluation of vitamin B-6 status in the United States. Specific population subgroups at particular risk of vitamin B-6 inadequacy can be identified.

F. **CONCLUDING STATEMENT**

Based on the foregoing discussion, the Panel recommends the following assessment for evaluation of vitamin B-6 status:

- plasma PLP concentration.
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V. VITAMIN C

A. INTRODUCTION

The consumption of fresh foods has been recognized for centuries as a preventive measure for scurvy (Hodges, 1980; Vilter, 1978). It was not until 1932 that the active compound, ascorbic acid, was discovered (King and Waugh, 1932; Svirbely and Szent-Györgyi, 1932). Since that time, research efforts have failed to establish the range of human requirement for vitamin C. This uncertainty is reflected in the varying recommended daily allowances advised by different countries (Sauberlich, 1984). Research efforts have focused on how much the recommended intake should exceed that required to prevent scurvy and allow for an appropriate reserve, and, whether tissue saturation provides any measurable health benefit. While 10 mg/day is sufficient to prevent scurvy, the RDA is set at 60 mg (National Research Council, 1980), which sustains a body pool of approximately 1500 mg and protects the adult male from clinical signs of scurvy for at least 1 month when consuming an ascorbate-free diet (Hodges et al., 1969, 1971).

Clinical manifestations of scurvy are rare in this country; affected individuals consist largely of the elderly consuming inadequate diets (National Research Council, 1980). Other conditions associated with inadequate vitamin C intake are poverty, alcoholism, and nutritional ignorance.

Attention has also focused on reported therapeutic benefits from routine high-level consumption of ascorbic acid (Cameron and Pauling, 1976; Pauling, 1971, 1974a,b). However, claims for unique therapeutic benefits are highly controversial and have not been supported by objective scientific studies (Anderson, 1975; Chalmers, 1975; Coulehan et al., 1976; Dykes and Meier, 1975; Karlowski et al., 1975; Moertel et al., 1985). Possible associations between vitamin C status and prevention of cancer are discussed in Chapter XX.

Vitamin C is highly water soluble and generally considered nontoxic. However, adverse effects of excess consumption have been reported. These include gastrointestinal distress (Sauberlich, 1981), uricosuria (Stein et al., 1976), excessive absorption of dietary iron by individuals with indiopathic hemochromatosis, thalassemia major or sideroblastic anemia (Cook and Monsen, 1977), impaired bactericidal activity of leukocytes (Shiotri and Bhat, 1977), and kidney stones (Sauberlich, 1981).
B. AVAILABLE INDICATORS

The biochemical defects that occur as the result of vitamin C deficiency do not lend themselves to the development of functional tests to assess nutritional status (Sauberlich, 1984). Thus, measurements considered to reflect body stores must be used.

1. Nutritional status

Plasma Ascorbic Acid. Plasma or serum ascorbic acid level is the most commonly used indicator of vitamin C status; however, it has several limitations and may not always reflect accurately the state of body reserves (Sauberlich et al., 1974). It may be influenced by recent intake of the vitamin, particularly high intakes (Omaye et al., 1979; Sauberlich et al., 1974). For this reason, measurements are usually made on samples obtained from subjects who have fasted overnight. Also, the level is reported to be lowered by stress (Irvin et al., 1978), cigarette smoking (Bailey et al., 1970; Brook and Grimshaw, 1968; Pelletier, 1968; Pelletier, 1970), oral contraceptive use (McLeroy and Schendel, 1973; Rivers and Devine, 1972), infections, and other disease states (Sahud and Cohen, 1971). However, these changes may not reflect changes in body pool size (Sauberlich et al., 1974).

Despite these limitations, the relationship between plasma level and ascorbate intake is essentially linear over the most critical range of intakes. Intakes below 20 mg/day are associated with plasma levels of 0.20 mg/dl or less and high intakes (80 mg/day or more) are associated with plasma levels approaching 1 mg/dl. With intermediate intakes plasma ascorbate concentrations tend to plateau at levels between 0.2 and 0.8 mg/dl (Sauberlich et al., 1974). In studies with five men who were deprived of ascorbate for more than 60 days, plasma ascorbate concentrations declined rapidly initially and then steadily as the body pool was depleted (Hodges et al., 1971).

Leukocyte Ascorbic Acid. Although leukocyte ascorbate concentration is more closely related to body stores than is plasma (Loh, 1972; Lowry et al., 1946), erythrocyte, or whole blood ascorbic acid (Omaye et al., 1979; Turnbull et al., 1981), samples are difficult to prepare, requiring a full-time field technician and a relatively large amount of blood (2-4 ml) (Omaye et al., 1979; Sauberlich, 1984; Sauberlich et al., 1974). Without adequate training of the technician and scrupulous preparation, great variation in results will occur. The Panel concludes that while useful as a diagnostic procedure in a clinical setting, leukocyte ascorbic acid analysis is not practical for NHANES III.
Body Pool Size. Isotope dilution or excretion techniques can be used to estimate the body pool size of ascorbic acid (Baker et al., 1969, 1971; Kallner et al., 1977). Although expensive, these methods are the most reliable measures of nutritional status of vitamin C. $^{14}$C-labeled ascorbic acid is given to the subject orally and within 24 to 48 hours the specific activity of blood or urine ascorbate is determined. Currently, efforts to utilize $^{13}$C (a stable isotope) for this procedure are being examined (Tolbert, 1985). The Panel notes that administration of a long-lived radioisotope ($^{14}$C) would not be feasible in a large survey and the $^{13}$C procedure, which may be useful in the future, has yet to be validated.

Urinary Ascorbitol. Studies are underway to evaluate urinary ascorbitol, an ascorbic acid metabolite, as an assessment tool for vitamin C status (Tolbert, 1985). The urinary level of ascorbitol appears to be proportional to body pool size, which is directly related to nutritional status. The stability of this compound, which is significantly greater than that of ascorbic acid, suggests possible utility of this measurement in a survey such as HANES (Tolbert, 1985). A major disadvantage of this assessment for population studies is the requirement for 24-hour urine samples. Logistical problems associated with collection of 24-hour urine samples from the survey population have already been discussed. At this time, the method has not been developed completely and reference data with which to compare measured values do not exist.

Tests for Capillary Fragility. Several tests have been developed to measure the appearance of petechial hemorrhages after measured pressure has been applied with a blood pressure cuff to the the veins of the upper arm (Göthlin, 1933; Hess and Fish, 1914). Although persons with naturally occurring scurvy frequently exhibit an abnormality of the capillaries, these tests are not necessarily positive in all individuals with vitamin C deficiency and other diseases that increase capillary fragility and permeability will also yield positive results (Vilter, 1967).

2. Excessive accumulation

Recent intakes of large doses of ascorbic acid are reflected in high plasma concentrations (Omaye et al., 1979). However, maximum plasma ascorbate concentration usually does not exceed 1.5 mg/dl as renal clearance of the vitamin rises sharply when intake is above 100 mg/day (Friedman et al., 1940). This measure does not, therefore, indicate long-term overload and cannot be used to identify individuals regularly consuming excessive amounts of vitamin C.
C. NHANES III APPROPRIATENESS

Serum vitamin C was determined in NHANES II for persons 3-74 years of age (Fulwood et al., 1982) and these data can be used in the future as baseline data. Subgroups of the population with inadequate vitamin C intake may exist, as may groups with excessive intake. Additionally, changes in dietary patterns, supplement use, and smoking since the NHANES II survey as well as the availability of the NHANES II data for comparison purposes make the inclusion of plasma vitamin C desirable for NHANES III.

D. RECOMMENDED APPROACHES

1. Indices

Measurement of plasma or serum ascorbate level is recommended for the assessment of vitamin C status in NHANES III.

2. Methods

Several methods are available for the determination of vitamin C (Pelletier, 1985). An adaptation of the method of Roe and Kuether (1943) was used in NHANES II. This original 2,4-dinitrophenylhydrazine (DNPH) method is a classic technique. It has been widely used for over 40 years to determine serum vitamin C; however, measurement by this method is subject to inaccuracies because of interfering compounds. The use of blanks, where total vitamin C is reduced to ascorbic acid to prevent formation of a DNPH osazone can eliminate this problem (Pelletier, 1985). HPLC methods may prove valuable in the future, but as yet, these are not adequately developed or validated for use in NHANES III. The method chosen for NHANES III should be tested in parallel against the method used in NHANES II to assure comparability between surveys.

3. Basis for interpretative criteria

The bases for interpreting plasma vitamin C results have been discussed in section B of this chapter and are further described in Irwin and Hutchins (1976), Sauberlich (1981), and Sauberlich et al. (1974).
The guidelines published by Sauberlich (1981) are widely accepted for interpretation of vitamin C values:

<table>
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<th>Serum Ascorbic Acid (mg/dl)</th>
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<tr>
<td>Acceptable (Low risk)</td>
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<tr>
<td>Low (Medium risk)</td>
</tr>
<tr>
<td>Deficient (High risk)</td>
</tr>
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</table>

4. **Target groups**

The Panel recommends assessment of vitamin C status on the entire NHANES III population.

5. **Other considerations**

Inasmuch as smoking has a substantial effect on serum ascorbate levels, the amount and history of smoking should be assessed in all individuals. Oral contraceptive use and recent infections should also be ascertained in all individuals.

E. **INTENDED USES OF DATA**

Serum or plasma ascorbate can be used to estimate the number of persons at risk for impaired vitamin C status. Serum ascorbate values cannot be used to identify persons who regularly consume very high doses of vitamin C. However, if blood samples are obtained from fasting subjects, serum ascorbate values may be useful in the examination of individuals consuming levels of vitamin C well in excess of the RDA.

Estimates of the vitamin C status of the U.S. population sample selected for NHANES III will be one of several factors (e.g., dietary intake, smoking, oral contraceptive use) that may be useful in follow-up studies that attempt to establish statistical associations between dietary intake and nutritional status with occurrence of cancer. Such follow-up studies, using the National Death Index, have been proposed for evaluating possible relationships between risk factors or protective effects with occurrence of cancer.
F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends the following measure for assessment of vitamin C nutritional status:

- serum or plasma vitamin C levels.
LITERATURE CITED


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VI. VITAMIN D

A. INTRODUCTION

In previous years, concern about vitamin D status has concentrated mainly on the possible occurrence of rickets in children. However, this condition has been largely eliminated in the United States as a consequence of vitamin D supplementation of dairy products. Nevertheless, nutritional, racial, and cultural factors have combined in certain subgroups of the population to produce isolated cases of vitamin D deficiency rickets (Arnaud et al., 1976; Bachrach et al., 1979; Edidin et al., 1980; O'Connor, 1977; Rudolf et al., 1980).

Recently, emphasis has been placed on the relationship of vitamin D to nutritional health in older populations (Parfitt et al., 1982). Elderly populations may be particularly prone to reduced vitamin D stores because of less efficient intestinal absorption, decreased sunlight exposure, and lowered dietary intake (Parfitt et al., 1982). Several reports from European countries have indicated inadequate vitamin D status in the elderly (Hodkinson and Hodkinson, 1980; Lamberg-Allardt, 1984; McKenna et al., 1985; Vir and Love, 1979). The vitamin D stores of one healthy, free-living population of elderly in the United States were found to be low (Omdahl et al., 1982).

A major ramification of inadequate vitamin D status in the elderly is the potential for increased loss of bone mineral. Decreased absorption of calcium caused by inadequate vitamin D is thought to be at least partially responsible for the decrease in bone mineral content during postmenopausal aging (Heaney et al., 1978; Horsman et al., 1980). The clinical manifestations of vitamin D deficiency are not restricted to rickets and osteomalacia. The latter is preceded by a prolonged period of secondary hyperparathyroidism (Rao et al., 1983). This period is usually asymptomatic but involves accelerated loss of cortical bone and, to some extent, trabecular bone. This phenomenon probably increases fracture risk in affected individuals (Kleeckoper et al., 1983). Because vitamin D is the major, if not sole, regulator of intestinal calcium absorption (DeLuca, 1985), lowered vitamin D levels may have a direct effect on calcium balance (Omdahl et al., 1982).

In adults, low dietary vitamin D intake and decreased exposure to solar radiation have been associated with colorectal cancer (Garland and Garland, 1980; Garland et al., 1985), but the basis for this finding is not known. Additionally, Sowers et al. (1985) observed an inverse relationship between estimated dietary intake of vitamin D and systolic blood pressure.

On the other hand, several investigators have questioned the prudence of vitamin D fortification of the food supply and animal feeds because of the potential for vitamin D intoxication.
(Holmes and Kummerow, 1983; Seelig, 1983; Taylor and Peng, 1980). Although acute toxicity of vitamin D is known to result from relatively low doses of vitamin D in individuals consuming additional hypercalcemic agents (Buysschaert et al., 1984; Crowe et al., 1984; Goodwin, 1982), it is frequently the result of indiscriminate self-dosing and is not widely believed to occur from the consumption of usual diets (Omaye, 1984). Excessive intake can result in hypertension (Blum et al., 1977), hypercalcemia, and extrasosseous calcification (Parfitt and Kleerekoper, 1980), but effects of chronic subacute overconsumption are unknown. Hypercalciuria occurs much earlier than hypercalcemia in the development of vitamin D toxicity, and may lead to renal function impairment (Parfitt, 1976). Although exogenous forms of vitamin D are generally in the form of calciferol, calcitriol (1,25-(OH)₂-D-3) is also available by prescription for the treatment of hypocalcemia of various etiologies (Physician's Desk Reference, 1985). Because this compound is thought to be approximately 10 times more potent in biological activity than vitamin D-3 itself in a normal individual (Boris et al., 1977; Tanaka et al., 1973), the risk of toxicity is greater. There is considerable individual variation in response to the degree and duration of vitamin D administration that results in signs of vitamin D toxicity (Parfitt et al., 1982). Thus, the definition of maximum limits on the intake of vitamin D and cutoff criteria for status determination is difficult. Many questions regarding normal and potentially hazardous ranges of vitamin D intake remain unanswered. With currently available techniques, it should be possible to relate supplemental vitamin D intake to blood metabolites and serum calcium to establish more clearly the levels associated with prolonged or excessive supplemental intake of vitamin D.

B. AVAILABLE INDICATORS

1. Nutritional status

25-Hydroxy-Vitamin D (25-OH-D). Plasma 25-OH-D is the most abundant circulating metabolite of vitamin D (Haddad and Hahn, 1973) and is also the most useful indicator of vitamin D status (Haussler and McCain, 1977a; Parfitt et al., 1982). Many assays for 25-OH-D do not discriminate between vitamin D-2 and vitamin D-3, and thus are indicators of the total vitamin supply from endogenous and exogenous sources (Parfitt et al., 1982). However, differentiation between the two forms is possible and would be useful in those states in the United States that fortify dairy products with vitamin D-2 (which can only be of exogenous origin). In states that supplement with vitamin D-3, this distinction cannot be made (Parfitt et al., 1982). The normal range for serum 25-OH-D is approximately 10-53 ng/ml (Rosen and Chesney, 1983). Low and high values for 25-OH-D concentration are referred to as hypo- and hypervitaminosis D, respectively (Parfitt et al., 1982). Vitamin D deficiency, on the other
hand, refers to an anatomical, physiological, or biochemical abnormality that can be corrected by nonpharmacological doses of vitamin D. This condition may or may not coexist with hypovitaminosis D (Parfitt et al., 1982).

**Urinary Calcium and Phosphorus.** These indices vary in response to dramatic changes in vitamin D stores; however, they are considered only gross indicators of vitamin D status (Parfitt et al., 1982; Sauberlich et al., 1974). Additionally, changes in these indices are not specific to vitamin D status (Sauberlich et al., 1974) because calcium and phosphorus intake may influence excretion of these nutrients.

**Serum Alkaline Phosphatase Activity.** Prior to the advent of direct measurements for vitamin D metabolites, the method of Bessey et al. (1946) for determining serum alkaline phosphatase activity was used as an indirect method for evaluating vitamin D nutritional status (Guzmán et al., 1961; Sauberlich et al., 1974). The activity of this enzyme usually increases with the onset of rickets, and generally increases proportionally to the severity of vitamin D depletion. However, alkaline phosphatase activity is not a specific measure of vitamin D status; protein-energy malnutrition and other diseases disrupt the conventional relationship of vitamin D to alkaline phosphatase (Sauberlich et al., 1974).

**1,25-Dihydroxy-Vitamin D.** Measurement of 1,25-(OH)₂-D levels is not recommended as a nutritional assessment of vitamin D status. 1,25-(OH)₂-D levels vary with dietary levels of calcium and phosphorus, parathyroid status, etc., and cannot be interpreted as indicative of vitamin D status (DeLuca and Schnoes, 1983; Haussler and McCain, 1977b; Rasmussen et al., 1980; Russell et al., 1984).

2. **Excessive accumulation**

**25-OH-D.** Normally, approximately 90% of circulating 25-OH-D is in the form of 25-OH-D-3 (Haddad and Hahn, 1973). Plasma 25-OH-D was approximately 15-fold greater in patients with varying degrees of hypervitaminosis D than in normal controls (Hughes et al., 1976). However, virtually all plasma metabolites existed in the D-2 form in the patients. Because hypervitaminosis D with concurrent hypercalcemia has been reported in anephric humans (Counts et al., 1975), and (with the exception of placenta) 1,25-(OH)₂-D is produced in vivo only in renal tissue, many investigators believe that elevated 1,25-(OH)₂-D is not a biological marker for hypervitaminosis D (Hughes et al., 1976; Russell et al., 1984; Shepard and DeLuca, 1980a).
Serum Calcium. Measurement of total serum calcium concentration is not appropriate for assessment of calcium or vitamin D status; but, it is a useful marker for identifying possible vitamin D intoxication. Hypercalcemia can exist without excessive vitamin D levels and hypervitaminosis D can occur without hypercalcemia; however, they frequently coexist. Hypercalcemia is also the most sensitive and rapid indicator of excessive intake of 1,25-(OH)₂-D. 1,25-(OH)₂-D is available only by prescription, and theoretically, overdosing should be uncommon.

C. NHANES III APPROPRIATENESS

Vitamin D status has not been measured in previous NHANES. Questions regarding possible deficiencies and toxic effects make it desirable to include measures of vitamin D status in NHANES III. Methods discussed in section D of this chapter are considered highly sensitive for the determination of vitamin D status (both deficiency and toxicity) and are feasible to perform under field conditions such as NHANES III.

D. RECOMMENDED APPROACHES

1. Indices

Serum 25-OH-D and total serum calcium concentration should be measured in NHANES III. Although other indices may be relatively easy to measure, for the purposes of evaluating vitamin D status, their usefulness is limited.

2. Methods

Two methods are frequently used to measure 25-OH-D: HPLC and competitive protein-binding assays (Edelstein et al., 1974; Haddad and Chyu, 1971; Preece et al., 1974). The latter method will probably be obsolete when NHANES III begins. Serum or plasma 25-OH-D should be measured by HPLC, for which several methods have been validated (Eisman et al., 1977; Shepard and Deluca, 1980b). Solid-phase extraction with disposable cartridges has been found to be rapid and simple in preparative chromatography for vitamin D metabolites (Aw et al., 1983).

Total serum calcium concentration should be measured by currently acceptable techniques (see Chapter XII).
3. Basis for interpretative criteria

Few studies have been performed which correlate clinical signs of vitamin D deficiency or toxicity with 25-OH-D levels. Preece et al. (1975) studied 35 Asian immigrants to the United Kingdom with overt rickets or osteomalacia and found serum 25-OH-D determined by the method of Preece et al. (1974) below 3.0 ng/ml in all cases and undetectable in 57%. Three elderly subjects with clinical osteomalacia were evaluated for vitamin D status and found to have 25-OH-D levels below 3.3 ng/ml. Patients were then given 3,000 IU of vitamin D-3 per day and all showed healing of radiologic lesions. 25-OH-D level increased in one patient from 3.2 ng/ml to 42.0 ng/ml within 244 days of treatment (Preece et al., 1975).

Mason and Posen (1979) studied 21 patients with chronic hypoparathyroidism who were consuming prescribed doses of ergocalciferol. Serum 25-OH-D, determined by the method of Mason and Posen (1977), was shown to correlate well ($r = 0.80$) with dose of ergocalciferol.

Hodkinson and Hodkinson (1980) attempted to identify the minimum level of 25-OH-D at which there was no evidence of osteomalacia in 36 elderly individuals. 25-OH-D values, determined by the method of Belsey et al. (1974), ranged from 1.5 ng/ml or less (the detection limit of the assay) to 16.8 ng/ml, showing a substantial overlap with findings in previously reported cases of osteomalacia. However, results of this study have been questioned as analyses were done without extraction of confounding metabolites (Haussler and McCain, 1977a). Other factors such as calcium and/or phosphate intake and age may mitigate the expression of pathology even when vitamin D deficiency is present. Additionally, as bone remodeling occurs in only approximately 5% of the mature skeleton each year, vitamin D deficiency may exist for several years in the adult before clinical signs are present (Russell et al., 1984). Haddad and Stamp (1974) found only a few subjects with vitamin D deficiency rickets, but all had serum 25-OH-D levels less than 3 ng/ml.

Even fewer data are available correlating clinical signs of vitamin D intoxication with biochemical indicators of vitamin D status. Haddad and Stamp (1974) reported three patients with clinical courses consistent with vitamin D intoxication whose 25-OH-D values, measured by the method of Haddad and Chyu (1971), were 206, 490, and 690 ng/ml. Hughes et al. (1976) reported that patients diagnosed as having hypervitaminosis D had 25-OH-D levels approximately 15 times higher than normal. Davies and Adams (1978) reported two patients with signs of vitamin D intoxication, one who had consumed 150,000 IU of vitamin D daily for 7 years and another who had consumed 100,000 IU daily for 10 years. Both patients were hypercalcemic and serum 25-OH-D levels were 450 and 400 ng/ml, respectively. All other patients with vitamin D intoxication described in this study presented with hypercalcemia. Belchetz et al. (1976)
reported the case of a 34-year-old woman with Munchausen syndrome who had surreptitiously consumed large doses of calciferol. Plasma calcium level exceeded 14 mg/100 ml, requiring urgent medical intervention. Serum 25-OH-D level was 520 ng/ml.

4. **Target groups**

Signs of deficiency or toxicity in children may not be found in a national population sample. However, it would be worthwhile to examine the entire NHANES III sample, including children, to evaluate 25-OH-D levels and determine the continuum of values in the general population. Additionally, because there is evidence of potentially low vitamin D status in the elderly, the Panel recommends individuals 65 years and older be oversampled in NHANES III for this assessment.

5. **Other considerations**

Multivariate analyses should be performed to determine the relationship of 25-OH-D levels to race, season, latitude, and other factors which may predispose individuals to lowered vitamin D status. Accurate assessment of use of vitamin D supplements should be obtained and efforts should be made to determine dietary intake of vitamin D.

E. **INTENDED USES OF DATA**

Collection of 25-OH-D values in NHANES III can be used to establish baseline data for the U.S. population. Deficiency is believed to be rare and, with the possible exception of the elderly, will probably not be detected in NHANES III. 25-OH-D values below 3 ng/ml will provide reasonably clear evidence of vitamin D deficiency; levels in the marginal range (3-10 ng/ml) must be more cautiously interpreted.

Individuals with extremely high 25-OH-D levels and concomitant hypercalcemia can be presumed to have excessive accumulation of vitamin D. A finding of hypercalcemia will be used primarily to confirm suspected vitamin D intoxication in persons with elevated 25-OH-D levels. Serum calcium levels in individuals with normal 25-OH-D levels will serve as control values. Additionally, the relationship between hypercalcemia and elevated levels of 25-OH-D can be compared in individuals who are users of vitamin D supplements. Intoxication from self-dosing is a potentially serious medical problem, but the statistical power of NHANES III may not be sufficient to determine reliable prevalence estimates for the U.S. population.
In epidemiological studies, associations have been reported between medical problems such as hypertension (see chapter XXII), osteoporosis (see chapter XXIII), and colorectal neoplasms (Garland and Garland, 1980; Garland et al., 1985) and vitamin D status. Information collected on vitamin D status in NHANES III may make it possible to examine potential relationships of vitamin D status with these conditions and subsequent mortality as recorded in the National Death Index.

F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that the following assessments of vitamin D nutritional status be made in NHANES III:

- serum or plasma 25-OH-D levels
- serum calcium levels.
LITERATURE CITED


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VII. VITAMIN E

A. INTRODUCTION

Vitamin E functions as an antioxidant, preventing cellular damage and enzyme inactivation caused by the action of oxidative processes on unsaturated lipids. In animals, vitamin E deficiency produces a variety of adverse effects on muscle, reproduction, neurological function, blood cell proliferation, and immune function, which differ from species to species. In humans, a well-defined syndrome resulting from lack of dietary vitamin E has not been recognized, and signs of vitamin E deficiency have been reported only in newborns, especially premature infants (Hassan et al., 1966; Johnson et al., 1974; Oski and Barness, 1967), and in persons with various disorders in which the absorption of dietary fat is greatly diminished (Bieri, 1976; Binder et al., 1965; Sokol et al., 1985).

Despite the lack of scientific evidence for demonstrable beneficial effects, self-supplementation with vitamin E has become popular because of anecdotal data and widely held beliefs concerning its beneficial effects on aging, sexuality, and prevention of cancer. Unlike the fat-soluble vitamins A and D, vitamin E consumed in excess seems to exhibit little toxicity. In a study of 28 adults who had voluntarily ingested 100 to 800 IU/day of tocopherol for an average of 3 years, Farrell and Bieri (1975) found no evidence of disturbance in liver, kidney, muscle, thyroid gland, erythrocytes, leukocytes, coagulation measures, or blood glucose.

B. AVAILABLE INDICATORS

1. Nutritional status

Circulating Vitamin E Levels. The most common assay for vitamin E nutritional status has been the measurement of alpha-tocopherol or total vitamin E activity in the serum or plasma. With currently available HPLC techniques, such measurements can be done routinely. Serum alpha-tocopherol was assayed successfully in HHANES by a reversed-phase HPLC method which simultaneously determined serum retinol (Bieri et al., 1979). Serum alpha-tocopherol levels less than 0.5 mg/dl have been considered to be indicative of deficiency (Sauberlich et al., 1974).

Although the measurement of serum or plasma tocopherol is feasible, its usefulness as an assessment technique to reflect either level of intake or tissue stores has been questioned (Bieri, 1976). Serum tocopherol is highly correlated with serum cholesterol and total lipids. Serum alpha-tocopherol may be a poor indicator of body vitamin E stores when the level of lipids in the serum is abnormal; falsely low or elevated tocopherol
levels may be present in the case of disorders characterized by alterations in serum lipids (Bieri, 1976; Russell et al., 1984). To overcome this problem, some investigators recommend reporting serum tocopherol values per unit of total serum lipid (Horwitt et al., 1972). Standards for adequacy of vitamin E status based on serum measurements expressed in this fashion have been suggested -- a ratio of 0.8 mg total tocopherols per gram of total serum lipids has been considered indicative of adequate nutritional status (Horwitt et al., 1972). Another approach to evaluating circulating vitamin E is to determine the ratio of alpha-gamma-tocopherol (Handelman et al., 1985). This ratio responds significantly more slowly to dietary variations in tocopherol than the alpha-tocopherol level alone, but requires an HPLC technique, such as the method of Hatam and Kayden (1979), capable of separating the tocopherols of interest. Standards of adequacy for this ratio have not been reported.

Theoretically, the assessment of tocopherol content of the red blood cells may provide a measure more closely associated with the recognized function of vitamin E in biomembranes. However, because serum and erythrocyte tocopherol are closely correlated, and there are greater technical difficulties in determining tocopherol in the erythrocytes, the usefulness of this assessment in a large survey is considered limited (Sauberlich et al., 1974).

**Tissue Vitamin E Levels.** Tocopherol content of a liver biopsy or adipose tissue sample has been suggested as a useful measure of body stores of the nutrient and long-term vitamin E status. Evaluation of adipose tissue measurements, including exploration of various denominators for normalizing adipose tocopherol for different biopsy sample sizes and different methods for obtaining subcutaneous tissue samples, is currently underway (Dratz, 1985). Such sampling appears too invasive for inclusion in NHANES III.

**Functional Tests.** One functional test of vitamin E status is the determination of the rate of hemolysis of red blood cells by hydrogen peroxide or dialuric acid (Horwitt et al., 1963; Russell et al., 1984; Sauberlich et al., 1974). The rate of hemolysis has been found by some investigators to correlate well with serum tocopherol levels and to be greatly increased in vitamin E deficiency. However, alterations in other nutrients (such as selenium) which influence the function of the cellular antioxidant system can also influence the rate of RBC hemolysis. Technical difficulties also limit the feasibility of applying this technique in the field because freshly prepared samples of erythrocytes must be used for this assay.

Another functional test of vitamin E status which has been suggested is measurement of the exhalation of ethane and pentane, peroxidation products of linoleic and linolenic acids (Russell et al., 1984). This technique has been used for several years to evaluate oxidative damage in animals, but has not yet
been studied extensively in humans. Techniques for collecting the expired gases have not yet been standardized, and the exhalation of these compounds is also affected by other anti-oxidants and pro-oxidant toxins such as carbon tetrachloride.

2. **Excessive accumulation**

   Plasma levels of vitamin E do not correlate well with intake. However, detection of high levels of tocopherol in tissues would provide an indication of excessive dietary intake of vitamin E.

C. **NHANES III APPROPRIATENESS**

   Assessment techniques for vitamin E status which adequately reflect body stores and which are feasible for application in a large survey are not available at this time. In addition, the scarcity of evidence of vitamin E deficiency in the United States (or any disease-free human population) and the relative lack of toxic consequences of excessive intake limit interest in the assessment of vitamin E nutrition status. Thus, assessment of vitamin E nutritional status is not recommended for NHANES III.

D. **CONCLUDING STATEMENT**

   Based on the foregoing discussion, the Panel recommends that assessment of vitamin E nutritional status not be included in NHANES III.
LITERATURE CITED


VIII. FOLACIN

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VIII. FOLACIN

A. INTRODUCTION

In recent years, surveys of selected groups and reviews of available dietary intake data have suggested that folacin nutritional status may be suboptimal in a substantial number of women in their reproductive years, especially pregnant and lactating women; elderly persons; and, adolescents of low socioeconomic status in the United States (Bailey et al., 1979, 1980, 1982; Clark and Gates, 1983; Daniel et al., 1975; Dawson and Disney, 1981; Herbert et al., 1975; Rosenberg et al., 1982; Tsui et al., 1983; Wagner et al., 1981). Serum and RBC folate levels were assessed and dietary intake was determined in many of these studies; these measurements served as the criteria for evaluating suboptimal status. The evidence of low circulating folate levels and/or low folate intake in several studies, as noted above, appears to merit assessment of folacin nutritional status in a national probability sample of the U.S. population.

B. AVAILABLE INDICATORS

1. Nutritional status

   Serum and Red Blood Cell (RBC) Folate. A fall in serum folate is the earliest biochemical indicator of developing folacin deficiency. However, serum folate level reflects recent folacin balance. Within 3 weeks after cessation of folacin consumption, serum folate levels fall to values associated with deficiency (frequently cited as <3 ng/ml), but major hematological manifestations may not appear until 3 to 4 months of folacin deprivation (Herbert, 1962, 1967).

   The RBC folate level is less sensitive than serum folate level to recent variations in intake and low values tend to reflect depletion of folate stores (Chanarin and Perry, 1977; Wu et al., 1975). Microbiological assays using Lactobacillus casei, competitive protein-binding radioassays, and HPLC techniques have been used for serum and RBC folate determinations.

   Polymorphonuclear (PMN) Leukocyte Lobe Counts. Evaluation of neutrophil hypersegmentation (5+ lobes) in smears of peripheral blood, or of the layer of white blood cells (buffy coat) obtained at the interface of serum or plasma and the sedimented red cells, can give an indication of early morphological changes in folacin deficiency (Herbert, 1962, 1967, 1970). This evaluation has been performed by calculating the neutrophil lobe average or the percentage of cells with five or more lobes, both labor-intensive techniques which may be difficult to apply to a large survey. However, a more practical evaluation may be a simple examination for any six-lobed cells, a feature of hypersegmentation reported in virtually all megaloblastic subjects.
(Lindenbaum and Nath, 1980). Hypersegmentation is considered an unreliable indicator of folacin deficiency during B-12 deficiency and during pregnancy, but is not affected in iron deficiency (Sauberlich, 1984).

**Formiminoglutamate (FIGLU) Excretion.** Measuring urinary FIGLU excretion after an oral histidine load (Luhby et al., 1959; Tabor et al., 1953) is another useful assessment of folacin deficiency. However, the use of a load test is unrealistic under survey conditions such as those contemplated for NHANES III.

2. **Excessive accumulation**

Folacin is generally considered a nontoxic vitamin (Preuss, 1978). However, one study has reported adverse reactions (gastrointestinal symptoms, sleep disorders, malaise, and irritability), accompanied by elevated serum folate levels, after consumption of excess folate (15 mg/day) (Hunter et al., 1970). However, attempts to replicate these findings failed (Hellström, 1971; Ralston et al., 1970). A recent report suggests that oral folate supplements may have a negative effect on zinc balance in humans by interfering with zinc absorption (Milne et al., 1984). Assessments of excess accumulation seem to be unnecessary from a public health point of view, and difficult to establish from measurements of tissue levels of folate.

C. **NHANES III APPROPRIATENESS**

Serum and RBC folate levels were measured in a subsample of subjects in NHANES II. Because of quality control problems with the assays used, the relatively small sample size, and the lack of clinical correlates for the biochemical measurements, a satisfactory assessment of the folacin nutritional status of the population was not possible (Sentí and Pilch, 1984). But the observation that approximately 10% of the subjects had low RBC folate values suggests that folacin status in the United States deserves further study.

With adequate sample size, improved assays, and clinical correlates, the measurement of serum and RBC folate levels would provide useful indices of folacin status. These measures of folacin status, and others, are amenable to a large survey. Therefore, assessment of folacin status is recommended for inclusion in NHANES III.
D. RECOMMENDED APPROACHES

1. Indices

Both serum and RBC folate levels should be assessed in NHANES III. Although RBC folate values are more indicative of folate stores than are serum folate values, assessment of both would be of interest in light of a recent report on differential uptake of supplemental folate by the serum and RBC in the elderly (Ettinger and Colman, 1985). The Panel recommends that the analyses be performed by a laboratory that has continuing access to a patient population with clinical folacin deficiency. In this manner, low RBC folate values may be correlated continually with marrow abnormalities being corrected by administration of folacin. The Panel recognizes that having access to such folate-deficient patients is not a simple matter. However, consideration should be given to possible establishment of a collaborative agreement among several clinical facilities to provide the contract laboratory with specific folate-deficient and control samples, and clinical information to be used for assay verification.

Evaluation of neutrophil hypersegmentation should be attempted in at least a subsample of the population and can be used to support the RBC folate data. Smears for the PMN lobe count could be made for all subjects, but counts should be made only for those with low RBC folate levels and selected controls with normal RBC folate values.

2. Methods

Either the microbiological or radiodilution assay for serum and RBC folate can be used [HPLC techniques are not presently developed for routine use (Sauberlich, 1984)], provided the technique is repeatedly verified against samples from persons known to be deficient or replete in folacin. In this manner, the goal of detecting folacin deficiency by relating RBC folate levels to clinical manifestations can be accomplished.

The microbiological assay is still regarded as the standard for assessing total folate in biological samples (Sauberlich, 1984) but has proved difficult to maintain with satisfactory quality control over the several years of NHANES II (Senti and Pilch, 1984). Quality control was also problematic with the commercial radioassay used in NHANES II, but the kit from the same manufacturer used in Hispanic HANES has proven more reliable (Gunter, 1985). If the radioassay is chosen for the measurement of folate, it should be recognized that the manufacturers of commercial folate assay kits have in the past, changed the technologies of the assays without notifying customers. For this reason, any contract should require the vendor to supply kits prepared in a uniform and identical manner for the duration of the survey.
Regardless of the method chosen, careful attention must be given to maintaining quality control and standardizing conditions of the assay. The use of lyophilized serum controls is recommended to monitor changes in response of the chosen assay with time. If whole blood control pools are used for RBC folate assays, they should be prepared from samples with a range of folate concentrations, rather than by diluting whole blood. Other standard factors such as ascorbate preservation, sample dilution, time and temperature of sample storage, etc., should be controlled. Any modification or variation in a previously validated method should be independently validated by the laboratory performing the assay.

Preparation of buffy coat smears is labor-intensive; therefore, peripheral blood smears are recommended for the evaluation of neutrophil hypersegmentation. The blood smears can be prepared and the PMN counts performed as described by Lindenbaum and Nath (1980). Because PMN lobe counts are subject to wide observer differences, the counts should be performed by a single individual. The slides could be evaluated in a single batch at the end of the survey.

3. Basis for interpretative criteria

The cutoff for RBC folate level indicative of folacin deficiency should be selected on the basis of the clinical experience of the laboratory performing the assays. With the data collected on samples from persons known to be deficient or replete in folacin, the sensitivity and specificity of a chosen cutoff value for RBC folate can be determined. Because the standard for evaluating folacin deficiency would be laboratory-specific, the results should probably be presented as the percentage below the designated cutoff.

One six-lobed PMN/100 leukocytes is suggested as the single criterion for labeling a smear positive. Alternatives are the presence of 5% or more cells with five or more lobes, or a lobe average greater than 3.5.

4. Target groups

The entire population should be evaluated with respect to folacin status. Sufficient representation of various geographic regions and ethnic groups will permit evaluation of subgroups considered to be at particular risk for folacin deficiency: pregnant and lactating women, adolescents (12-18 years), and persons over 65 years, especially those of low socioeconomic status.
Assessment of the folacin status of elderly persons (including those over 74 years of age) should be emphasized because of their increased exposure to diseases and drugs which affect folate absorption and/or metabolism (Rosenberg et al., 1982).

5. Other considerations

Serum ferritin level, hemoglobin concentration, mean corpuscular volume, transferrin saturation, and erythrocyte protoporphyrin level should also be measured in subjects whose folacin status is to be assessed. These measurements will aid in interpretation of the folate data by eliminating iron deficiency as a potential cause of anemia, and revealing relationships of folacin status and hematological variables. Ideally, serum vitamin B-12 measurements would be desirable on subjects with low folate levels and appropriate controls.

Accurate description of drug (both prescription and over-the-counter) intake should be obtained so that effects of specific drugs on folate blood levels can be controlled in multivariate analyses. Alcohol consumption should also be determined with respect to folacin status.

E. INTENDED USES OF DATA

Data collected as described should permit the determination of national probability estimates of the prevalence of unsatisfactory folacin status in the United States and the identification of subgroups at particular risk of deficiency. If clinical correlates of given laboratory-specific RBC folate levels are obtained as suggested, prevalence estimates can be made with considerable confidence. Serum folate values will be useful for screening the population. Evaluation of neutrophil hypersegmentation will permit assessment of hematological changes associated with low RBC folate values.
F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that folacin status of the population be assessed in NHANES III by determining:

- RBC folate levels for the total population, and for patients with clinical folacin deficiency
- Serum folate levels for the total population
- Neutrophil hypersegmentation for persons with low RBC folate levels and a random sample of individuals to serve as a control group.
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IX. NIACIN

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IX. NIACIN

A. INTRODUCTION

Nicotinamide and nicotinic acid (referred to collectively as niacin) have similar but not identical properties. Both are products of tryptophan metabolism. Niacin is present in foods of both plant and animal origin, but can also be obtained by conversion from tryptophan after ingestion and absorption. Approximately 60 mg of dietary tryptophan yields 1 mg of niacin. Flour for bread and bakery products in the United States is enriched with 24 mg niacin per pound [21 CFR 137.165] (Office of the Federal Register, 1984).

The niacin deficiency state, pellagra, is rare in the United States, generally occurring only in persons, such as alcoholics, whose diets are grossly inadequate (Sauberlich, 1984). On the other hand, large doses of niacin can have pharmacological effects on the central nervous system, cardiovascular system, blood lipids, and blood sugar. High levels of nicotinic acid, but not nicotinamide, (e.g., 3 g/day) have been used to lower blood cholesterol, but such treatment is accompanied by several vascular, gastrointestinal, and biochemical side effects (Margolis, 1978).

B. AVAILABLE INDICATORS

1. Nutritional status

Methods for assessment of niacin nutriture have been reviewed recently by Sauberlich (1984). The most practical assessment of niacin nutriture is measurement of urinary levels of N¹-methylnicotinamide and its pyridone, N¹-methyl-2-pyridone-5-carboxyamine (2-pyridone) (Sauberlich et al., 1974). N¹-methylnicotinamide excretion falls to a minimum level as clinical manifestations of niacin deficiency begin to appear; however, 2-pyridone excretion is absent weeks before clinical indications of impaired niacin status are evident (Goldsmith et al., 1952; Vivian et al., 1958; Walters et al., 1955). Thus, the ratio of these two metabolites is commonly used in assessment. In normal adults, a ratio of 1.3 to 4.0 exists between 2-pyridone:N¹-methylnicotinamide excretion (de Lange and Joubert, 1964; Holman and de Lange, 1950). A value below 1.0 is considered indicative of niacin deficiency (de Lange and Joubert, 1964; Sauberlich et al., 1974; Viteri, 1983).

Unlike many urinary measurements which require 24-hour collections, the ratio of 2-pyridone to N¹-methylnicotinamide can be determined in random fasting urine samples because it is not affected by duration of the collection period (Sauberlich et al., 1974). Age does not affect the ratio of these metabolites; thus, a single cutoff value of the ratio (<1.0) has been
used to evaluate nutritional status in all groups (Sauberlich et al., 1974). Several methods of measuring plasma and urinary concentrations of niacin and niacin derivatives using high pressure liquid chromatography (HPLC) have been developed recently (Sauberlich, 1984). The HPLC methods simplify the analysis and enhance the speed, accuracy, and sensitivity of determining the ratio of urinary N¹-methylnicotinamide and its pyridone.

Other biochemical assessments available for determination of niacin status including load tests, levels of niacin compounds or derivatives in plasma, or urinary nicotinic acid would be of limited value in large scale surveys such as NHANES III. These assessments, accomplished by HPLC or by the previously developed fluorimetric, radiometric, or microbiologic methods, do not provide a useful measure of niacin nutritional status because they reflect recent intake rather than body pool.

2. Excessive accumulation

Niacin toxicity usually only results from pharmacological uses of the vitamin, especially the nicotinic acid form. Biochemical tests to detect excessive accumulation are not available.

C. NHANES III APPROPRIATENESS

Although the N¹-methylnicotinamide:2-pyridone ratio can be assessed conveniently by HPLC in random, fasting urine samples and low values are considered indicative of niacin deficiency, this index does not measure the body pool of niacin and, therefore, will not be likely to identify subgroups of the population at risk of deficiency. For these reasons the Panel concludes that there is little basis for including niacin assessment in NHANES III.

D. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that assessment of niacin status not be included in NHANES III.


X. RIBOFLAVIN

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Advantages of the EGRAC test are that it requires only a small amount of blood, does not require a fasting sample, and is independent of age and sex (Komindr and Nichoalds, 1980). Although the EGRAC value is influenced by recent intake, the effect is less pronounced than the change in urinary riboflavin value observed after a change in dietary intake and the ratio is considered a useful measure of impaired riboflavin nutritional status (McCormick, 1985; Sauberlich, 1984). The degree of stimulation of EGR activity is dependent on the FAD saturation of the apoenzyme, which in turn is dependent on the availability of riboflavin; however, an elevated EGRAC cannot indicate the degree of riboflavin deficiency. For example, the EGRAC did not continue to increase in response to continuing depletion of tissue riboflavin in a controlled study (Sterner and Price, 1973).

Certain disease states, drugs, and endocrine disorders may interfere with riboflavin status. For example, EGRAC cannot be used as an indicator of riboflavin status in persons with glucose-6-phosphate dehydrogenase deficiency, a condition affecting approximately 10% of American blacks (Frischer et al., 1973; Schrier et al., 1958), because of increased avidity of the reductase for FAD in persons with this metabolic condition (Flatz, 1970; Thurnham, 1972).

**Urinary Analysis.** Urinary riboflavin values tend to reflect recent dietary intake rather than status of the vitamin. Problems associated with urinary riboflavin determinations are similar to those discussed for thiamin. Collection of 24-hour urine samples is most desirable, but not feasible. Expressing riboflavin values obtained for a casual urine sample per gram of creatinine may not be satisfactory because of the variation in creatinine excretion which occurs in a single day.

**Blood Riboflavin.** Various microbiological assays have been used to determine riboflavin levels in blood (as well as urine), including assays with Lactobacillus arabinosus and Tetrahymena pyriformis. However, microbiologic assays lack specificity as to which flavin is measured, and antibiotic therapy during the weeks before the blood sample is obtained may affect the accuracy of the analyses. In addition, the level of riboflavin in blood components is relatively insensitive to changes in riboflavin status.

2. **Excessive accumulation**

There is little evidence of human riboflavin toxicity. As a water-soluble substance, it is eliminated rapidly. There appears to be little reason to be concerned about possible excessive intake or accumulation.
C. NHANES III APPROPRIATENESS

Riboflavin nutritional status of the U.S. population has never been systematically evaluated. Because suitable methods now exist for such assessment, it seems appropriate for inclusion in NHANES III even though deficiency is not thought to be prevalent in the U.S. population. For this reason, it is recommended that riboflavin status of the total sample population be assessed in NHANES III. There is no evidence that excessive accumulation of riboflavin should be of concern.

D. RECOMMENDED APPROACHES

1. Indices

Riboflavin status can be assessed conveniently in NHANES III by determining erythrocyte glutathione reductase activity coefficient (EGRAC). While this biochemical indicator tends to reflect recent intake, in combination with food intake information and the medical examination, an indication of inadequate riboflavin nutruture is possible (Komindr and Nichoalds, 1980).

2. Methods

EGR activity is measured spectrophotometrically using samples prepared from venous blood usually, but not necessarily, obtained from fasting subjects (McCormick, 1985; Sauberlich et al., 1972). Blood samples are treated with ethylenediaminetetraacetic acid or heparin as an anticoagulant and kept in ice until erythrocytes are removed after washing by centrifugation. Cells are kept frozen until analysis. Duplicate samples are prepared for spectrophotometric determinations at 340 nm. After equilibration, the activity ratio is determined by relating the change in absorbance with added FAD to the change in absorbance without added FAD.

3. Basis for interpretative criteria

McCormick (1985) has established guidelines for interpretation of EGRAC as follows:

- <1.2 — acceptable
- 1.2-1.4 — low
- >1.4 — deficient

In addition, information on riboflavin intake should be obtained, specifically, intake of dairy food (food frequency) and use of dietary supplements containing riboflavin. Interpretation of EGRAC should also take into account the use of drugs, level of physical activity, occurrence of endocrine disorders, and glucose-6-phosphate dehydrogenase deficiency (see section B-1).
4. **Target groups**

The Panel suggests that riboflavin status be determined on a representative sample of the U.S. population. Inasmuch as the protocol for NHANES III may call for three 2-year cycles of national probability samples, the Panel suggests that riboflavin, along with vitamin B-6 and thiamin, be determined together on persons included in either the first or second cycle of NHANES III.

5. **Other considerations**

Washed erythrocytes for the assay of EGR activity must be prepared shortly after the blood samples are drawn.

E. **INTENDED USES OF DATA**

The EGRAC data would provide an evaluation of impaired riboflavin status in a representative sample of the U.S. population. The EGRAC results should be related to dietary intake, use of drugs such as tranquilizers and antidepressants, level of physical activity, and occurrence of specific diseases and disorders. Such data would provide an overview of the range of status of the U.S. population and could identify population subgroups which are possibly at risk. There is neither a method nor a need to evaluate excess accumulation.

F. **CONCLUDING STATEMENT**

The Panel recommends that the riboflavin status of the U.S. population be assessed in the NHANES III by determining:

- erythrocyte glutathione reductase activity coefficient.
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XI. THIAMIN

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XI. THIAMIN

A. INTRODUCTION

Thiamin (vitamin B-1) has an essential role as a coenzyme in carbohydrate metabolism and energy production. Dietary sources of the vitamin include whole grain cereals, legumes, and foods of animal origin, as well as certain fresh fruits and vegetables. In the United States, thiamin enrichment of flours for use in bakery products, buns, rolls, and breads has been practiced for several decades. For example, government regulations stipulate that "enriched flour" should contain 1.8 mg thiamin per pound (Office of the Federal Register, 1984).

While more common in parts of the world where rice is the staple food, thiamin deficiency is rare in the United States. It appears confined to selected subgroups of the population such as chronic alcoholics, persons with disorders characterized by chronic emesis and diarrhea, and socially isolated persons such as the elderly (Viteri, 1983). Recent studies in Japan have noted that adolescents and college students whose diets may be nutritionally inadequate can exhibit clinical manifestations of thiamin deficiency (Hatanaka and Ueda, 1981; Kawai et al., 1980). The apparent hypovitaminosis was reversed by modification of lifestyle and dietary practices.

Thiamin status was assessed in the Ten-State Nutrition Survey (U.S. Department of Health, Education and Welfare, 1972a,b) and in NHANES I (National Center for Health Statistics, 1979) using analysis of thiamin excreted in the urine (μg/g creatinine).

B. AVAILABLE INDICATORS

A number of techniques for analyzing thiamin and its metabolites in body tissues have been developed for use in assessing nutritional status and identifying deficiencies. The review by Sauberlich (1984) of current laboratory methods for assessing the B-complex vitamin status of individuals and populations should be consulted for detailed information.
1. **Nutritional status**

**Transketolase Thiamin Pyrophosphate (TPP) Effect.** Transketolase is a TPP-requiring enzyme which catalyzes the reactions:

a. xylulose-5-phosphate + ribose-5-phosphate → sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate

b. xylulose-5-phosphate + erythrose-4-phosphate → fructose-6-phosphate + glyceraldehyde-3-phosphate

Measurement of erythrocyte transketolase activity, with and without added TPP, has been shown to be a useful indicator of early insufficiency of thiamin (Sauberlich et al., 1974). Values obtained without the addition of TPP represent the endogenous enzyme activity and are dependent on the amount of coenzyme present in the red blood cells. The percent enhancement of enzyme activity caused by the added TPP is called the "TPP effect." The TPP effect is small when thiamin status is adequate, but is elevated in thiamin deficiency. This measurement has been widely used in surveys to assess thiamin status, including field studies in developing countries (Bamji, 1970; Vir and Love, 1977). Methods for determining transketolase activity have been improved and automated with considerable success (Sauberlich, 1984).

**Urinary Thiamin.** Experimental studies have shown that determinations of thiamin in 24-hour urine collections are useful in confirming thiamin status of patients suspected of deficiency on the basis of clinical signs and symptoms (Viteri, 1983). Under survey conditions, 24-hour urine collections are not feasible. Random and/or fasting urine collection and analysis has been utilized with thiamin content related to creatinine content as in NHANES I. Although a correlation between urinary excretion of thiamin per gram of creatinine and thiamin intake has been observed, interpretation of such excretion data is subject to errors, particularly when used as normative data in comparison with values of individual subjects (Sauberlich et al., 1974). In addition, children have a markedly higher level of thiamin excretion when expressed on a creatinine basis than adults (Sauberlich et al., 1974). Because of difficulties in interpretation and problems associated with expressing values on a creatinine basis, urinary thiamin was not measured in NHANES II or HHANES. Regardless of the type of sample collected, measurement of urinary thiamin does not adequately assess the status of body reserves.

**Blood, Serum, and Erythrocyte Thiamin.** Levels of thiamin in serum, red blood cells, and whole blood have been used as indicators of thiamin status (Tanphaichitr and Wood, 1984). Most detection methods are based on the fluorometric thiochrome procedure and require careful sample preparation. According to Sauberlich (1984), methodological problems are compounded by the


observation that decreases in blood thiamin levels are modest, even with frank beriberi. Thus, such determinations are imprecise measures of nutritional status.

2. Excessive accumulation

As with most water-soluble vitamins, excessive intake merely results in increased urinary excretion. According to Viteri (1983) massive doses of thiamin, even intravenously, are essentially devoid of adverse effects, except for occasional anaphylactoid responses. Because excess intake does not result in excessive body stores, but rather, elevated levels of excretion, urinary excretion is the only method to assess excess intake.

C. NHANES III APPROPRIATENESS

NHANES III would be an appropriate vehicle to determine the thiamin status of the U.S. population. The measurement of erythrocyte transketolase activity and its stimulation by thiamin pyrophosphate is simple, convenient, and can provide a clear indication of the prevalence of inadequate thiamin nutrition in this country. Although inadequate thiamin intake is not a serious problem in the United States, impaired thiamin status is known to occur in alcoholics and the elderly. Assessment of excessive intakes of thiamin by measurement of urinary excretion does not appear to be justified.

D. RECOMMENDED APPROACHES

1. Indices

The measurement of erythrocyte transketolase activity and its in vitro stimulation by addition of thiamin pyrophosphate (TPP effect) is recommended as the method for thiamin status evaluation in NHANES III.

2. Methods

Waring et al. (1982) have developed a semi-automated continuous-flow procedure using the Technicon AutoAnalyzer II® (Technicon Instruments Corp., Tarrytown, NY) which provides data on transketolase activity before and after stimulation with TPP. This system incorporates procedures to eliminate hemoglobin interference and to allow use of glyceraldehyde-3-phosphate as an internal standard. These aspects of the assay procedure provide for increased sensitivity, reliability, and precision as well as improved quality control among laboratories (Sauberlich, 1984).
Several other techniques have been developed to measure transketolase activity and the TPP effect. For example, Bayoumi and Rosalki (1976) have described an ultraviolet spectrophotometric procedure for measuring transketolase activity and Basu et al. (1974) published a micromethod for measuring transketolase activity in erythrocytes from 50 μl of whole blood. Possible problems with these and other techniques have been reviewed by Batory et al. (1982).

If assessment of thiamin nutritional status is to be included in NHANES III, the Panel recommends that additional methodological studies be undertaken. In addition to determining the prevalence of elevated values for TPP effect, the Panel recommends that statistical evaluation of the shape of the TPP effect distribution curve be conducted.

3. Basis of interpretative criteria

Criteria for determining thiamin status from transketolase-TPP effect assessments have been published (Brin, 1967; Sauberlich, 1967; Sauberlich et al., 1974). These are as follows:

<table>
<thead>
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<th>TPP Effect</th>
<th>Interdepartmental Committee on Nutrition for National Defense*</th>
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<tr>
<td>Normal (adequate)</td>
<td>0-14%</td>
<td>Acceptable (low risk) 0-15%</td>
</tr>
<tr>
<td>Marginally deficient (marginal)</td>
<td>15-24%</td>
<td>Low (medium risk) 16-20%</td>
</tr>
<tr>
<td>Severely deficient</td>
<td>≥25%</td>
<td>Deficient (high risk) &gt;20%</td>
</tr>
</tbody>
</table>

* See Brin, 1967; Sauberlich, 1967; Sauberlich et al., 1974.

4. Target groups

The Panel suggests that thiamin status be determined on a representative sample of the U.S. population. Inasmuch as the protocol for NHANES III may call for three 2-year cycles of national probability samples, the Panel suggests that thiamin, along with vitamin B-6 and riboflavin, be determined together on persons included in either of the first or second cycle of NHANES III.
5. **Other considerations**

Kjøsen and Seim (1977) have noted that patients with diabetes mellitus or polyneuritis had decreased transketolase activity levels while those with pernicious anemia have elevated enzyme activity. The medical history and clinical examination to be employed in NHANES III may assist in identifying the possible occurrence of these diseases in the population to be surveyed.

E. **INTENDED USES OF DATA**

Data on TPP effect would provide an evaluation of the extent of inadequate thiamin status of a representative sample of the U.S. population. There does not appear to be a need to evaluate excess accumulation.

F. **CONCLUDING STATEMENT**

The Panel recommends that thiamin status be assessed in NHANES III by measuring:

- erythrocyte transketolase TPP effect.
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XII. CALCIUM

A. SPECIAL CONSIDERATIONS

Greater than 99% of body calcium is stored in bone (Schuette and Linkswiler, 1984) and evaluations of calcium status are generally concerned with the metabolic endpoints of negative calcium balance and osteopenia. Although these conditions are usually considered a problem of the elderly, evidence indicates that the peak adult bone mass attained may have the greatest influence on subsequent fracture susceptibility, a characteristic of osteoporosis (Heaney et al., 1982; Johnston et al., 1981). Early nutrition, particularly calcium intake, is thought to be important in determining peak adult bone mass (Heaney et al., 1982). Calcium intake has been determined from dietary data obtained in previous HANES (Abraham et al., 1977, 1979; Carroll et al., 1983) as well as the U.S. Department of Agriculture Nationwide Food Consumption Survey (NFCS) (U.S. Department of Agriculture, 1980). Recommendations regarding assessment of osteoporosis and evaluation of potential relationships to calcium intake (past and current) are discussed in detail in Chapter XXIII of this document.

Serum calcium was determined in NHANES I (National Center for Health Statistics, 1979). Data are unpublished by NCHS but are available on tape. Serum calcium remains remarkably stable under most conditions (Schuette and Linkswiler, 1984) and is not thought to be an indicator of calcium nutritional status. Recently, the hypothesis has been advanced that calcium may be involved in the development of hypertension (Gruchow et al., 1985; Kesteloot and Geboers, 1982; McCarron, 1982). This theory has been discussed in Chapter XXII of this document and recommendations for its evaluation have been included therein.

Vitamin D is associated with calcium status as it is the primary regulator of intestinal calcium absorption (DeLuca, 1985). Impairment in vitamin D status may result in a concomitant negative calcium balance (Omdahl et al., 1982). Additionally, hypercalcemia is considered a marker for vitamin D intoxication. Further discussion of this subject and methods for the determination of vitamin D status have been discussed in Chapter VI of this document.

Although the evidence is somewhat controversial (Schuette and Linkswiler, 1984), several other factors may have a negative influence on calcium status and, if possible, should be identified in NHANES III. High protein (Allen et al., 1979; Hegsted et al., 1981; Margen et al., 1974) or phosphorus (Goldsmith et al., 1976; Hegsted et al., 1981; Spencer et al., 1978) intakes have been shown to alter urinary calcium levels, but the combined effects of protein and phosphorus levels of normal diets are unclear.
Calcium consumed in the diet is considered essentially nontoxic. Hypercalcemia is generally caused by hyperparathyroidism or excess vitamin D and is unlikely to be caused by dietary calcium (Venugopal and Luckey, 1978).

The Panel notes that methods for the assessment of calcium status which are considered feasible for inclusion in a field survey are not available. However, if the recommendations contained in Chapters XXII, XXIII, and VI are adopted for use in NHANES III, the data collected will provide an opportunity to advance current knowledge on the relationships among calcium and vitamin D nutriture and osteoporosis and hypertension.

B. CONCLUDING STATEMENT

The Panel does not recommend specific analyses of serum or urinary calcium for the assessment of calcium nutriture. However, if collected, data on serum calcium will aid in the interpretation of vitamin D intoxication, and data on serum ionized calcium will permit testing of hypotheses on the relationship of calcium and hypertension.
LITERATURE CITED


XIII. CHROMIUM

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XIII. CHROMIUM

A. INTRODUCTION

Evidence of chromium essentiality for normal growth and development has been derived primarily from animal studies. Investigations with chromium-deprived rats and squirrel monkeys, genetically diabetic mice, and other animals have shown that chromium administration or dietary supplementation reversed manifestations of chromium deficiency such as abnormal glucose tolerance or reduced growth rates (Pi-Sunyer and Offenbacher, 1984). Malnourished children, pregnant women, elderly, and diabetic persons that have exhibited abnormal glucose tolerances or elevated lipid levels responded to administration of chromium-rich brewer's yeast, or inorganic or organic chromium compounds. For example, Jeejeebhoy et al. (1977) found a chromium-responsive deficiency syndrome in a patient on long-term total parenteral nutrition. When 250 μg of chromium were added to the daily parenteral nutrition infusate for 2 weeks, the glucose tolerance test and respiratory quotient became normal and peripheral neuropathy disappeared. Subsequently, 20 μg chromium were administered daily in the infusate and signs and symptoms did not reappear.

The Food and Nutrition Board has recommended a chromium intake of 50-200 μg/day, based in part on the lack of evidence for chromium deficiency in the U.S. population which consumes on the average 60 μg/day (National Research Council, 1980). Meat products, cheese, dairy products, and whole grain are considered good sources of bioavailable chromium (Solomons, 1983).

To date, there has not been any extensive effort to determine the chromium status of the U.S. population or that of another large population. Lack of knowledge on the specific metabolic functions of chromium beyond its recognized role in glucose metabolism, insulin activity, and energy utilization has contributed to the scarcity of data on nutritional status. Further, clinical assessment of chromium status is difficult because the deficiency state has not been defined precisely. Environmental contamination interferes with the accuracy of assay methods for chromium because of the minute amounts being determined. In addition, available assay methods are prohibitively expensive for widespread use.
B. AVAILABLE INDICATORS

1. Nutritional status

Tissue and Excretion Measurements. Measurements of the chromium content of plasma, red cells, whole blood, urine, and hair have been utilized to assess chromium status. However, the majority of values reported in the older literature are significantly higher than those obtained by newer techniques of neutron activation analyses, electrothermal atomic absorption spectroscopy, gas chromatography, or mass spectroscopy. Mertz (1979) noted that chromium is environmentally ubiquitous, requiring stringent procedures for control of contamination during sample collection, storage, and analysis. Losses during sample preparation and analysis coupled with possible increases from contamination suggest previously reported normal values may be unreliable (Slovensk, 1983). Comparison of rates at which radio-labeled chromium ($^{51}$Cr) doses are removed from the blood with rates of $^{51}$Cr uptake by tissues suggests that plasma levels do not reflect tissue levels or metabolic pools (Mertz, 1969). Urinary excretion can be expressed as a chromium:creatinine ratio (Anderson et al., 1982), but attempts to correlate values with clinical measures have been inconclusive partially because normal excretion levels are low (1.0 μg/day) (Anderson et al., 1983). Hair analyses have relatively poor reproducibility.

Functional Tests. Restoration of normal values for glucose tolerance and insulin levels when chromium supplementation is given to diabetic patients in the parenteral nutrition infusate has been described as a clinically useful test for chromium depletion (Jeejeebhoy et al., 1977). In practice, normalization of previously impaired glucose tolerance tests after 3 to 6 weeks of supplementation with 150-200 μg/day chromium is an indicator of a previous deficiency state.

Although not attempted on a large population to date, collection of data on postprandial plasma glucose values and chromium:creatinine urinary excretion ratios would be of interest. However, such a trial would require critical validation of the methodology to be used.

2. Excessive accumulation

Chromium is ubiquitous in soil, water, and biota. There is little or no evidence of accumulation from the environment or toxicity from oral ingestion of chromium. Schroeder et al. (1962) cite several studies reporting no toxicity from chronic ingestion by fish, rodents, and cats. Pi-Sunyer and Offenbacher (1984) indicate that oral ingestion of pharmacologic doses (50-1000 mg of trivalent chromium) by mice, rats, and cats for 1 to 3 months was essentially nontoxic. They also reported that malnourished children given 250 μg chromium intravenously did not exhibit toxic responses (Pi-Sunyer and Offenbacher,
1984). Contact dermatitis and chronic bronchitis in response to exposure and inhalation of dust or fumes containing hexavalent chromium are known from occupational medicine (Nelder, 1983).

C. NHANES III APPROPRIATENESS

The Panel recognizes that the chromium status of the U.S. population is unknown. There are few data on occurrence of deficiency status or toxicity from excessive consumption of dietary items high in chromium. Nevertheless, the Panel concludes that inclusion of tests for chromium nutriture in NHANES III is inappropriate. Methodological techniques for assessing chromium status amenable to a large scale survey such as NHANES III are not available. Functional tests, such as carbohydrate loading and chromium administration, are clinically useful but cannot be undertaken in NHANES III because of the time, equipment, and expense that would be required as well as the need for subject cooperation.

D. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that assessment of chromium nutritional status not be included in NHANES III.
LITERATURE CITED


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XIV. COPPER

A. INTRODUCTION

In 1980, the Food and Nutrition Board concluded that 2 to 3 mg/d of dietary copper was sufficient to maintain copper balance in normal adults (National Research Council, 1980a). Whether changes in estimated intake of copper over the past few decades are the result of more sophisticated analytical methodology or a decline in absolute intake is unclear (National Research Council, 1980a). Danks (1980) speculated that copper intake may be falling due to prepackaging of foods; that is, copper is removed from processed foods because its oxidant properties limit shelf life of many products. Klevay et al. (1980) have expressed concern that current dietary intakes of copper may not be adequate for all individuals. Consumption of large amounts of zinc has been shown to give rise to hypocupremia and signs of copper-deficiency anemia and neutropenia in animals (Bremner, 1979; Pfeiffer and Jenney, 1978; Prasad et al., 1978). Excessive supplemental zinc (50 mg/d) has also been reported to decrease copper status as evidenced by decreased erythrocyte superoxide dismutase (SOD) (Fischer et al., 1984).

Recently, impaired copper status has been suggested as a potential factor in development of ischemic heart disease in humans (Klevay et al., 1984; Reiser et al., 1985). Clinical studies have shown a negative correlation between copper status and intake of fructose and sucrose (Fields et al., 1983; Reiser et al., 1985). High intake of these sugars may exacerbate the effects of copper deficiency, including cardiac abnormalities. Data supporting this theory have been derived from studies of animals that were rendered much more copper-deficient than considered possible to achieve in humans (Fields et al., 1983; Reiser et al., 1983).

These recent observations suggest copper status may warrant further investigation in the U.S. population. Therefore, its assessment should be included in NHANES III if appropriate methods are validated prior to initiation of the survey.

B. AVAILABLE INDICATORS

1. Nutritional status

Plasma Copper. Serum or plasma levels of copper can be determined readily by atomic absorption spectrophotometry using either simple dilution (Sprague and Slavin, 1965) or initial trichloroacetic acid treatment (Olson and Hamlin, 1968; Parker et al., 1967). Animal studies have shown hypocupremia to be consonant with experimental copper depletion, usually appearing as an early indication of deficiency (Suttle and Angus, 1976). Human subjects on copper-deficient total parenteral
nutrition solutions show decreases in plasma copper levels of approximately 11 µg/dl per week (Fleming et al., 1976; Solomons et al., 1976a); copper levels return to normal following dietary or parenteral copper repletion.

Hypocupremia is not necessarily indicative of dietary inadequacy because low serum or plasma copper can be elicited by a number of factors unrelated to dietary copper intake. There are many potential causes of hypocupremia including: protein-energy malnutrition (Lahey et al., 1958; Lehmann et al., 1971), malabsorption syndromes (Butterworth et al., 1958; Solomons et al., 1976b; Sternlieb and Janowitz, 1964), ulcerative colitis (Chachaj et al., 1974), and the nephrotic syndrome (Cartwright et al., 1954). Circulating copper levels may also be affected by infection (Pekarek et al., 1972), corticosteroids (Henkin et al., 1969), diurnal variation (Lifschitz and Henkin, 1971), pregnancy (Hambidge and Droegemueller, 1974), and use of oral contraceptive agents (Horwitt et al., 1975).

**Ceruloplasmin.** Most of circulating copper exists in the form of ceruloplasmin, although reports differ on the exact percentage (Solomons, 1979). Danks (1980) reported that the quantity of this metalloenzyme in normal individuals varies greatly. Therefore, it is not recommended as an indicator of copper status in cross-sectional surveys such as HANES. The response of ceruloplasmin to oral copper supplementation may provide a useful indicator of impaired copper status; ceruloplasmin levels can be measured before and several days after administration of physiologic oral replacement doses of copper. As copper excess does not induce over-production of copper proteins, only subjects with copper deficiency will respond with increased ceruloplasmin (Danks, 1980).

**Erythrocyte Superoxide Dismutase (SOD).** The enzyme superoxide dismutase is a copper- and zinc-dependent metalloenzyme ubiquitous in animal cells. Because deficiency of copper (Bohnenkamp and Weser, 1976; Morgan and O'Dell, 1977), but not zinc (Bettger et al., 1978), depresses the activity of SOD in rats, chicks (Bettger et al., 1979), and pigs (Williams et al., 1975), it is a potential indicator of copper status (Bettger et al., 1979).

**Other Biochemical Measures.** Although hair (Hambidge, 1973; Jacob et al., 1978), fingernail (Martin, 1964), and red blood cell (Robbins et al., 1975) copper content have been proposed as potential indicators of copper stores, their relationship to copper nutritional status is currently unclear and the Panel does not recommend their use in NHANES III.
2. Excessive accumulation

Copper poisoning is usually the result of industrial accidents, consumption of acidic beverages transferred through copper pipes, or deliberate ingestion of copper-containing solutions (Venugopal and Luckey, 1978). Fecal copper is considered a better indicator of acute copper overload than blood copper levels in animals (National Research Council, 1980b), which vary greatly in their tolerance to excess copper (Underwood, 1977). Very little information is available on chronic, subacute over-consumption of copper by humans, but the Panel concludes that it is not a public health problem in this country.

C. NHANES III APPROPRIATENESS

Serum copper levels were measured for subjects aged 3-74 years in NHANES II. Serum copper and all other measures of copper status, with the possible exception of erythrocyte SOD, are not sensitive or accurate enough to be considered as a measure of copper nutritional status in a national population survey. Erythrocyte SOD shows promise as a valuable indicator of impaired copper status and it is recommended that studies to evaluate erythrocyte SOD as an index of copper nutritional status be conducted prior to NHANES III. Validation studies should be similar in design to the study of Reiser et al. (1985), but examine the erythrocyte SOD levels in individuals on unrestricted diets consuming a normal range of copper intakes. Methods to establish evidence of excessive copper accumulation in a survey such as NHANES III are not available.

D. RECOMMENDED APPROACHES

1. Indices

Erythrocyte SOD should be measured in NHANES III provided studies show it to be a reliable indicator (see section C). An added advantage of this assay is that it can utilize red cells which might otherwise not be used during HANES analyses.

2. Methods

Erythrocyte SOD activity can be measured by several methods (Marklund and Marklund, 1974; Misra and Fridovich, 1977; Winterbourn et al., 1975). Current methods are time-consuming and laborious. Additionally, an extraction procedure to remove hemoglobin must be performed at the site of collection. For these reasons, the analysis of SOD is recommended for only a subsample of the NHANES III population.
3. **Basis for interpretative criteria**

Criteria for interpreting erythrocyte SOD values must be based primarily on results of the clinical validation trials recommended above. Few studies examining SOD have been conducted in human beings known to be copper deficient. Klevay et al. (1984) showed erythrocyte SOD activity declined during experimental copper depletion of a young man and increased during repletion. Values were approximately 1000 units/g hemoglobin after 105 days of a copper-restricted diet, which compared with 2338 units/g hemoglobin, the low value of the 95% confidence limit for male staff members of the metabolic ward. SOD activity was not measured in the subject during the control phase of the experiment. Milne et al. (1982) found erythrocyte SOD activity decreased from 3212 to 2667 units/g hemoglobin in four men after 4 months on a diet marginal in copper (0.8 mg/d). Values increased to 4253 units/g hemoglobin after 2 weeks of repletion and then leveled at 3254 units/g hemoglobin after 4 weeks of copper supplements. Okahata et al. (1980) found erythrocyte SOD activity in a 7-month-old infant, with bone marrow findings consistent with copper deficiency, was 52% of that of age-matched controls. After 4 weeks of copper supplementation, values were similar to controls.

Fischer et al. (1984) conducted experiments to confirm the detrimental effect of excess dietary zinc on copper status. After 6 weeks, erythrocyte SOD levels were significantly lower in individuals consuming supplemental zinc (50 mg/d) than in controls. Plasma zinc levels were higher in the supplement group after 2 weeks, but plasma copper levels were similar throughout the trial.

4. **Target groups**

The Panel recommends that if erythrocyte SOD is determinated, it should be made on a random subsample of the adult population. Because the validation may not be completed by 1988, plans should be made to include erythrocyte SOD in the second or third survey cycle, if appropriate.

5. **Other considerations**

Individuals taking supplemental zinc should be identified to aid in the interpretation of copper nutritional status.
E. INTENDED USES OF DATA

Interpretation of these data, if collected, will depend on further trials to establish clinical correlates. Whether erythrocyte SOD values will allow determination of prevalence estimates of copper deficiency is not known; however, they should improve currently available baseline data.

F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that assessment of copper status in NHANES III be performed by the following measurement in a random sample of the adult population only if validation studies can be completed before the appropriate cycle:

- erythrocyte SOD activity.
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XV. IRON

A. INTRODUCTION

Iron deficiency has been characterized as the most common single nutritional deficiency in both the developed and developing countries. Nutritional iron deficiency is the result of inability to meet normal physiological needs through diet, but iron deficiency may also be caused by blood loss. Although the manifestations of iron deficiency and iron-deficiency anemia are generally subtle rather than overt (Finch and Cook, 1984), effects such as impairments in work performance and cognitive function are nonetheless of public health significance. An additional public health consideration is that of potential acceleration of iron overload in susceptible individuals by high levels of iron intakes.

B. AVAILABLE INDICATORS

1. Nutritional status

Serum Ferritin. In normal adults, the concentration of serum ferritin parallels the total amount of storage iron in the tissues; in the range 20 to 300 ng/ml, 1 ng/ml serum ferritin is equivalent to approximately 120 μg/kg of storage iron (Cook and Skikne, 1982). A reduction in serum ferritin below a critical level is an early indicator of depletion of iron stores and developing iron deficiency. In adults, values less than 12 ng/ml are considered to indicate depletion of stores (Jacobs et al., 1972).

In NHANES II, serum ferritin was assayed in a subsample of the population by the two-site immunoradiometric assay (IRMA) of Miles et al. (1974). The need to perform repeated iodinations of antisera greatly increased the variability of results with this method. However, a recently developed commercial kit, based on the same assay principle, was used successfully for Hispanic HANES. In addition, an enzyme-linked immunosorbent assay (ELISA) is currently being tested as an improvement over the IRMA method (see section 02).

Serum Iron, Total Iron-binding Capacity (TIBC), and Transferrin Saturation. Transferrin saturation is used more frequently than serum iron concentration or TIBC as an indicator of iron status, particularly as a measure of iron supply to the erythroid bone marrow. A transferrin saturation of less than 16% in adults is widely regarded as indicative of iron-deficient erythropoiesis (Bainton and Finch, 1964). Children and infants exhibit age-related changes in the normal level of transferrin saturation, and thus require age-specific cutoffs for the interpretation of iron status as indicated by transferrin saturation values. In addition to the changes caused by iron deficiency,
transferrin saturation is influenced by diurnal variations in serum iron concentration and may decline in response to infection or inflammation (Cook, 1982).

In NHANES II, serum iron concentration was determined by a modification of the Automated Technicon AAI-25 method, based on procedures described by Ramsay (1957) and Giovanniiello et al. (1968). This colorimetric assay employed ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] as the chromogen which reacts with Fe (II) to form a violet complex. The TIBC was determined by saturating serum with excess iron and adding magnesium carbonate to remove the iron not bound to serum transferrin. After centrifugation to remove the magnesium carbonate, the bound iron remaining in the supernatant was assayed as above. Transferrin saturation was calculated from the formula:

\[
\text{Transferrin saturation (\%) = \frac{\text{serum iron (\mu g/dl)}}{\text{TIBC (\mu g/dl)}} \times 100}
\]

Erythrocyte Protoporphyrin. Erythrocyte protoporphyrin is another useful indicator of iron-deficient erythropoiesis; the lack of iron in the developing red cell limits heme synthesis and results in the accumulation of protoporphyrin IX in the erythrocyte. In adults, a transferrin saturation less than 16% has been found to correspond to an erythrocyte protoporphyrin level greater than 70 \mu g/dl RBC (Langer et al., 1972), but erythrocyte protoporphyrin is more stable and responds more gradually to changes in iron supply than does transferrin saturation. Erythrocyte protoporphyrin level is elevated also in infection and lead poisoning.

In NHANES II, erythrocyte protoporphyrin was measured by a modification of the method of Sassa et al. (1973) in which protoporphyrin was extracted from whole blood containing an anticoagulant, and then determined spectrofluorometrically. The concentration was expressed per dl of RBC by dividing the whole blood concentration by hematocrit.

Mean Corpuscular Volume (MCV). A subnormal value for MCV is observed when iron deficiency becomes severe (and the number of microcytic erythrocytes is great enough to influence the measurement). A low MCV is a fairly specific indicator of iron deficiency once thalassemia trait and the effects of inflammation have been excluded.

In NHANES II, the MCV was calculated from the formula:

\[
\text{MCV (femtoliters) = } \frac{\text{hematocrit (\%)}}{\text{RBC per liter}} \times 100
\]
The hematocrit was determined by the spun micro-
hematocrit method performed at the examination site (Davidsohn
and Henry, 1969). The RBC counts were performed on a Coulter
Model FN counter (Coulter Electronics, Hialeah, FL) on venous
blood anticoagulated with EDTA.

Some investigators indicate that the preferred technique
for determining MCV is direct electronic calculation, a measure-
ment performed by newer cell counters. However, use of this
technique in NHANES III would be impractical because of the
fragility of the required instrument.

Hemoglobin. Low hemoglobin concentration is associated
with more severe degrees of iron deficiency, but no cutoff value
can delineate a clean separation between normal and iron-defic-
ient individuals because of the substantial overlap of "normal"
and "anemic" values. In addition, interpretation of hemoglobin
concentration is complicated by differences in hemoglobin distri-
butions in blacks and whites. Low hemoglobin concentration can
also result from infection/inflammation, thalassemia minor, fol-
ate and vitamin B-12 deficiency, hemoglobinopathies, pregnancy,
and other states in which there is overhydration or acute plasma
volume expansion. Some investigators regard an increase in
hemoglobin concentration following a trial of iron supplemen-
tation as the best test for evaluating iron deficiency. Such a
test is not feasible in a cross-sectional survey.

In NHANES II, hemoglobin concentration was determined on
a Coulter hemoglobinometer (Coulter Electronics, Hialeah, FL)
(Coulter Electronics, 1970; Davidsohn and Henry, 1969).

Reticulocyte Counts. Reticulocytes are immature
erythrocytes that represent a stage of development between a
normoblast and an adult cell; they retain basophilic substances
that have the appearance of a reticulum when exposed to vital
stains (Wolf et al., 1973). Assessing the reticulocyte count
can indicate whether a period of rapid erythropoiesis is occur-
rting and perhaps aid in the interpretation of iron status in
children and adolescents. However, a single count has limited
value unless it is markedly abnormal. Thus, inclusion of this
measure in NHANES III would not be cost effective.

Red Cell Distribution Width (RDW). Even before red
blood cells show evidence of becoming microcytic, iron-deficient
erythropoiesis can give rise to increased variation in red cell
size. Measurement of the RDW, usually reported by automated
blood cell counters as the coefficient of variation of red cell
size, is considered by some investigators an indicator of very
early iron deficiency (McClure et al., 1985). Elevated RDW
values may be useful in distinguishing between thalassemia and
iron deficiency as the cause of low MCV. However, the require-
ment for a more sophisticated cell counter to perform this
measurement makes its inclusion in NHANES III impractical.
Moving such an instrument from location to location would make
proper maintenance and calibration virtually impossible.
2. **Excessive accumulation**

Substantially elevated transferrin saturation, serum iron concentration, and serum ferritin concentration can occur in iron overload states and may be used to identify suspected cases. However, these changes are also seen in other conditions, particularly liver disease.

C. **NHANES III APPROPRIATENESS**

In the previous HANES, several hematological and biochemical measurements were performed successfully and have proven useful for assessing the prevalence of impaired iron status in U.S. population groups. However, some age groups have not yet been studied in sufficient detail, some refinements in analytical techniques are now available, and auxiliary information could be collected which would aid in the interpretation of the iron status data. For these reasons, plus the desirability of assessing changes in iron status over time, determination of the prevalence of impaired iron status is appropriate for NHANES III.

To address the question of the prevalence of iron overload disorders, a nationwide survey such as the HANES is not an appropriate vehicle. Use of biochemical indicators (elevated serum ferritin concentration and transferrin saturation) permitted identification of a few individuals in NHANES II whose clinical evaluations and medical histories were consistent with a diagnosis of idiopathic hemochromatosis (Pilch and Senti, 1984). However, for a condition as rare as hereditary hemochromatosis [a recent estimate of its frequency is 0.003 (Edwards et al., 1981)], the statistical power of HANES may be too limited to make reliable estimates of disease prevalence. In addition, the biochemical indicators are not sufficiently specific. They would, for example, show the same changes more frequently as a result of parenchymal liver disease. Hemosiderosis, or excessive total-body accumulation of iron from exogenous sources, including accumulation from parenteral administration, can occur in individuals without a genetic predisposition to hemochromatosis. This condition also produces elevated serum iron and ferritin values, but from information obtained from the subjects, nutritional causes can be excluded. Although estimates obtained from the biochemical indicators may not be reliable for determining the prevalence of iron overload, their assessment is still important to permit the further investigation of individuals who show such changes. Even if the cause of elevated values is not iron overload, other causes will be important to pursue for the subject's welfare.
D. RECOMMENDED APPROACHES

1. Indices

As in NHANES II, serum ferritin level, transferrin saturation, erythrocyte protoporphyrin concentration, MCV, and hemoglobin concentration should be measured.

2. Methods

In general, the methodology used for the indicators previously assessed in NHANES II and Hispanic HANES should not be altered greatly in order to achieve optimal comparability with previous HANES data. The following exceptions should be noted.

Because the chromogen ferrozine can react with copper as well as iron and artificially elevate serum iron values, thiourea should be added to the reaction mixture to chelate copper if the same colorimetric assay is to be used.

Consideration should be given to the electrochemical quantification of serum iron and TIBC. This method is currently under investigation in several laboratories and results appear to be comparable to those obtained by other methods. It has the advantage of requiring a small amount of sample and is not sensitive to copper interference.

The IRMA procedure used for serum ferritin should be replaced by an ELISA (Cook, 1984). The enzyme-tagged immunologic reagent may be stored up to 2 years, minimizing the need to reprepare the indicator and greatly enhancing the precision of the assay. In addition, a standardized procedure using monoclonal reagents should be established rather than relying on commercial kits. A proposed ELISA technique for serum ferritin will appear in a manual to be published by the Nutrition Foundation as an activity of the International Nutritional Anemia Consultative Group (INACG) and will be available to members of the Expert Iron Panel of the International Committee for Standardization in Hematology. This group's evaluation would be of value to NCHS if this method is to be used in NHANES III.

3. Basis for interpretative criteria

The approach used to assess iron nutritional status can be similar to that described in the Expert Scientific Working Group report on iron status in NHANES II (Pilch and Senti, 1984). The determination of impaired iron nutritional status can be based on finding abnormal values for two or three of the following: serum ferritin (or MCV for infants and small children), transferrin saturation, and erythrocyte protoporphyrin. Differences in hemoglobin distribution between groups identified
as having normal and impaired iron status can be determined. "Abnormal" values may be interpreted using cutoffs derived by the Expert Scientific Working Group (Pilch and Senti, 1984) or by a modification of their procedure. (Some uncertainty still exists in defining criteria of normalcy for MCV, transferrin saturation, erythrocyte protoporphyrin, and ferritin for children.)

4. **Target groups**

   The entire population should be evaluated for iron status, including persons over 74 years of age.

   Children aged 6 through 12 months, 13 through 18 months, and 19 through 36 months should be sampled in large enough numbers to obviate the necessity of pooling data across these three age groups.

   In NHANES II, an unexpectedly high prevalence of impaired iron status was encountered in males 11 through 14 years of age. This finding needs to be reexamined in NHANES III. The prevalence in this age group may reflect a discrepancy between iron absorption and the need for iron occasioned by a rapidly expanding erythrocyte mass. Interest in examining in greater detail the high prevalence of impaired iron status detected earlier led the Panel to recommend that special attention be paid to this age group (see following section).

5. **Other considerations**

   Although capillary blood collected from small children has been used in the past, combining data from these samples with venipuncture data leads to difficulty in interpretation. Every effort should be made in NHANES III to collect a venous sample from all participants, regardless of age. The Panel does not recommend collection of capillary blood samples.

   Because there was difficulty in distinguishing between iron deficiency and infection/inflammation in NHANES II, inclusion of questions about recent (within the preceding month) infections or immunizations, as well as the measurement of erythrocyte sedimentation rate or acute phase reactant, would aid in interpretation of the data.

   To improve interpretation of results, blood sampling in the morning is preferable for transferrin saturation analysis but is not essential. However, time of blood drawing should be noted.

   History of blood donations (and if feasible, menstrual history) should be included in the health questionnaire to evaluate frequency and amount of blood loss.

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For infants and young children, detailed information should be obtained on birth weight, neonatal blood loss, iron supplementation (and age started), diet history, and number of infections in the prior 3 months.

For pregnant women, the following information is desirable: a detailed history of diet; iron, folic acid, and vitamin B-12 supplementation; duration of pregnancy; and previous pregnancies and complications.

The peak of the adolescent growth spurt in males generally occurs in the age range 11 through 14 years of age (Roche and Himes, 1980; Tanner et al., 1976, 1981). Because the adolescent growth spurt is more closely related to sexual maturity than to age, iron deficiency manifestations may be more prevalent during or soon after the year of maximal linear growth than at other periods. Although the year of maximum linear growth cannot be determined in a cross-sectional survey, the stage of sexual maturity is likely to be correlated with rate of linear growth. Therefore, assessment of the stage of sexual maturity in males 11 through 14 years of age is desirable. Two procedures should be used: grading testicular size using an orchidometer, and grading the developmental stages of pubic hair and penis (Tanner grades).

E. INTENDED USES OF DATA

The data obtained can be used to develop estimates of the prevalence of impaired iron status in U.S. population groups and to assess factors and population characteristics associated with the occurrence of impaired iron status. The data should be especially helpful in assessing changes in iron status over time, because baseline data are available from NHANES II. In view of the importance of this latter use for the iron data, extensive method comparison studies should be conducted prior to their use in NHANES III if changes in analytical methodology for the iron status assessments are contemplated. The Panel does not recommend use of these data to assess the prevalence of iron overload states. However, the collection of data concerning "potential iron overload" based on elevated serum ferritin and transferrin saturation, affords the opportunity for additional studies or referral to personal physicians when abnormal values are found.
F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that the following indices of iron nutritional status be assessed in the entire population in NHANES III:

- serum ferritin level
- transferrin saturation
- erythrocyte protoporphyrin level
- mean corpuscular volume
- hemoglobin concentration.
LITERATURE CITED


### XVI. MAGNESIUM

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XVI. MAGNESIUM

A. INTRODUCTION

Because magnesium is widely distributed in plant and animal tissues used as food, inadequate magnesium status in humans is considered rare in individuals with normal organ function (Shils, 1984). Decreases in dietary intake or absorption of magnesium lead rapidly to retention of magnesium by the kidney (Venugopal and Luckey, 1978), permitting magnesium homeostasis in normal individuals with widely varying intakes of dietary magnesium (Shils, 1984).

However, dietary surveys (U.S. Department of Agriculture, 1972, 1980) suggest that many adults may be consuming levels of dietary magnesium lower than the current RDAs of 300 and 350 mg/day for adult females and males, respectively (National Research Council, 1980). Additionally, Lakshmanan et al. (1984) recently followed 334 adult men and women consuming self-selected diets for 1 year; on average, magnesium balances were negative in this group on the days metabolic studies were conducted.

Magnesium deficiency may occur as a response to long-term loop diuretic therapy (Reyes and Leary, 1983; Wester and Dyckner, 1984) and may lead to arrhythmias (Cohen and Kitzes, 1983) and other cardiac abnormalities (Reyes and Leary, 1984).

Magnesium toxicity via parenteral routes is usually acute, resulting in nausea, depression, and paralysis. Oral MgSO₄ is poorly absorbed. Excessive intake causes diarrhea and dehydration and generally occurs only when kidney function is impaired (Venugopal and Luckey, 1978). Serum magnesium levels do not change significantly when oral or intravenous supplementation is instituted in magnesium-sufficient individuals with normal renal function (Heaton, 1969).

B. AVAILABLE INDICATORS

Serum Magnesium. Serum magnesium can be measured easily and conveniently by atomic absorption; however, the relationship between this indicator and body stores is considered weak (Dyckner and Wester, 1978; Seelig, 1980). Wide ranges of serum or plasma magnesium are accepted as "within normal limits" and only severe cases of deficiency will result in unquestioned hypomagnesemia (Seelig, 1980).

Leukocyte Magnesium Content. Leukocyte magnesium content has been evaluated for estimating tissue magnesium stores in humans (Elin and Johnson, 1982). During magnesium depletion, the quantity of magnesium lost from lymphocytes in the rat is similar to that lost from cardiac and skeletal muscles (Ryan
and Ryan, 1979). This method requires large sample volumes and lengthy preanalytical separation procedures. Methodological problems, confounded with the probability that leukocyte magnesium content may be dependent on the age distribution of the blood mononuclear cells, lead to a coefficient of variation in excess of 25% for this assay (Elin and Johnson, 1982).

Magnesium Load Test. The magnesium load test is regarded as the most reliable test for determining magnesium status (Seelig, 1980). A timed preload collection of urine is made for approximately 24 hours, at which time magnesium is given intravenously or intramuscularly. Timed postload collections of urine are made for approximately 24 hours. The percentage of magnesium load retained is calculated comparing data from the base period and net excretion after the load. Forty percent retention of magnesium has been arbitrarily set as the upper limit of normal (Caddell et al., 1975). This method is not amenable to a large scale survey because a 48-hour urine collection is required.

C. NHANES III APPROPRIATENESS

Serum magnesium levels were obtained in NHANES I (National Center for Health Statistics, 1979). Results have not been published, but are available on tape for use as baseline data. Although better predictive methods for determination of magnesium status exist, they are either unvalidated in a large population or analytically complex. Although magnesium may be an important correlate for other diseases (e.g., hypertension) (Kaplan, 1982; McCarron, 1983) and adequate intake may be of concern in the adult population, it is not feasible to measure magnesium nutritional status in NHANES III.

D. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends that analyses for the determination of magnesium nutritional status should not be included in NHANES III.


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A. INTRODUCTION

Manganese deficiency has not been observed in a free-living human population (National Research Council, 1980). The Food and Nutrition Board estimated a "safe and adequate" daily intake to be 0.5 to 1.0 mg/day for infants, 1.0 to 3.0 mg/day for children and 2.5 to 5.0 mg/day for adults (National Research Council, 1980).

Plants consumed as food provide ample levels of manganese because plants have a higher requirement for this element than animals. Thus, even crop plants growing on soil low in available manganese generally contain sufficient quantities of manganese to supply human needs (Underwood, 1981). Only one purported instance of clinical manganese deficiency has been reported. This resulted from consumption of a vitamin K-deficient purified diet which inadvertently omitted manganese from the mineral supplement (Daisy, 1972). The individual experienced hypocholesterolemia, weight loss, transient dermatitis, nausea, and changes in the growth rate and color of hair.

Manganese is among the least toxic of the trace elements (Underwood, 1977); manganese toxicity resulting from dietary intake is not considered to be a nutritional problem (Keen et al., 1984). Although manganese toxicity via inhalation occurs in industrial settings, particularly mining (Underwood, 1977), reports of oral overload are rare. Kawamura et al. (1941) described toxicity symptoms in individuals who had consumed well water contaminated with manganese leaking from discarded batteries. Elevated tissue manganese levels were reported in an individual who consumed "large" quantities of minerals for 4 to 5 years (Banta and Markesbery, 1977). The patient exhibited neuropsychiatric symptoms similar to those of individuals with known manganese intoxication. The range between dietary requirement and toxicity appears to be large (Hurley, 1984), which may account for the relative infrequency of excessive accumulation.

B. AVAILABLE INDICATORS

Serum or Plasma Manganese. Serum manganese levels are reported to vary widely in normal humans (Underwood, 1977), but this may result from methodological deficiencies rather than biological variation (Versieck, 1985). The manganese concentration in serum or plasma is difficult to measure without the use of neutron activation analysis or x-ray fluorescence spectroscopy (Keen et al., 1984) and serum or plasma manganese concentration has not been validated as an assessment tool for manganese.
status. Low serum levels of trace elements may be indicative of profound deficiency, but are not considered adequate to detect mild deficiencies (Favier and Ruffieux, 1983).

Whole-Blood Manganese. Whole-blood manganese level of rats correlates well with liver manganese concentration in manganese deficiency (Keen et al., 1983). Rats fed deficient diets for 60 days had blood manganese values 40% lower than controls. These low values persisted even after diets had been interchanged for 1 week, indicating that blood manganese levels reflect body pools and not recent intake. Measurement of whole-blood manganese requires flameless atomic absorption spectrophotometry which is not automated at the present time. The Panel notes that the technique has not been validated in humans.

Hair Manganese. Hair manganese, as analyzed by atomic absorption, has been evaluated as a tool for assessment of manganese status and is known to vary with the individual, season, and color of hair (Papavasiliou et al., 1979; Underwood, 1977). Although hair manganese may relate somewhat to the manganese level of the diet (Underwood, 1977), hair analysis is not considered reliable for evaluating the nutritional status of individuals (Barrett, 1985).

C. NHANES III APPROPRIATENESS

Manganese has not been measured in a HANES survey. Little or no evidence exists indicating occurrence of manganese deficiency or toxicity states in the U.S. population. Additionally, at this time, there is no validated method for the determination of manganese status in a population survey. Therefore, the Panel does not recommend manganese assessment for inclusion in NHANES III.

D. CONCLUDING STATEMENT

Based on the foregoing discussions, the Panel recommends that assessment of manganese nutritional status not be included in NHANES III.
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XVIII. SELENIUM

A. INTRODUCTION

Interest in selenium status is increasing rapidly as more information accumulates on the essentiality of selenium to human health. Keshan disease, a childhood cardiomyopathy occurring in China, has been associated with low dietary selenium intakes (Chen et al., 1980) and various signs and symptoms have been reported in patients receiving total parenteral nutrition or consuming restricted diets low in selenium (Johnson et al., 1981; van Rij et al., 1979). In animal studies, supplemental selenium has been shown to inhibit chemical carcinogenesis and the growth of both virally induced and transplantable tumors (Ip, 1985; Milner, 1985). Epidemiological studies have suggested that low selenium intakes may be associated with increased incidence of cancer and cardiovascular disease (Clark, 1985; Shamberger et al., 1975).

The Food and Nutrition Board (National Research Council, 1980) has set a "safe and adequate range" of selenium intake of 50 to 200 μg/day. The range of selenium intakes between those supporting nutritional adequacy and those producing signs of toxicity is fairly narrow (10- to 100-fold) (Underwood, 1977), causing concern about the possibility of deleterious effects in individuals using excess selenium supplements. Recently manifestations of toxicity were reported in 13 individuals who had consumed a selenized yeast product (Helzlsouer et al., 1985).

B. AVAILABLE INDICATORS

1. Nutritional status

Selenium Levels in Blood. The measurement of the selenium concentration in blood and blood components has been used to assess the long-term selenium nutritional status of animals and human subjects. In rats, erythrocyte and plasma selenium levels were found to be indicators of the selenium content of muscle and liver (Behne et al., 1981), the major sites of storage (Behne and Wolters, 1983). Skeletal muscle is also a major storage site for selenium in humans (Schroeder et al., 1970). Burk (1976) noted that low blood selenium levels reliably indicate inadequate selenium intake because homeostatic mechanisms act to conserve the element, but that low selenium levels are not invariably associated with signs of selenium deficiency. Low blood selenium levels have been reported in children with protein-calorie malnutrition (Burk et al., 1967; Levine and Olson, 1970), and in patients with gastrointestinal cancers (McConnell et al., 1975). Recent evidence from China (Yang et al., 1983) suggests preliminary levels for defining various degrees of selenium exposure based on values for blood selenium
(see table below). The extreme values for selenium indices reported in China can provide useful reference points for comparing widely diverging selenium exposures, but are of limited use in defining the physiological relevance of smaller deviations in selenium status. Blood selenium values of 19-25 µg/dl have been reported for the U.S. population (Burk, 1984).

<table>
<thead>
<tr>
<th>Area</th>
<th>Dietary Se intake, µg/d</th>
<th>Blood Se level, µg/dl</th>
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<td>Low-Se area with Keshan disease</td>
<td>11</td>
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<tr>
<td>Low-Se area</td>
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<td>2.7</td>
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<tr>
<td>Se-adequate area</td>
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<tr>
<td>High-Se area</td>
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<td>44</td>
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<tr>
<td>High-Se area with chronic selenosis</td>
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Erythrocyte selenium level is an indicator of total body selenium status in most individuals on relatively stable diets (Levander, 1985a). However, selenium levels do not change as rapidly in the erythrocytes as they do in the muscle pool during acute selenium intoxication (Levander and Morris, 1980). Most currently recommended assays for selenium in erythrocytes are complicated by the need for wet ashing of the samples. Serum or plasma selenium level provides a shorter-term index of selenium status than does erythrocyte level, but has the advantage of a much simpler assay procedure. Selenium levels in serum or plasma respond more quickly than muscle levels to short-term shifts in selenium intake (Levander, 1985a).

Selenium Content of Other Tissues. Muscle biopsy directly assesses the selenium content of a large (50% of total body selenium), long-term pool, but is clearly not feasible for survey work. Hair selenium has been reported to be an excellent indicator of muscle and liver selenium in rats (Behne et al., 1981) and to correlate well with blood selenium levels of persons in China (Chen et al., 1980). However, hair analysis for selenium is probably compromised in the United States and Europe by the use of selenium-containing shampoos (Davies, 1982). Hair color is another complicating variable; while human data are unavailable, selenium content is known to vary in the hair of different colored pigs (Wahlstrom et al., 1984). Toenails have been suggested as a noninvasive tissue for assay which presents fewer problems due to environmental contamination than hair.
(Morris et al., 1983). Selenium is incorporated into nails as they grow and clippings from all ten toes can reflect tissue selenium status integrated over an extended period of time (Morris et al., 1983). However, little information on the relationship of toenail selenium to total body selenium has been published.

**Selenium Excretion in Urine.** Selenium exposure in an industrial setting has been monitored by measuring selenium excretion in the urine (Glover, 1967). Also, a good correlation has been found between blood selenium levels and 24-hour urinary excretion in New Zealanders (Griffiths and Thomson, 1974). However, the selenium content of random urine samples is greatly affected by recent dietary intake (Levander, 1985a) and 24-hour urine samples are exceedingly difficult to obtain, suggesting that urinary selenium is not a useful assessment tool in a survey situation.

**Glutathione Peroxidase Activity.** The activity of this selenoenzyme in whole blood, serum or plasma, erythrocytes, and platelets has been measured as a functional indicator of selenium status. Good correlations between selenium content and glutathione peroxidase activity in tissues are generally found in populations in which low selenium status is present (Rea et al., 1979; Thomson et al., 1977). However, enzyme activity is probably saturated in most North Americans; thus, its usefulness in differentiating various degrees of selenium status is questionable. The enzyme assay is easier to perform than chemical analysis for selenium, but this advantage may be offset by the greater care needed in sample handling and storage to avoid denaturation of the enzyme. Glutathione peroxidase activity can be influenced by variables such as age, sex, exposure to oxidants and toxicants, deficiencies of iron and vitamin B-12, and the choice of substrate used in the assay (Ganther et al., 1976). In addition, standard enzyme activity values are not available to interpret the results.

2. **Excessive accumulation**

The levels of selenium present in the blood (erythrocytes and serum or plasma), accumulating in tissues, and excreted in the urine are known to increase in response to consumption of large amounts of selenium (Burk, 1976). Blood selenium values characteristic of the selenotic state have been reported. Elevated tissue levels should raise concern over toxicity, but may not reliably predict the occurrence of clinical toxicity (Burk, 1976) because some forms of selenium (such as selenomethionine) may accumulate without producing toxic signs and concomitant administration of certain substances (for example, arsenic and linseed meal) can modify selenium toxicity.
C. NHANES III APPROPRIATENESS

Serum or plasma selenium concentration is a relatively simple, single index of selenium nutritional status which provides information about both deficient and excess levels of consumption. It can be measured by methods amenable to a large field survey. In addition, there is currently a great deal of interest in assessing the selenium status of the U.S. population. For these reasons, assessment of selenium nutritional status is recommended for inclusion in NHANES III.

D. RECOMMENDED APPROACHES

1. **Indices**

   The measurement of serum or plasma selenium is recommended for the assessment of selenium status in NHANES III. Although serum or plasma selenium is not as good an indicator of long-term status in individuals as is erythrocyte selenium, its analysis is considerably simpler, making it preferable for a large survey. Levander (1985a) notes that plasma selenium levels are probably satisfactory for assessing long-term status in population groups. If simpler analytical techniques for the measurement of erythrocyte selenium are developed, this assessment should be considered for inclusion in the survey.

2. **Methods**

   Several techniques are available for the measurement of serum or plasma selenium level. Methods based on direct atomic absorption using Zeeman background correction show particular promise. With one such procedure, Pleban et al. (1982) reported within-run and between-run coefficients of variation for plasma selenium of 6.4 and 7.4%, respectively, and obtained values in good agreement with those determined by the more difficult and time-consuming fluorometric analysis. Such procedures are currently being evaluated for routine use and should be ready by 1987; they would allow automated instrumental analysis without the necessity of acid digestion (Levander, 1985b).

3. **Basis for interpretative criteria**

   Because serum or plasma selenium values can reflect body stores fairly well, these data will permit the evaluation of relative selenium status, although more work is required to establish the significance of small differences in selenium levels. Levander (1985b) has suggested that, although it is inappropriate at present to select cutoff values to designate
selenium deficiency or toxicity based on serum or plasma selenium, it would be appropriate to examine the diets of persons whose serum or plasma selenium values fall outside the range 5-30 μg/dl.

4. Target groups

If assessments of selenium nutritional status were included in NHANES III, they would represent the first such data for the U.S. population as a whole. There is relatively little information on age-, sex- and race-differences in selenium status in the United States. For these reasons, assessment of selenium status should be conducted for all subjects in NHANES III.

5. Other considerations

As with all trace elements, scrupulous care must be taken in sample collection and handling and trace metal-free apparatus must be used to avoid contamination of samples intended for selenium analysis.

Although reliable data for the selenium content of food are not currently available, they should be at the time NHANES III is conducted. At present, the U.S. Department of Agriculture is evaluating existing data on selenium content for inclusion in nutrient composition tables and analyzing the selenium content of foods for which adequate data do not exist (Holden, 1985). Depending upon the dietary methodology chosen for inclusion in the survey, it may be possible to obtain estimates of usual, or at least average, consumption of selenium for various population groups.

Assessment of factors (such as exposure to heavy metals and protein-calorie malnutrition) which interact with selenium status should also be considered.

E. INTENDED USES OF DATA

Collection of serum or plasma selenium values for a representative sample will permit the evaluation of selenium status by virtue of this indicator's ability to reflect body stores. If appropriate cutoffs can be established, the prevalence of selenium deficiency and dietary excess can be estimated. If follow-up studies of the NHANES III population are conducted, data on serum or plasma selenium and dietary selenium may permit the testing of hypotheses relating the incidence of cancer to selenium status and intake. In testing such diet/cancer relationships, other dietary factors which can interact with selenium (vitamin A, vitamin E, and polyunsaturated fat) should also be evaluated.
F. CONCLUDING STATEMENT

Based on the preceding information, the Panel concludes that selenium nutritional status should be assessed in NHANES III by measuring:

- serum or plasma selenium concentration.
LITERATURE CITED


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XIX. ZINC

A. INTRODUCTION

The assessment and occurrence of zinc deficiency have been reviewed (Hambidge, 1981; Prasad 1977, 1983; Sandstead, 1981; Sandstead et al., 1982; Solomons, 1979). As indicated in these reports, overt and severe zinc deficiency, in the absence of underlying disease or inappropriate enteral or parenteral feeding, is rare in the United States. However, some surveys of groups presumed to be vulnerable to zinc deficiency (children, pregnant women, the elderly) in which zinc status has been assessed by dietary intake, plasma/serum zinc levels, and/or hair zinc, have been interpreted as suggesting that zinc nutritional status is less than optimal in subgroups of the population. In some of these studies, supplementation of the diet with additional zinc has been reported to produce beneficial effects on physiological functioning and growth, and even on health. Despite the preliminary nature of these observations, self-supplementation with zinc is becoming increasingly popular. Excess dietary zinc can interfere with the absorption of copper (Bremner, 1979; Prasad et al., 1978) and may decrease serum HDL cholesterol levels (Hooper et al., 1980). Klevay (1975) has hypothesized that a high ratio of dietary zinc to dietary copper contributes to coronary heart disease (Klevay, 1975). Based on these considerations, the assessment of zinc nutritional status appears to be an issue of public health significance.

B. AVAILABLE INDICATORS

1. Nutritional status

Serum/Plasma Zinc. The level of zinc in the serum or plasma can be easily and accurately measured by atomic absorption spectrophotometry. As is true for all assessments of trace elements, zinc contamination of samples must be scrupulously avoided. In addition to the potential for falsely elevated values caused by contamination, serum zinc values can be influenced by nonnutritional factors including pregnancy (Halsted and Smith, 1970; Halsted et al., 1968); oral contraceptive agent use (Halsted et al., 1968; Smith and Brown, 1976); serum albumin concentration (Solomons, 1979); and infection or acute stress (Beisel et al., 1976). Serum zinc levels are subject to fluctuation caused by diurnal variation, fasting, and meal consumption (Sandstead, 1981). Although low circulating zinc values must be interpreted as representing zinc depletion or redistribution of zinc within the body, levels within the normal range do not preclude deficiency (Sandstead, 1981).
Other Biochemical Assessments. Measurements of the concentration of zinc in hair, red blood cells, and leukocytes have been investigated as possible tools for the assessment of zinc deficiency, but difficulties in sample preparation and questions about their relation to zinc nutritional status have prevented acceptance of their routine use (Baer and King, 1984; Hambidge, 1982; Klevay, 1970; Milne et al., 1984; Solomons, 1979).

Functional Tests. Some investigators have suggested that assessment of impaired physiological functions affected by zinc deficiency, including taste and smell acuity, growth, immunocompetence, dark adaptation, and neuropsychological function, etc., may be useful in evaluating zinc nutritional status (Pilch and Senti, 1984; Solomons, 1984). However, each of these functions may be influenced by factors other than dietary zinc, and whether changes in them occur consistently in response to zinc depletion is not yet known.

2. Excessive accumulation

Serum/Plasma Zinc. Circulating zinc levels increase in response to increased intake of zinc, but the use of these values for assessing toxicity is subject to some of the same limitations as their use for assessing deficiency. A quantitative relationship between serum zinc level and clinical/functional consequences of zinc excess has not been established.

C. NHANES III APPROPRIATENESS

The serum zinc level for persons aged 3 through 74 years was assessed in NHANES II. It was the only indicator related to zinc status included in that survey. Attempts to use these data to determine the prevalence of zinc deficiency and toxicity in the U.S. population were restricted by the limitations of the indicator itself (Pilch and Senti, 1984). Functional impairments were not detected in the data available on persons with low or high serum zinc levels.

Serum (or plasma) zinc levels are the only measure of zinc status readily available for use in a large-scale survey; this measure lacks the specificity to reflect body stores or to identify the deficient or toxic state. Serum metallothionein level may prove to be useful in the future, but at present it is not validated as an index of zinc nutritional status. Zinc deficiency might be assessed best by a functional correlate (Zn-dependent enzyme activity, immune function, taste acuity, etc.) that shows improved response after intervention with zinc, a method not suited to cross-sectional surveys such as the HANES. Therefore, assessment of zinc nutritional status is not recommended for inclusion in NHANES III.
D. CONCLUDING STATEMENT

Based on the considerations above, the Panel recommends that assessment of zinc nutritional status not be included in NHANES III.
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A. INTRODUCTION

Since the turn of the century, morbidity and mortality from contagious diseases have declined markedly in the United States. Similarly, improved diagnosis, prevention, and treatment have led to decreasing mortality and morbidity from various forms of cardiovascular disease (Riggan et al., 1983; Horm et al., 1984). Concomitantly, there has been increased public attention and biomedical research focus on causes of cancer and factors associated with various types of cancers in various populations. The role of diet and dietary factors in the etiology or prevention of cancer is a topic of continuing scientific debate. In a review of causes of cancer, Doll and Peto (1981) stated that if suitable modifications of national dietary practices were adopted, there is strong but indirect evidence that the death rates from the most common types of cancers could be reduced. However, they indicated that neither laboratory studies nor epidemiological observations may be able to provide evidence for causal or protective relationships in human populations because of the difficulty in control of confounding factors. Nevertheless, Doll and Peto (1981) estimated the a 35% reduction of the U.S. death rate from the major types of cancer might be possible by dietary modifications.

More recently, a committee of the National Research Council (NRC) has published a comprehensive review entitled, "Diet, Nutrition, and Cancer" (National Research Council, 1982). This report reviewed, in detail, the then current state of knowledge on associations between dietary factors and occurrence of various types of human cancer. The report draws on experimental animal studies, epidemiological investigations, and other sources of data and information. While cautioning "... it is not yet possible to make firm scientific pronouncements about the association between diet and cancer", the NRC committee noted a positive association between cancer occurrence and high dietary intakes of fat and protein, as well as an inverse relationship between cancer and dietary intakes of vitamin A and its carotenoid precursors, vitamin C, and selenium. The NRC Committee observed that data were not sufficient to reach firm conclusions on the association of the B vitamins, vitamin E, iron, copper, zinc, molybdenum, iodine, arsenic, lead, or cadmium with human carcinogenesis even though some animal studies did provide data on either positive or negative associations. The NRC Committee concluded that there is scientific evidence to suggest most common types of cancer are influenced by dietary patterns, but precise estimates of the contribution of diet and its components as well as the percent reduction of risk for certain cancers that might be afforded by dietary modifications were not possible (National Research Council, 1982).
B. AVAILABLE INDICATORS

The collection and analysis of data on cancer morbidity and mortality are major concerns of the National Cancer Institute and the National Center for Health Statistics. These agencies have ongoing programs to identify cancer incidence and mortality data from various death records, population records, national census data, hospital records, and related medical records collected both locally and nationally (Horm et al., 1984; Riggan et al., 1983). A number of these epidemiological programs have been collecting and publishing estimates of cancer morbidity and mortality for several decades. These publications include data on cancer incidence and mortality by sex, age, race, ethnic origin, geographical regions, and other factors using standardized statistical approaches. For example, Burbank (1971) analyzed patterns of cancer mortality in the United States from 1950 to 1967; Mason et al. (1975, 1976) prepared atlases of geographic patterns of cancer mortality for several subsets of the U.S. population by county and type of cancer. Such data provide far more extensive information on the occurrence of cancer in the United States than would be provided by prevalence estimates that could be obtained in NHANES III.

However, these reports do not relate incidence of cancer or types of cancer to nutritional status or the intake of specific nutrients. As implied by Doll and Peto (1981) and the recent report on diet, nutrition, and cancer (National Research Council, 1982), indicators of nutritional status that can be taken together to identify a direct association between nutrient deficiency or excess are not available. Animal studies and epidemiological investigations have provided information on possible protective effects of retinoids and carotenoids, vitamin C, and selenium. However, the relationship between levels that may be considered nutritionally adequate and those at which there are possible protective effects, remains to be established.

The data collected in NHANES III can also be used for surveillance of risk factors associated with the development of cancer such as dietary practices, smoking, use of alcohol, and possibly occupational exposure to carcinogens.

The Panel notes that current experimental and epidemiological efforts may result in development of clinical measures of exposure to carcinogens which may be appropriate for consideration in future surveys of the U.S. population. For example, human exposure to carcinogenic N-nitroso compounds has been related to ingestion of such substances and to nitrosation of amine precursors in the gastrointestinal tract. Bartsch et al. (1983) have developed a technique for measuring urinary N-nitrosoproline following ingestion of proline. In a pilot study, these investigators found that subjects from an area where esophageal and stomach cancer incidence are high had higher levels of excretion of N-nitrosoproline than subjects from an
area where esophageal and stomach cancer incidence are low. Ingestion of vitamin C by subjects reduced levels of nitrosated substances in the urine.

C. NHANES III APPROPRIATENESS

Currently, evidence linking certain dietary components with increased or decreased risk of some types of cancer is available; however, these studies often provide conflicting or inconsistent data. There are data suggesting certain nutrients and dietary factors such as retinoids, carotenoids, vitamin C, and selenium may have protective effects against cancer. Establishing clear associations between individual nutrients and such effects requires longer periods of data collection than are contemplated for the cross-sectional portion of NHANES III.

Nevertheless, the Panel concludes that collection of data in NHANES III that relate to both cancer and the status of various nutrients will be useful. Such data will be essential both as baseline data for prospective studies in general and for follow-up studies of persons with and without cancer in 1988-1994. Current cases should be determined to exclude prevalent cases from prospective incident cases. Individuals examined in NHANES III will be identifiable in the National Death Index in the future. Thus, the opportunity to relate cancer mortality data with information on nutrient status (1988-1994) will be available. In addition, the nutritional status data collected in NHANES III would be useful in establishing reference distributions of nutrients thought to be associated with cancer prevention. The Panel suggests that evaluation of various risk factors such as dietary and lifestyle habits, and possible protective factors such as vitamin A and carotenoids, vitamin C, and selenium nutritional status may have utility when follow-up studies are performed.

D. RECOMMENDED APPROACHES

1. Indices and methods

Information on cancer occurrence or absence may be derived from questions in the medical histories. Undiagnosed cancers may be detected occasionally in subjects (for example, leukemia may be identified during examination of blood smears), but such events are likely to be rare.

Nutritional status at the time of the survey with respect to vitamin A, vitamin C, and selenium should be assessed as described in Chapters III, V, and XVIII, respectively. Additionally, serum carotenoids may be measured by the method of Bieri et al. (1985).
Questionnaires should provide information on such lifestyle factors as usual dietary intake, smoking, and alcohol use, as well as current and past occupational history.

2. **Target groups**

Inasmuch as possible, data should be collected on the entire population to be surveyed in NHANES III.

E. **INTENDED USES OF DATA**

The Panel concludes that while NHANES III will not provide meaningful data on the cross-sectional association of various nutrients with occurrence of cancer at the time of collection and data analysis, it may provide surveillance data on risk factors and baseline data on protective factors for future longitudinal studies. For example, linkage with the National Death Index may provide information that could be useful in the examination of relationships between nutrient status in various population subgroups in 1988-1994 and subsequent cause of death. Identification of persons with cancer at the time of the survey is important for prospective studies of incident cases.

F. **CONCLUDING STATEMENT**

Based on the foregoing discussion, the Panel recommends that NHANES III include:

- collection of medical history information on cancer
- evaluation of vitamin A and carotenoids, vitamin B6, and selenium status
- collection of information on diet and lifestyle factors
- planning for follow-up studies.
LITERATURE CITED


XXI. CHOLESTEROL AND BLOOD LIPIDS

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XXI. CHOLESTEROL AND BLOOD LIPIDS

A. INTRODUCTION

Coronary heart disease (CHD) is a major medical problem in the United States. It results from insufficient coronary blood flow due to atherosclerotic narrowing produced by plaque formation (Robbins, 1967). These plaques, consisting of deposits of lipids, particularly cholesterol, and connective tissue within the intima of the arterial walls, project into the lumen of the vessel. The plaques are vulnerable to additional pathological changes such as ulceration, hemorrhage, and calcification, and predispose to the formation of thrombi at the involved sites. Although atherosclerotic plaques have been observed in younger persons, CHD is rarely manifest before age 30 years and at least 85% of deaths from myocardial infarction occur in subjects more than 55 years old (Herbert, 1983).

Epidemiological studies have shown a strong positive association between serum cholesterol level and risk of heart attack (Kannel et al., 1971; Keys, 1970; Keys et al., 1972; McGill, 1968; Paul et al., 1963; The Pooling Project Research Group, 1978; Yano et al., 1978). In controlled clinical studies, it has been demonstrated that the amount and kind of fat in the diet influence the level of cholesterol in the blood (Grande et al., 1970, 1972; Keys et al., 1965a,b,c). Saturated fatty acids, C_{12} - C_{16} chain length, were shown to be hypercholesterolemic, polyunsaturated fatty acids hypcholesterolemic, and oleic and stearic acids neutral in effect on serum cholesterol levels when substituted isocalorically for dietary carbohydrate. Dietary cholesterol also contributed to serum cholesterol levels. Hegsted et al. (1965) reported similar findings; however, they noted that the contribution of lauric acid was small. In a recent study, dietary oleic acid (high oleic safflower oil) was reported to be hypcholesterolemic relative to palmitic acid (palm oil) in patients with normal plasma triglyceride levels (Mattson and Grundy, 1985).

Reduction in CHD associated with drug-induced reduction in serum cholesterol levels was observed in the Lipid Research Clinics Coronary Primary Prevention Trials in which asymptomatic middle-aged men with primary hypercholesterolemia were treated with cholestyramine resin and the control group received a placebo for an average of 7.4 years (Lipid Research Clinics Program, 1984a,b). Both groups followed a moderate cholesterol-lowering diet. The cholestyramine group experienced average plasma total (TC) and low-density lipoprotein cholesterol (LDL-C) reductions of 13.4 and 20.3%, respectively; corresponding reductions for the placebo group were 4.9 and 7.7%, respectively. The cumulative 7-year incidence of the primary end point (definite CHD death and/or definite nonfatal myocardial infarction) was 7% in the treated group and 8.6% in the placebo group. When taking into account the stratification of participants at entry and their
different lengths of follow-up, the cholestyramine group experienced a 19% reduction in risk ($p < 0.05$) at the primary endpoint with a 8.5% lower total serum cholesterol level. However, no significant differences were observed in overall mortality.

Intervention studies in which diets were modified to reduce saturated fat and cholesterol and increase polyunsaturated fat content have produced sustained decreases in serum cholesterol of 8 to 15% in disparate study groups (Dayton et al., 1969; Frantz et al., 1975; Leren, 1970; Miettinen et al., 1972). Most of these studies showed some decrease in rates of coronary heart disease incidence and mortality in the populations surveyed.

Arntzenius et al. (1985) reported no coronary lesion growth in 18 of 39 patients with stable angina pectoris after 2 years on a vegetarian diet with a polyunsaturated/saturated fatty acid (P/S) ratio >2 that provided less than 100 mg cholesterol per day. Coronary lesion growth was not observed in patients who had values <6.9 for the ratio TC/high density lipoprotein cholesterol (HDL-C) throughout the trial, or who initially had values >6.9 that were significantly lowered by dietary intervention.

Shekelle et al. (1981) evaluated 1900 middle-aged men participating in the Western Electric Study to determine relationships between diet, serum cholesterol, and the 19-year risk of death from coronary heart disease. Results showed a positive correlation prospectively between mean baseline Keys diet scores (a higher score indicates a higher intake of saturated fatty acids and/or cholesterol and a lower intake of polyunsaturated acids) and the 19-year risk of death from coronary disease in this group.

Correlation of dietary information on three cohorts of middle-aged men (1001 total) collected 20 years ago with mortality from heart disease over that period showed that those who died of CHD had higher Keys dietary scores ($p=0.06$) than those who did not (Kushi et al., 1985). The higher Keys score carried a relative increased risk of 1.6 for CHD. The authors concluded that the results tend to support the hypothesis, albeit weakly, that diet is related to the development of heart disease.

There is continuing controversy over interpretation of studies attempting to evaluate diet-heart disease relationships, particularly those associated with the correlation between diet, lipidemia, and subsequent clinical endpoint (Harper, 1981; Kronmal, 1985; Mann, 1977). Proponents of the diet-heart disease connection concede that other risk factors may play a much greater role in the etiology of this disease.
B. AVAILABLE INDICATORS

1. Plasma lipids and lipoproteins

LDL-C, HDL-C, and TC plasma concentrations are indicators of risk of CHD (Gordon et al., 1977a). TC and LDL-C levels are directly related to CHD risk and HDL-C is inversely related. LDL-C levels are highly correlated with TC levels because, on the average, two-thirds of TC is carried by LDL. In the Framingham study, the correlation coefficient was 0.84 for men (Kannel and Gordon, 1976). HDL-C was only weakly correlated with LDL-C and TC levels indicating that it provides a net contribution to the identification of CHD risk over and above that provided by TC and/or LDL-C. In the Framingham study, TC had little correlation with risk of CHD in the age range 49-82 years. However, even at very advanced ages, HDL-C was the most potent lipid risk factor, significantly inversely associated with CHD, while a weaker association of LDL-C with CHD was observed (Gordon et al., 1977b).

Recent studies suggest that HDL₂ may be the major antiatherogenic component of HDL (Anderson et al., 1979; Fellin et al., 1985; Miller et al., 1981). The Panel notes, however, that methodology for HDL₂ determination may be too labor-intensive for inclusion in NHANES III.

2. Plasma apoproteins

LDL-B apoprotein levels discriminated patients with angiographically defined CHD from those without CHD significantly better than did LDL-C or TC plasma levels (Fruchart et al., 1982; Sniderman et al., 1980; Whayne et al., 1981). The majority of those with CHD had elevated levels of LDL-B apoprotein whereas their LDL-C levels were within the normal range. Similarly, results from some studies suggest that plasma levels of apo-lipoprotein A-I, the major apoprotein of HDL, provide additional information over that given by HDL alone in discriminating patients with coronary atherosclerosis (De Backer et al., 1982; Riesen et al., 1980).

3. Plasma triglycerides

Although plasma triglyceride level may be a risk factor for subsequent fatal and nonfatal cardiovascular events (Hjermann et al., 1981), evidence that plasma triglyceride level is an independent risk factor for CHD is considered unconvincing by many investigators (Hulley et al., 1980).
4. Serum linoleate/oleate cholesterol ester ratio

Recently, the ratio of linoleate/oleate cholesterol esters in the serum has been proposed as an indicator of dietary patterns of lipid consumption (Bernert et al., 1982). The Panel also discussed the possibility of using red blood cell membranes for this measurement. If validated, this method may serve as an important tool in assessing recent dietary intake of fat.

C. NHANES III APPROPRIATENESS

Measurements of plasma TC and HDL-C are reasonably simple and accurate and are thus feasible for inclusion in a large-scale survey such as NHANES III. Plasma TC concentration was measured in individuals in all age groups in NHANES I. Because of the relatively small number of blood samples collected for children under 4 years of age, data have been published only for subjects aged 4-74 years. In NHANES II and Hispanic HANES, plasma TC and HDL-C were measured in all subjects 20-74 years of age; triglycerides were measured in blood samples drawn from fasting subjects in the same group. Data from these surveys have not been published by NCHS.

Newer methodology allows the determination of various apoprotein fractions of cholesterol which may prove to be valid predictors of CHD. For this reason, it would be of interest to have values for these indices in a representative national sample.

D. RECOMMENDED APPROACHES

1. Indices

The Panel recommends that plasma TC, HDL-C, and apoproteins B and A-I be measured in NHANES III. If it is not feasible to include all of these indices, they should be collected in the order given. TC is a surrogate for LDL-C, the lipoprotein cholesterol subfraction directly correlated with incidence of CHD. HDL-C, inversely correlated with CHD incidence, is particularly useful in discriminating risk among subjects in older age groups. Apoprotein B and A-I concentrations have shown discrimination beyond that of LDL-C and HDL-C in some cases; baseline data in a representative U.S. population are lacking. Serum linoleate/oleate cholesterol ester ratio should be included provided adequate data are available prior to NHANES III to support their usefulness as an appropriate measure. Plasma triglyceride concentration does not appear to be an independent predictor of CHD. Measurement of triglyceride in NHANES III is of questionable utility and is not recommended.
2. Methods

The Technicon Autoanalyzer I® (Technicon Instruments Corp., Tarrytown, NY) was used for cholesterol measurement in NHANES I, II, and Hispanic HANES. However, kits based on enzymatic methods are now commercially available and have been widely used for several years (such as Dow Diagnostics, Indianapolis; and Boehringer Mannheim Cat. No. 236691). Prior treatment of the sample is not required in the determination of total plasma cholesterol and only a single aqueous reagent is used. Comparison studies (Allain et al., 1974) indicate good correlation of the results with those obtained with Autoanalyzer II® (Technicon Instruments Corp., Tarrytown, NY) and with the reference method used in previous HANES of Abell et al. (1952). The analysis requires 100 µl of plasma.

HDL is not completely stable to freezing and erroneous results may be obtained if HDL-C is measured in the supernatant of frozen and thawed whole plasma samples after precipitation with manganese chloride and sodium heparin. Suggested alternatives to sending the frozen whole plasma samples from field collection laboratories to a central laboratory for analysis are to (1) ship the plasma samples in wet ice and perform the HDL-C analysis at the central laboratory within 4-5 days after sample collection, or (2) perform the HDL fractionation in the field and ship the frozen supernatant. A plasma volume of 0.5 ml is required for determination of HDL-C.

Radial immunodiffusion assays are available for the direct measurement of apoproteins B (Sniderman et al., 1975) and A-I (Cheung and Albers, 1977) in plasma.

If measured, serum linoleate/oleate cholesterol ester ratio could be determined by a method such as that of Bernert et al. (1982).

3. Basis for interpretative criteria

On the basis of their review of available data, the NIH Consensus Conference (Steinberg, 1985) concluded that individuals with elevated plasma cholesterol levels are at increased risk of developing premature CHD. Moderate risk cholesterol levels were defined as those between the 75th and 90th percentiles and high risk levels as those above the 90th percentile. By age, these levels are:

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<th>High risk</th>
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<td>30-39</td>
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<tr>
<td>≥40</td>
<td>&gt;240</td>
<td>&gt;260</td>
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4. **Target groups**

TC and HDL-C analyses should be performed for the entire NHANES III population. However, in view of the small blood volume drawn from preschool children and relatively large sample required for HDL-C analyses, it is recognized that HDL-C analyses for this age group may have lower priority than other analyses.

It is recommended that analyses for apoproteins B and A-I be conducted on a random subsample of adults in the NHANES III population.

5. **Other considerations**

Alcohol consumption, cigarette smoking, body weight, oral contraceptive agent use and estrogen replacement therapy, drug use, and physical activity affect HDL-C levels. Efforts should be made to identify these confounding variables in assessing HDL-C as an indicator of CHD.

The Panel notes that HDL-C concentration appears to be related to pubertal development in adolescents. Measurement of HDL-C in this population will provide an opportunity to relate this index to Tanner scores, if measured.

E. **INTENDED USES OF DATA**

Comparison of data on TC and HDL-C from NHANES III with that from NHANES I, II, and Hispanic HANES may show changes that can be associated with secular trends in incidence of diseases, especially coronary heart disease, and with diets and lifestyle factors. A thorough investigation of the comparability of the currently recommended and previously used methods is necessary so that the validity of determining secular trends can be assured.

The Panel supports the proposal that social security numbers and maiden names be recorded in NHANES III. This will enable the use of these data and the National Death Index in succeeding years to correlate cholesterol levels in individuals with CHD mortality.
F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends inclusion of the following measurements for cholesterol and blood lipids in order of priority:

- plasma total cholesterol concentration
- plasma high density lipoprotein cholesterol levels
- plasma apoproteins B and A-I
- serum linoleate/oleate cholesterol esters provided they are shown to be a useful indicator prior to NHANES III.
LITERATURE CITED


## XXII. HYPERTENSION

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A. INTRODUCTION

Kaplan (1982) concluded that high blood pressure is quantitatively the greatest single risk for premature death and disability. Race appears to be a factor in the development of hypertension; blacks generally have higher blood pressure than whites after the age of 25 years (Roberts and Maurer, 1977). Both elevated diastolic and systolic blood pressure are independently predictive of morbidity and mortality (Kannel et al., 1981). With increasing age, blood pressure increases at a greater rate in individuals with higher initial blood pressure (Svärdsudd and Tibblin, 1980; Wu et al., 1980).

The etiology of hypertension is considered to be multifactorial, but its relationship to the intake of various dietary constituents continues to be a subject of scientific controversy. Several dietary constituents have been associated (either positively or negatively) with blood pressure, particularly calcium, potassium, sodium, chloride, and magnesium (Ackley et al., 1983; Gruchow et al., 1985; Khaw and Thom, 1982; Kurtz and Morris, 1983; McCarron, 1982; McCarron et al., 1983; Resnick et al., 1983; Simpson, 1984; Sowers et al., 1985; Stanton et al., 1982; Tobian, 1975; Whitescarver et al., 1984). Alcohol intake has also been correlated with differences in blood pressure (Criqui et al., 1981; Gruchow et al., 1985; Kannel and Sorlie, 1975; Klatsky et al., 1977; Kromhout et al., 1985). Other dietary constituents postulated to be associated with hypertension include dietary fat, fiber, and protein (Anderson, 1983, 1985; Hodges and Rebello, 1985; Iacono et al., 1982; McCarron et al., 1983).

The findings of epidemiological and clinical studies on the relationships between diet and hypertension have been somewhat inconsistent. Hypotheses regarding the importance of intake of various dietary constituents on blood pressure have been questioned because of lack of control of possible confounding factors.

Many questions on the complex etiology of hypertension remain. While a survey such as NHANES will not address all issues, it can assess blood pressure distributions in the population and can identify associations between dietary factors and hypertension for further investigation in a clinical setting. The public health implications of elevated blood pressure and profound ramifications of this condition warrant its continued evaluation in the United States population. Knowledge of blood pressure trends in the population may aid in devising strategies for public health approaches to the problem.
B. AVAILABLE INDICATORS

1. Direct measurements

Brachial Artery Pressure. The measurement of brachial artery pressure by the mercury sphygmomanometer method is the accepted standard procedure for measuring blood pressure. "Random-zero" devices are the ideal method to reduce observer bias and digit preference (the tendency to record blood pressure readings ending with a particular number); however, quality control of equipment and observers has proved difficult to maintain in the field. Although blood pressure readings taken with a random-zero device would not be fully comparable to previous NHANES, the reduction in observer bias and digit preference may justify the addition of this technique for NHANES III.

Left Ventricular Mass (LVM). Left ventricular hypertrophy (LVH) may be an important indicator of organ damage resulting from hypertension. In adults, increased echocardiographic voltage is taken as clinical evidence for left ventricular hypertrophy (LVH) (Schieken et al., 1982). Echocardiography (ECG) was used to determine the occurrence of LVH in the Framingham study and LVH was highly associated with elevated blood pressure (Dawber, 1980). In all age groups, the annual incidence of coronary heart disease was nearly five times higher in individuals with LVH. Similarly, myocardial infarction, angina pectoris, and sudden death occurred more commonly in subjects diagnosed with LVH. Echocardiography has advantages over ECG in that it can determine LVM over a continuum, including LVH. Savage et al. (1979) used echocardiographic techniques to measure LVM and found that more than 60% of a group of 234 asymptomatic adults with mild to moderate hypertension demonstrated various cardiac abnormalities, including LVH. Echocardiographic measures of LVM in 181 children demonstrated that the LVM was significantly increased in children in the highest blood pressure group (Schieken et al., 1982). Echocardiography has also been useful in providing evidence of past elevations of blood pressure in individuals who exhibit similar blood pressures at the time of testing. Retrospective studies show that the level of blood pressure over time is significantly higher for subjects with LVH demonstrated by echocardiography than for individuals who do not have LVH (Savage et al., 1983).

2. Urinary electrolytes

The Panel concludes that evaluations of sodium, chloride, and potassium excretion in multiple 12- or 24-hour urine collections are more accurate measures of intake of these electrolytes than dietary recall. Dai et al. (1984) randomly sampled 1939 individuals aged 34-57 years and found that high blood pressure was associated with increased urinary sodium: potassium ratio and decreased urinary potassium concentration. The Panel recognizes that collection of samples other than casual
urine samples is not feasible in a survey such as HANES because of logistical problems in the complete collection and storage of 12- or 24-hour urine samples from such a large population group. However, recent research indicates that casual urine samples may provide useful information on the relationships between blood pressure and urinary excretion of electrolytes (Khaw, 1983; Khaw and Rose, 1982; Simpson and Paulin, 1981). Urinary calcium has also been shown to be related to blood pressure (Kesteloot and Geboers, 1982). Use of sodium:potassium and sodium:calcium ratios for interpreting electrolyte data would obviate the need for timed collection periods or for expressing excretion values per unit of creatinine.

3. Blood calcium

Although the interpretations are controversial, blood pressure appears to be directly related to total blood calcium and inversely related to ionized calcium (Kesteloot and Geboers, 1982; McCarron, 1982; Resnick et al., 1983). Total blood calcium can be measured easily and conveniently on a small quantity of blood. Ionized calcium, which is considered the physiologically active form of calcium, has been difficult to measure, but a calcium-specific electrode is now available which gives reliable and consistent results and is less fragile than previous models (Smith et al., 1983; Wandrup and Kvetny, 1985).

C. NHANES III APPROPRIATENESS

Blood pressure distribution was determined in all three previous HANES and in the earlier National Health Examination Surveys (NHES). Between NHES (1960) and NHANES II (1976-1980), mean systolic blood pressure declined 5 and 10 mm Hg for white and black adults, respectively (Dannenberg et al., 1985). The HANES are extremely useful for estimating long-term trends in blood pressure distribution and the prevalence of hypertension in the U.S. population. This is particularly important because of the large increase in drug treatment of high blood pressure since NHANES II. For this reason, methods to evaluate blood pressure in NHANES III should be as similar as possible to those used in previous HANES.

D. RECOMMENDED APPROACHES

1. Indices and methodology

Blood pressure measurements should be performed using a standard mercury sphygmomanometer to assure comparability with previous HANES. Additionally, a random-zero device should be used if adequate quality control can be maintained in the
field. Cuff size is an important variable in the determination of blood pressure measurements and should be as similar as possible to previous HANES. However, cuff size has not been comparable throughout HANES surveys: in NHANES I and NHANES II two cuff sizes (child and adult) were used, and in HHANES five cuff sizes (infant, child, adult, large, and thigh) were used. Because of the increased reliability of determinations resulting from the use of appropriate cuff size, the Panel recommends using the five sizes used in HHANES. The Panel realizes that the ability to estimate change in the prevalence of hypertension in the total population may be somewhat impaired by altering the cuff sizes from those used in NHANES I and II, but the sensitivity of measurements will be greatly improved.

At least two, and preferably three, sphygmomanometer determinations should be taken. The first measurement should be made by standard mercury sphygmomanometer in all subjects. Subsequent measurements can be done using the same technique or using a random-zero device. The Panel suggests that the protocol for blood pressure determination followed in HHANES be repeated in NHANES III. That is, measurements should be taken at two or three different times following 5 minutes of relative immobility in a sitting position. Procedures for placement of the cuff should be as in previous HANES.

The Panel recommends that Korotkoff phases I, IV, and V be reported by the examiner in NHANES III. Measurement of all three phases will increase accuracy and reduce digit preference. Systolic blood pressure (SBP) should be taken as Korotkoff phase I and diastolic blood pressure (DBP) as Korotkoff phase V in adults. Korotkoff phase IV best represents DBP in children as Korotkoff phase V may not occur (Kirkendall, 1980).

Each subject should have blood pressure measurements taken at the same stage of the examination (preferably before venipuncture); however, the Panel recognizes that the logistics of survey operations may preclude this possibility.

Assessment of LVH should be performed in NHANES III to aid in providing evidence of organ damage associated with hypertension, as determined by a single casual blood pressure reading. The Panel recommends that electrocardiography, if included, be done using the same protocol as in previous HANES. However, the Panel also recognizes that newer methodology, such as echocardiography, may be preferable for the determination of LVM. Echocardiography recording techniques, screening criteria for acceptability, and measurement guidelines can be standardized to achieve high within- and between-observer reproducibility (Schieken et al., 1979). The Panel defers to the judgment of expert cardiologists on which of these methods should be used for the determination of LVH in NHANES III.
Serum ionized calcium determination is recommended using the ion-selective electrode procedure. Urinary sodium, potassium, and calcium should also be measured by currently acceptable techniques. The Panel recommends that these data be expressed as the ratio of sodium:potassium and sodium:calcium.

2. Basis for interpretative criteria

Differences exist as to interpretation of blood pressure values. Various organizations (National High Blood Pressure Coordinating Committee, 1984; World Health Organization, 1978), as well as independent investigators (Kaplan, 1982), have proposed diagnostic criteria for defining hypertension. Defining the term "hypertension" implies that there is an arbitrary cutoff point, above which individuals are considered to be in a disease state and below which the values are considered normal or disease-free (Dawber, 1980). This may lead to problems when reporting prevalence estimates for "hypertension" in a population. For example, based on NHANES II data, the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure has reported that more than 60 million individuals aged 6-74 years have blood pressure in excess of 140/90 mm Hg, or have been told by a physician that they have hypertension (National High Blood Pressure Coordinating Committee, 1984). Alternatively, in NHANES I (1971-1974), 23.2 million adults (18-74 years) were estimated to have hypertension, defined as SBP at least 160 mm Hg or DBP at least 95 mm Hg on a single reading, or were normotensives being treated for hypertension (Roberts and Maurer, 1977). Although it is necessary to establish cutoff values for determination of hypertensive status, most experts agree that there is a continuous quantitative relationship of blood pressure to morbidity and mortality. That is, as blood pressure increases, so does the risk of cardiovascular disease and stroke (Kaplan, 1982). The Panel accepts the following classification scheme for persons aged 18 years or older as recommended by the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (National High Blood Pressure Coordinating Committee, 1984):

<table>
<thead>
<tr>
<th>DBP (mm Hg)</th>
<th>Category*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;85</td>
<td>Normal BP</td>
</tr>
<tr>
<td>85-89</td>
<td>High Normal BP</td>
</tr>
<tr>
<td>90-104</td>
<td>Mild Hypertension</td>
</tr>
<tr>
<td>105-114</td>
<td>Moderate Hypertension</td>
</tr>
<tr>
<td>&gt;115</td>
<td>Severe Hypertension</td>
</tr>
</tbody>
</table>

182
SBP (mm Hg)  
(when DBP < 90)  
< 140  
140–159  
> 160  

Category*  
Normal BP  
Borderline Isolated Systolic Hypertension  
Isolated Systolic Hypertension  

* A classification of borderline isolated systolic hypertension (SBP 140–159 mm Hg) or isolated systolic hypertension (SBP > 160 mm Hg) takes precedence over a classification of high normal blood pressure (DBP 85–89 mm Hg) when both occur in the same individual. A classification of high normal blood pressure (DBP 85–89 mm Hg) takes precedence over a classification of normal blood pressure (SBP < 140 mm Hg) when both occur in the same person.

In children, reliable determination and evaluation of blood pressure measurements are difficult. Sustained high blood pressure appears to be rare in children (Rames et al., 1978); however, children with blood pressures in the upper percentiles are likely to demonstrate hypertension as adults (Berenson et al., 1983; Hofman, 1984; Hofman et al., 1985). The following upper limits of normal blood pressure for children have been suggested by the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (National High Blood Pressure Coordinating Committee, 1984):

<table>
<thead>
<tr>
<th>Age (in years)</th>
<th>Arterial Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14–18</td>
<td>&lt; 135/90</td>
</tr>
<tr>
<td>10–14</td>
<td>&lt; 125/85</td>
</tr>
<tr>
<td>6–10</td>
<td>&lt; 120/80</td>
</tr>
<tr>
<td>&lt; 6</td>
<td>&lt; 110/75</td>
</tr>
</tbody>
</table>

Interpretative criteria for LVH will depend on methods used for analysis.

3. Target groups

All subjects 6 years and older should have blood pressure measured. Urinary electrolytes and serum ionized calcium should also be measured in this population group. The presence of LVH should be evaluated in all adult subjects with moderate or severe hypertension, as previously defined, plus a random sample of adults to serve as a control group.
4. **Other considerations**

Pertinent lifestyle factors such as alcohol and tobacco use, as well as dietary intake, will be assessed in the general health and dietary questionnaires. Ancillary information that should be obtained includes current hormone use (including oral contraceptive agents), antihypertensive medication, salt- and fluid-retaining drugs, as well as past history of hypertension.

The stage of the examination at which blood pressure is measured should be recorded. Season of year during which measurements are taken should be identified.

E. **INTENDED USES OF DATA**

Because of the cross-sectional nature of this survey, data collected will have limited value in improving understanding of the etiology of hypertension. Nevertheless, sphygmomanometer measurements obtained in NHANES III can be used to monitor the blood pressure status of the U.S. population. The first blood pressure measurement, taken with a standard sphygmomanometer in a sitting position, can be used for comparison with the first measurement taken in NHANES I and II to determine if trends are evident in population subgroups. Subsequent measurements with a random-zero device, can be used to provide a more reliable assessment of the prevalence of elevated blood pressure.

The prevalence of LVH in the general adult population and its relationship to current blood pressure status can also be assessed. Data can be used to partition subjects into two groups: individuals with elevated blood pressure with evidence of organ damage (LVH) and individuals with elevated blood pressure without evidence of organ damage. Through subsequent follow-up in the National Death Index, the possible relationships between blood pressure status and the cause of death may be identified.

Measurement of serum ionized calcium and urinary electrolytes will be useful in assessing relationships between these electrolytes and blood pressure.
F. CONCLUDING STATEMENT

Based on the foregoing discussion, the Panel recommends assessment of blood pressure status in NHANES III by the following measurements:

- brachial artery pressure by mercury sphygmomanometer, with and without a random-zero device, in individuals 6 years and older
- LVM or LVH in adults with hypertension plus a random subsample
- serum ionized calcium in individuals 6 years and older
- urinary sodium:potassium ratio and sodium:calcium ratio in a casual urine sample in individuals 6 years and older.
LITERATURE CITED


XXIII. OSTEOPOROSIS

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<th>Title</th>
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</table>
XXIII. OSTEOPOROSIS

A. INTRODUCTION

Osteoporosis is one of several syndromes involving development of osteopenia. Osteopenia is manifested by decreases in the absolute levels of bone mass and develops over a time continuum. These manifestations may be produced by a variety of different pathophysiologic mechanisms. Osteoporosis is not a discrete disease state; discussion of the syndrome must include consideration that the amount of bone mineral decreases and the fracture incidence increases with increasing age after age 35 years in the general population (Parfitt, 1984a).

Maximum bone mass is attained around 35 years of age for cortical bone and probably at a slightly younger age for trabecular bone (Parfitt, 1984a). Males attain a peak bone mass about 30% greater than that of females and blacks reach a peak bone mass about 5 to 10% greater than that of whites. Individual differences in cortical thickness are great within each demographic group; the coefficient of variation is about 10 to 15% (Garn, 1981).

Peak adult bone mass accrued early in adulthood may be an important determinant of bone mass remaining later in life. After attainment of peak bone mass, bone is lost at varying rates over the remainder of the lifespan with an average annual loss rate of about 1%. There is loss of both cortical and trabecular bone from the skeleton with age and the loss may vary significantly from one site to another (Parfitt, 1984b). The distribution and determinants of these losses may be influenced by different factors. In addition to age, decreased bone density is related to sex and race and is most common in white postmenopausal women and elderly men. Although not conclusively ascertained, risk factors for bone mineral loss may include sedentary lifestyle, lighter body weight, smoking, alcohol abuse, and certain diseases or drug treatments (Johnston, 1984; Melton and Riggs, 1983). Nutritional factors may also play a role in the development of osteoporosis although a firm link of dietary intake with development of osteoporosis has not been established. Among nutrients receiving greatest attention in this respect are calcium (Matkovic et al., 1979), phosphorus (Anderson et al., 1977), and protein (Allen et al., 1979).
B. AVAILABLE INDICATORS

1. Noninvasive examination of bone

Single Photon Absorptiometry. The bone mineral content (BMC) of the radius is highly correlated with total body calcium (Chesnut et al., 1975), skeletal weight (Mazess 1971, 1979), and ash weight of bone (Sorenson and Cameron, 1967). Forearm BMC may be assessed by single photon absorptiometry, a technique that is applicable only to the appendicular skeleton and is considered the most practical method to assess bone mineral content of the limbs (Wahner et al., 1983). According to Kimmel (1984), the technique takes approximately 5 minutes and involves a radiation exposure to local tissues of 2 to 5 mrad. (Kimmel, 1984) has compared techniques for measuring bone mineral content and reported that single photon absorptiometry has a precision of 3 to 5% on repeated measurements and a reliability of 1 to 4% in relation to values determined by ashing (taken as the reference standard). This method was reported to be a "precise method for documenting bone mineral content in population surveys and for determining the rate of bone loss in prospective longitudinal studies" (Khairi et al., 1976).

For determination of bone mineral content, single photon absorptiometry appears greatly superior to simple radiographic assessment techniques which have severe limitations with regard to intraobserver variability, polychromatic radiation, and variable film response (Adams et al., 1969; Naor et al., 1972). Single photon absorptiometry also exposes the subject to smaller amounts of radiation (2 to 5 mrad) than radiographic assessment (30 mrad for a chest x-ray). BMC is determined by the amount of transmitted radiation, measured by a scintillation counter, which is inversely related to the quantity of mineral positioned in the beam path (Boyd et al., 1974). BMC (g/cm) is usually expressed on a per bone width basis (BMC/BW) in g/cm², in order to standardize measurements among persons with widely varying bone sizes (Harper et al., 1984). Alternatively, BMC normalized by cortical bone area has been suggested as a standardization procedure to reflect changes in geometric remodeling (Hayes and Gerhart, 1985).

Bone mineral content of the forearm has been measured most often at the distal radius and at one-third of the distance from the distal end. Methods of locating sites for measurement by single photon absorptiometry were discussed by Parfitt et al. (1985). Aubrey et al. (1984) compared use of a distal radius site at which the radius and ulna were 5 mm apart with use of a midradius site one-third of the distance from the distal end of the radius. The correlation coefficients of lumbar spine bone mineral content to distal radius bone mineral content at the 5 mm site and to the midradial site was approximately 0.50. Aubrey et al. (1984) also reported that the 5 mm site contained more than 50% trabecular bone, similar to the proportion of trabecular bone in the spine; however, other investigators report that the
distal radius contains about 25% trabecular bone (Wahner et al., 1983). The trabecular bone of the distal radius has no hemato-
poietic marrow and may have a slower rate of turnover than
trabecular bone at some other sites (Parfitt et al., 1985).
Thus, radius measurements may not accurately indicate bone
mineral content or density of lumbar and thoracic vertebrae,
particularly in patients with metabolic bone diseases (Mazess
et al., 1984a).

**Dual Photon Absorptiometry.** Bone mineral content of the
axial skeleton can be determined by dual photon absorptiometry.
This technique permits direct measurement of two common fracture
sites of osteoporosis patients, the lumbar spine and the proximal
femur (Gotfredsen et al., 1984; Wahner et al., 1983). The tech-
nique takes 15 to 20 minutes and involves a radiation exposure to
local tissues of 5 to 15 mrad. A peak skin dose of 18 mrad was
recently reported for commercial instruments (Dunn and Wahner,
1985). The precision of dual photon absorptiometry is 1 to 3%
and the reliability of the method is 4 to 6% (Kimmel, 1984).

The L2 to L4 vertebrae are preferred for dual photon
absorptiometry measurements. Because dual photon absorptiometry
measures all bone in the path, the sternum interferes with mea-
surement of vertebrae in the thoracic spinal column and calcifi-
cations in the aorta and osteoarthritis of the spine can also
introduce error into the determination of spinal bone mineral
density. Compression fractures are more common in the T12 and
L1 vertebrae than in the L2-L4 vertebrae. Such fractures can
change the density values of the vertebrae, making evaluation
difficult. The L5 vertebra is difficult to distinguish by dual
photon absorptiometry. The correlation between bone mineral
content of thoracic and lumbar vertebrae is 0.85 (Trotter et al.,
1960; Wilson, 1972).

Measures of bone mineral content in axial and appen-
dicular locations suggest that loss of trabecular bone from
the axial skeleton is greater than loss of cortical bone from
appendicular sites in younger osteoporosis patients with com-
pression fractures (Riggs et al., 1981, 1982; Wahner et al.,
1983). However, losses of cortical and trabecular bone have
been shown to be similar in older osteoporosis patients with
femoral fractures (Riggs et al., 1982). Little association
was shown between BMC/BW of the radius and spinal bone mineral
content in patients with osteoporosis. Spinal bone mineral
content predicted from the BMC of the distal third of the radius
was overestimated by 12 to 15% for osteoporosis patients and
by 6 to 10% for normal subjects (Mazess et al., 1984a). Bone
mineral content of spine distinguished between patients with
osteoporosis (diagnosed by presence of vertebral fracture) and
age- and sex-matched normal subjects to a greater extent than
bone mineral content of mid- or distal radius (Riggs et al.,
1981; Wahner et al., 1983). BMC of both mid- and distal radius
of almost all osteoporosis patients fell within the 90% confi-
dence limits for BMC of age- and sex-matched normal subjects.
(Riggs et al., 1981). Because prediction of bone mineral content of the axial skeleton from bone mineral content of the appendicular skeleton is of limited accuracy, direct measurement of axial and appendicular fracture sites is required for either diagnosis or long-term monitoring of bone disease (Mazess et al., 1984a; Parfitt et al., 1985).

Roentgenography. The Panel was apprised by NCHS that radiological examinations would not be allowed as a part of the NHANES III protocol. It should be noted, however, that x-ray measurements of the spinal column would reveal the occurrence of crush fractures of the vertebrae and thus could be useful as an index of the occurrence of spinal osteoporosis.

Computed Tomography. Computed tomography (CT) can be used to measure bone mass of both the appendicular and axial components of the skeleton (Mazess, 1981; Orphanoudakis et al., 1979; Parfitt, 1983). X-ray CT, like conventional roentgenographic measures, will not be allowed for use in NHANES III (see above). 125I-CT can be used to measure trabecular bone of the distal radius (Rüegsegger et al., 1974). However, the accuracy of CT measures of trabecular bone is reduced because a dual-energy procedure is needed for accuracy and because precision is diminished by the large variations occurring in bone structure. Therefore, small position changes can result in markedly different results during examination (Cann et al., 1980; Mazess, 1981; Weissberger et al., 1978). Increased bone marrow fat content may falsely lower CT measurements 20 to 25% in the elderly (Mazess, 1983). The dual energy CT method would not be feasible for NHANES III because the equipment is not portable and the procedure involves a high radiation dose (200 to 250 mrad) (Kimmel, 1984).

Ultrasound. Use of ultrasound has been investigated as a method for measuring physical characteristics of bone. Langton et al. (1984) summarized data showing that the slope of the ultrasonic attenuation of the calcaneus differed for postmenopausal women with and without fractures. However, the relationship of changes in the calcaneus to changes in bones likely to fracture in osteoporotic patients has not been established. Velocity of ultrasound has also been investigated as an accessory measure to BMC (measured by single photon absorptiometry) of the proximal radius (Greenfield et al., 1981). Abnormal ultrasound values alone were less closely associated than BMC with spinal radiographs showing evidence of osteoporosis. The investigators considered that velocity of ultrasound measures might provide an adjunct measure to improve the association of BMC of the radius with incidence of osteoporosis (Greenfield et al., 1981). Neither type of ultrasound measure has yet been validated as a useful means of detection of bone changes indicative of osteoporosis.
2. Selected factors associated with calcium homeostasis

1,25-Dihydroxy-Vitamin D. Although one study of vitamin D metabolites in postmenopausal osteoporosis patients and age-matched control subjects showed no difference in plasma levels of 1,25-dihydroxy-vitamin D (Crilly et al., 1981), other studies have indicated that levels of circulating 1,25-dihydroxy-vitamin D are lower in women with postmenopausal osteoporosis and elderly men with osteoporosis than in normal age-matched control groups (Gallagher et al., 1979; Loré et al., 1984; Sørensen et al., 1982). Studies have shown that the mean concentrations of 1,25-dihydroxy-vitamin D for osteopenic patients having at least one spinal compression fracture may be low: 19.4 ± 2.8 pg/ml (Sørensen et al., 1982), 25.9 ± 1.5 pg/ml (Gallagher et al., 1979), and 27 ± 9.9 pg/ml (Loré et al., 1984). In these same studies, normal age- and sex-matched control subjects' mean concentrations were 34.8 ± 2.8 pg/ml, 33.2 ± 2.3 pg/ml, and 41.2 ± 19.4 pg/ml, respectively. Although the differences between the means were statistically significant in all three studies, the distributions of the individual values for the three groups overlapped considerably. Data on 1,25-dihydroxy-vitamin D levels in normal Japanese subjects and patients with osteoporosis were similar; i.e., there was a significant difference between the means for normal subjects and patients having the most severe cases of osteoporosis but not for those classified as having milder forms of osteoporosis (Okano et al., 1979). Although considerable overlap was shown between the distributions of values for the normal subjects and patients with osteoporosis, classification of severity of osteoporosis may aid in interpretation of 1,25-dihydroxy-vitamin D levels in osteoporosis patients.

Any valid interpretation of serum levels of 1,25-dihydroxy-vitamin D will require knowledge of serum levels of 25-hydroxy-vitamin D, parathyroid hormone, and phosphorus as well as calcifiable bone mass ascertained from bone biopsy samples.

25-Hydroxy-Vitamin D. Of three studies (Gallagher et al., 1979; Loré et al., 1984, Sørensen et al., 1982) comparing serum levels of 25-hydroxy-vitamin D in subjects with osteoporosis and normal age- and sex-matched controls, two reported significant elevations in 25-hydroxy-vitamin D concentrations. Loré et al. (1984) found that osteoporosis patients had serum levels of 25-hydroxy-vitamin D of 29.2 ± 11.5 ng/ml while normal subjects had serum levels of 8.2 ± 5.7 ng/ml. Estimation from graphic data of Sørensen et al. (1982) suggests that mean serum concentration of 25-hydroxy-vitamin D of osteoporosis patients was approximately 31 ng/ml and that of control subjects was approximately 12 ng/ml. All patients in the study of Sørensen et al. (1982) were taking vitamin D supplements (dosages not specified). Use of vitamin D supplements by subjects in the study of Loré et al. (1984) was not stated. However, in another
study of hospitalized elderly women no correlation was found between circulating levels of 25-hydroxy-vitamin D and bone mineral content determined by single photon absorptiometry (Ohata and Fujita, 1979).

Calcitonin. Calcitonin deficiency has been implicated in the development of postmenopausal osteoporosis (Taggart et al., 1982). However, others have reported no decrease in calcitonin after menopause and there is an increase in calcitonin after oophorectomy (Milhaud et al., 1983). While circulating calcitonin levels are easily measured by radioimmune assay (RIA), the role of this measurement as an indicator of the occurrence of osteoporosis or degree of its development is as yet unclear.

Dietary Calcium. The influence of dietary calcium intake on development of osteoporosis has not been established. Matković et al. (1979) reported that lower calcium intakes in persons of the same ethnic background in Yugoslavia were associated with decreased bone mass of the metacarpal and higher fracture rate of the proximal femur. The data of these investigators suggested that calcium intake is an important determinant of bone mass in young adults although it may have little influence on age-related bone loss. Differences in bone density of postmenopausal women appeared to be related to self-reported prior milk consumption during childhood and adolescence (Sandler et al., 1985). Other investigators have not found a relationship between reported short-term or habitual calcium intake and bone mass (Donath et al., 1975; Sowers et al., 1985).

Dietary Phosphorus. Diets adequate in calcium (1.2% by weight) and containing a relatively high ratio of phosphorus to calcium (1:1) have been shown to increase bone loss in aging mice (Krishnarao and Draper, 1972). Similarly, supplements of phosphorus to diets adequate in calcium (1.1 g Ca/d, 2.8 g P/d) resulted in increased bone resorption from ulna and iliac crest in adult mongrel dogs (Laflamme and Jowsey, 1972) and diets containing adequate calcium and high levels of phosphorus were associated with increased bone loss and lameness in horses (Krook, 1968). In contrast, bones of monkeys (Cebus albifrons) fed diets containing either low or adequate levels of calcium and high levels of phosphorus (Ca:P ratios of 1:2.2 and 1:4, respectively) for 3 to 88 months developed only minimal histologic differences in bones and no changes detected by radiography or 125I photon absorptiometry (Anderson et al., 1977). Also, African Bantus, a group that has not shown evidence of nutritional osteopenia, consume diets low in calcium and high in phosphorus in comparison to white groups who consume higher levels of calcium (Walker, 1972). Some evidence from animal studies suggests that reduced calcium excretion following administration of phosphate is mediated by parathyroid hormone (PTH) (Laflamme and Jowsey, 1972). Large oral doses of phosphates resulted in transitory increases in serum PTH in humans in two studies (Reiss et al., 1970; Van Den Berg et al., 1980) but not in a third (Goldsmith et al., 1976). Effects of these doses of
phosphate on bone were not determined. A relationship between dietary phosphorus intake and bone integrity has not been examined extensively by epidemiologic studies. High phosphorus diets usually also contain high levels of protein which may also affect calcium and bone metabolism.

**Dietary Protein.** High protein intakes by humans result in increased urinary excretion of calcium (Chu et al., 1975; Linkswiler et al., 1974; Margen et al., 1974). These effects were observed when diets were fed containing 142 to 560 g protein/day. Customary protein intake of western population is about 100 g/day (Crim and Munro, 1984). Such effects have persisted for experimental periods as long as 60 days (Hegsted and Linkswiler, 1981; Johnson et al., 1970). Protein composition may affect calcium excretion. Proteins having a high content of sulfur amino acids have been associated with increased hypercalciuria in humans (Zemel and Linkswiler, 1981). Protein sources having a high phosphorus content (meat) did not result in increased urinary calcium excretion (Spencer et al., 1981). Effects of varying either protein or phosphorus intakes singly, with calcium intake held constant at 500 mg/day, were analyzed by Hegsted et al. (1981). Higher phosphorus intake in this study was associated with decreased urinary loss of calcium whereas higher protein intake was associated with increased urinary calcium excretion, particularly when phosphorus intake was low.

The effect of high protein intake on bone mineral content is not known. One study suggested that bone mineral content of female vegetarians aged 60-87 years was greater than that of age- and sex-matched omnivores (Sanchez et al., 1980); however, that study and others did not find differences in bone mineral content or calcium balance of younger subjects (Anand and Linkswiler, 1974; Ellis et al., 1974; Sanchez et al., 1980).

C. **NHANES III APPROPRIATENESS**

The prevalence of osteoporosis in the U.S. population is not known. Sites most likely to fracture with increasing age are the proximal femur, distal radius, proximal humerus, pelvis, and vertebrae (Melton and Riggs, 1983). Based on roentgenograms of the dorso-lumbar spine, Iskrant and Smith (1969) attributed about 70% of fractures in persons age 45 years and older to osteoporosis. Application of this factor to the approximately 1.9 million fractures occurring each year in the United States in persons age 45 years and older results in an estimate of 1.3 million fractures that may occur as a result of osteoporosis (Kelsey, 1984). Use of age- and sex-specific fracture rates of the population of Rochester, Minnesota allowed estimation of excess fracture rates among United States whites age 40 years or older compared with fracture rates of persons age 30-39 years (Melton and Riggs, 1983). The excess of fractures varied considerably with site, but, overall, these estimates suggest that at least half of all adult fractures may be related to osteoporosis (Melton and Riggs, 1983).
Because nutritional factors may be involved in attainment of peak adult bone mass and the subsequent development of osteoporosis, it is recommended that NHANES III make a strong effort to determine in a national population sample the age at which maximum bone mass is attained. Peak adult bone mass may be one of the most important determinants of bone mass remaining later in life. Knowledge of the amount of bone mass present in the adult population based on a national population sample for which risk factors of bone loss are also evaluated would aid in further characterization of patterns of loss among subsets of the adult population.

D. RECOMMENDED APPROACHES

1. Indices

At the present time, measures of bone mineral content of both the axial and appendicular skeleton are necessary for monitoring of bone status. Based upon current methodology, the Panel recommends that forearm BMC be determined in all subjects 18 years and older. Lumbar spine BMC should be determined in male subjects 30 years of age and older as well as in all women carefully defined as postmenopausal. However, the Panel recognizes that state-of-the-art equipment and methodology are undergoing development and further advances in assessment and interpretation of bone mineral content may be made by the time of NHANES III. For this reason, the Panel recommends that selection of specific indices for determination of bone mineral content be reevaluated in late 1986 in order that the methodology considered optimal at the time of NHANES III may be considered for use. The Panel recommends further that such methods be investigated in a pilot study for feasibility of inclusion in the survey and that a reexamination of available methods should be made prior to the pilot study. Of the factors associated with calcium homeostasis discussed in Section B-2, determination of current dietary calcium intake and, if a feasible method is developed, a history of dietary calcium intake during childhood, adolescence, and young adulthood is recommended as an adjunct measurement in all subjects. Information concerning water consumption, the calcium content of the water supply, and the length of time of residence in the community might also supply useful information about calcium intake.

2. Methods

The Panel defers recommendation of specific methods for determination of bone mineral content and anatomic sites for such determinations in order that improved methodology be considered. At the time of selection of indices to be included in NHANES III, appropriate experts should be consulted on specific methodology and equipment.
3. **Basis for interpretative criteria**

Low levels of bone mass, i.e., the lower limit of the normal range in bone mass of young adults, have been the most often used criterion for diagnosis of osteoporosis (Horsman et al., 1981; Parfitt, 1984a). Examples of studies employing such criteria follow. A 10-15% deficit in BMC of the radius adjusted for bone width, age, height, and body weight was considered an indicator of osteopenia in Eskimos compared with a control population of Wisconsin white adults (Harper et al., 1984; Mazess, 1979). Studies are available on bone mineral content in the midshaft and distal end of the radius in normal U.S. whites (763 children ages 15-19 years, 538 adults ages 20-49 years, and 550 adults over age 50) (Mazess and Cameron, 1974) and in normal white children and young adults ages 6-39 years from Wisconsin and Indiana (Hui et al., 1985). Bone analysis in the above studies was done by single photon absorptiometry. In a study of total body and regional bone mineral measured by dual photon absorptiometry, patients with osteoporosis were found to have bone mineral content at least 20% less than that of normal males and females of the same age (Mazess et al., 1984b).

Evidence from recent biomechanical studies of bone suggests that fracture risk is associated with absolute levels of bone mass (Hayes and Gerhart, 1985; Melton et al., 1985; Parfitt, 1984b). Prediction of fracture risk may be possible based on bone mass measured in NHANES III and subsequent follow-up of a cohort of NHANES III participants.

4. **Target groups**

Target groups for methods considered most feasible at this time were identified in Section D-1. Oversampling of specific groups is not recommended. Depending on the methods selected for NHANES III, specific measures on subsets of the population may be appropriate.

5. **Other considerations**

History of bone fracture in adults in the absence of severe trauma should be ascertained. History of diseases affecting bone status and use of medications affecting calcium balance, including diuretics and calcium-containing antacids, should be determined. Additionally, questions concerning lifestyle factors thought to affect bone mineral content, such as extent of participation in weight-bearing exercise and use of alcohol and tobacco, should be included.
E. INTENDED USES OF DATA

Potential uses for these data include: 1) description of bone mineral content in the adult population; 2) provision of baseline data on bone mineral content in relation to age; 3) provision of baseline data for follow-up studies of changes in bone mineral content of a cohort over time; and, 4) possible development of criteria for prediction of bone loss over time. In combination with the medical history and information obtained by questionnaire including a history of extent of participation in weight-bearing exercise, determinants of peak adult bone mass and risk factors for osteoporosis may be identified on a population basis. Dietary data relating present calcium intake to bone status should be interpreted cautiously because current calcium intake has not been found to correlate well with determinations of bone status. If it proves feasible to obtain a reasonable estimate of prior intake of calcium during childhood and adolescence, NHANES III could provide a means of examining the relationship of calcium intake early in life to bone mineral content later in life on a population basis.

F. CONCLUDING STATEMENT

Based upon the foregoing discussion, the Panel recommends that NHANES III include assessment of bone mineral content in the U.S. population. The measures listed below are currently considered appropriate based on the present status of methodology for bone mineral assessment. The Panel concludes that single photon absorptiometry and dual photon absorptiometry have considerable promise for the determination of the bone mineral content of the forearm and lumbar vertebrae, respectively in NHANES III; however, because changes in methodology and equipment and advances in knowledge are occurring rapidly in this field, the Panel recommends that final selection of specific methods for determination of bone mineral content be deferred until late 1986. The Panel recommends further that the methods selected be evaluated in a pilot study to ensure that they are feasible to include in the full 6-year period contemplated for NHANES III:

- bone mineral content of the forearm in all subjects 18 years of age and older
- bone mineral content of the lumbar vertebrae in male subjects 30 years of age and older and in all women carefully defined as postmenopausal; and
- history of calcium intake (if feasible) during childhood, adolescence, and young adulthood, as well as current calcium intake, of all subjects.
LITERATURE CITED


Chesnut et al., 1975. (See Chestnut et al., 1975).


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