

RELATIONSHIP BETWEEN BEEF CONSUMPTION AND VITAMIN B12 INTAKE
AND STATUS OF HEALTHY MEN AND WOMEN

By

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TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS.....	ix
ABSTRACT.....	xi
CHAPTER	
1 INTRODUCTION.....	1
Hypotheses.....	2
Specific Objectives	2
2 LITERATURE REVIEW	3
Vitamin B12.....	3
Chemistry	3
Dietary Sources	4
Absorption	6
Transport.....	7
Storage.....	7
Excretion.....	8
Biochemical Functions	8
Dietary Reference Intakes	10
Vitamin B12 Deficiency.....	11
Etiology	11
Clinical symptoms.....	14
Health related risks of a vitamin B12 deficiency	15
Vitamin B12 Status Assessment.....	20
Serum Vitamin B12 Concentration	20
Methylmalonic Acid Concentration	20
Holotranscobalamin Concentration.....	21
Serum Homocysteine Concentration.....	22
Megaloblastic Anemia.....	22

Dietary Intake Assessment in Adults	22
Twenty-four hour recall method	22
Multiple-day food record	24
Food frequency questionnaire	25
Assessment of vitamin B12 intake	26
Vitamin B12 Status in the United States	30
Vegetarianism	31
Definitions of Vegetarianism and Prevalence in the United States.....	31
Assessment of Dietary Adequacy of Vegetarian Diets	32
Assessment of Beef Consumption and Health.....	33
Assessment of Dietary Adequacy of Beef Consumption	35
Research Rational and Potential Application of Findings	35
3 MATERIALS AND METHODS	38
Study Design and Methods Overview	38
Diet History Questionnaire	39
Overview	39
Modifications Made to the Paper Version of the Diet History Questionnaire	39
Modifications Made to Diet*Calc Analysis Software.....	40
Data Generation.....	41
Diet History Questionnaire Instruction Pretest.....	41
Human Subjects Procedures	42
Diet History Questionnaire Instructions	42
Sample Collection and Processing.....	43
Processing of Plasma for Vitamin B12 Analysis	43
Processing the Diet History Questionnaire.....	44
Analytical Methods.....	44
Formation of Dietary Groups	44
Identification of Food Groups from the Diet History Questionnaire	45
Analysis of Additional Nutrients.....	45
Plasma Vitamin B12 Concentration	46
Statistical Methods.....	47
4 RESULTS	49
Demographic Characteristics of the Study Population	49
Subjects.....	49
Demographic Characteristics.....	49
Dietary Vitamin B12 Intake.....	50
Beef, Poultry, Pork, Seafood, and Mixed Foods	52
Dairy and Eggs	53
Cereal.....	53
Fortified Soy Products, Meal Replacements, and Other Sources.....	54
Naturally-Occurring and Fortified Sources	54
Dietary Intake of Macronutrients and Micronutrients	58
Energy Intake.....	58

Protein Intake.....	58
Carbohydrate Intake	59
Fat Intake	59
Saturated Fat.....	59
Folate Intake	60
Vitamin B6 Intake	60
Iron Intake	60
Zinc Intake.....	60
Plasma Vitamin B12 Concentration	61
Vitamin B12 Status.....	61
Vitamin B12 Intake and Status.....	64
5 DISCUSSION AND CONCLUSION	65
APPENDIX	
A SUBJECT DATA COLLECTION FORM.....	73
B DIET HISTORY QUESTIONNAIRE.....	79
C SCRIPT FOR DIET HISTORY QUESTIONNAIRE INSTRUCTIONS	119
D DIRECTIONS AND SURVEY FOR DIET HISTORY QUESTIONNAIRE PRETEST	125
E ADDITIONAL INSTRUCTIONS PACKET	128
LIST OF REFERENCES.....	134
BIOGRAPHICAL SKETCH	143

LIST OF TABLES

<u>Table</u>	<u>page</u>
2-1 Dietary vitamin B12 sources	6
3-1 Daily and weekly beef intake frequency	44
3-2 Foods categorized within each food group	46
4-1 Demographic characteristics of dietary groups	50
4-2 Daily total dietary intake of vitamin B12	51
4-3 Daily mean vitamin B12 contribution by dietary sources	55
4-4 Daily mean dietary intake of macronutrients	59
4-5 Daily mean dietary intake of micronutrients	61
4-6 Plasma vitamin B12 concentration	61
4-7 Plasma vitamin B12 concentration among non-vegetarians and vegetarians	63

LIST OF FIGURES

<u>Figure</u>	<u>page</u>
2-1 Structure of cobalamin	4
2-2 Vitamin B12 function in the remethylation pathway of homocysteine to methionine.....	9
2-3 Role of cobalamin in the formation of succinyl-CoA.....	10
4-1 Mean vitamin B12 intake compared to recommended intakes.....	52
4-2 Percent of total vitamin B12 intake from food sources.....	56
4-3 Percent of total vitamin B12 intake from cereal sources	56
4-4 Percent of total vitamin B12 intake from shellfish and fish.....	57
4-5 Percent of total vitamin B12 intake from naturally-occurring and fortified sources of vitamin B12.....	58
4-6 Plasma vitamin B12 concentration.....	62
4-7 Vitamin B12 status among dietary groups	63
4-8 Percent of individuals deficient among non-vegetarians and vegetarians.....	64

LIST OF ABBREVIATIONS

Abbreviation	Meaning
AMPM	automated-multiple-pass method
ANOVA	analysis of variance
BMI	body mass index
CARDIA	coronary artery risk development in young adults
CNS	central nervous system
CSFII	Continuing Survey of Food Intakes by Individuals
DHQ	Diet History Questionnaire
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EAR	Estimated Average Requirement
EDTA	ethylenediaminetetraacetic acid
EPIC-Oxford	European prospective investigation into cancer and nutrition-Oxford
FFQ	food frequency questionnaire
FIN	food identification number
g	gram
IF	intrinsic factor
kcal	kilocalories
kg	kilogram
L	liter
LDL	low-density lipoprotein
m	meter
MCV	mean corpuscular volume
mg	milligram
mL	milliliter
MMA	methylmalonic acid
MS	methionine synthase
NCI	National Cancer Institute
NDS-R	Nutrient Data System for Research
NFCS	Nationwide Food Consumption Survey
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NTD	neural tube defects
OSC	Optimal Solutions Corporation
oz	ounce
pmol	picomole
RDA	Recommended Dietary Allowance

SAH	<i>s</i> -adenosylhomocysteine
SAM	<i>s</i> -adenosylmethionine
SD	standard deviation
TC	transcobalamin
THF	tetrahydrofolate
UL	Tolerable Upper Intake Level
UNC	University of North Carolina, Chapel Hill
USDA	United States Department of Agriculture
wk	week
μg	microgram
μmol	micromole
*qdd	Questionnaire Data Dictionary
¹²⁵ I	iodine-125
⁵⁷ Co	cobalt-57

Abstract of Thesis Presented to the Graduate School
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Major Department: Food Science and Human Nutrition

Vitamin B12 is an essential nutrient required in the diet to ensure normal cell division and nervous system function. Dietary sources of naturally-occurring vitamin B12 are limited to those of animal origin; therefore diets restricting animal products are likely to be vitamin B12 deficient. Beef, a highly concentrated source of vitamin B12 that is often restricted in the diet of both meat-consumers and vegetarians for health reasons, is hypothesized to be a major contributor to dietary vitamin B12 intake among meat-consumers. It is important to assess the effect of beef consumption on total dietary intake of vitamin B12 and plasma vitamin B12 concentration, which is the focus of this investigation.

Vitamin B12 intake and plasma concentration were assessed among frequent beef consumers (beef intake >1 time/wk) (n=97), seldom beef consumers (beef intake ≤ 1 time/wk) (n=42), and never beef consumers (n=42), and compared to a vegetarian group (n=121). The dietary vitamin B12 intake (mean ± SD μg/1000 kcal/d) of the never beef

group (2.0 ± 1.4) and vegetarian group (1.9 ± 1.5) was both lower ($p < 0.0001$) than the frequent beef group (3.3 ± 1.4) and the seldom beef group (3.7 ± 2.2). In the frequent beef group, the largest contributors to vitamin B12 intake were seafood (30%), beef (28.5%), and dairy products (16%). Among the seldom beef group, the largest contributors to vitamin B12 intake were seafood (33.5%), cereal (20%), and dairy (13.8%). Sources in the never beef group that contributed the largest proportion of vitamin B12 were seafood (41%), dairy (21.3%), and soy products (12%). There were no differences ($p = 0.70$) in mean plasma vitamin B12 concentration (range 263 to 289 pmol/L) among the four dietary groups. The percentage of individuals who were deficient based on plasma vitamin B12 concentration (<148 pmol/L) was approximately two-fold higher in the non-beef consumers [never beef (12%); and vegetarians (17%)] compared to beef consumers [frequent beef (6%); and seldom (5%)].

The present study was the first to stratify subjects based on their frequency of consumption of beef products and to compare the dietary vitamin B12 intake and food sources to vitamin B12 concentration among beef consumption groups. This study also was the first to use a dietary history questionnaire modified specifically for vitamin B12-containing foods and to use a nutrient database where 100% of foods have information on vitamin B12 content. These data indicate that consumption of beef at least one time per week plays an important role in providing adequate amounts of vitamin B12 in the diet of meat consumers. In addition to beef, seafood and dairy products were major contributors to dietary vitamin B12 intake. Dietetics practitioners and nutrition educators can use these data to promote beef, seafood, and dairy consumption for optimizing vitamin B12 status through dietary means.

CHAPTER 1 INTRODUCTION

Vitamin B12, a water-soluble vitamin, plays an important role in the remethylation of homocysteine to methionine (1) and the formation of succinyl CoA, a Krebs's cycle intermediate, from L-methylmalonyl CoA (2). Vitamin B12 is an essential nutrient that is naturally present only in dietary sources of animal origin. Foods that are naturally rich sources of vitamin B12 are seafood, organ meats (especially liver), beef, poultry, pork, dairy products (e.g., milk, cheese, yogurt), and eggs (especially egg yolk) (3). Compared to other meat products (e.g., poultry, pork, and some types of fish), beef has the highest concentration of vitamin B12 per 3 ounce (oz) serving. Dairy products and eggs have smaller amounts of vitamin B12 per serving than meat products (4).

Consumption of a vegetarian diet, which restricts animal sources, may limit dietary intake of vitamin B12 and impair vitamin B12 status (5). Vitamin B12 deficiency has been related to elevated homocysteine concentration, a risk factor for cardiovascular disease, impaired fetal development, and neurological abnormalities (6-8).

Dietary intake of vitamin B12 has been assessed in meat-consumers and vegetarians by using a number of different assessment methodologies including 24-hour food recalls, multiple-day food records, and food frequency questionnaires (FFQ). Dietary vitamin B12 intake has been reported to be consistently lower in vegetarians than meat-consumers when the vegetarian group does not consume supplements or vitamin B12-fortified products (9-11). The impact of frequent beef consumption on total dietary

vitamin B12 intake and plasma vitamin B12 concentration relative to other food sources among meat-consumers has not been previously investigated.

Hypotheses

1. High frequency of beef intake is associated with a greater intake of dietary vitamin B12 and higher plasma vitamin B12 concentration compared to other food sources.
2. Among meat eaters, beef and beef-containing foods are the largest contributors to dietary vitamin B12 intake relative to other food sources.

Specific Objectives

The overall goal of this study was to assess vitamin B12 intake and status in healthy individuals who consumed beef more frequently compared to those who consumed vitamin B12 from other food sources. The objectives of this research study were as follows:

1. To determine the differences in mean dietary vitamin B12 intake [micrograms (μg) per 1000 kilocalories (kcal)] among all dietary groups.
2. To characterize the daily contribution of dietary vitamin B12 intake from beef sources ($\mu\text{g}/1000$ kcal) relative to the total dietary vitamin B12 intake from all food sources and other types of animal-based foods.
3. To assess the relationship between dietary vitamin B12 intake and plasma vitamin B12 concentration within each dietary group.

CHAPTER 2 LITERATURE REVIEW

Vitamin B12

Chemistry

The chemical properties of vitamin B12, also known as cobalamin, have been previously reviewed (12). Cobalamin, a water-soluble vitamin has a molecular mass of 1,580 daltons. Cobalamin is a general term for a group of corrinoids that are cobalt-containing compounds (12). The structure of cobalamin consists of a macrocyclic corrin ring, a 5,6-dimethylbenzimidazole nucleotide, and an aminopropanol group (Figure 2-1). The macrocyclic corrin ring consists of four reduced pyrrole rings linked to one cobalt atom at the center. Attached to the central cobalt atom is a lower (alpha) ligand 5,6-dimethylbenzimidazole nucleotide (13). Numerous upper (beta) ligands such as $-\text{CN}$, $-\text{OH}$, $-\text{H}_2\text{O}$, $-\text{NO}_2$, 5'-deoxyadenosyl, and $-\text{CH}_3$ can be covalently bound to the central cobalt atom. Addition of a ligand creates various forms of cobalamin, such as cyanocobalamin, hydroxocobalamin, aquocobalamin, nitritocobalamin, adenosylcobalamin, and methylcobalamin, respectively (12).

Methylcobalamin and adenosylcobalamin are active coenzymes and can be derived from other cobalamin forms (2). Cyanocobalamin is the commercial form produced by industry (12). In tissue, a "coenzyme synthetase" system converts cyanocobalamin and hydroxocobalamin to adenosylcobalamin. In this reaction, ATP provides a 5'-deoxy-5'-adenosyl moiety that is moved to the cobalamin. Hydroxocobalamin can be methylated in the cytosol to form methylcobalamin (2).

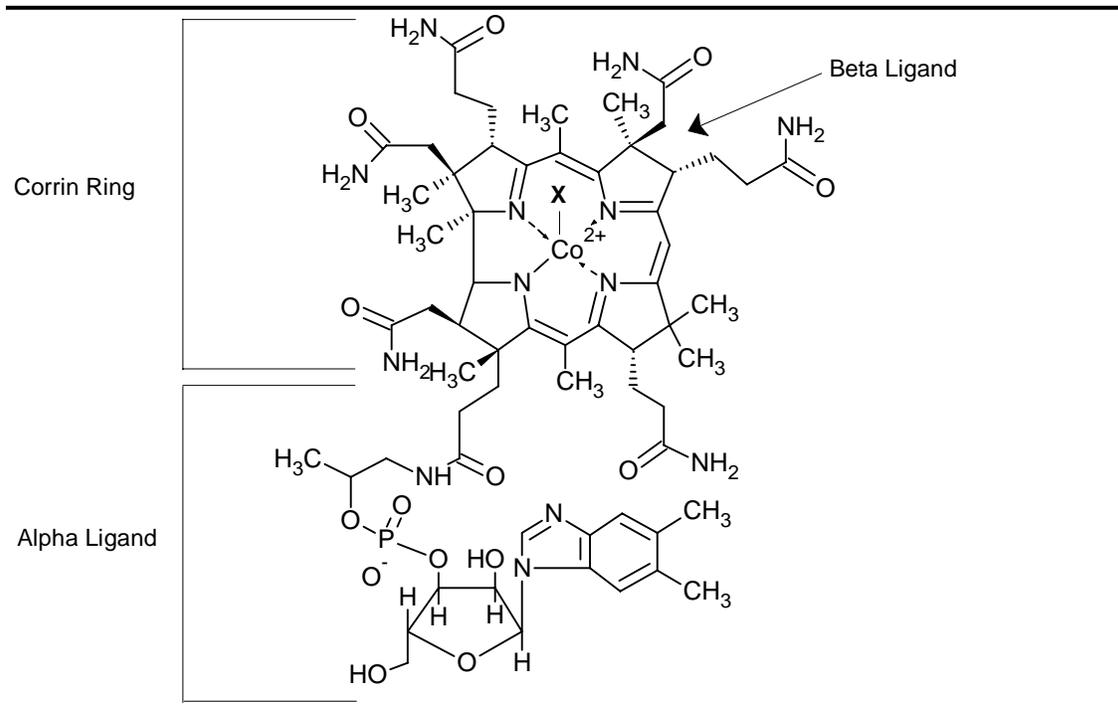


Figure 2-1. Structure of cobalamin. Adapted from Martens et al. (2002), p. 276 (12).

During exposure to light, methylcobalamin, adenosylcobalamin, and cyanocobalamin are unstable. Light disrupts the carbon-cobalt bond and decreases enzyme activity (12). Cyanocobalamin is stable in aqueous solutions, low pH, and high temperatures (14).

Dietary Sources

Cobalamin, which will be referred to subsequently as vitamin B12, is an essential nutrient that is naturally present only in dietary sources of animal origin (3). Only certain microorganisms (members of Archea and some eubacteria) can synthesize vitamin B12 as reviewed by Raux et al. (2000) (15). Animal sources rich in vitamin B12 have obtained the vitamin from these microorganisms. Sources rich in vitamin B12 are seafood (e.g., fish and shellfish), organ meats (especially liver), beef, poultry, pork, dairy products (e.g., milk, cheese, yogurt), and eggs (especially egg yolk) (16). It may be possible to

acquire vitamin B12 from plant sources if they are contaminated with vitamin B12 producing-microorganisms (12).

Beef has the potential to provide a large amount of dietary vitamin B12 since one 3 oz serving contains approximately 2.2 μg of vitamin B12 (4), almost providing the Recommended Dietary Allowance (RDA) for vitamin B12 (RDA = 2.4 μg ; non-pregnant, non-lactating adults). Compared to all other meat products (e.g., poultry, pork, and some types of fish), beef has the highest concentration of vitamin B12 per 3 oz serving (Table 2-1) (4). Dairy products and eggs have smaller amounts of vitamin B12 per serving compared to meat. Seafood provides a higher amount of vitamin B12 in one 3 oz serving than beef. Specifically, one 3 oz serving of shellfish (e.g., clams, mussels, and oysters) provides approximately 20 μg per serving of vitamin B12 (4). Although, according to a report by the United States Department of Commerce, seafood, including shellfish, is consumed in much smaller amounts per year (per capita 16.6 pounds) compared to beef (per capita 65 pounds) (17-19).

Adenosylcobalamin and hydroxocobalamin are the predominant vitamin B12 forms found in meat, fish, poultry, pork, and eggs (16). Methylcobalamin and hydroxocobalamin are the predominant forms of vitamin B12 found in dairy products (16). The form of vitamin B12 in commercial supplements and fortified foods such as breakfast cereals, soy products, and meal replacement formulas is predominately cyanocobalamin (20).

Table 2-1. Dietary vitamin B12 sources

Dietary Source	Vitamin B12 Content (μg) per 100 grams (g)	Vitamin B12 Content per serving (μg)	Serving Size
Beef	2.50	2.2	3 ounce
Chicken	0.34	0.29	3 ounce
Turkey	0.34	0.29	3 ounce
Pork	0.75	0.64	3 ounce
Milk	0.45-0.53	1.1-1.3	1 cup
Cheese	0.35-1.55	0.10-0.44	1 ounce
Yogurt	0.47	1.07	8 ounce
Egg	1.42	0.64	1 large
Seafood			
Shellfish			
Clams, mussels, oysters	24-49	20-42	3 ounce
Shrimp, lobster	1.4-3.1	1.2-2.6	3 ounce
Fish			
Cod, tuna, salmon	1.05-2.8	0.89-2.38	3 ounce

Adapted from United States Department of Agriculture, National Nutrient Database for Standard Reference, Release 17 (2005) (4).

Absorption

The mechanism of dietary vitamin B12 absorption has been reviewed previously (21). In the stomach, dietary vitamin B12 bound to protein is disassociated by pepsin and hydrochloric acid. The acid environment increases the vitamin B12 binding affinity for R proteins (i.e., cobalophilins or haptocorrins), which are secreted by salivary glands and the gastric mucosa (3, 21). These R proteins prevent denaturation of the vitamin from chemicals produced in the stomach. Intrinsic factor (IF), a glycoprotein secreted by the parietal cells of the stomach in response to stimulation by food, is released but does not bind to free vitamin B12 in the stomach (21). The vitamin B12-R protein complex and IF then travel to the small intestine. Vitamin B12 is released from the R protein complex in the duodenum by pancreatic proteases. These proteases hydrolyze the R proteins thereby releasing vitamin B12 (21). The alkaline environment enhances the binding of vitamin B12 to IF, and the IF-vitamin B12 complex then travels to the ileum for receptor-mediated uptake (21). The intestinal cells of the ileum have receptors for vitamin B12

called cubilins. It is unclear whether both IF and vitamin B12 are absorbed during this process or if only vitamin B12 is absorbed. It is known that this is a calcium dependent process (14). Vitamin B12 is absorbed throughout the ileum, but the greatest amount is in the distal third. Most absorption of vitamin B12 is receptor-mediated, but approximately 1% is absorbed by passive diffusion (22).

Transport

Once vitamin B12 is internalized into the enterocyte it can take 3 to 4 hours before the vitamin is in circulation (2). Vitamin B12 is bound in the plasma to two proteins: haptocorrin and transcobalamin (TC) (23). Haptocorrin accounts for 80% of all vitamin B12 bound in circulation, while TC accounts for 20% of all vitamin B12 bound in circulation. Transcobalamin carries vitamin B12 in a one-to-one ratio (24). Tissue cells have receptors only for TC, making it the major transport protein for cellular uptake. Once vitamin B12 crosses the cell membrane of peripheral tissues, vitamin B12 is released from TC into the cytosol (3).

Storage

Unlike most water-soluble vitamins, vitamin B12 can be stored in the body for a considerable length of time due to enterohepatic circulation via continuous bile secretion (25). The liver is the main storage tissue followed by the muscles accounting for 60% and 30% of total body stores, respectively. Vitamin B12 also can be stored in small amounts in other tissues such as bone, kidneys, heart, muscle, brain, and spleen (26). Approximately 2 to 5 milligrams (mg) of vitamin B12 can be stored in the body (2) predominately in the form of adenosylcobalamin and methylcobalamin (26).

Excretion

Vitamin B12 is excreted primarily through feces originating primarily from unabsorbed bile (27). Excess vitamin B12 losses through urine occur when the serum vitamin B12 concentration surpasses the binding ability of the transport proteins in the blood (22). There is an inverse relationship between fecal and urinary losses and storage. If storage of vitamin B12 decreases, then excretion of vitamin B12 will decrease and more will be conserved. Approximately, 0.1% to 0.2% of stored vitamin B12 is lost each day (28).

Biochemical Functions

Vitamin B12 plays an important role in two enzymatic processes. The first is a methylcobalamin dependent pathway that remethylates homocysteine to methionine (Figure 2-2). This reaction occurs in the cytoplasm of the cell. Cobalamin bound to the enzyme methionine synthase (MS) acts as an acceptor of a methyl group donated from 5-methyltetrahydrofolate (5-methyl THF). Methionine synthase acts as a catalyst during this remethylation reaction by removing the methyl group from 5-methyl THF and adding it to cob(I)alamin, forming methylcobalamin and regenerating THF. Methylcobalamin donates the methyl group to homocysteine, resulting in remethylation of homocysteine to methionine (1). Methionine can further be converted to S-adenosylmethionine (SAM). SAM functions to provide methyl groups for over 100 methylation reactions including deoxyribonucleic acid (DNA), protein, and phospholipid synthesis (29). In a vitamin B12 deficiency, the human body lacks sufficient amounts of cobalamin to accept the methyl group from 5-methyl THF. Folate is trapped in the 5-methyl THF form preventing the regeneration of THF. Since THF is required to produce 5,10 methylene THF used for DNA synthesis, a vitamin B12 deficiency leads to decreased DNA synthesis and

impaired cell division (30). Homocysteine cannot be remethylated to methionine, resulting in a build-up of homocysteine in the blood (5).

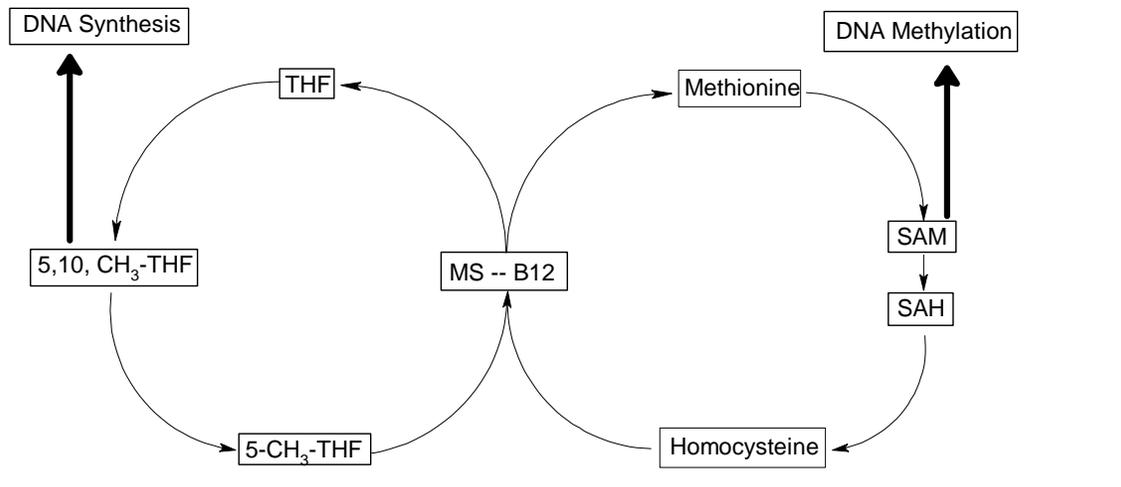


Figure 2-2. Vitamin B12 function in the remethylation pathway of homocysteine to methionine. Adapted from Scott (1999), p. 442 (21). THF = tetrahydrofolate; 5,10-CH₃-THF = 5, 10 methylenetetrahydrofolate; 5-CH₃-THF = 5-methyltetrahydrofolate; MS = methionine synthase; SAM = *s*-adenosylmethionine; SAH = *s*-adenosylhomocysteine.

The second vitamin B12-dependent reaction is an adenosylcobalamin dependent pathway, in which L-methylmalonyl CoA is converted to succinyl CoA (Figure 2-3). This reaction occurs in the mitochondria. The oxidation of methionine, threonine, isoleucine, and odd chain fatty acids results in the formation of propionyl CoA. Propionyl CoA is then converted to D-methylmalonyl CoA by a biotin, ATP, and magnesium dependent enzyme, propionyl CoA carboxylase. Methylmalonyl CoA racemase converts D-methylmalonyl CoA to L-methylmalonyl CoA. Methylmalonyl CoA mutase is adenosylcobalamin dependent and requires two adenosylcobalamin molecules to transform L-methylmalonyl CoA to succinyl CoA (2). Succinyl CoA is a key Krebs's cycle intermediate. In a deficiency of adenosylcobalamin, methylmalonyl

CoA builds up, is hydrolyzed to methylmalonic acid (MMA), and MMA ultimately accumulates in the blood (12).

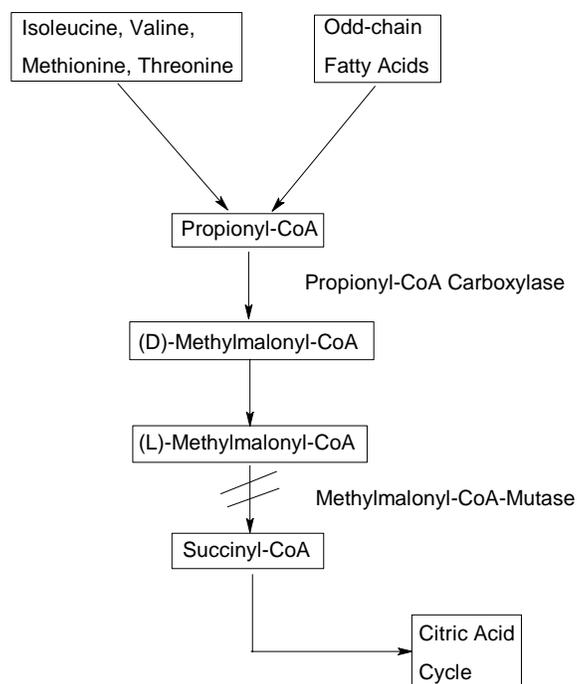


Figure 2-3. Role of cobalamin in the formation of succinyl-CoA. Adapted from Horster and Hoffmann (2004), p. 1073 (31).

Dietary Reference Intakes

The RDA for vitamin B12 is based on the amount of vitamin B12 required for maintenance of normal hematological status and serum vitamin B12 concentration considering estimates of intake and turnover (20). The RDA for male and female adults is 2.4 $\mu\text{g}/\text{day}$ (non-pregnant, non-lactating) (20). During pregnancy and lactation, the vitamin B12 RDA increases to 2.6 and 2.8 $\mu\text{g}/\text{day}$, respectively (20). Adults greater than 51 years of age frequently malabsorb food-bound vitamin B12, therefore, it is recommended that their vitamin B12 RDA (2.4 $\mu\text{g}/\text{day}$) be derived from consumption of foods fortified with vitamin B12 or from vitamin B12 supplements (20).

Dietary data from the National Health and Nutrition Examination Survey (NHANES) 1999-2000 indicated that Americans of all age groups are consuming well above the RDA for vitamin B12. The mean vitamin B12 intake for males and females aged 20 to 39 years was 6.1 and 4.0 $\mu\text{g}/\text{day}$, respectively, and the intakes for ages 40 to 59 years was 6.1 and 4.1 $\mu\text{g}/\text{day}$, respectively. Individuals over 60 years of age had a slightly lower vitamin B12 intake, although the intake still surpassed the RDA. In this age group, reported intake of vitamin B12 for males was 5.3 $\mu\text{g}/\text{day}$ and for females was 3.9 $\mu\text{g}/\text{day}$ (32). Data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1995 was used to estimate the percent that specific dietary sources contributed to vitamin B12 intake. For men, the sources that contributed the greatest percentage of dietary vitamin B12 were mixed foods (18.5%), beef (15%), and milk and milk drinks (10%). For women, the greatest percentage of total dietary vitamin B12 intake was from mixed foods (16.4%), milk and milk drinks (14.6%), and beef (12%) (20).

To date, no adverse effects of large doses of vitamin B12 have been reported from food or supplements. No adverse or toxic effects have been reported in studies of patients who receive very large doses of vitamin B12 for the treatment of pernicious anemia (33). There is no Tolerable Upper Intake Level (UL) established for vitamin B12 (20).

Vitamin B12 Deficiency

Etiology

In addition to dietary inadequacy of vitamin B12, there are numerous other causes contributing to a vitamin B12 deficiency including pernicious anemia, food-bound malabsorption, pancreatic insufficiency, jejunal bacterial overgrowth, tropical sprue, or gastric or ileal resection (22). Drugs such as nitrous oxide, metformin, and stomach acid blockers can also impair vitamin B12 status (34-36). Only vitamin B12 deficiency

associated with pernicious anemia, food-bound malabsorption, and dietary deficiency will be discussed in the following sections.

Pernicious anemia. Pernicious anemia is an autoimmune disease that occurs when the body produces auto-antibodies against the parietal cells. This causes loss of parietal cells and a subsequent decrease in IF. Other auto-antibodies can bind to IF and block the vitamin B12 binding site. Lack of IF due to the absence or reduction of parietal cell mass or blockage of the IF-B12 binding site results in vitamin B12 malabsorption (21). The exact number of Americans with pernicious anemia is unknown. Although, based on studies of elderly it has been estimated that 1.9% of the population over the age of 65 years has pernicious anemia (37).

Historically, a Shilling's test was the primary test used to diagnose pernicious anemia. This test involves giving an individual a radioactive oral dose and a flushing dose of crystalline cobalamin and measuring the amount of radioactive cobalamin excreted in the urine over time (38). This test is not used as widely by clinicians as it was in the past (39). Alternative methods for diagnosing pernicious anemia include testing an individual's serum for IF antibodies or testing for parietal cell antibodies (38).

In 1926, Minot and Murphy used raw liver to cure pernicious anemia in humans. Vitamin B12 was eventually isolated and termed the "anti-pernicious anemia factor" (40). Individuals with pernicious anemia can be initially treated with weekly intramuscular injections of 1,000 µg of cyanocobalamin for up to 2 months, preceded by monthly injections for life (29). Kuzminski et al. (1998) reported that 2,000 µg per day of oral cobalamin may be an effective alternative to intramuscular injections (41).

Food-bound malabsorption. Vitamin B12 absorption requires secretion of stomach acid to disassociate the vitamin from any bound components. With inadequate amounts of gastric acid, vitamin B12 will not be released from the protein moiety resulting in decreased vitamin absorption and increased excretion (21). Vitamin B12 consumed in a crystalline form is not bound, so regardless of stomach acid secretion, crystalline vitamin B12 will be absorbed normally (42). Drugs such as antacids and proton-pump inhibitors may contribute to food-bound malabsorption of vitamin B12 by decreasing gastric acid production (34).

To determine if vitamin B12 deficiency is due to food-bound malabsorption, research laboratories use a protein-bound cobalamin absorption test (43). In this technique, radiolabelled vitamin B12 is mixed with powdered egg yolk and made into an omelet. The omelet is consumed by the patient and 1.5 hours later the patient is given an unlabelled dose of hydroxocobalamin. Urinary excretion of vitamin B12 is then measured (43).

Individuals over the age of 51 are at risk for food-bound vitamin B12 malabsorption due to the age-related decrease in stomach acid or achlorhydria (42, 44). As a part of the RDA recommendations, individuals older than 51 years of age are advised to obtain vitamin B12 from fortified foods sources or from vitamin B12 supplements (20). These sources are not protein bound, therefore these sources can be absorbed through the normal mechanism (45).

Dietary deficiency. Those who restrict their intake of vitamin B12 containing foods, such as vegetarians or vegans, are at risk for vitamin B12 deficiency, unless they consume vitamin B12 from fortified foods or supplements (5). Since vitamin B12 can be

stored in the body, a decrease in dietary intake may not affect vitamin B12 concentration initially (9). Those who restrict their intake of vitamin B12 containing foods (naturally occurring, fortified, or supplements) for longer durations are at higher risk for a vitamin B12 deficiency due to depletion of body stores (9, 10).

Clinical symptoms

Megaloblastic anemia. Vitamin B12 deficiency occurs in stages as previously reviewed (5, 38). Vitamin B12 deficiency begins with a reduction in serum concentration followed by decreased cellular vitamin B12 concentration. Metabolic abnormalities occur next as indicated by increased homocysteine and MMA concentrations and decreased DNA synthesis (5, 38). The last stage is indicated by the presence of megaloblastic anemia (5). Megaloblastic anemia results in large abnormally shaped erythrocytes and elevated mean corpuscular volume (MCV). Similar to a folate deficiency, leukocytes become enlarged and hypersegmentation of the nuclei occurs. Treatment with vitamin B12 can reverse changes in the erythrocytes and leukocytes (2). Presence of megaloblastic anemia may indicate a possible deficiency of vitamin B12, although it is not a strong diagnostic tool since a folate deficiency results in the same type of anemia (38).

Neurological disorders. According to a review by the Institute of Medicine (1998), neurological abnormalities occur in 75 to 90% of individuals with vitamin B12 deficiency. They generally appear long after the deficiency has occurred making it a poor assessment tool for vitamin B12 status (20). Neurological abnormalities are caused by demyelination of the central nervous system (CNS). The myelin sheath and spinal cord near the brainstem are affected first. Degradation of the CNS can occur before a vitamin B12 deficiency is recognized in an individual. Lindenbaum et al. (1988) found that 28%

of individuals with neurological abnormalities presented with normal hematological indices. Tingling and numbness in extremities, memory loss, disorientation, and dementia are common neurological signs of a deficiency (46). These symptoms are not specific for a vitamin B12 deficiency since they also can be present among individuals who have other neurological conditions. Therefore, an individual can not be diagnosed with a vitamin B12 deficiency based only on presentation of neurological symptoms (38).

Health related risks of a vitamin B12 deficiency

As a person restricts more animal food sources, the risk of inadequate intake of vitamin B12 increases (5). Since vegetarians restrict various animal food sources, thereby restricting naturally-occurring food sources of vitamin B12, they are at risk for a deficiency. This is especially the case if vitamin B12-fortified foods or supplements are not consumed. Data continue to support a relationship between vegetarian diets and increased risk for birth defects, neurological abnormalities, and elevated homocysteine concentration related to a vitamin B12 deficiency (6-8).

Birth defects. Women of childbearing age who consume a vitamin B12 deficient diet are at an increased risk for impaired fetal development if they are pregnant. Folic acid is generally the vitamin associated with birth defects, specifically neural tube defects (NTD) such as spina bifida. There is, however, strong evidence supporting a role for low vitamin B12 status as an NTD risk factor, which may be related to the interdependent role of folic acid and vitamin B12 in one carbon metabolism (8, 47-49). Kirke et al. (1993) compared maternal folate and vitamin B12 concentrations before the birth of a child. After delivery, these women were grouped based on presence of an NTD in the child. Both plasma folate and vitamin B12 concentrations were lower in women who had a child with an NTD compared to those who did not give birth to a child with an NTD.

Low vitamin B12 status was considered an independent risk factor for NTD (49).

Groenen et al. (2004) investigated maternal vitamin B12 status and the risk for spina bifida in offspring. In this study, serum vitamin B12 concentrations of mothers and their children who had spina bifida were compared to those of control mothers. The mothers with children who had spina bifida had a mean serum vitamin B12 concentration that was 21% lower [<185 picomoles per liter (pmol/L)] than that of the control mothers and was associated with a three and a half-fold increase in spina bifida risk (8).

van Rooij et al. (2003) evaluated vitamin B12 status of mothers and infants with nonsyndromic orofacial clefts. Mothers who had decreased serum vitamin B12 concentration (<185 pmol/L) had a three-fold increased risk of having offspring with a nonsyndromic orofacial cleft. There were no differences between median serum vitamin B12 concentrations in the affected versus the control infants (48). Afman et al. (2001) investigated the association between vitamin B12 binding by TC and the risk of NTD. They found that mothers with children who are affected with an NTD had a significantly higher mean homocysteine concentration and lower TC and vitamin B12 concentrations than mothers with normal children. They hypothesized that decreased binding of vitamin B12 by transcobalamin, which resulted in a vitamin B12 deficiency as indicated by increased homocysteine concentration, was associated with increased risk for birth defects. They concluded that vitamin B12 supplementation may be needed along with folic acid supplementation in women of childbearing age (47).

Neurological abnormalities. Neurological abnormalities can occur in individuals with a vitamin B12 deficiency (20). Depending on the time at which a neurological disorder persists, some can be reversed. Pittock et al. (2002) discussed a case-report of a

34-year old man with reversible myelopathy who suffered from pernicious anemia. He was treated with intramuscular injections of cyanocobalamin every two weeks. After one month of treatment, all clinical symptoms had completely reversed to normal (7).

Louwman et al. (2000) investigated the effect of marginal cobalamin status on adolescent cognitive functioning when following a macrobiotic diet. Data from 28 adolescents on vegan diets and 24 adolescents on non-vegetarian diets indicated that adolescents on macrobiotic diets performed worse on psychological tests than adolescents on non-vegetarian diets (50).

There are numerous published studies that show neurological abnormalities in infants who are exclusively breast-fed from mothers following a vegetarian or vegan diet and who have poor maternal vitamin B12 status (51-55). The first report (Jadhav et al. 1962) included six cases occurring in India. None of the infants had absorption problems, but all had megaloblastic anemia attributed to poor vitamin B12 intake due to low maternal vitamin B12 breast milk concentrations. Half of the mothers had inadequate vitamin B12 breast milk concentrations because of their restriction of animal products, the other half suffered from vitamin B12 malabsorption. All infants were born without complications, but began to display abnormal skin pigmentation, apathy, anemia, involuntary movements, and developmental regression around the ages of 7 to 12 months. Upon diagnosis, they were treated with oral cobalamin and the symptoms were reversed (56).

Elevated homocysteine concentration. As discussed previously, with inadequate amounts of vitamin B12, homocysteine cannot be remethylated and homocysteine can build up in the blood (5). There are many proposed mechanisms by which an elevated

homocysteine concentration may increase the risk for cardiovascular disease, as reviewed by Ueland et al. (2000) (57). One hypothesis is that homocysteine may increase endothelial permeability by decreasing tumor necrosis factor and increasing thrombin formation, which may exacerbate the development of atherosclerotic plaque and contribute to the progression of cardiovascular disease (58).

Since vegetarians limit or exclude animal products, which are the only naturally-occurring sources of dietary vitamin B12, they are at an increased risk for a vitamin B12 deficiency and hyperhomocysteinemia (59). Data from NHANES III showed that two-thirds of United States men and women with elevated homocysteine concentrations had low serum vitamin B12 concentrations (60). Gao et al. (2003) used an FFQ to investigate dietary patterns and serum homocysteine concentration in a Chinese population. Individuals were categorized into three dietary groups based on greater intake of calories from: (1) milk and fruit, (2) red meat, and (3) unbleached, refined cereals (rice and flour products). Forty percent of subjects in the refined cereals group had a high homocysteine concentration and 67% had a low serum vitamin B12 concentration. This group was four to five times more likely than individuals in the milk and fruit group or red meat group to have these hematological factors. They concluded that dietary patterns where the majority of calories were from low vitamin B12 sources contributed to the increase in homocysteine concentration in Chinese adults (61).

Obeid et al. (2002) measured homocysteine and MMA concentrations among individuals following various types of vegetarian diets. It was shown that vegans, followed by the combined lacto/lactoovovegetarian group, had the highest median homocysteine concentration, lowest median serum vitamin B12 concentration, and

highest median MMA concentration (62). A report by Herrmann et al. (2003) further supported these findings of an association between vitamin B12 intake and increased homocysteine concentration in vegetarians. They reported that 67% of vegans, 38% of vegetarians, and 16% of omnivores had elevated homocysteine concentrations. These researchers concluded that the vitamin B12 status of vegetarians and vegans could be described as marginal to severely deficient compared to a more normal vitamin B12 concentration in the omnivore control group. These data indicated that decreased serum vitamin B12 concentration was directly correlated with elevated homocysteinemia in vegetarians (63). Krajcovicova-Kudlackova et al. (2000) measured homocysteine concentration in vegetarians and reported that 53% of vegans, 29% of vegetarians, and 5% of omnivores had hyperhomocysteinemia. Serum vitamin B12 concentrations were significantly lower in 28% of vegetarians, 78% vegans, and none of the omnivores (64).

Research findings support the conclusion that vitamin B12 supplements can correct hyperhomocysteinemia. Mezzano et al. (2000) studied vegetarians with poor serum vitamin B12 status. After one intramuscular injection of cyanocobalamin (10,000 µg), serum vitamin B12 concentration increased significantly above normal from 110 ± 46 pmol/L to 393 ± 151 pmol/L) and mean total homocysteine concentration decreased significantly [from 12.4 ± 4.7 micromoles per liter (µmol/L) to 7.9 ± 31 µmol/L)] (6). Recently, Bor et al. (2006) reported that a daily intake of at least 6 µg of vitamin B12 (from food or supplements) reduced blood homocysteine, MMA, and vitamin B12 concentrations in postmenopausal women (65).

Vitamin B12 Status Assessment

Serum Vitamin B12 Concentration

Serum vitamin B12 is generally the first diagnostic test performed in a clinical setting on patients suspected of having a vitamin B12 deficiency (2). Established standards that have been used in both clinical and research settings are defined as follows: (a) deficient (<148 pmol/L); (b) marginally deficient (148-221 pmol/L); and (c) normal (>221 pmol/L) (66); although depending on the method of analysis, the normal range can vary from laboratory to laboratory. Ranges of vitamin B12 values using radioassay methods are found to be higher and less accurate compared to microbiological assays, which are generally lower and more accurate (2). The use of serum vitamin B12 concentration as an indicator of vitamin B12 status is currently under much scrutiny because of the potential for false normals (2). Lindenbaum et al. (1990) found that MMA concentration predicted a vitamin B12 deficiency two times more than serum vitamin B12 concentration, and five percent of individuals who were diagnosed with a clinical vitamin B12 deficiency had a normal serum vitamin B12 concentration (67). In contrast, Bolann et al. (2000) reported that serum vitamin B12 concentration was the best indicator of vitamin B12 status and should be used first in determining if the symptoms experienced by an individual are vitamin B12 related (68). Currently other metabolites of vitamin B12 metabolism such as MMA, are considered a better determinate of vitamin B12 status. Holo-TC is under investigation as a potentially more sensitive vitamin B12 status assessment indicator.

Methylmalonic Acid Concentration

Serum MMA concentration is considered a functional measure of intracellular vitamin B12 status (20). Vitamin B12 is required to convert L-methylmalonyl CoA to

succinyl CoA. In the event of a vitamin B12 deficiency, this pathway is disrupted due to decreased enzyme activity. Consequently, the concentration of MMA increases in the blood and in response to a vitamin B12 deficiency in the urine (12). The normal serum concentration range for MMA is 73 to 271 nanomoles per liter (nmol/L) (2). Urinary MMA may be an earlier status indicator than other status indicators since MMA can become elevated before serum vitamin B12 concentration drops below 148 pmol/L (2). Renal insufficiency is associated with an increase in the concentration of MMA independent of vitamin B12 deficiency (38). MMA concentration also can be elevated in the elderly regardless of vitamin B12 status (69). The photometric methods used to determine serum MMA concentration are very costly. For this reason serum MMA concentration is not used routinely as a clinical diagnostic test (2). Gultepe et al. (2003) developed a modified procedure for measuring urinary MMA concentration that is more sensitive than the older photometric method (70).

Holotranscobalamin Concentration

Serum holo-TC is a biologically active complex of vitamin B12 and transcobalamin. Since serum vitamin B12 concentration may not always be a reliable indicator of vitamin B12 status, holo-TC is currently being investigated as a more sensitive test for vitamin B12 assessment (71). Our research group is currently investigating the influence of the polymorphism transcobalamin 776C→G (TC 776C→G) on the concentration of serum holo-TC, and the reliability of holo-TC as an indicator of vitamin B12 status. von Castel-Dunwoody et al. (2005) reported an inverse relationship between plasma homocysteine and serum holo-TC, and plasma vitamin B12 concentrations. These findings correlated to previous studies investigating the relationship between homocysteine and holo-TC concentrations. Holo-TC concentration

may be helpful in characterizing vitamin B12 status in marginally deficient individuals (72).

Serum Homocysteine Concentration

Vitamin B12 and folate are required for the remethylation of homocysteine to methionine (1), and vitamin B6 is required for the transulfuration of homocysteine to cystathionine (12). Serum homocysteine concentration may provide an indication of intracellular vitamin B12 status (20). Serum homocysteine concentration below 12 $\mu\text{mol/L}$ is considered normal (60). In a vitamin B12 deficiency, serum homocysteine concentration can be elevated. Since there are three vitamins (e.g., vitamin B12, folate, and vitamin B6) that play a role in converting homocysteine to other compounds in the body, homocysteine concentration is not a specific determinant for vitamin B12 deficiency (73).

Megaloblastic Anemia

Megaloblastic anemia is diagnosed when hematocrit and hemoglobin are low, but MCV is high. Vitamin B12 and folate deficiencies, among other disorders, can cause this type of anemia. Therefore, megaloblastic anemia is not a definitive status indicator of a vitamin B12 deficiency. If folic acid is given to treat the condition, the anemia will reverse to normal, even if the underlying cause is due to vitamin B12 deficiency. However, the neurological complications of a deficiency will not resolve and can result in further damage (38).

Dietary Intake Assessment in Adults

Twenty-four hour recall method

The twenty-four hour recall method of dietary intake assessment can be administered in-person or over the phone and can be used for large epidemiological

studies. This method is inexpensive, requires no literacy, and has a high response rate. Twenty-four hour recalls rely heavily on memory and individuals may omit food items or even report food items not consumed that day (74). The three-pass method is generally used to obtain complete dietary recalls (75). In this method, an individual will simply start listing all the foods they ate without interruption. After the individual has given a brief list of the foods they ate, the interviewer asks specific questions regarding each food item eaten, portion size, and the meal time. For example, if the person said they had a piece of bread, the interviewer should ask, the type of bread (e.g., multi-grain, white, etc), additional products added, the amount, and time of day consumed. Next, the interviewer repeats the foods that were reportedly consumed at that meal or snack, to see if any additional foods were omitted. The interviewer should address snacks between meals, beverages, and food consumed during the night. Individuals generally think of their main meals and disregard daytime or evening snacks. Food models and measuring cups can be used with the individual to help them correctly identify their portion size (75).

The United States Department of Agriculture (USDA) recently revised their method from three-steps to five-steps for interviewing during a 24-hour dietary recall. They have developed a very comprehensive automated-multiple-pass method (AMPM) (75). It was developed to keep the attention of individuals during the interview and to correct for under-reporting (75). This five-step method has more passes and increased opportunities to add forgotten foods. The computerized method uses a database with predetermined questions, possible follow-up questions, and instructions for the interviewer. The AMPM contains 2,400 questions and 21,000 response options (75, 76). The AMPM is directly linked to the USDA Food and Nutrient Database for Dietary

Studies for nutrient analysis. Moshfegh et al. (2001) pilot tested this method in 700 individuals and found a higher average daily caloric intake and more foods reported than the CSFII (1996) that used a three-step method (77).

Multiple-day food record

The multiple-day food record involves instruction of an individual to write everything they ate on a food record that is provided (78). A food record can be self-administered or administered by an interviewer. If self-administered, instructions should be provided with the diet record. Example instructions would include information on food description, preparation methods, brand names, and ingredients in mixed foods (78). Kolar et al. (2005) provided a 12-page serving size booklet with pictures to illustrate and quantify serving sizes during their study. Records are reviewed for completeness and entered into a nutrient database program such as Nutrient Data System for Research (NDS-R), University of Minnesota (78). Since they require review and manual correction, multiple-day diet records are generally discouraged for large epidemiological studies (78). Dietary records do not rely on memory, but can be tedious for the individual completing the record (79). The reliability of the nutrient data from the food record increases with the numbers of days recorded (80). Craig et al. (2000) measured the effect of atypical days, as described by participants, on nutrient intake estimates in 1,090 women. Those who reported an atypical day as less than normal or higher than normal had lower intakes or higher intakes, respectively, of all nutrients. They concluded that atypical days in an individual's diet can affect estimated nutrient intakes in research studies that use the multiple-day food record (80).

Food frequency questionnaire

A FFQ is used to determine usual dietary intake (81). This type of questionnaire is commonly used for large epidemiological studies (81). A FFQ can be self-administered and is less expensive than other dietary assessment methods (82). Twenty-four hour recall methods and multiple-day food records are more detailed than a FFQ. A FFQ uses general food groupings, such as high-fiber cereal, whereas a 24-hour recall method or multiple-day food record provides the specific brand name of cereal that was consumed. A FFQ has preset questions and portion sizes to which an individual responds. These types of questionnaires are developed to be scanned by a computer and uploaded into a software package (81). There generally is no option for the individual to add a personal response not listed on the questionnaire. Food frequency questionnaires need to be validated before using them for a large epidemiological study to ensure that the questionnaire is capturing true dietary intake (82).

The National Cancer Institute (NCI) has validated a FFQ referred to as the Diet History Questionnaire (DHQ) (81). Frequency of intake and portion size for 124 individual food items is captured in this FFQ. Forty-four of these questions have embedded questions that ask about seasonal intake, type of fats added, and whether the food item was low-fat or fat free (81). The original DHQ also includes ten questions regarding dietary supplements, and four summary questions (81). Portion size choices were determined from analyses of data from the CSFII (1994-1996). Based on responses from 10,019 adults during this study, portion size choices were changed in the questionnaire. Instead of using portion sizes such as small, medium, and large, this questionnaire provides portion sizes specific to the food item such as “less than 1 cup,” “1-2 cups,” and “more than 2 cups”. Also, based on data from the CSFII (1994-1996),

5,261 individual foods were categorized into 170 food groups (83). These food groups are the basis of the 124 food items used in the DHQ (81). Nutrient estimations are calculated for each food item listed in the DHQ by multiplying the daily frequency by a nutrient value specific for the gender of the individual and the portion size selected.

The NCI DHQ was validated in 2001 against repeated 24-hour recalls and compared to the Block and Willett FFQ (81). The Block FFQ is an eight-page questionnaire that covers 106 food items. Portion size choices are described as small, medium, or large (81). The questionnaire has thirteen dietary supplement questions, eight questions regarding use of added fats, five summary questions, and six questions regarding restaurant eating. The Willett FFQ is a four page questionnaire with 126 food items. The questionnaire is unique in that it does not have a separate portion size question. Individuals select one response in which the frequency and portion size are combined together in one response. The questionnaire also includes ten dietary supplement questions and ten questions about added fats (81). The validation study for the DHQ consisted of 1,301 men and women who completed four telephone 24-hour recalls during one session. The subjects were then randomized to receive the DHQ and Block FFQ or the DHQ and Willett FFQ. Compared to the 24-hour recall method, it was found that the DHQ was more accurate in determining nutrient intake than either the Block or Willett FFQs (81).

Assessment of vitamin B12 intake

The assessment of vitamin B12 intake among vegetarians and non-vegetarians has generally been assessed along with other nutrients to provide an overall profile of dietary adequacy of a vegetarian diet in contrast to a non-vegetarian diet. Studies where only

vitamin B12 intake has been assessed (generally in relationship to serum concentration status) have largely been conducted with vegans compared to non-vegetarians.

Haddad and Tanzman (2003) used CSFII 1994-1996 data to compare the dietary intake of various nutrients including vitamin B12 among self-defined vegetarians and non-vegetarians in the United States. Dietary data was collected using two 24-hour dietary recalls. Men and women ages 20 years and above who reported consuming no meat had a vitamin B12 intake lower than those who consumed meat (84). Two separate studies by Barr and Broughton (2000) and Janelle and Barr (1995) reported findings in Canadian women that were similar to those reported in a United States study. Both studies compared nutrient intake, including vitamin B12, among vegetarian and non-vegetarian women in Canada. The study by Barr and Broughton (2000) assessed dietary intake using the 24-hour recall method, and the study conducted by Janelle and Barr (1995) assessed dietary intake using 3-day food frequency questionnaires. Among women, vitamin B12 intakes of the vegetarians were lower than non-vegetarians (85, 86).

Haddad et al. (1999) also assessed vitamin B12 intake and status of vegans and non-vegetarians. To measure dietary intake, a 24-hour recall and 4-day food records were used. Vitamin B12 intake among the female vegans was lower than the vitamin B12 intake among the female non-vegetarians. No differences in vitamin B12 intake were reported among the male vegan and non-vegetarian groups. Serum vitamin B12 concentration (male and female combined) did not differ among the vegan and non-vegetarian groups (87).

Miller et al. (1991) assessed vitamin B12 intake in relation to serum vitamin B12 status among individuals who consumed a macrobiotic diet but sometimes included dairy,

eggs, and seafood in their diet. Dietary intake was obtained although the specific assessment tool used (i.e., food record, FFQ, or dietary recall) was not indicated. To determine dietary vitamin B12 intake, the frequency of intake was multiplied by the vitamin B12 content of the foods. This number was then added to a frequency score that was based on how often specific foods (e.g., dairy, eggs, seafood) were consumed. This study compared the frequency intake of dairy, eggs, and seafood in relation to serum vitamin B12 concentration. Those who consumed dairy products ≤ 1 time/week or > 1 time/week had a significantly higher serum vitamin B12 concentration than those who never consumed dairy products. No differences were found in frequency of egg or seafood intake on serum vitamin B12 concentration between intake groups. Serum vitamin B12 concentration was then compared to tertiles of vitamin B12 intake score. Vitamin B12 intake scores of 0, 0.01 to 0.15, and 0.16 to 1.7 were defined as low, medium, and high, respectively. Adults who had a low vitamin B12 intake score had significantly lower serum vitamin B12 concentration than those with a score of medium or high. (9).

Rauma et al. (1995) used 5-day food records to determine vitamin B12 intake among vegans who followed a strict uncooked diet and non-vegetarians. The mean vitamin B12 intake from the vegan group was significantly lower than the non-vegetarian group (1.8 versus 6.2 $\mu\text{g}/\text{day}$, respectively). Although, the authors stated that dietary assessment of vitamin B12 of the vegan group was underreported because their database did not contain data for the foods consumed on the “uncooked diet”. Serum vitamin B12 concentration was correlated ($r = 0.63$; $p < 0.01$) with dietary vitamin B12 intake among

the vegans. Contribution of total vitamin B12 from various food sources consumed were not determined in this study (10).

Larsson and Johansson (2002) compared dietary intake of various nutrients including vitamin B12 among 16 to 20 year old vegans and omnivores in Sweden. In this study, participants were interviewed for 1 to 2-hours, 1 to 2 weeks apart to determine dietary intake. Dietary vitamin B12 intakes for female and male vegans (0.0 ± 0.1 , 0.1 ± 0.03 , respectively) were significantly lower ($p < 0.0001$) than female and male omnivores (5.0 ± 2.5 , 5.9 ± 1.5 , respectively) (88). Larsson and Johansson (2005) published additional data comparing dietary sources of vitamin B12, in addition to other nutrients. Among the vegans, 100% of vitamin B12 intake was from dietary supplements. In contrast, the majority of vitamin B12 in the diet of omnivores came from animal products, defined as meat, fish, seafood, dairy products, and eggs, followed by dietary supplements (89).

Dunn-Emke et al. (2005) assessed dietary intake of vitamin B12 among vegans with prostate cancer following a well-planned, low-fat diet. Subjects were given specific dietary guidelines for this diet along with a soy protein powder that contained isoflavones (potential inhibitor of cancer growth). After 6 months, 3-day food records were obtained. The mean vitamin B12 intake for these subjects was $3.7 \mu\text{g}/\text{day}$, with 81% of the vitamin B12 intake from supplemental soy protein and 19% from fortified plant foods (90).

Leblanc et al. (2000) determined vitamin B12 intake among lactoovovegetarians, lactovegetarians, and vegetarians following a macrobiotic diet. Participants recorded dietary intake using a 5-day food record. The nutrient software used to analyze the food records contained additional foods found in a vegetarian diet. Only 80% of these foods

contained data on vitamin B12 content. After analysis, it was determined that the women who followed a macrobiotic diet had a significantly lower ($P<0.05$) dietary vitamin B12 intake compared to the women in the other vegetarian groups. There were no differences in vitamin B12 intake among vegetarian men. The median intakes of vitamin B12 for all three groups (men and women) were below the RDA for vitamin B12 (range of 0-1.73 $\mu\text{g}/\text{day}$) (11).

Vitamin B12 Status in the United States

Based on data from NHANES III (1994-1998), mean serum vitamin B12 was 382 pmol/L for individuals 4 years and older (91). Younger males and females (4-5 years) had the highest median serum vitamin B12 concentration for all age groups. Men and women 70 years and over, had the lowest median serum vitamin B12 concentrations (i.e., 286 pmol/L and 322 pmol/L, respectively). Based on ethnicity, non-Hispanic black Americans had the highest median concentration (419 pmol/L) followed by Mexican Americans (368 pmol/L). In contrast to non-Hispanic white Americans had the lowest median concentration (329 pmol/L) (91).

Data from the NHANES III (1998-94) indicated that the prevalence of Americans with a serum vitamin B12 concentration less than 148 pmol/L increased with age. Thirteen percent of individuals 70 years and older, 12% of individuals 60 to 69 years, 9% of individuals 50 to 59 years, 8% of individuals 40 to 49 years, and 2% of individuals 30 to 39 had a serum vitamin B12 concentration less than 148 pmol/L. Serum vitamin B12 concentration less than 74 pmol/L was found in 1% of the total population over 4 years of age (91).

Vitamin B12 deficiency related to protein-bound vitamin B12 malabsorption in the elderly is estimated between 10 to 30% (45). Carmel et al. (2002) conducted a study

evaluating the prevalence of elevated homocysteine concentration in young Asian Indians in the United States. They found that a large percentage of this population had elevated plasma homocysteine concentration compared to healthy controls. Fifty-nine percent of men and 23.8% of women had low vitamin B12 status as defined by a vitamin B12 concentration <180 pmol/L. They concluded that elevated homocysteine concentration was possibly caused by low dietary vitamin B12 intake (92). Refsum et al. (2001) also studied an Asian Indian population and found that 47% had vitamin B12 deficiency, and 77% had hyperhomocysteinemia regardless of whether they followed a vegetarian or non-vegetarian diet (93).

Vegetarianism

Definitions of Vegetarianism and Prevalence in the United States

There are many definitions of the term vegetarian. Vegetarians may exclude all animal products (vegan); exclude meat and egg products, but include dairy (lactovegetarian); or exclude meat products but include dairy and eggs (lactoovovegetarian). Some vegetarians (pesco-vegetarian) may restrict beef, poultry, and pork consumption, but consume fish, dairy, and eggs.

In 1979, the USDA reported that 1.7% of Americans were vegetarians (94). A 2003 national Harris Interactive survey reported that approximately 2.5% of Americans describe themselves as vegetarians (84, 95). According to data obtained from the CSFII 1994-1996, 1998, more individuals in the 20 to 29 age group define themselves as vegetarian. The age group with the lowest number of people defining themselves as vegetarians was the 60 to 69 year old group (84). There are many factors which motivate individuals to become vegetarian such as health, ethical, or religious attitudes (96).

Assessment of Dietary Adequacy of Vegetarian Diets

Haddad et al. (1999) used a 24-hour recall and 4-day food records to determine the dietary adequacy of vegetarian diets among 45 healthy adults. In this small study, vegans had lower total protein, fat, saturated fat, monounsaturated fat, cholesterol, and higher dietary fiber intake (87). The European Prospective Investigation into Cancer and Nutrition-Oxford (EPIC-Oxford) study, which included approximately 66,000 meat-eaters and vegetarians, including vegans, in the United Kingdom confirmed the results of Haddad et al. (1999) in a much larger study. This study used 7-day food records and a FFQ developed for the United States Nurses' Health study to determine differences among dietary intake of meat-consumers, fish-consumers, vegetarians, and vegans. Subjects were categorized based on their answers to questions regarding meat (i.e., beef, poultry, and pork), fish, dairy or egg intake. The differences between the meat-consumers and the vegans were more pronounced than between the meat-consumers and vegetarians. The meat-consumers reported higher mean calorie, protein, fat, saturated fat, and vitamin B12 intake, and lower carbohydrate, fiber, folate, and iron intake than the vegetarians and vegans. The intake of vitamin B6 was relatively similar across groups (<10% difference). Serum vitamin B12 concentration was not determined in this study (97).

The American Dietetic Association and Dietitians of Canada have recognized the possible limitations of a vegetarian diet, but have taken the position that a carefully planned vegetarian diet can provide the necessary needed nutrients (98). To better determine the adequacy of vegetarian diets, more data are needed related to the intake of macronutrients (i.e., carbohydrate, protein, and fat) and micronutrients (i.e., vitamin B12, folate, vitamin B6, zinc, and iron).

Assessment of Beef Consumption and Health

There is an increasing trend for Americans to exclude or limit beef intake from their diets for health reasons, as reviewed by White and Frank (1994) (99). The Food Marketing Institute in 2000 reported that 68% of American consumers felt their diet could be healthier. In doing so, 68% percent were increasing their fruit and vegetable intake, 22% were decreasing beef consumption, and 9% were increasing their intake of chicken and turkey (100).

In recent years, beef consumption has been linked to cardiovascular disease (17, 101, 102). The literature regarding beef intake and various markers of cardiovascular disease are conflicting. In 1991, data from the Coronary Artery Risk Development in Young Adults (CARDIA) study were used to determine relationships between meat (i.e., red meat and poultry) consumption and lipid profiles. Frequency of meat consumption was assessed using a FFQ. The data suggested that those who ate meat less than one time per week had lower total cholesterol and low-density lipoprotein (LDL) concentrations compared to those who consumed meat more than one time per week (102). Nicklas et al. (1995) analyzed 24-hour dietary recalls in 504 young adults. Those whose meat consumption fell at the 75th percentile or higher did not meet the Dietary Guidelines for Americans for total fat, saturated fat, and cholesterol. In contrast to the Slattery et al. (1991) study, even though the percent of calories from fat was higher than the recommended amount, there were no significant differences in blood lipid profiles in any of the meat consumption quartiles (103). Fraser (1999) used a FFQ to assess dietary intake and mortality in approximately 34,000 non-Hispanic white California Seventh-Day Adventists. This FFQ included 51 different food types. The participants were categorized into three groups; vegetarian (i.e., no meat, poultry, and/or fish), semivegetarian (i.e.,

poultry and fish < 1 time per week), and nonvegetarian (beef, poultry, and fish consumers). Males who consumed red meat more frequently than 3 times per week had a relative risk of 2.5 for developing fatal ischemic heart disease. Those who consumed red meat more than 1 time per week had a relative risk of 1.86 for developing colon cancer (101).

The following studies promote lean beef intake by providing supporting data relative to the role of fat content in beef and other meats on lipid profiles. The O'Dea study in 1990 confirmed that high frequency of beef consumption was not associated with poor health status, but beef fat was. Ten participants ate a controlled diet of very-low fat lean beef (i.e., 9% energy from fat) for 3 weeks. During these 3 weeks, total LDL and cholesterol significantly decreased from baseline. Beef drippings were added to the diet during weeks 4 and 5, increasing total fat intake to 20% of total calories at week 4 and 30% at week 5. Total LDL and cholesterol significantly increased by week 5. It was concluded that lean beef could be incorporated into diets instead of reducing overall beef intake (104). Beauchesne-Rondeau et al. (2003) compared the effects of incorporating lean beef, poultry, and fish on lipid profiles in hypercholesterolemic patients. These patients recorded 3-day food diaries before each 26-day treatment diet. All three treatments were associated with a reduction in total cholesterol and LDL concentration. It was concluded that regardless of the protein source, decreased saturated fat in the diet could decrease cardiovascular disease risk (105). Snetselaar et al. (2004) used three 24-hour recalls to assess dietary intake and compared that to serum blood lipid data in adolescents. Subjects were randomized to a lean beef diet or a lean poultry and fish diet. No differences were determined in total cholesterol concentration and saturated fat intake

between the two diets (106). However, adolescents on the lean poultry and fish group were found to have significantly lower iron status after 3 months on this diet.

Assessment of Dietary Adequacy of Beef Consumption

Beef is the most commonly consumed meat product in the American diet. Per capita, Americans consume approximately 65 pounds of beef per year, followed by 55 pounds of poultry, and 50 pounds of turkey (17). Combined data from the Nationwide Food Consumption Survey (NFCS) 1977-78 and the CFSII 1989-91 and 1994-95 indicate that individuals eating separate cuts of beef have decreased, but beef consumption from meat mixtures has increased (107). The fat composition of beef has been reported to be 50% monounsaturated fatty acids, 46% saturated fatty acids, and 4% polyunsaturated fatty acids. Compared to other protein sources, beef provides the highest concentration of zinc and iron per 3 oz serving. Beef also is rich in protein, vitamin B12, and choline (17). The current 2005 Dietary Guidelines for Americans continue to maintain that beef is important in a well-rounded diet as long as leaner cuts of meat and sensible portion sizes are consumed (108).

Research Rational and Potential Application of Findings

Total dietary vitamin B12 intake has been assessed among meat-consumers and vegetarians. The majority of these studies assessing vitamin B12 intake have focused on vegetarian intakes using meat-consumers as the relative control group. These studies are focused on measuring the outcomes related to a vegetarian lifestyle. It has been confirmed that the more restrictive the diet the lower the dietary vitamin B12 intake and a lower serum vitamin B12 concentration. Beef, a highly concentrated source of vitamin B12, is often restricted in the diet of meat-consumers and vegetarians for health reasons. Restriction of beef could result in lower dietary vitamin B12 intake and lower status,

which may have multiple adverse health consequences such as neurological abnormalities, impaired fetal development, and risk for cardiovascular disease.

Therefore, it is important to assess the effect of beef consumption on total dietary intake of vitamin B12, which has not been the focus of previous investigations.

The present study categorized subjects based on frequency of beef intake. This type of categorization has been done only in studies assessing the association between beef consumption and cardiovascular disease, but not how beef intake is associated with vitamin B12 intake and status. In addition, the present study compared the vitamin B12 contribution from dietary sources of vitamin B12 consumed by different beef-consumption groups. In this comparison, the contribution of vitamin B12 from specific animal sources, including beef, were estimated. Yoshino et al. (2005) recently published estimates of vitamin B12 intake and the proportion of vitamin B12 contributed from various food sources, excluding vitamin B12 from fortified sources, among Japanese adults over a 25 year period using an FFQ (109). Unlike the study by Yoshino et al. (2005) the FFQ used in the current study has been modified to contain all vitamin B12 sources including current fortified soy-based sources that vegetarians commonly consume. The assessment methods and nutrient databases used in previous studies to calculate dietary vitamin B12 intake were not completely tailored for vegetarian foods. There are many vitamin B12-fortified products available for vegetarians consumers. Manufacturers such as Worthington, Loma Linda, Morning Star Farms, and Boca produce a large number of vitamin B12 fortified soy products such as soy burgers, soy crumbles, soy bacon, soy chicken patties, and vegetarian cold cuts. Unlike previous

studies, the present study uses a database that had been modified to assess the contribution of these fortified products to dietary vitamin B12 intake.

The present study is the first to stratify subjects based on their frequency of consumption of beef products and to compare the vitamin B12 intake and food sources to vitamin B12 concentration among the beef consumption groups. This study also is the first to use a modified dietary history questionnaire specifically for vitamin B12-containing foods and use a nutrient database where 100% of foods have information on vitamin B12 content.

The data from this study provides valuable information regarding frequency of beef consumption and beef's role as a contributor to total dietary vitamin B12 intake in the diet. The data from this study will assist consumers in making educated decisions regarding the frequency of consumption of beef related to the potential impact on vitamin B12 status. Data from this study provides new information to nutrition educators and dietetic practitioners regarding the relationship between consumption of foods within specific food groups and the impact on vitamin B12 status. This new information can be incorporated into educational materials for consumers and practitioners related to optimizing vitamin B12 status through dietary means.

CHAPTER 3 MATERIALS AND METHODS

Study Design and Methods Overview

Healthy adult male and female volunteers (n = ~1,000) were recruited by flyers, newspaper, and radio advertisements for 1 year to participate in the study. Prospective subjects were screened by phone using a screening questionnaire (Appendix A) to determine if they met the following inclusion criteria: (a) age 18 to 49 years, (b) non-smoking, (c) no use of prescription medications (birth control was allowed), (d) low alcohol intake (< one drink per day), (e) no history of chronic diseases, (f) non-pregnant, (g) non-lactating, (h) no use of vitamin B12 supplements within the last 6 months; and (i) no major dietary changes within the last 3 years. An attempt was made to screen out consumers of highly-fortified cereal as well. Eligible males and females (n = 388) were selected to participate in the study.

Subjects were scheduled to have fasting blood samples drawn and to receive detailed instructions regarding the completion of the DHQ (Appendix B). Following the collection of 70 milliliters (mL) of blood that was processed for multiple analyses including plasma vitamin B12, subjects were provided with a comprehensive instruction lasting 15 to 20 minutes on the procedures to complete the DHQ (Appendix C). Subjects took the DHQ forms with them to complete at home and mail back to the primary investigator within 2 weeks of instruction.

Total dietary vitamin B12 intake, in addition to other nutrients, and plasma vitamin B12 concentration were assessed. Subjects were compensated \$50 for their participation

in the study. The University of Florida, Institutional Review Board approved this protocol and all subjects signed approved informed consent forms.

Diet History Questionnaire

Overview

The contribution of beef and beef-containing foods to total vitamin B12 intake was assessed by the NCI DHQ. This study was a collaborative effort between investigators at the University of Florida and the University of North Carolina, Chapel Hill (UNC). The original DHQ from NCI was modified with added questions for beef containing foods and vitamin B12-fortified foods. The Diet*Calc Analysis program, also provided by NCI, was used to analyze the questionnaire responses. This program is a free software program that can be downloaded from the NCI website (www.riskfactor.cancer.gov). The nutrient database within the software is from the USDA Survey Nutrient Database and NDS-R, University of Minnesota. The Diet*Calc Analysis program nutrient database was last updated in August 2004.

Modifications Made to the Paper Version of the Diet History Questionnaire

The DHQ from NCI was modified in such a way that vitamin B12-containing foods could be isolated in the data analysis. A comprehensive review of the original DHQ was conducted to determine if vitamin B12-containing foods were missing from the DHQ. Fifteen questions (including embedded questions) were added and twenty-six questions were modified to distinguish between vitamin B12-containing food items and other food items. For example, an original DHQ question may have referred to the frequency of lasagna consumption. The DHQ was modified to determine the frequency of consumption of lasagna made with beef, lasagna made with meat other than beef, and lasagna made without meat. Seven questions were changed to reflect beef containing

foods. For example, the original DHQ asked about cold cuts. Questions like these were modified to be more specific, and in this example, asked only about beef containing cold cuts. Eight questions were added to reflect soy substitute products. These questions referred to commercial products available to consumers that are possibly fortified with vitamin B12. The ten supplement questions from the original DHQ were deleted since this study population did not consume any supplements.

Modifications Made to Diet*Calc Analysis Software

Since modifications were made to the paper version of the DHQ, corresponding modifications needed to be made to the Diet*Calc Analysis software. The software changes were imperative so the DHQ would be compatible with the Diet*Calc Analysis software. The modifications to the Diet*Calc Analysis software were completed by staff in the Nutrition Epidemiology Core at UNC. The Questionnaire Data Dictionary (*qdd) file within the Diet*Calc Analysis program was modified to be compatible with the changes made to the DHQ. The *qdd file contained coding information for interpreting scanned DHQs. Once the *qdd file was updated allowing the program to correctly interpret new questions, the Nutrient and Food Group database within the program also was modified. The nutrient content of vitamin B12-containing foods that were added to the DHQ were extensively reviewed using the USDA National Nutrient Database for Standard Reference (4) and manufacturer websites for specific product information. For example, if the old question asked about cold cuts and the new revised question was regarding only beef cold cuts, then the Nutrient and Food Group database was updated to only reflect nutrients that were provided from beef cold cuts.

Data Generation

When the paper version of the DHQ was scanned (procedure for scanning discussed later), food items or questions were linked to a predetermined Food Identification Number (FIN). Every FIN had defined nutrients for gender and serving size. FINs were found in the Diet*Calc Nutrient and Food Group database. Diet*Calc used the FIN associated with each response to assess the nutrient intake of the individual. When Diet*Calc identified a FIN from the ASCII text file, it used the database to calculate an individual's nutrient intake. This Nutrient and Food Group database was converted into a Microsoft Excel spreadsheet with all the FINs and corresponding nutrient values per serving size and gender. This spreadsheet was used to identify the beef and beef-containing foods assessed in this study.

Diet*Calc produced three data files available for analysis. The Details.txt file was an expanded version of the data calculated from the ASCII text file. This file allowed the investigator to compare FINs to individual nutrient intake and daily frequency of intake. The second file was a Results.txt file, which was a condensed version of the Detail.txt file. This file provided combined daily nutrient intake values for each individual regardless of the food sources. The third file was a Report.txt file, which provided a summary document of daily nutrient intake for each individual. This file was not used for data analysis, but for individual feedback when a participant indicated they wanted their dietary intake results from the DHQ.

Diet History Questionnaire Instruction Pretest

Prior to beginning the study, a pretest of the DHQ instruction and scanning was conducted with a group of 20 Master of Science and Doctoral students in addition to five vegetarian and vegan consumers. This pretest was done to ensure that the instructions

and handouts provided to the subjects for completing the DHQ were clear and understandable. Those who participated in the pretest were instructed using the same script that would be used for the study population. They were expected to follow these instructions and complete the DHQ as if they were a participant in the study. This group was given an additional survey asking various questions regarding length of time to complete the DHQ, if any frequently consumed foods were missing from the DHQ, and if they had additional comments that might allow for the DHQ instruction to be better understood (Appendix D). Comments from this pretest group were reviewed and incorporated into the DHQ and into the DHQ instructions.

Human Subjects Procedures

Qualified subjects reported to the Food Science and Human Nutrition Building during their scheduled appointment following an overnight fast. The subjects were required to be fasting for 8 hours prior to having their blood drawn. The study was explained to the subjects, and if they agreed to continue, they were asked to sign the approved informed consent form. Subjects who granted consent were assigned a subject identification number.

A phlebotomist drew 70 mL of blood for analysis. During the blood draw, subjects were asked again if they were taking a vitamin B12-containing supplement. Subjects received a snack after their blood was drawn prior to receiving the DHQ instructions.

Diet History Questionnaire Instructions

After completion of the blood draw, each participant was given a 20-minute group instruction reviewing the procedures to follow when completing the questionnaire. During this time, the instructions were read aloud and explained in detail using examples of questions and scenarios. Subjects were again asked if they consumed a vitamin B12-

containing supplement 6 months prior to the study. If a subject indicated that they did, specific information regarding supplement use was recorded.

To ensure that portion sizes were reported accurately, subjects were taught how to use food labels to determine portion sizes, and instructed on how to use measuring cups and spoons for visual representation of a portion size. Subjects also were given additional information that aided in completion of the DHQ. This information, which included a schematic drawing of the proper procedure to mark or change an answer, and handouts on “Caffeinated vs. Non-caffeinated Beverages,” “Fortified Cereals,” and “Seasonal Fruits and Vegetables,” were reviewed during the group instruction (Appendix E). The purpose of these handouts was to ensure that the DHQ would be scanned correctly, and that subjects would be better prepared to consistently answer the questions presented in the DHQ. Subjects also were given two sample questions reviewing the procedure to average their intake over 12 months. These examples were based on usual dietary intake and referred to specific questions in the DHQ. Subjects were given the opportunity to ask further questions if anything was unclear.

Sample Collection and Processing

Processing of Plasma for Vitamin B12 Analysis

Blood samples were drawn into Vacutainer® tubes (Vacutainer® Blood Collection Set; Becton Dickinson, Vacutainer® Systems; Franklin Lakes, NJ) containing K₃ ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and immediately placed on ice. Following centrifugation at 2000 x g for 30 minutes at 4°C, aliquots were stored at -20°C until plasma vitamin B12 analysis.

Processing the Diet History Questionnaire

Subjects were encouraged to return their DHQ within 2 weeks. If they did not return the DHQ within 2 weeks, they were contacted at the phone number or email address provided during the initial screening. All returned DHQs were reviewed for completeness. The purpose of the review was to ensure that the DHQ had been completed and that no question had a missing response. To prevent bias, marked responses to questions were not reviewed. Questions for which a response was missing were noted, and the subject was contacted to obtain the missing information. If the participant was phoned, the questions for which responses were missing were read aloud to the participant and their response was recorded on the DHQ. If the participant was contacted via email, then the question and answer responses were typed.

The DHQ was sent to Optimal Solutions Corporation (OSC), Lynbrook, New York, in groups of 100 to be scanned electronically. Once the scanning process was completed for each group of 100, OSC mailed the DHQs and electronic data in the form of an ASCII text file to UNC. The ASCII text file was uploaded at UNC into the Diet*Calc Analysis program modified for this version of the DHQ.

Analytical Methods

Formation of Dietary Groups (Objective 1)

Subjects who consumed meat were grouped based on their frequency of consumption of beef (e.g., steak, roast, hamburger, etc) as reported from the DHQ results (Table 3-1).

Table 3-1. Daily and weekly beef intake frequency

Daily Frequency	Weekly Frequency	Diet Group
0.286 – 1.0	2.0 – 7.0	Frequent Beef
0.0001 – 0.285	0.0007 – 1.995	Seldom Beef
<0.0001	<0.0007	Never Beef

If the daily frequency of consumption of these foods was greater than or equal to a daily frequency of 0.286 (equivalent to > 1 time a week), the subjects were grouped in the frequent beef group. If the daily frequency was greater than 0.0001 but less than 0.286 (equivalent to ≤ 1 time a week), subjects were grouped into the seldom beef group. Individuals who never consume beef foods but who consumed other types of meat including seafood were placed in the never beef group. Subjects were categorized as vegetarians only if their frequency of consumption of all meat products (i.e., beef, poultry, pork, mixed dishes, and seafood) was zero.

Identification of Food Groups from the Diet History Questionnaire (Objective 2)

All foods included in the DHQ were categorized into specific food groups (i.e., beef, pork/other meat, poultry, dairy, eggs, seafood, cereal, soy, meal replacement, mixed dishes with meat type unknown, non-dairy fats, beans, rice/pasta, soups/sauces, breads/cracker/cakes/pies, nuts/seeds, vegetables, fruit, syrup/honey/gelatin/candy, and beverages). Dietary vitamin B12 intake was estimated from the following food groups: (1) beef, (2) pork, (3) poultry, (4) dairy, (5) eggs, (6) seafood, (7) soy, (8) cereal, (9) meal replacement, and (10) mixed dishes with meat type unknown. The remaining food groups were grouped together as (11) “other”. Table 3.2 indicates the types of foods found within each food group.

Analysis of Additional Nutrients

Total calorie, carbohydrate, fat, saturated fat, and protein intake were determined using information reported from the DHQ. Total dietary intake of other micronutrients (folate and vitamin B6) relevant to vitamin B12 metabolism also was evaluated. Key minerals (e.g., iron and zinc) known to be concentrated in beef, but possibly limited in the diet of vegetarians, also were analyzed.

Table 3-2. Foods categorized within each food group

Food Group	Types of Food Included
Beef	Steak, roast, hamburger, ground beef, beef hotdog, beef sausage, beef cold cuts, mixed dishes made with beef
Poultry	All cuts of chicken and turkey (breast, wing, etc), chicken and turkey cold cuts, ground chicken and turkey
Pork and other meats	Ham, pork (all cuts), bacon, ham cold cuts, veal, venison, lamb, shortribs, liver
Dairy	Milk, cheese, yogurt, cream, pudding, ice cream, butter
Eggs and egg mixtures	Eggs, mayonnaise
Seafood*	
Fish	Tuna, fried and not fried fish
Shellfish	Oysters, clams, muscles, shrimp, crab, lobster
Cereals**	
Highly-fortified	All-Bran, Multigrain Cheerios, Complete, Just Right Fruit and Nut, KASHI Heart-to-Heart, Mueslix, Product 19, Smart Start, Special K, Total (all types)
Low-fortified	Oatmeal, grits, all other types of cereals not listed above
Soy	Tofu, soymilk, soy substitute products (Morningstar Farms, Worthington/Loma Linda), egg substitutes
Meal replacement	Power bars, Nutri-grain bars, Balance bar, Zone bar
Mixed dishes with meat type unknown	Pasta with meat/fish sauce, Mexican food, lasagna, chili or pizza with meat other than beef
Other	Non-dairy fats, beans, rice/pasta, soups/sauces, breads/cracker/cakes/pies, nuts/seeds, vegetables, fruit, syrup/honey/gelatin/candy, and beverages

* Seafood was analyzed as one food group and as separate food groups of fish and shellfish.

**Cereal was analyzed as one food group and as separate food groups of highly-fortified and low-fortified cereals. Highly-fortified cereals contained >25% of the Daily Value for vitamin B12. Low-fortified cereals contained <25% of the Daily Value for vitamin B12.

Plasma Vitamin B12 Concentration (Objective 3)

The plasma vitamin B12 concentration of all subjects was determined using MP Biomedicals, Inc SimulTRAC-S B12/Folate Radioassay kit (Orangeburg, NY). This is a competitive protein binding assay using cobalt-57 (^{57}Co). Dithiothreitol (DTT) solution was added to the vitamin B12/folate tracer (^{57}Co , ^{125}I , borate buffer with human serum albumin, dextran, potassium cyanide, dye and preservative). This mixture was added to

the plasma samples and boiled for 15 minutes. The boiling process inactivates endogenous binding proteins and converts all cobalamin forms to cyanocobalamin. Once cooled, purified IF was added to the mixture and incubated for 1 hour. During incubation, endogenous cyanocobalamin and ^{57}Co compete for binding sites to IF. The mixture was centrifuged. Bound cobalamin (labeled and unlabelled) accumulated at the bottom in a pellet, while unbound cobalamin was in the supernant. The radioactivity of the pellet was measured using a gamma counter. Vitamin B12 concentration was inversely related to the measured radioactivity. During the assay, samples were covered with aluminum foil to decrease exposure to light.

A plasma vitamin B12 concentration above 221 pmol/L was considered normal. Plasma vitamin B12 concentrations between 148 and 221 pmol/L were considered marginally deficient, and a concentration below 148 pmol/L was considered deficient (66).

Statistical Methods

The statistical analysis was performed using SAS, version 9.1, SAS Institute Inc. Cary, NC, USA. An initial analysis was conducted to determine whether the four dietary groups differed significantly with respect to the following demographic variables: gender, age, body mass index (BMI), ethnicity, marital status, employment status, student status, and educational level. For the continuous demographic variables, a one-way analysis of variance (ANOVA) was used to determine whether the mean responses differed among the dietary groups. A chi-square test was used to determine whether the proportion of responses in each of the categories differed among groups when considering categorical response variables. Significant differences in gender, age, BMI, marital status, and student status were found among the four dietary groups. BMI and gender were highly

correlated. As a consequence, after adjusting for gender, mean BMI did not differ significantly among dietary groups. Similarly age was highly correlated with student and marital status. After adjusting for age, neither student status nor marital status differed significantly among dietary groups. Therefore, only gender and age were used as covariates in the analysis. For each of the dependent variables, a one-way ANOVA was conducted with age and gender as the covariates and allowing for differences in variances among the four dietary groups. Plasma vitamin B12 concentration was log transformed for normality. Reported plasma concentrations are back-log transformed. Pearson's correlation was used to determine the relationship between vitamin B12 intake and concentration.

CHAPTER 4 RESULTS

Demographic Characteristics of the Study Population

Subjects

Three hundred eighty-eight subjects were enrolled in the study. After enrollment, 62 of the subjects were excluded due to admission during the study interview that a vitamin B12-containing supplement had been taken within the past 6 months. Six subjects were excluded because they failed to return their completed DHQ. Data from 18 subjects were not included because the total calorie intake reported from the DHQ was less than 600 calories or greater than 5,000 calories, which are deemed to be implausible. Therefore, 302 (137 males, 165 females) eligible subjects were included and the meat consumers in this group were further categorized into three groups based on frequency of beef consumption. The dietary beef intake groups were as follows: (1) frequent beef consumers ($n = 97$), (2) seldom beef consumers ($n = 42$), and (3) never beef consumers ($n = 42$). Those who excluded all meat products (i.e., beef, poultry, pork, mixed dishes containing meat, and seafood) were grouped into a fourth dietary group referred to as the vegetarian group ($n = 121$). The vegetarian group consisted of lactoovovegetarians ($n = 66$), lactovegetarians ($n = 47$), and vegans ($n = 8$).

Demographic Characteristics

The demographic characteristics of the study population are presented in Table 4-1. The mean age and BMI (kg/m^2) (mean \pm SD) for all the participants were 25 ± 14 years and 24 ± 9 , respectively. The mean education level was 15 ± 5 , which is equivalent to

the junior level at a university. Gender and age were significantly different ($p < 0.0001$); as were marital status ($p = 0.02$), student status ($p = 0.001$), and BMI ($p = 0.03$) among the four groups.

Table 4-1. Demographic characteristics of dietary groups^{1,5}

Demographic Variable	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)	p-value
Age ²	23.2 ± 6.1	23.7 ± 5.1	26.0 ± 7.2	28.1 ± 8.8	<0.0001
BMI ^{2,3}	24.5 ± 5.4	24.0 ± 3.7	22.7 ± 2.9	23.0 ± 4.3	0.0333
Education level ²	14.6 ± 2.1	15.1 ± 2.7	15.2 ± 1.7	15.3 ± 2.2	0.1336
Gender (%) ⁴					<0.0001
Female	34	69	79	55	
Male	66	31	21	45	
Ethnicity (%) ⁴					0.1039
Asian	10	14	12	24	
White	60	64	74	56	
African American	8	5	2	5	
Hispanic	19	10	7	9	
Other	2	7	5	5	
Marital status (%) ⁴					0.0177
Single	89	88	86	76	
Married	11	12	14	24	
Student status (%) ⁴					0.0007
Full-time	79	69	60	55	
Part-time	4	10	12	3	
Not a student	16	21	29	41	
Employed (%) ⁴					0.2484
Yes	56	60	74	63	
No	44	40	26	37	

¹Means expressed as mean ± standard deviation (SD).

²One-way ANOVA was used for statistical comparisons between groups.

³Body Mass Index (BMI) calculated as kg/m².

⁴Chi-square test was used for statistical comparisons between groups.

⁵Due to rounding, percentages will not always sum to 100.

Dietary Vitamin B12 Intake (Objectives 1 and 2)

Total vitamin B12 intake (mean ± SD) among the four dietary groups is presented in Table 4-2. Vitamin B12 intake is expressed in this table as total vitamin B12 intake (µg/day) consumed and as µg/1000 calories consumed. Unadjusted for calories, total vitamin B12 intake in the seldom beef group was lower ($p = 0.001$) than the frequent beef group. Vitamin B12 intake in the never beef group and vegetarian group also were lower ($p < 0.0001$) than the frequent beef group. The unadjusted vitamin B12 intake of the never

beef group was lower ($p = 0.05$) than the seldom beef group. The vitamin B12 intake unadjusted for calories in the vegetarian group was lower ($p < 0.0001$) than the seldom beef group and lower ($p = 0.02$) than the never beef group.

After adjusting for calorie intake ($\mu\text{g}/1000$ kcals), the never beef and vegetarian groups had lower ($p < 0.0001$) vitamin B12 intakes than the frequent beef and seldom beef groups. There were no differences ($p = 0.27$) in vitamin B12 intake ($\mu\text{g}/1000$ kcals) among the frequent beef and seldom beef groups. The vitamin B12 intake of the vegetarian and never beef intake groups did not differ ($p = 0.60$).

Table 4-2. Daily total dietary intake of vitamin B12¹

	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)
Vitamin B12 (μg)	8.2 ± 4.0	5.9 ± 3.5^2	$4.5 \pm 2.7^{2,3}$	$3.3 \pm 2.8^{2,3,4}$
Vitamin B12 ($\mu\text{g}/1000$ kcals)	3.3 ± 1.4	3.7 ± 2.2	$2.0 \pm 1.4^{2,3}$	$1.9 \pm 1.5^{2,3}$

¹Means expressed as mean \pm SD. One-way ANOVA was used for statistical comparisons between groups adjusted for age and gender.

²Significantly lower than frequent beef ($p < 0.05$).

³Significantly lower than seldom beef ($p < 0.05$).

⁴Significantly lower than never beef ($p < 0.05$).

The total vitamin B12 intake (μg) among the four dietary groups was compared to the Estimated Average Requirement (EAR) as illustrated in Figure 4-1. The frequent beef group consumed 4.1 times more vitamin B12 than the EAR. The seldom beef group consumed 3 times more vitamin B12 than the EAR. The never beef group consumed 2.3 times more than the EAR, and the vegetarian group consumed 1.7 times more vitamin B12 than the EAR (Figure 4-1).

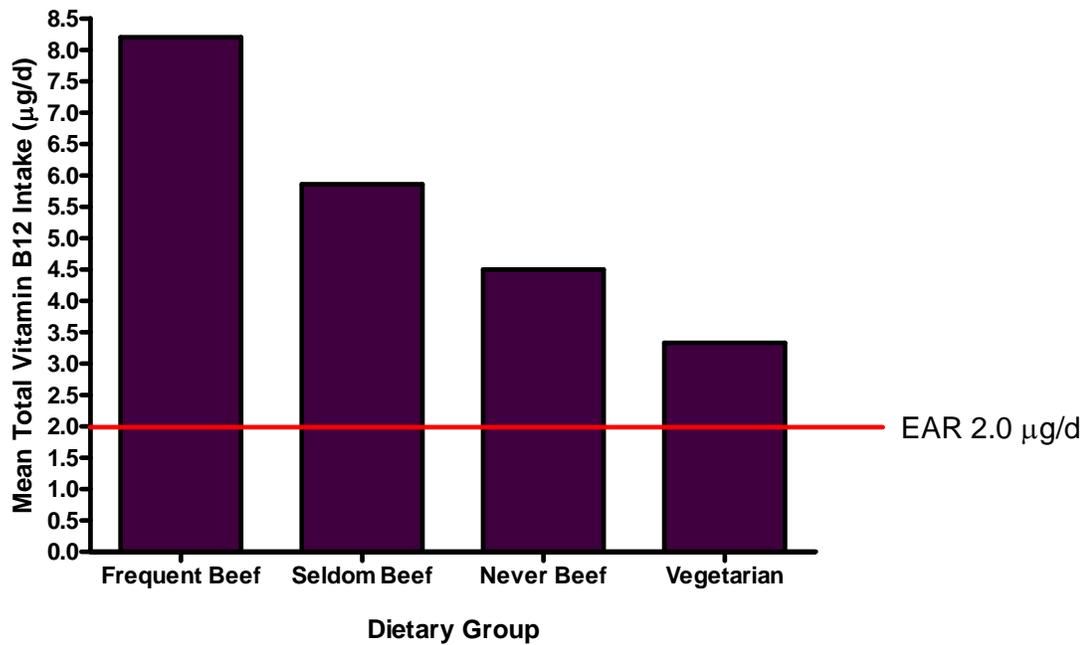


Figure 4-1. Mean vitamin B12 intake compared to recommended intakes. EAR (2.0 µg/day) is based on recommendation for non-pregnant, non-lactating healthy adults.

The daily mean vitamin B12 intake (µg/1000 kcals) contributed by various food sources is presented in Table 4-3.

Beef, Poultry, Pork, Seafood, and Mixed Foods

Among meat consumers (i.e., frequent beef, seldom beef, and never beef groups), vitamin B12 from beef in the seldom beef group was lower ($p < 0.0001$) than vitamin B12 from beef in the frequent beef group. Vitamin B12 from poultry in the never beef group was lower ($p = 0.004$) than vitamin B12 from poultry in the frequent beef group and lower ($p = 0.03$) than the vitamin B12 from poultry in the seldom beef group. There were no differences ($p = 0.90$) in vitamin B12 from poultry among the frequent beef and seldom beef groups. There were no differences in vitamin B12 intake from pork or seafood, including shellfish and fish, among the four dietary groups. Vitamin B12 from mixed foods that contained meat (type undeterminable) in the never beef group was

lower ($p < 0.0001$) than the vitamin B12 intake from mixed foods in the frequent beef group and the seldom beef group. There were no differences ($p = 0.18$) in vitamin B12 intake from mixed foods among the frequent beef and seldom beef group.

Dairy and Eggs

There were no differences in vitamin B12 intake from dairy products among all dietary groups. Vitamin B12 intake from eggs in the vegetarian group was lower ($p = 0.001$) than the frequent beef, lower ($p = 0.001$) than the seldom beef, and lower ($p = 0.0004$) than the never beef groups.

Cereal

Vitamin B12 intake from all types of cereal was lower ($p = 0.01$) in the frequent beef and lower ($p = 0.003$) in the never beef group compared to the seldom beef group. The vitamin B12 intake from cereal (all types) in the never beef group also was lower ($p = 0.03$) than that in the vegetarian group. There were no differences in vitamin B12 intake from all types of cereal among the frequent beef group and vegetarians ($p = 0.13$); frequent beef group and never beef group ($p = 0.10$); and seldom beef group and vegetarians ($p = 0.14$). Cereal was further separated to determine differences in mean vitamin B12 intake from highly-fortified cereals and low-fortified cereals. Vitamin B12 intake from highly-fortified cereals in the frequent beef and never beef group was lower ($p < 0.01$) than the seldom beef group. Vitamin B12 intake from highly-fortified cereals in the never beef group also was lower ($p = 0.02$) than the vitamin B12 intake from highly-fortified cereals in the vegetarian group. There were no differences in mean vitamin B12 intakes from low-fortified cereal across all groups.

Fortified Soy Products, Meal Replacements, and Other Sources

Vitamin B12 intake from fortified soy products in the frequent beef and seldom beef groups was lower ($p < 0.0001$) than observed in the vegetarian group. Vitamin B12 intake from fortified soy products in the frequent beef group also was lower ($p < 0.0001$) than the never beef group. Vitamin B12 intake from fortified soy products in the never beef group was lower ($p = 0.04$) than the vegetarian group. No differences were found for vitamin B12 intake from meal replacement sources among the dietary groups.

Vitamin B12 intake from other sources in the frequent beef group was lower ($p = 0.03$) than the vitamin B12 intake from other sources in the never beef group and ($p = 0.001$) the vegetarian group.

Naturally-Occurring and Fortified Sources

Vitamin B12 intakes from food sources in which vitamin B12 occurs naturally was compared with vitamin B12 intake from fortified foods. Naturally-occurring vitamin B12 intake in the never beef group was lower ($p < 0.0001$) than the frequent beef group and ($p = 0.01$) the seldom beef group. The vegetarian group had lower ($p < 0.0001$) vitamin B12 intake from naturally-occurring sources than the frequent beef, seldom beef, or never beef groups. The frequent beef group had a lower ($p = 0.0004$) intake of vitamin B12 from fortified foods than the seldom beef group, never beef group ($p = 0.03$), and vegetarian groups ($p < 0.0001$). Vitamin B12 from fortified foods in the never beef group was lower ($p = 0.04$) than the vegetarian group.

Table 4-3. Daily mean vitamin B12 contribution by dietary sources ($\mu\text{g}/1000$ kcals)¹

Dietary Source	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)
Beef	0.94 \pm 0.5	0.16 \pm 0.2 ²	--	--
Poultry	0.07 \pm 0.1	0.07 \pm 0.1	0.03 \pm 0.1 ^{2,3}	--
Pork	0.10 \pm 0.1	0.14 \pm 0.6	0.05 \pm 0.3	--
Seafood	1.00 \pm 1.2	1.24 \pm 1.5	0.83 \pm 1.0	--
Shellfish	0.78 \pm 1.1	0.86 \pm 1.1	0.58 \pm 0.7	--
Fish	0.21 \pm 0.3	0.34 \pm 0.4	0.27 \pm 0.3	--
Mixed	0.08 \pm 0.1	0.06 \pm 0.1	0.01 \pm 0.00 ^{2,3}	--
Dairy	0.53 \pm 0.3	0.51 \pm 0.4	0.43 \pm 0.3	0.51 \pm 0.4
Eggs	0.07 \pm 0.1	0.10 \pm 0.1	0.10 \pm 0.1	0.03 \pm 0.1 ^{2,3,4}
Cereal	0.25 \pm 0.5 ³	0.75 \pm 1.2	0.15 \pm 0.4 ^{3,5}	0.44 \pm 1.1
High-fortified cereal	0.21 \pm 0.5 ³	0.71 \pm 1.2	0.11 \pm 0.4 ^{3,5}	0.41 \pm 1.1
Low-fortified cereal	0.03 \pm 0.00	0.02 \pm 0.1	0.03 \pm 0.1	0.04 \pm 0.1
Soy products	0.03 \pm 0.1 ^{4,5}	0.12 \pm 0.3 ⁵	0.24 \pm 0.3 ⁵	0.39 \pm 0.6
Meal replacement	0.08 \pm 0.2	0.10 \pm 0.3	0.06 \pm 0.1	0.13 \pm 0.3
Other	0.15 \pm 0.1 ^{4,5}	0.18 \pm 0.1	0.20 \pm 0.1	0.22 \pm 0.2
Naturally-occurring products	2.93 \pm 1.3	2.40 \pm 1.9	1.49 \pm 1.1 ^{2,3}	0.70 \pm 0.6 ^{2,3,4}
Fortified products	0.41 \pm 0.6 ^{3,4,5}	1.20 \pm 1.4	0.75 \pm 1.0 ⁵	1.17 \pm 1.5

¹Means expressed as mean \pm SD. One-way ANOVA was used for statistical comparisons between groups adjusted for age and gender.

²Significantly lower than frequent beef group ($p < 0.05$).

³Significantly lower than seldom beef group ($p < 0.05$).

⁴Significantly lower than never beef group ($p < 0.05$).

⁵Significantly lower than vegetarian group ($p < 0.05$).

The proportion of vitamin B12 intake provided by different dietary sources to total vitamin B12 intake ($\mu\text{g}/1000$ kcals) is illustrated in Figure 4-2. In the frequent beef group, the largest contributors to vitamin B12 intake were seafood (30%), beef (28.5%), and dairy products (16%). Among the seldom beef group, the largest contributors to vitamin B12 intake were seafood (33.5%), cereal (20%), and dairy (13.8%). Sources in the never beef group that contributed the largest proportion of vitamin B12 were seafood (41%), dairy (21.3%), and soy products (12%). Dairy (27%), cereal (23.4%), and soy products (20.7%) were the largest contributors to vitamin B12 intake among the vegetarians (Figure 4-2).

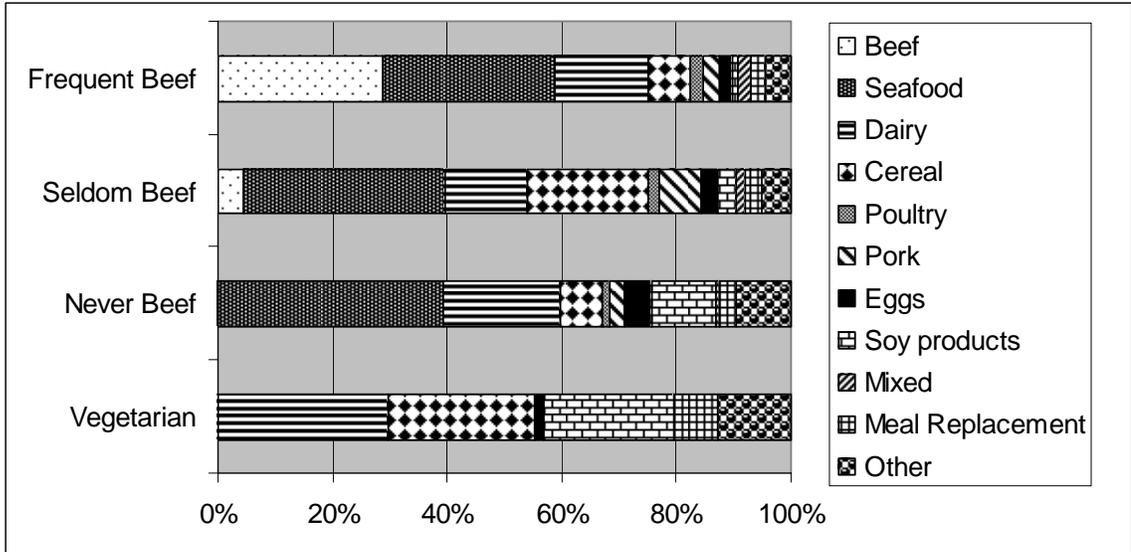


Figure 4-2. Percent of total vitamin B12 intake from food sources. Proportions were determined based on adjusted mean intakes of vitamin B12 from each food source.

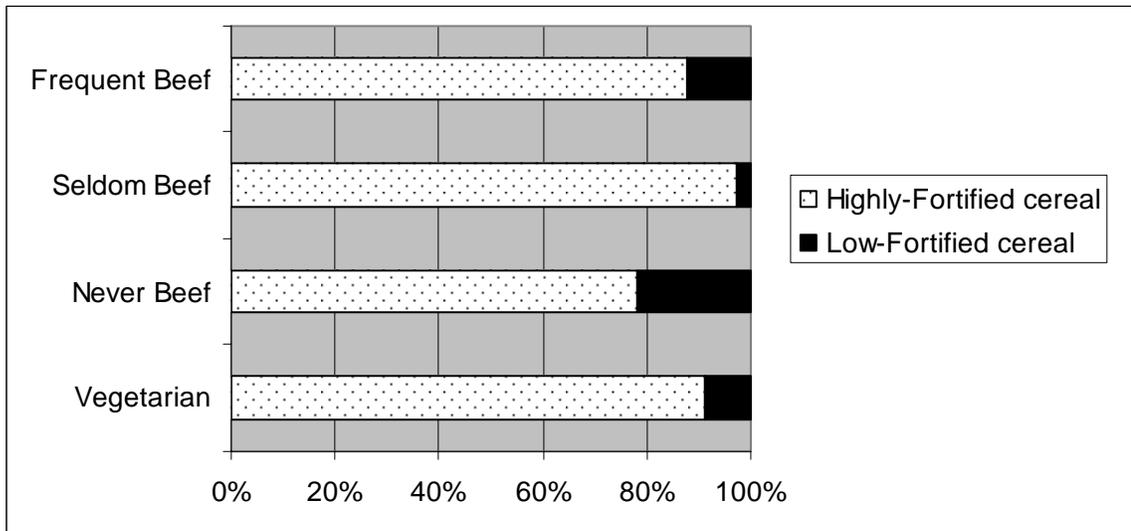


Figure 4-3. Percent of total vitamin B12 intake from cereal sources. Proportions were determined based on adjusted mean intakes of vitamin B12 from each cereal source.

The proportion of vitamin B12 provided by sub-groupings of foods within a category is shown in Figures 4.3 and 4.4. Highly-fortified cereals provided the majority of vitamin B12 derived from cereal sources in all dietary intake groups (Figure 4-3). Vitamin B12 derived from a subgroup of seafood (i.e., shellfish and fish) revealed that

shellfish provided a larger proportion of vitamin B12 among the groups who consumed seafood (Figure 4-4).

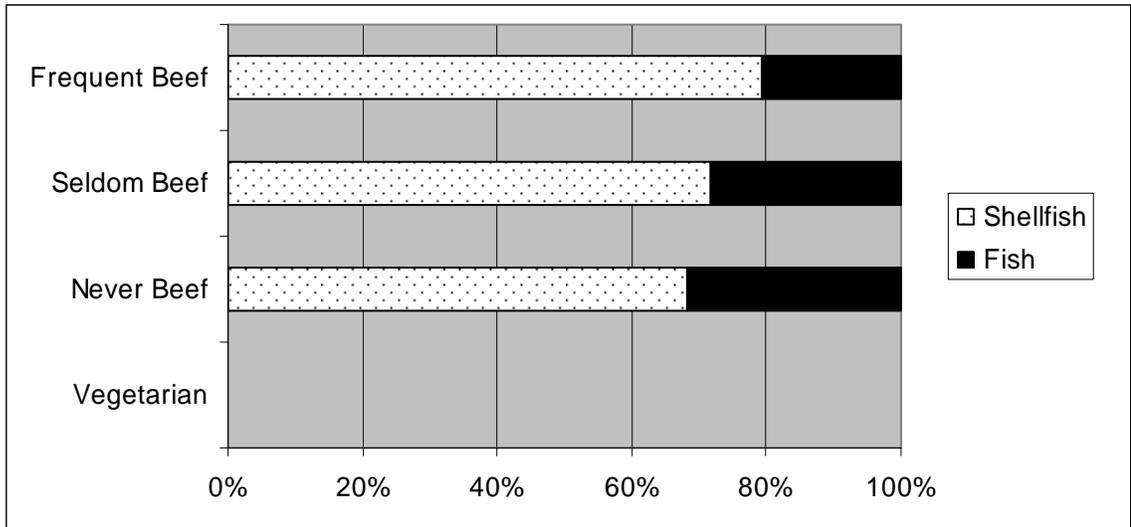


Figure 4-4. Percent of total vitamin B12 intake from shellfish and fish. Proportions were determined based on adjusted mean intakes of vitamin B12 from each seafood source.

The proportion of vitamin B12 intake provided from naturally-occurring or fortified foods is illustrated in Figure 4.5. When the vitamin B12 content of foods consumed was categorized as naturally-occurring or fortified, it was found that naturally-occurring sources of vitamin B12 provided a larger proportion of this nutrient compared to fortified products in the frequent beef, seldom beef, and never beef groups. In the vegetarian group, fortified products provided a larger proportion of the vitamin B12 content of the diet (Figure 4-5).

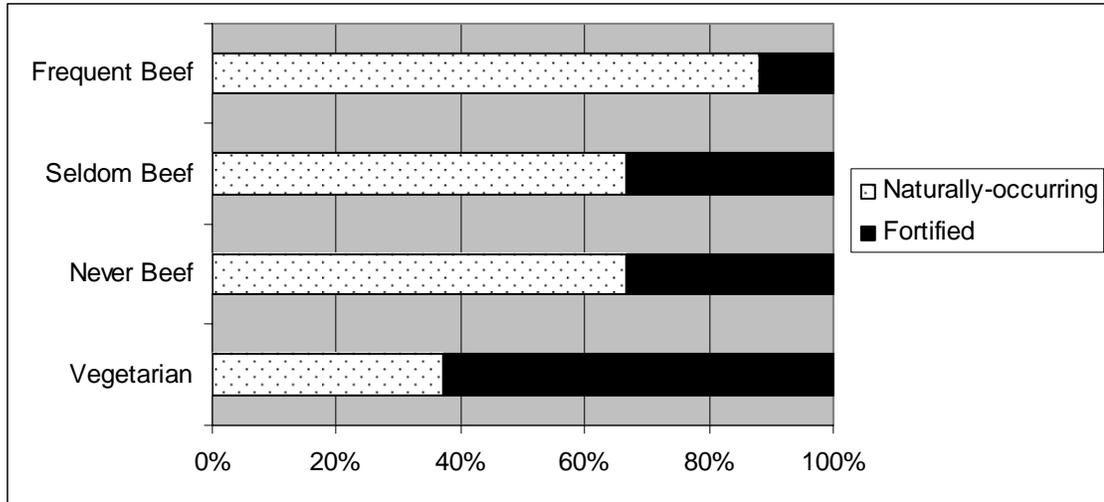


Figure 4-5. Percent of total vitamin B12 intake from naturally-occurring and fortified sources of vitamin B12. Proportions were determined based on adjusted mean intakes of vitamin B12 from naturally-occurring or fortified food sources.

Dietary Intake of Macronutrients and Micronutrients

Table 4-4 presents the energy and macronutrient intakes (mean \pm SD) among the four dietary groups reported from the DHQ. To control for differences in energy intake, protein, carbohydrate, fat, and saturated fat were standardized and expressed as $\mu\text{g}/1000$ kcals consumed.

Energy Intake

The energy or calorie intake of the seldom beef, never beef, and vegetarian groups was lower ($P < 0.0001$) than the calorie intake of the frequent beef group. There were no differences in energy intake among the seldom beef, never beef, and vegetarian groups.

Protein Intake

The protein intake of the seldom beef, never beef, and vegetarian groups also was lower ($p < 0.05$) than the protein intake of the frequent beef group. The protein intake of the vegetarian group was lower ($P < 0.0001$) than the protein intake of the seldom beef and never beef group.

Carbohydrate Intake

The carbohydrate intake of the frequent beef group was lower ($p < 0.0001$) than the carbohydrate intake of the seldom beef, never beef, and vegetarian groups. The carbohydrate intake of the seldom beef and never beef groups was lower ($p < 0.05$) than the carbohydrate intake of the vegetarian group.

Fat Intake

Fat intake was lower ($p = 0.01$) in the seldom beef group and lower ($p = 0.0001$) in the never beef group compared to the frequent beef group. Fat intake in the vegetarian group also was lower ($p < 0.0001$) than the frequent beef group. There were no differences in fat intake among the seldom beef, never beef, and vegetarian groups.

Saturated Fat

Saturated fat intake in the seldom beef, never beef, and vegetarian groups was lower ($P < 0.0001$) than the intake of saturated fat in the frequent beef group. There were no differences in saturated fat intake among the seldom beef, never beef, and vegetarian groups.

Table 4-4. Daily mean dietary intake of macronutrients (per 1000 kcals consumed)¹

Nutrient	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)
Energy (kcal)	2489 ± 1026	1729 ± 650 ²	1856 ± 731 ²	1862 ± 809 ²
Protein (g)	41 ± 9	39 ± 7 ²	37 ± 9 ²	33 ± 9 ^{2,3,4}
Carbohydrate (g)	112 ± 21 ^{3,4,5}	129 ± 19 ⁵	132 ± 15 ⁵	140 ± 21
Fat (g)	40 ± 7	37 ± 8 ²	36 ± 5 ²	36 ± 8 ²
Saturated Fat (g)	12 ± 3	9 ± 3 ²	9 ± 3 ²	10 ± 3 ²

¹Means expressed as mean ± SD. One-way ANOVA was used for statistical comparisons between groups adjusted for age and gender.

²Significantly lower than frequent beef ($p < 0.05$).

³Significantly lower than seldom beef ($p < 0.05$).

⁴Significantly lower than never beef ($p < 0.05$).

⁵Significantly lower than vegetarian ($p < 0.05$).

Table 4-5 presents the daily intakes of micronutrients (mean \pm SD) (i.e., folate and vitamin B6) relevant to vitamin B12 metabolism and key minerals known to be concentrated in beef, but possibly limited in the diet of vegetarians (i.e., iron and zinc). To control for differences in energy intake among the four dietary groups these nutrients also were standardized and expressed as $\mu\text{g}/1000$ kcals consumed.

Folate Intake

The frequent beef group had a lower ($p < 0.0001$) folate intake than the seldom beef, never beef, and vegetarian groups. There were no differences in folate intake among the seldom beef, never beef, and vegetarian groups.

Vitamin B6 Intake

The frequent beef group had a lower ($p = 0.0001$) intake of vitamin B6 than the seldom beef, never beef ($p = 0.03$), the vegetarian groups ($p = 0.001$). There were no differences in vitamin B6 intake among the seldom beef, never beef, and vegetarian groups.

Iron Intake

The frequent beef group had a lower ($p < 0.0001$) intake of iron than the seldom beef, never beef, and vegetarian groups. The seldom beef and the never beef groups had a lower intake of iron ($p = 0.003$; $p = 0.001$, respectively) compared to the vegetarian group. There were no differences in iron intake between the seldom beef and never beef groups ($p = 0.72$).

Zinc Intake

The zinc intake of the never beef group was lower than the zinc intake of the frequent beef group ($p = 0.001$), seldom beef group ($p = 0.002$), and vegetarian groups ($p = 0.02$).

Table 4-5. Daily mean dietary intake of micronutrients (per 1000 kcals consumed)¹

Nutrient per 1000 kcals	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)
Folate (μg)	180 \pm 48 ^{3,4,5}	271 \pm 85	273 \pm 93	287 \pm 107
Vitamin B6 (mg)	1.0 \pm 0.3 ^{3,4,5}	1.3 \pm 0.4	1.2 \pm 0.4	1.2 \pm 0.6
Iron (mg)	7.4 \pm 1.9 ^{3,4,5}	10.4 \pm 3.8 ⁵	10.1 \pm 3.8 ⁵	13.1 \pm 7.6
Zinc (mg)	5.6 \pm 1.8	6.1 \pm 2.7	4.5 \pm 1.5 ^{2,3,5}	5.4 \pm 2.6

¹Means expressed as mean \pm SD. One-way ANOVA was used for statistical comparisons between groups adjusted for age and gender.

²Significantly lower than frequent beef ($p < 0.05$).

³Significantly lower than seldom beef ($p < 0.05$).

⁴Significantly lower than never beef ($p < 0.05$).

⁵Significantly lower than vegetarian ($p < 0.05$).

Plasma Vitamin B12 Concentration (Objective 3)

Plasma vitamin B12 concentrations (mean \pm SD) for the four groups are presented in Table 4-6. There were no differences in the plasma vitamin B12 concentration among the dietary groups ($p = 0.70$). As illustrated in Figure 4-6, mean plasma vitamin B12 concentration was above 221 pmol/L, the limit set as normal, for all dietary groups.

Table 4-6. Plasma vitamin B12 concentration¹

	Frequent Beef (n = 97)	Seldom Beef (n = 42)	Never Beef (n = 42)	Vegetarian (n = 121)
Plasma vitamin B12 (pmol/L)	280 \pm 114	289 \pm 114	279 \pm 139	263 \pm 143

¹Means expressed as mean \pm SD. One-way ANOVA was used on log-transformed means for statistical comparisons between groups adjusted for age and gender. Plasma concentrations are based on back-log transformed means.

Vitamin B12 Status

Vitamin B12 status was based on plasma vitamin B12 concentration and defined as normal (>221 pmol/L), marginally deficient (148-221 pmol/L), or deficient (<148 pmol/L). The percent of individuals who were considered normal, marginally deficient, and deficient is shown in Figure 4-7. Vitamin B12 status was normal in 74% of the frequent beef consumers, 74% of the seldom beef consumers, 69% of the never beef

consumers, and 61% of the vegetarians. Vitamin B12 status was marginally deficient in 20% of the frequent beef consumers, 21% of the seldom beef, 19% of the never beef consumers, and 22% of the vegetarians. Vitamin B12 status was deficient in 6% of the frequent beef consumers, 5% of the seldom beef consumers, 12% of the never beef consumers, and 17% of the vegetarians. There were no differences in the percentage of individuals who were normal, marginally deficient, or deficient among the four groups ($p = 0.18$). The percentage of individuals who were deficient based on plasma vitamin B12 concentration (<148 pmol/L) was approximately two-fold higher in the non-beef consumers [never beef (12%); and vegetarians (17%)] compared to beef consumers [frequent beef (6%); and seldom (5%)].

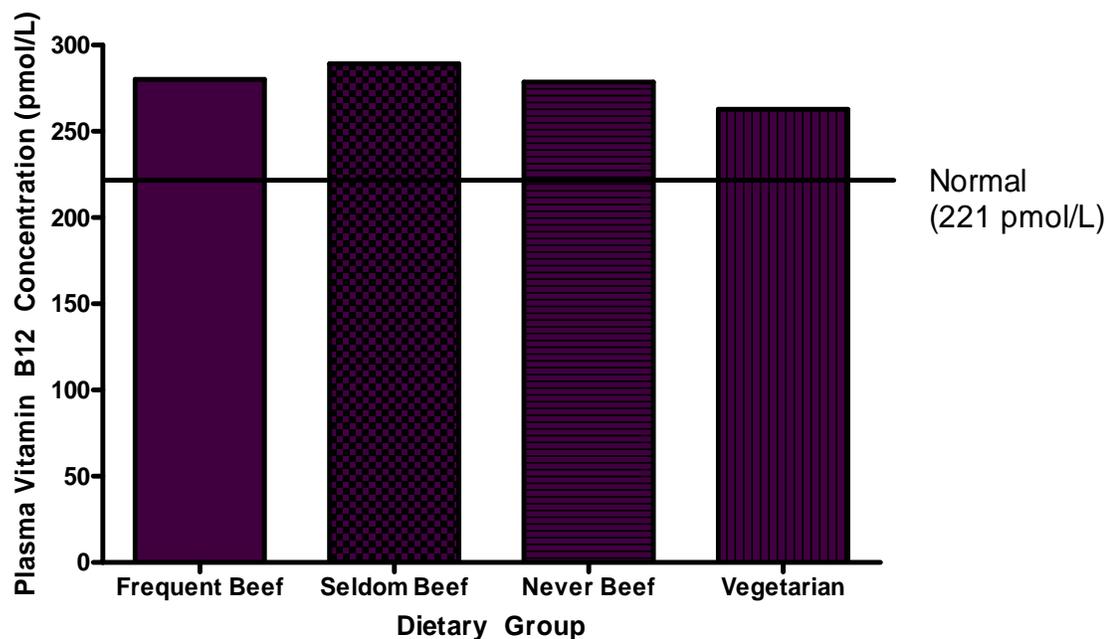


Figure 4-6. Plasma vitamin B12 concentration (pmol/L). Normal vitamin B12 status was defined as a plasma vitamin B12 concentration greater than 221 pmol/L.

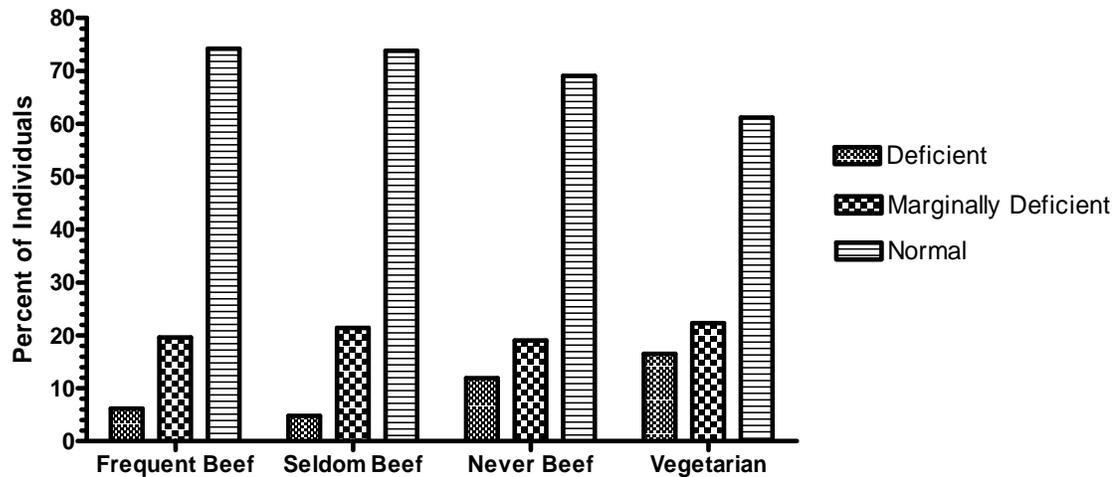


Figure 4-7. Vitamin B12 status among dietary groups. Statistical comparisons were determined using a Chi square test. Vitamin B12 status was defined as follows: (1) deficient (<148 pmol/L); (2) marginally deficient (148-221 pmol/L); and (3) normal (>221 pmol/L).

The mean plasma vitamin B12 concentration for the non-vegetarian and vegetarian groups are presented in Table 4-7. The mean plasma vitamin B12 concentration (mean \pm SD) was lower ($p = 0.01$) in the vegetarian group than the non-vegetarian group.

Table 4-7. Plasma vitamin B12 concentration among non-vegetarians and vegetarians¹

	Non-Vegetarians (n = 181)	Vegetarians (n = 121)
Plasma vitamin B12 (pmol/L)	313 \pm 124	280 \pm 146 ²

¹Means expressed as mean \pm SD. One-way ANOVA was used for statistical comparisons controlled for BMI.

²Significantly lower than non-vegetarians ($P = 0.01$)

The percent of individuals who were deficient in the non-vegetarian and vegetarian groups are presented in Figure 4-8. The percent of individuals who were deficient in the non-vegetarian group (7%) was lower ($p = 0.01$) than the percent of individuals who were deficient in the vegetarian group (17%).

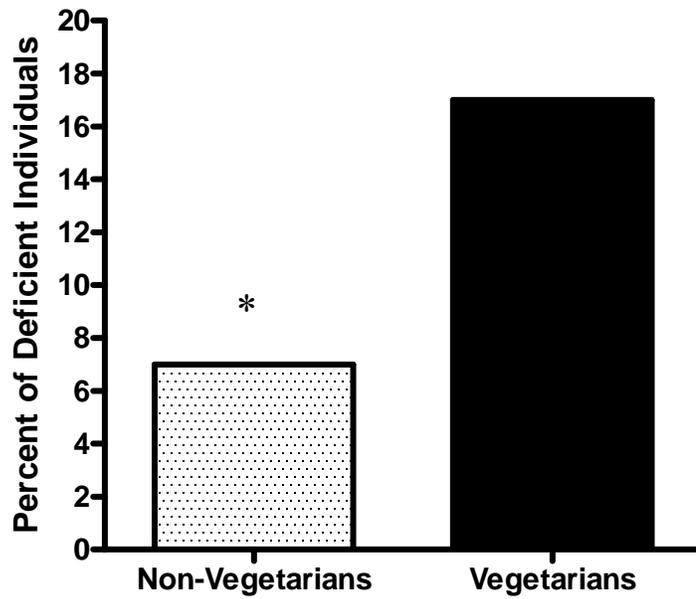


Figure 4-8. Percent of individuals deficient among non-vegetarians and vegetarians. Statistical comparisons were determined using a Chi Square test ($p = 0.01$). Vitamin B12 deficiency was defined as <148 pmol/L.

Vitamin B12 Intake and Status

The association between total vitamin B12 intake and plasma vitamin B12 concentrations for all individuals was assessed. Dietary vitamin B12 intake was weakly but significantly correlated with plasma vitamin B12 concentration ($r = 0.23$, $p < 0.0001$).

CHAPTER 5 DISCUSSION AND CONCLUSION

The focus of this study was to assess vitamin B12 intake and status in healthy men and women who consumed beef at variable frequencies and to estimate the relative contribution of specific foods to vitamin B12 intake. Previous vitamin B12 intake assessment has primarily focused on the adequacy of vitamin B12 intake in individuals who consume vegetarian diets compared to that of individuals who consume non-vegetarian diets. Beef, a highly concentrated source of vitamin B12 and often restricted in the diets of meat-consumers and vegetarians for health reasons, is hypothesized to be an important contributor of dietary vitamin B12 intake among meat-consumers. Prior to the current study, the role of beef in contributing to vitamin B12 intake had not been explored.

Consumption of beef at least one time a week or more resulted in higher dietary vitamin B12 intake than that observed in the other dietary intake groups. Exclusion of beef from the diet resulted in lower vitamin B12 intake compared to the vitamin B12 intake of beef consumers. These data indicate that frequent beef consumption plays an important role in providing vitamin B12 in the diet of meat consumers. Miller et al. (1991) assessed frequency of intake of other vitamin B12 containing foods such as dairy, eggs, and seafood among vegetarians in relation to serum vitamin B12 concentration. They did not, however, assess the relationship between frequency of intake of these foods and dietary intake of vitamin B12. Since this study only involved vegetarians, frequency of beef consumption and the relation to vitamin B12 intake and status could not be

assessed. Until this current study, the effect of beef intake frequency on total dietary intake of vitamin B12 had not been addressed.

Previous investigations have reported that mean daily vitamin B12 intake among meat-consumers was consistently above 2.0 $\mu\text{g}/\text{day}$ (84-86, 97). National data from NHANES 1999-2000 indicate that Americans of all age groups are consuming well above the EAR (2.0 $\mu\text{g}/\text{day}$) for vitamin B12 (32). In the current study, daily vitamin B12 intake for all meat consumption groups exceeded the EAR. Data from the present study indicated that as beef intake frequency increased so did the intake of vitamin B12 as illustrated by the fact that the vitamin B12 intake among the frequent beef consumers was 4.1 times above the EAR compared to 2.3 times the EAR in the individuals who never consumed beef, but included all other animal products.

Analysis of the CSFII 1995 data for men and women indicated that the top three foods contributing to the vitamin B12 intake were mixed foods, milk and milk drinks, and beef (20). Beef contributes approximately 2.2 $\mu\text{g}/\text{serving}$ of vitamin B12. Therefore, when categorizing subjects based on their frequency of beef intake, as done in the current study, one might assume that beef would provide the largest proportion of vitamin B12 among the beef consumers. Unexpectedly, this was not found among all beef consumption groups. Beef contributed 28.5% among the frequent beef consumers, but only 4% among the seldom beef consumers. In all three meat consumption groups (frequent, seldom, and never) seafood and dairy consistently contributed a substantial percentage of dietary vitamin B12 in the diet. Seafood contributed at least 30% (range 30-41%) of the total dietary vitamin B12 intake and dairy provided no less than 13.8% (range 13.8-21.3%) of the total dietary vitamin B12 intake among these groups.

Differences in data from the current study and the CSFII 1995 data may be attributed to differences in the categorization of food groups. In the current study, the type of meat found in the mixed foods determined the category in which it was placed. For this reason, an independent category for mixed foods was not created in the current study.

In the current study, beef intake among the frequent beef consumers was the only meat (compared to poultry and pork) that provided a substantial amount of vitamin B12 in the diet. In contrast, in the seldom beef group, beef did not contribute a large proportion to dietary vitamin B12 intake. Yoshino et al. (2005) investigated dietary sources of vitamin B12 among Japanese adults irrespective of diet type. It was reported that seafood, meats, and dairy provided a larger proportion of vitamin B12 in the diet than other sources of vitamin B12. Larsson and Johansson (2005) separated their study population into vegetarians and non-vegetarians in a similar manner as was done in the current study. All animal products were combined together when assessing the contribution of vitamin B12 from dietary sources by these investigators (89). Therefore, the contribution of dietary vitamin B12 intake from various animal sources could not be assessed. In the current study, specific food groups that provided vitamin B12 to the diet were characterized to a greater extent than was done in previous investigations. For example, the contribution of vitamin B12 from different types of seafood (i.e., shellfish and fish) was determined, as well as, the contribution of vitamin B12 from highly-fortified cereal versus low-fortified cereals. In addition, the overall vitamin B12 intake was characterized as naturally-containing sources or fortified food sources. Among meat consumers, the vitamin B12 provided by seafood was predominately from shellfish; the

vitamin B12 from cereal was from highly-fortified cereals; and a larger proportion of vitamin B12 intake of meat consumers was from naturally-occurring food sources.

In comparison to beef consumers, vegetarians had a lower intake of vitamin B12. These findings are consistent with previous investigations (85, 86). Even with lower intakes of vitamin B12 among the vegetarians, the intake of vitamin B12 among the vegetarian group was 1.7 times above the EAR in the current study. The addition of so many fortified products in the marketplace that are acceptable to vegetarians has made it possible for individuals who follow a vegetarian diet pattern to consume enough vitamin B12 to maintain an adequate intake. Several studies that have assessed vitamin B12 intake of vegetarian and vegan populations have reported that dietary intake of vitamin B12 may have been low because the nutrient database used to assess intake did not contain foods frequently consumed by vegetarians or because the nutrient database was not complete, in that information regarding vitamin B12 content of all consumed foods was not included (10, 11). To address this research need, the FFQ used to assess dietary intake of vitamin B12 in the current study was modified to contain vitamin B12-fortified foods that are commonly consumed by vegetarians. Vegetarians get a large proportion of their vitamin B12 from dairy, highly-fortified cereals, and fortified soy products, which likely explains why low dietary vitamin B12 intake in the vegetarian group was not observed in the current study.

In addition to a low vitamin B12 intake, the intake of calories, protein, fat, and saturated fat were lower in vegetarians compared to frequent beef-consumers. The vegetarians also had higher intakes of carbohydrates, folate, vitamin B6, and iron than the

frequent beef-consumers. This nutrient intake pattern was similar to what was reported in the EPIC-Oxford study by Davey et al. (2003).

Beef is a rich source of iron and zinc, therefore, it has been assumed that increased beef consumption would be associated with a higher intake of iron and zinc. This was not always true in our study population. Iron was actually lower in all of the meat consuming groups compared to the vegetarian group. This may occur because a higher proportion of the vitamin B12 intake among vegetarians was from highly-fortified cereals, products that are also enriched with iron. Zinc intake did not differ among the vegetarian and the frequent or seldom beef groups. The group that never consumed beef but consumed other animal products had a lower zinc intake than the frequent beef, seldom beef, and vegetarian groups. These findings suggest that in meat consumers, beef may play an important role in maintaining adequate dietary zinc intake.

Mean plasma vitamin B12 concentration in our study population did not differ among the dietary groups. This is surprising since previous investigations support the conclusion that vegetarians, especially vegans, have a lower vitamin B12 concentration compared to non-vegetarians (10, 87, 88). It may be possible that our vegetarian population consumed enough vitamin B12 from animal and fortified foods to maintain a normal plasma vitamin B12 concentration. NHANES III (1994-1998) indicated that the mean vitamin B12 concentration of all Americans was 382 pmol/L. In the current study, the mean vitamin B12 concentration of each dietary intake group was lower than that observed in this national survey. The means of each group did not exceed 290 pmol/L, however, this concentration is still considered clinically normal. It is important to note that conclusions regarding status can not be based on mean values alone since means can

be affected by outliers. It is more appropriate to use the percentage of individuals with concentrations below the accepted normal range to assess vitamin B12 status. In the current study, no significant difference was found among the percent of individuals who were normal, marginally deficient, or deficient. Although, it is striking that 17% of vegetarians and 12% of never beef consumers were deficient compared to 6% of the frequent beef and 5% of the seldom beef groups.

A strength of this study is that supplement users were excluded from participation making it possible to assess intake of vitamin B12 from only dietary sources. This study also used a modified FFQ that contained a comprehensive number of vitamin B12-containing foods and vitamin B12-fortified food products commonly consumed by vegetarians. The addition of these foods allowed for better dietary assessment of vitamin B12 intake. Another strength of this study was that it was possible to directly compare the frequency of beef consumption to dietary vitamin B12 intake. No other study has grouped subjects in this manner, therefore previous investigations have been unable to determine the effect of beef consumption on dietary vitamin B12 intake.

Differences within the vegetarian group with respect to the various subgroups of vegetarian food patterns may be considered a weakness of the study. For example, when comparing the contribution of vitamin B12 intake from eggs, the results of the vegetarian group may be different if the lactoovovegetarian, lactovegetarian, and vegan subcategories classifications were evaluated separately. Since vegans and lactovegetarians do not consume eggs, this may be one reason why the vegetarian group as a whole had a lower vitamin B12 intake from eggs compared to the meat-consumers.

The small sample size of the meat-consuming dietary groups is another notable weakness. This may reflect why no differences were found for plasma vitamin B12 concentration. Due to the limitations and current scrutiny of the reliability of the plasma vitamin B12 assay, this method of assessment may not truly reflect vitamin B12 status. Other vitamin B12 status indicators that are more reflective of functional status are being measured by other investigators involved in the current research study and will be reported separately.

Due to a small sample size among the frequent beef group, seldom beef group, and never beef group, these groups were pooled to evaluate the relative difference between non-vegetarian and vegetarian consumers regardless of beef intake. These comparisons were performed as part of a separate analysis. The mean plasma vitamin B12 concentration of the non-vegetarian group was higher than the vegetarian group, this was not found with the previous four dietary groups. Also, the percent of individuals who were deficient in the non-vegetarian group were lower than the vegetarians, which again, were not observed with the four dietary groups. These data indicate that the small sample size of the beef intake subcategories may explain why no differences were detected in vitamin B12 status among the four dietary groups.

To conclude, this study used a modified FFQ for vitamin B12 food sources to compare vitamin B12 intake among beef consumers and vegetarians. The hypothesis that high dietary intake frequency of beef would be associated with a greater intake of total dietary vitamin B12 was supported by the data in this study. Increased frequency of beef intake was not associated with a higher mean plasma vitamin B12 concentration. However, the percentage of individuals who were deficient among non-vegetarians was

lower than vegetarians. Beef did not provide a larger proportion of vitamin B12 intake among all meat consumers. In the frequent beef group, seafood provided a comparable proportion of total vitamin B12 intake as beef. Overall, seafood and dairy provided a substantial proportion of vitamin B12 among meat consumers. Dietetic practitioners and nutrition educators can use these data to promote beef, seafood, and dairy consumption for optimizing vitamin B12 status through dietary means. Future studies should evaluate the impact of frequency of intake of seafood and dairy food sources on total vitamin B12 intake.

APPENDIX A
SUBJECT DATA COLLECTION FORM

Introduction

I am calling in regard to your interest in our nutrition study; do you have a few minutes right now?

This is a UF Nutrition department study and involves coming in one morning for about 1 hour for a fasting blood sample, we take about 1 ½ ounces of blood, and you only need to fast 8 hours. We will give you a breakfast snack right afterward, and then give a brief explanation of a food frequency questionnaire you will be taking home. You will be asked to mail it back in the provided envelope, and once we receive the questionnaire you would get paid the \$50. I just have to ask you some questions to see if you are eligible for our study and to get background information, OK?

How old are you? 18-49

Do you smoke? no

Are you pregnant or breastfeeding? no

Do you take any prescription medications other than oral contraceptives? no

If not within the age range or if they answer yes to any question:

I am very sorry, but you do not meet our exclusion criteria, but thank you for your interest.

Now I just have a few questions about your diet to see what specific category of our study you would fit in to. Please answer as best you can, estimates are ok and consider all instances of when you might eat the items I will ask about, even if only occasionally.

Do you take a multi-vitamin, complex, red star nutritional yeast, or any other supplement or additive ever?

If they take a multivitamin, B complex, red star nutritional yeast, complete the session through all diet info but do not record. Conclude by confirming their name and saying “This has been a preliminary screening call, your information will be reviewed by the principal investigator based on need, and our selection criteria at this time. If you are chosen you will be called again to schedule an appointment over the next two weeks. Thank you very much for your interest and your time.

Do you eat breakfast cereals? (If so) What Kind do you eat mostly?

If they eat a 100% fortified cereal or eats a 50% cereal daily complete the through all diet info but do not record. Conclude by confirming their name and saying “This has been a preliminary screening call, your information will be reviewed by the principal investigator based on need, and our selection criteria at this time. If you are chosen you will be called again to schedule an appointment over the next two weeks. Thank you very much for your interest and your time.

If the interviewee fulfills all selection criteria continue with the questionnaire, record info on moderate/non-fortified cereal consumption below.

Do you eat breakfast cereals?

- Yes
- No

Name/Brand	Quantity	Frequency

Are you a vegan, vegetarian or meat eater?					
Vegan – this means you eat NO animal derived foods intentionally (if they eat small amt like in cake then OK)					
Vegetarian – this means you eat NO beef, chicken, turkey, pork, or fish					
How often do you eat ...					
	Never	Rarely (<1 x/mo)	Occasionally (1-4 x/mo)	Frequently (2-4 x/wk)	Always (5-7 x/wk)
Beef					
Chicken					
Turkey					
Pork					
Fish					
Eggs					
Cheese					
Cow’s Milk					
Yogurt					
Other Dairy					

Do you follow a restricted diet such as

- No red meat
- Lactose-free
- Kosher
- Weight loss
- Weight gain
- Low salt
- Low fat
- Low cholesterol
- Low carbohydrate
- Hypoallergenic

(If so) How long have you consumed this type of diet?

Have you made any major dietary changes within the last 3 years?

- No
 - Yes; How long ago did you make changes and what changes did you make?
-

	NO	YES	
Do you consume alcoholic beverages?			
➤ How often/quantity			

Health Information

I am going to ask you a few questions about your health to determine if you are eligible for our study. I will be recording this information, but it will be kept confidential and is this ok with you? _____

Height:	Weight:		
Have you do you currently have any of the following?	NO	YES	
Alcoholism			
Anemia			
Blood clots			
Bronchitis			
Cystic Fibrosis			
Dermatitis			
Diabetes			
Eating disorders/Chronic nausea or vomiting			
Food allergy			
Gall bladder disease			
GI problems/ Lactose intolerance			
Gout			
Migraines			
Hemorrhoids			
Hepatitis/Liver disease			
Heart disease/High cholesterol/High blood pressure			
HIV			
Kidney disease			
Neurological disorder			
Obesity			
Seizures/Stroke			
Thyroid problem			
Tumors/Cancer			
Ulcers			
Other			
Have you been hospitalized within the last 5 years?			
➤ Cause			

Do you have a history of more than 1 miscarriage?			
<input type="radio"/> Yes <input type="radio"/> No			
If you are selected to participate in this study are you willing to sign an informed consent understanding we have access to medical information on you?			
<input type="radio"/> Yes <input type="radio"/> No			

Demographic Information

What is your birth date? _____ / _____ / _____
 Month Day Year

How would you describe your race or ethnic background?

- White
- Black or African American
- American Indian or Alaska Native
- Hispanic or Latino
- Asian
- Native Hawaiian or Other Pacific Islander
- Other _____

What is the highest level of school or training that you have completed? [Circle only one response]

Grade school	01	02	03	04	05	06	07	08
High school	09	10	11	12				
Technical school or college	13	14	15	16				
Graduate or professional	17	18	19	20+				
Don't know	X							

Marital status?

- Single/never married
- Married
- Separated
- Divorced
- Widowed

Are you a full-time or part-time student?	Are you employed?
<input type="radio"/> Full time	<input type="radio"/> Yes
<input type="radio"/> Part time	<input type="radio"/> No
<input type="radio"/> Not a student	<input type="radio"/> Student employee

Contact Information

Name			
M / F	Last	First	Middle
Address			
	Street		Apt. #
	City		Zip code
Phone			
	Day	Evening	Cell
E-mail			

Name of person and phone number to call in case of an emergency if you are invited to participate in this study:

If we need to contact you, and can not reach you where/with who can a message be left?

How did you hear about our study?

APPENDIX B
DIET HISTORY QUESTIONNAIRE

NATIONAL INSTITUTES OF HEALTH

Diet History Questionnaire



GENERAL INSTRUCTIONS

- Answer each question as best you can. Estimate if you are not sure. A guess is better than leaving a blank.
- Use only a black ball-point pen. Do not use a pencil or felt-tip pen. Do not fold, staple, or tear the pages.
- Put an X in the box next to your answer.
- If you make any changes, cross out the incorrect answer and put an X in the box next to the correct answer. Also draw a circle around the correct answer.
- If you mark NEVER, NO, or DON'T KNOW for a question, please follow any arrows or instructions that direct you to the next question.

BEFORE TURNING THE PAGE, PLEASE COMPLETE THE FOLLOWING QUESTIONS.

Today's date:

MONTH	DAY	YEAR
01 <input type="checkbox"/> Jan	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> 2002
02 <input type="checkbox"/> Feb	<input type="checkbox"/> 0 <input type="checkbox"/> 0	<input type="checkbox"/> 2003
03 <input type="checkbox"/> Mar	<input type="checkbox"/> 1 <input type="checkbox"/> 1	<input type="checkbox"/> 2004
04 <input type="checkbox"/> Apr	<input type="checkbox"/> 2 <input type="checkbox"/> 2	<input type="checkbox"/> 2005
05 <input type="checkbox"/> May	<input type="checkbox"/> 3 <input type="checkbox"/> 3	<input type="checkbox"/> 2006
06 <input type="checkbox"/> Jun	<input type="checkbox"/> 4	
07 <input type="checkbox"/> Jul	<input type="checkbox"/> 5	
08 <input type="checkbox"/> Aug	<input type="checkbox"/> 6	
09 <input type="checkbox"/> Sep	<input type="checkbox"/> 7	
10 <input type="checkbox"/> Oct	<input type="checkbox"/> 8	
11 <input type="checkbox"/> Nov	<input type="checkbox"/> 9	
12 <input type="checkbox"/> Dec		

In what month were you born?

01 <input type="checkbox"/> Jan
02 <input type="checkbox"/> Feb
03 <input type="checkbox"/> Mar
04 <input type="checkbox"/> Apr
05 <input type="checkbox"/> May
06 <input type="checkbox"/> Jun
07 <input type="checkbox"/> Jul
08 <input type="checkbox"/> Aug
09 <input type="checkbox"/> Sep
10 <input type="checkbox"/> Oct
11 <input type="checkbox"/> Nov
12 <input type="checkbox"/> Dec

In what year were you born?

19

<input type="checkbox"/> 0	<input type="checkbox"/> 0
<input type="checkbox"/> 1	<input type="checkbox"/> 1
<input type="checkbox"/> 2	<input type="checkbox"/> 2
<input type="checkbox"/> 3	<input type="checkbox"/> 3
<input type="checkbox"/> 4	<input type="checkbox"/> 4
<input type="checkbox"/> 5	<input type="checkbox"/> 5
<input type="checkbox"/> 6	<input type="checkbox"/> 6
<input type="checkbox"/> 7	<input type="checkbox"/> 7
<input type="checkbox"/> 8	<input type="checkbox"/> 8
<input type="checkbox"/> 9	<input type="checkbox"/> 9

Are you male or female?

Male
 Female

BAR CODE LABEL OR SUBJECT ID
HERE

<input type="checkbox"/>									
--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

1. Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

- NEVER (GO TO QUESTION 2)
- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

- 1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

- Less than $\frac{3}{4}$ cup (6 ounces)
 $\frac{3}{4}$ to $1\frac{1}{4}$ cups (6 to 10 ounces)
 More than $1\frac{1}{4}$ cups (10 ounces)

2. Over the past 12 months, how often did you drink **orange juice**?

- NEVER (GO TO QUESTION 3)
- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

- 2a. Each time you drank **orange juice**, how much did you usually drink?

- Less than $\frac{3}{4}$ cup (6 ounces)
 $\frac{3}{4}$ to $1\frac{1}{4}$ cups (6 to 10 ounces)
 More than $1\frac{1}{4}$ cups (10 ounces)

3. Over the past 12 months, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, grapefruit or others)?

- NEVER (GO TO QUESTION 4)
- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

- 3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

- Less than $\frac{3}{4}$ cup (6 ounces)
 $\frac{3}{4}$ to $1\frac{1}{2}$ cups (6 to 12 ounces)
 More than $1\frac{1}{2}$ cups (12 ounces)

Over the past 12 months...

4. How often did you drink other **fruit drinks** (such as cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)?

- NEVER (GO TO QUESTION 5)
- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

- 4a. Each time you drank **fruit drinks**, how much did you usually drink?

- Less than 1 cup (8 ounces)
 1 to 2 cups (8 to 16 ounces)
 More than 2 cups (16 ounces)

- 4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

- Almost never or never
 About $\frac{1}{4}$ of the time
 About $\frac{1}{2}$ of the time
 About $\frac{3}{4}$ of the time
 Almost always or always

5. How often did you drink **milk, including lactose-free milk** (but NOT milk substitutes) **as a beverage** (NOT in coffee, NOT in cereal)? (Please include chocolate milk, flavored milk like Ovaltine or Quick, and hot chocolate.)

- NEVER (GO TO QUESTION 6)
- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

- 5a. Each time you drank **milk as a beverage**, how much did you usually drink?

- Less than 1 cup (8 ounces)
 1 to $1\frac{1}{2}$ cups (8 to 12 ounces)
 More than $1\frac{1}{2}$ cups (12 ounces)

Please continue on next page.

5b. What kind of **milk** did you usually drink?

- Whole milk (including lactose-free variety)
- 2% fat milk (including lactose-free variety)
- 1 % fat milk (including lactose-free variety)
- Skim, nonfat, or ½% fat milk (including lactose-free variety)

6. How often did you drink a **milk substitute such as soy or rice milk as a beverage** (NOT in coffee, NOT in cereal)? (Please include milk substitute used to make other beverages such as chocolate "milk", Ovaltine, Quick and hot chocolate.)

NEVER (GO TO QUESTION 7)

- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

6a. Each time you drank a milk substitute as a beverage, how much did you usually drink?

- Less than 1 cup (8 ounces)
- 1 to 1½ cups (8 to 12 ounces)
- More than 1½ cups (12 ounces)

6b. What kind of **milk substitute** did you usually drink?

- Soy milk (8th Continent)
- Soy milk (Whitewave - Silk)
- Soy milk (VitaSoy)
- Soy milk (other brand)
- Rice milk

Over the past 12 months...

7. How often did you drink **meal replacement, energy, or high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, AdvantEdge, Boost or others?

NEVER (GO TO QUESTION 8)

- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

7a. Each time you drank **meal replacement, energy, or high protein beverages**, how much did you usually drink?

- Less than 1 cup (8 ounces)
- 1 to 1½ cups (8 to 12 ounces)
- More than 1½ cups (12 ounces)

8. Over the past 12 months, did you drink **soft drinks, soda, or pop**?

NO (GO TO QUESTION 9)

YES

8a. How often did you drink **soft drinks, soda, or pop IN THE SUMMER**?

NEVER

- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

8b. How often did you drink **soft drinks, soda, or pop DURING THE REST OF THE YEAR**?

NEVER

- | | |
|---|--|
| <input type="checkbox"/> 1 time per month or less | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 2-3 times per day |
| <input type="checkbox"/> 1-2 times per week | <input type="checkbox"/> 4-5 times per day |
| <input type="checkbox"/> 3-4 times per week | <input type="checkbox"/> 6 or more times per day |
| <input type="checkbox"/> 5-6 times per week | |

8c. Each time you drank **soft drinks, soda, or pop**, how much did you usually drink?

- Less than 12 ounces or less than 1 can or bottle
- 12 to 16 ounces or 1 can or bottle
- More than 16 ounces or more than 1 can or bottle

8d. How often were these soft drinks, soda, or pop **diet or sugar-free**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

- 8e. How often were these soft drinks, soda, or pop **caffeine-free**? (See list of caffeine and caffeine-free sodas provided by the researcher.)

- Almost never or never
 About 1/4 of the time
 About 1/2 of the time
 About 3/4 of the time
 Almost always or always

9. Over the past 12 months, did you drink **beer**?

NO (GO TO QUESTION 10)

YES

- 9a. How often did you drink **beer IN THE SUMMER**?

- NEVER
 1 time per month or less
 2-3 times per month
 1-2 times per week
 3-4 times per week
 5-6 times per week
 1 time per day
 2-3 times per day
 4-5 times per day
 6 or more times per day

- 9b. How often did you drink **beer DURING THE REST OF THE YEAR**?

- NEVER
 1 time per month or less
 2-3 times per month
 1-2 times per week
 3-4 times per week
 5-6 times per week
 1 time per day
 2-3 times per day
 4-5 times per day
 6 or more times per day

- 9c. Each time you drank **beer**, how much did you usually drink?

- Less than a 12-ounce can or bottle
 1 to 3 12-ounce cans or bottles
 More than 3 12-ounce cans or bottles

Over the past 12 months...

10. How often did you drink **wine** or **wine coolers**?

- NEVER (GO TO QUESTION 11)
 1 time per month or less
 2-3 times per month
 1-2 times per week
 3-4 times per week
 5-6 times per week
 1 time per day
 2-3 times per day
 4-5 times per day
 6 or more times per day

- 10a. Each time you drank **wine** or **wine coolers**, how much did you usually drink?

- Less than 5 ounces or less than 1 glass
 5 to 12 ounces or 1 to 2 glasses
 More than 12 ounces or more than 2 glasses

11. How often did you drink **liquor** or **mixed drinks**?

- NEVER (GO TO QUESTION 12)
 1 time per month or less
 2-3 times per month
 1-2 times per week
 3-4 times per week
 5-6 times per week
 1 time per day
 2-3 times per day
 4-5 times per day
 6 or more times per day

- 11a. Each time you drank **liquor** or **mixed drinks**, how much did you usually drink?

- Less than 1 shot of liquor
 1 to 3 shots of liquor
 More than 3 shots of liquor

12. Over the past 12 months, did you eat **oatmeal**, **grits**, or **other cooked cereal**?

NO (GO TO QUESTION 13)

YES

- 12a. How often did you eat **oatmeal**, **grits**, or **other cooked cereal IN THE WINTER**?

- NEVER
 1-6 times per winter
 7-11 times per winter
 1 time per month
 2-3 times per month
 1 time per week
 2 times per week
 3-4 times per week
 5-6 times per week
 1 time per day
 2 or more times per day

12b. How often did you eat **oatmeal, grits, or other cooked cereal DURING THE REST OF THE YEAR?**

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

12c. Each time you ate **oatmeal, grits, or other cooked cereal**, how much did you usually eat?

- Less than ¼ cup
 ¼ to 1¼ cups
 More than 1¼ cups

13. How often did you eat **cold cereal?**

- NEVER (GO TO QUESTION 14)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

13a. Each time you ate **cold cereal**, how much did you usually eat?

- Less than 1 cup
 1 to 2½ cups
 More than 2½ cups

13b. How often was the cold cereal you ate a **fortified cereal?** (See list of **fortified cereals** provided by the researcher.)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

13c. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Fruit Loops, Cap'n Crunch, or others)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

13d. Was **milk** or a **milk substitute** added to your cold cereal?

- NO (GO TO QUESTION 14)
 YES

13e. What kind of **milk** or **milk substitute** was usually added?

- Whole milk (including lactose-free variety)
 2% fat milk (including lactose-free variety)
 1% fat milk (including lactose-free variety)
 Skim, nonfat, or ½% fat milk (including lactose-free variety)
 Soy milk (8" Continent)
 Soy milk (Whitewave - Silk)
 Soy milk (VitaSoy)
 Soy milk (other brand)
 Rice milk
 Other

13f. Each time **milk** or a **milk substitute** was added to your cold cereal, how much was usually added?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

14. How often did you eat **applesauce?**

- NEVER (GO TO QUESTION 15)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Please continue on next page.

14a. Each time you ate **applesauce**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 cup
 More than 1 cup

15. How often did you eat **apples**?

- NEVER (GO TO QUESTION 16)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

15a. Each time you ate **apples**, how many did you usually eat?

- Less than 1 apple
 1 apple
 More than 1 apple

16. How often did you eat **pears** (fresh, canned, or frozen)?

- NEVER (GO TO QUESTION 17)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

16a. Each time you ate **pears**, how many did you usually eat?

- Less than 1 pear
 1 pear
 More than 1 pear

17. How often did you eat **bananas**?

- NEVER (GO TO QUESTION 18)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

17a. Each time you ate **bananas**, how many did you usually eat?

- Less than 1 banana
 1 banana
 More than 1 banana

18. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

- NEVER (GO TO QUESTION 19)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

18a. Each time you ate **dried fruit**, how much did you usually eat (not including dried apricots)?

- Less than 2 tablespoons
 2 to 5 tablespoons
 More than 5 tablespoons

19. Over the **past 12 months**, did you eat **peaches, nectarines, or plums**?

- NO (GO TO QUESTION 20)
 YES

19a. How often did you eat **fresh peaches, nectarines, or plums WHEN IN SEASON?** (See list for description of "in season".)

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

19b. How often did you eat **peaches, nectarines, or plums** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR?**

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

19c. Each time you ate **peaches, nectarines, or plums**, how much did you usually eat?

- Less than 1 fruit or less than ½ cup
 1 to 2 fruits or ½ to ¾ cup
 More than 2 fruits or more than ¾ cup

20. How often did you eat **grapes**?

- NEVER (GO TO QUESTION 21)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

20a. Each time you ate **grapes**, how much did you usually eat?

- Less than ½ cup or less than 10 grapes
 ½ to 1 cup or 10 to 30 grapes
 More than 1 cup or more than 30 grapes

21. Over the **past 12 months**, did you eat **cantaloupe**?

- NO (GO TO QUESTION 22)
 YES

21a. How often did you eat **fresh cantaloupe WHEN IN SEASON?** (See list for description of "in season".)

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per season | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

21b. How often did you eat **fresh or frozen cantaloupe DURING THE REST OF THE YEAR?**

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the **past 12 months**...

21c. Each time you ate **cantaloupe**, how much did you usually eat?

- Less than ¼ melon or less than ½ cup
 ¼ melon or ½ to 1 cup
 More than ¼ melon or more than 1 cup

22. Over the **past 12 months**, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

- NO (GO TO QUESTION 23)
 YES

22a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON?** (See list for description of "in season".)

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per season | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

22b. How often did you eat **fresh or frozen melon, other than cantaloupe** (such as watermelon or honeydew) **DURING THE REST OF THE YEAR?**

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

22c. Each time you ate **melon other than cantaloupe**, how much did you usually eat?

- Less than ½ cup or 1 small wedge
 ½ to 2 cups or 1 medium wedge
 More than 2 cups or 1 large wedge

23. Over the past 12 months, did you eat **strawberries**?

NO (GO TO QUESTION 24)

YES

23a. How often did you eat **fresh strawberries WHEN IN SEASON?** (See list for description of "in season".)

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

23b. How often did you eat **fresh or frozen strawberries DURING THE REST OF THE YEAR?**

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

23c. Each time you ate **strawberries**, how much did you usually eat?

- Less than ¼ cup or less than 3 berries
 ¼ to ¾ cup or 3 to 8 berries
 More than ¾ cup or more than 8 berries

24. Over the past 12 months, did you eat **oranges, tangerines, or tangelos**?

NO (GO TO QUESTION 25)

YES

24a. How often did you eat **fresh oranges, tangerines, or tangelos WHEN IN SEASON?** (See list for description of "in season".)

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

24b. How often did you eat **oranges, tangerines, or tangelos** (fresh or canned) **DURING THE REST OF THE YEAR?**

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

24c. Each time you ate **oranges, tangerines, or tangelos**, how many did you usually eat?

- Less than 1 fruit
 1 fruit
 More than 1 fruit

25. Over the past 12 months, did you eat **grapefruit**?

NO (GO TO QUESTION 26)

YES

25a. How often did you eat **fresh grapefruit WHEN IN SEASON?** (See list for description of "in season".)

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

25b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR?**

NEVER

- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

25c. Each time you ate **grapefruit**, how much did you usually eat?

- Less than ½ grapefruit
 ½ grapefruit
 More than ½ grapefruit

26. How often did you eat **other kinds of fruit** such as pineapple, mangoes, blueberries, or others?

- NEVER (GO TO QUESTION 27)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

26a. Each time you ate **other kinds of fruit**, how much did you usually eat?

- Less than ¼ cup
 ¼ to ¾ cup
 More than ¾ cup

27. How often did you eat **COOKED greens** (such as spinach, turnip, collard, mustard, chard, or kale)?

- NEVER (GO TO QUESTION 28)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

27a. Each time you ate **COOKED greens**, how much did you usually eat?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

28. How often did you eat **RAW greens** (such as spinach, turnip, collard, mustard, chard, or kale)?
(We will ask about lettuce later.)

- NEVER (GO TO QUESTION 29)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

28a. Each time you ate **RAW greens**, how much did you usually eat?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

Over the past 12 months...

29. How often did you eat **coleslaw**?

- NEVER (GO TO QUESTION 30)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

29a. Each time you ate **coleslaw**, how much did you usually eat?

- Less than ¼ cup
 ¼ to ¾ cup
 More than ¾ cup

30. How often did you eat **sauerkraut or cabbage** (other than coleslaw)?

- NEVER (GO TO QUESTION 31)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

30a. Each time you ate **sauerkraut or cabbage**, how much did you usually eat?

- Less than ¼ cup
 ¼ to 1 cup
 More than 1 cup

31. How often did you eat **carrots** (fresh, canned, or frozen)?

- NEVER (GO TO QUESTION 32)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

31a. Each time you ate **carrots**, how much did you usually eat?

- Less than ¼ cup or less than 2 baby carrots
 ¼ to ½ cup or 2 to 5 baby carrots
 More than ½ cup or more than 5 baby carrots

32. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

- NEVER (GO TO QUESTION 33)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

32a. Each time you ate **string beans** or **green beans**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 cup
 More than 1 cup

33. How often did you eat **peas** (fresh, canned, or frozen)?

- NEVER (GO TO QUESTION 34)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

33a. Each time you ate **peas**, how much did you usually eat?

- Less than 1/4 cup
 1/4 to 3/4 cup
 More than 3/4 cup

34. Over the past 12 months, did you eat **corn**?

- NO (GO TO QUESTION 35)
 YES

34a. How often did you eat **fresh corn WHEN IN SEASON**? (See list for description of "in season".)

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

34b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

34c. Each time you ate **corn**, how much did you usually eat?

- Less than 1 ear or less than 1/2 cup
 1 ear or 1/2 to 1 cup
 More than 1 ear or more than 1 cup

35. Over the past 12 months, how often did you eat **broccoli** (fresh or frozen)?

- NEVER (GO TO QUESTION 36)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

35a. Each time you ate **broccoli**, how much did you usually eat?

- Less than 1/4 cup
 1/4 to 1 cup
 More than 1 cup

36. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

- NEVER (GO TO QUESTION 37)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

36a. Each time you ate **cauliflower** or **Brussels sprouts**, how much did you usually eat?

- Less than 1/4 cup
 1/4 to 1/2 cup
 More than 1/2 cup

37. How often did you eat mixed **vegetables**?

NEVER (GO TO QUESTION 38)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

37a. Each time you ate **mixed vegetables**, how much did you usually eat?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

38. How often did you eat **onions**?

NEVER (GO TO QUESTION 39)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

38a. Each time you ate **onions**, how much did you usually eat?

- Less than 1 slice or less than 1 tablespoon
 1 slice or 1 to 4 tablespoons
 More than 1 slice or more than 4 tablespoons

39. Now think about all the **cooked vegetables** you ate in the **past 12 months** and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? (*Please do not include potatoes.*)

NEVER (GO TO QUESTION 40)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the **past 12 months**...

39a. Which fats were usually added to your vegetables **DURING COOKING**? (*Please do not include potatoes. Mark all that apply.*)

- | | |
|--|---|
| <input type="checkbox"/> Margarine (including low-fat) | <input type="checkbox"/> Canola or rapeseed oil |
| <input type="checkbox"/> Butter (including low-fat) | <input type="checkbox"/> Oil spray, such as Pam or others |
| <input type="checkbox"/> Lard, fatback, or bacon fat | <input type="checkbox"/> Other kinds of oils |
| <input type="checkbox"/> Olive oil | <input type="checkbox"/> None of the above |
| <input type="checkbox"/> Corn oil | |

40. Now, thinking again about all the **cooked vegetables** you ate in the **past 12 months**, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE**? (*Please do not include potatoes.*)

NEVER (GO TO QUESTION 41)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 2 times per day |
| <input type="checkbox"/> 1–2 times per week | <input type="checkbox"/> 3 or more times per day |

40a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE**? (*Please do not include potatoes. Mark all that apply.*)

- | | |
|--|---------------------------------------|
| <input type="checkbox"/> Margarine including low-fat | <input type="checkbox"/> Cheese sauce |
| <input type="checkbox"/> Butter (including low-fat) | <input type="checkbox"/> White sauce |
| <input type="checkbox"/> Lard, fatback, or bacon fat | <input type="checkbox"/> Other |
| <input type="checkbox"/> Salad dressing | |

40b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- Did not usually add these
 Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

40c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- Did not usually add these
 Less than 1 tablespoon
 1 to 3 tablespoons
 More than 3 tablespoons

41. Over the past 12 months, how often did you eat **sweet peppers** (green, red, or yellow)?

- NEVER (GO TO QUESTION 42)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

41a. Each time you ate **sweet peppers**, how much did you usually eat?

- Less than 1/8 pepper
 1/8 to 1/4 pepper
 More than 1/4 pepper

42. Over the past 12 months, did you eat **fresh tomatoes** (including those in salads)?

- NO (GO TO QUESTION 43)
 YES

42a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON?** (See description of "in season".)

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per season | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per season | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

42b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR?**

- NEVER
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

42c. Each time you ate **fresh tomatoes**, how much did you usually eat?

- Less than 1/4 tomato
 1/4 to 1/2 tomato
 More than 1/2 tomato

Over the past 12 months...

43. How often did you eat **lettuce salads** (with or without other vegetables)?

- NEVER (GO TO QUESTION 44)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

43a. Each time you ate **lettuce salads**, how much did you usually eat?

- Less than 1/4 cup
 1/4 to 1 1/4 cups
 More than 1 1/4 cups

44. How often did you eat **salad dressing** (including low-fat) on salads?

- NEVER (GO TO QUESTION 45)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

44a. Each time you ate **salad dressing** on salads, how much did you usually eat?

- Less than 2 tablespoons
 2 to 4 tablespoons
 More than 4 tablespoons

45. How often did you eat **sweet potatoes or yams**?

- NEVER (GO TO QUESTION 46)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

45a. Each time you ate **sweet potatoes or yams**, how much did you usually eat?

- 1 small potato or less than 1/4 cup
 1 medium potato or 1/4 to 3/4 cup
 1 large potato or more than 3/4 cup

46. How often did you eat **French fries, home fries, hash browned potatoes, or tater tots**?

- NEVER (GO TO QUESTION 47)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

46a. Each time you ate **French fries, home fries, hash browned potatoes, or tater tots** how much did you usually eat?

- Less than 10 fries or less than ½ cup
 10 to 25 fries or ½ to 1 cup
 More than 25 fries or more than 1 cup

47. How often did you eat **potato salad**?

- NEVER (GO TO QUESTION 48)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

47a. Each time you ate **potato salad**, how much did you usually eat?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

48. How often did you eat **baked, boiled, or mashed potatoes**?

- NEVER (GO TO QUESTION 49)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

48a. Each time you ate **baked, boiled, or mashed potatoes**, how much did you usually eat?

- 1 small potato or less than ½ cup
 1 medium potato or ½ to 1 cup
 1 large potato or more than 1 cup

Over the **past 12 months...**

48b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- Almost never or never (GO TO QUESTION 48d)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

48c. Each time **sour cream** was added to your potatoes, how much was usually added?

- Less than 1 tablespoon
 1 to 3 tablespoons
 More than 3 tablespoons

48d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

48e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

48f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

- Never added
 Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

48g. How often was cheese or cheese sauce added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

- Almost never or never (GO TO QUESTION 49)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

48h. Each time **cheese** or **cheese sauce** was added to your potatoes, how much was usually added?

- Less than 1 tablespoon
 1 to 3 tablespoons
 More than 3 tablespoons

49. How often did you eat **salsa**?

- NEVER (GO TO QUESTION 50)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

49a. Each time you ate **salsa**, how much did you usually eat?

- Less than 1 tablespoon
 1 to 5 tablespoons
 More than 5 tablespoons

50. How often did you eat **catsup**?

- NEVER (GO TO QUESTION 51)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

50a. Each time you ate **catsup**, how much did you usually eat?

- Less than 1 teaspoon
 1 to 6 teaspoons
 More than 6 teaspoons

51. How often did you eat **stuffing, dressing, or dumplings**?

- NEVER (GO TO QUESTION 52)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

51a. Each time you ate **stuffing, dressing, or dumplings**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 cup
 More than 1 cup

Over the past 12 months...

52. How often did you eat **chili made with beef**? (DO NOT include chili made with soy or vegetable protein substitute. We will ask about this later.)

- NEVER (GO TO QUESTION 52b)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

52a. Each time you ate **chili made with beef**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 3/4 cups
 More than 1 3/4 cups

52b. How often did you eat **chili made with meat other than beef**? (DO NOT include chili made with soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 52d)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

52c. Each time you ate **chili made with meat other than beef**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 3/4 cups
 More than 1 3/4 cups

52d. How often did you eat **chili without meat**? (DO NOT include chili made with a soy or vegetable protein meat substitute. We will ask about meat substitutes later.)

- NEVER (GO TO QUESTION 53)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

52e. Each time you ate **chili made without meat**, how much did you usually eat?

- Less than ½ cup
 ½ to 1¾ cups
 More than 1¾ cups

53. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas) **made with beef**? (*DO NOT include Mexican foods made with soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 53b)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

53a. Each time you ate **Mexican foods made with beef**, how much did you usually eat?

- Less than 1 taco, burrito, etc.
 1 to 2 tacos, burritos, etc.
 More than 2 tacos, burritos, etc.

53b. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas) **made with meat other than beef**? (*DO NOT include Mexican foods made with soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 53d)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

53c. Each time you ate **Mexican foods made with meat other than beef** how much did you usually eat?

- Less than ½ cup
 ½ to 1¾ cups
 More than 1¾ cups

53d. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas) **made without meat**? (*DO NOT include Mexican foods made with a soy or vegetable protein meat substitute.*)

- NEVER (GO TO QUESTION 54)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

53e. Each time you ate **Mexican foods made without meat**, how much did you usually eat?

- Less than ½ cup
 ½ to 1¾ cups
 More than 1¾ cups

54. How often did you eat **cooked dried beans** (such as baked beans, pintos, kidney, blackeyed peas, lima, lentils, soybeans, refried beans, or chick peas/garbanzo beans, including hummus)? (*Please don't include bean soups or chili.*)

- NEVER (GO TO QUESTION 55)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

54a. Each time you ate **beans**, how much did you usually eat?

- Less than ½ cup
 ½ to 1 cup
 More than 1 cup

54b. How often were the beans you ate **refried beans, beans prepared with any type of fat, or with meat added**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

55. How often did you eat **other kinds of vegetables** such as asparagus, mushrooms, zucchini or others ?

- NEVER (GO TO QUESTION 56)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

55a. Each time you ate **other kinds of vegetables**, how much did you usually eat?

- Less than ¼ cup
 ¼ to ½ cup
 More than ½ cup

56. How often did you eat **rice or other cooked grains** (such as bulgur, cracked wheat, or millet)?

- NEVER (GO TO QUESTION 57)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

56a. Each time you ate **rice or other cooked grains**, how much did you usually eat?

- Less than ½ cup
 ½ to 1½ cups
 More than 1½ cups

56b. How often was **butter, margarine, or oil** added to your rice **IN COOKING OR AT THE TABLE**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Over the past 12 months...

57. How often did you eat **pancakes, waffles, or French toast**?

- NEVER (GO TO QUESTION 58)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

57a. Each time you ate **pancakes, waffles, or French toast**, how much did you usually eat?

- Less than 1 medium piece
 1 to 3 medium pieces
 More than 3 medium pieces

57b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

57c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

57d. Each time **margarine** or **butter** was added to your pancakes, waffles, or French toast, how much was usually added?

- Never added
 Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

57e. How often was **syrup** added to your pancakes, waffles, or French toast?

- Almost never or never (GO TO QUESTION 58)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

57f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

- Less than 1 tablespoon
 1 to 4 tablespoons
 More than 4 tablespoons

58. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made with beef?** (DO NOT include spaghetti or other pasta or products made with soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 58b)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

58a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made with beef**, how much did you usually eat?

- Less than 1 cup
 1 to 2 cups
 More than 2 cups

58b. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made with meat other than beef?** (DO NOT include spaghetti or other pasta or products made with soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 58d)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

58c. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made with meat other than beef**, how much did you usually eat?

- Less than 1 cup
 1 to 2 cups
 More than 2 cups

58d. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made without meat?** (DO NOT include spaghetti or other pasta or products made with a soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 59)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

58e. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli, or tortellini made without meat**, how much did you usually eat?

- Less than 1 cup
 1 to 2 cups
 More than 2 cups

59. How often did you eat **macaroni and cheese?**

- NEVER (GO TO QUESTION 60)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

59a. Each time you ate **macaroni and cheese**, how much did you usually eat?

- Less than 1 cup
 1 to 1¹/₂ cups
 More than 1¹/₂ cups

60. How often did you eat **pasta salad or macaroni salad?**

- NEVER (GO TO QUESTION 61)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

60a. Each time you ate **pasta salad or macaroni salad**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 cup
 More than 1 cup

61. Other than the pastas listed in Questions 58, 59, and 60, how often did you eat **pasta, spaghetti, or other noodles**?

- NEVER (GO TO QUESTION 62)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

61a. Each time you ate **pasta, spaghetti, or other noodles**, how much did you usually eat?

- Less than 1 cup
 1 to 3 cups
 More than 3 cups

61b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce or spaghetti sauce made with meat other than beef**? (*DO NOT include tomato or spaghetti sauce made with soy or vegetable protein substitutes. We will ask about these later.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

61c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce or spaghetti sauce made with meat other than beef**? (*DO NOT include tomato or spaghetti sauce made with soy or vegetable protein substitutes. We will ask about these later.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

61d. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce or spaghetti sauce made WITHOUT meat**? (*DO NOT include tomato sauce or spaghetti sauce made with a soy or vegetable protein meat substitute. We will ask about these later.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

61e. How often did you eat your pasta, spaghetti, or other noodles with **margarine, butter, oil, or cream sauce**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

62. How often did you eat **bagels or English muffins**?

NEVER (GO TO INTRODUCTION TO QUESTION 63)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

62a. Each time you ate **bagels or English muffins**, how many did you usually eat?

- Less than 1 bagel or English muffin
 1 bagel or English muffin
 More than 1 bagel or English muffin

62b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

62c. How often was **butter** (including low-fat) added to your bagels or English muffins?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

62d. Each time **margarine or butter** was added to your bagels or English muffins, how much was usually added?

- Never added
 Less than 1 teaspoon
 1 to 2 teaspoons
 More than 2 teaspoons

62e. How often was **cream cheese** (including low-fat) spread on your bagels or English muffins?

- Almost never or never (GO TO INTRODUCTION TO QUESTION 63)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Over the past 12 months...

62f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

- Less than 1 tablespoon
 1 to 2 tablespoons
 More than 2 tablespoons

The next questions ask about your intake of breads other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

63. How often did you eat **breads or rolls AS PART OF SANDWICHES** (including burger and hot dog rolls)?

- NEVER (GO TO QUESTION 64)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

63a. Each time you ate **breads or rolls AS PART OF SANDWICHES**, how many did you usually eat?

- 1 slice or ½ roll
 2 slices or 1 roll
 More than 2 slices or more than 1 roll

63b. How often were the breads or rolls that you used for your sandwiches **white bread** (including burger and hot dog rolls)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

63c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or rolls?

- Almost never or never (GO TO QUESTION 63e)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

63d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or rolls, how much was usually added?

- Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

63e. How often was **margarine** (including low-fat) added to your sandwich bread or rolls?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

63f. How often was **butter** (including low-fat) added to your sandwich bread or rolls?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

63g. Each time **margarine** or **butter** was added to your sandwich breads or rolls, how much was usually added?

- Never added
 Less than 1 teaspoon
 1 to 2 teaspoons
 More than 2 teaspoons

64. How often did you eat **breads or dinner rolls, NOT AS PART OF SANDWICHES**?

- NEVER (GO TO QUESTION 65)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

64a. Each time you ate **bread**s or **dinner rolls**, **NOT AS PART OF SANDWICHES**, how much did you usually eat?

- 1 slice or 1 dinner roll
 2 slices or 2 dinner rolls
 More than 2 slices or 2 dinner rolls

Over the past 12 months...

64b. How often were the breads or rolls you ate **white bread**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

64c. How often was **margarine** (including low-fat) added to your breads or rolls?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

64d. How often was **butter** (including low-fat) added to your breads or rolls?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

64e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

- Never added
 Less than 1 teaspoon
 1 to 2 teaspoons
 More than 2 teaspoons

64f. How often was **cream cheese** (including low-fat) added to your breads or rolls?

- Almost never or never (GO TO QUESTION 65)
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

64g. Each time **cream cheese** was added to your breads or rolls, how much was usually added?

- Less than 1 tablespoon
 1 to 2 tablespoons
 More than 2 tablespoons

65. How often did you eat **jam, jelly, or honey** on bagels, muffins, bread, rolls, or crackers?

- NEVER (GO TO QUESTION 66)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

65a. Each time you ate **jam, jelly, or honey**, how much did you usually eat?

- Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

66. How often did you eat **peanut butter** or **other nut butter**?

- NEVER (GO TO QUESTION 67)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

66a. Each time you ate **peanut butter** or **other nut butter**, how much did you usually eat?

- Less than 1 tablespoon
 1 to 2 tablespoons
 More than 2 tablespoons

67. How often did you eat **roast beef** or **steak IN SANDWICHES, SUBS or WRAPS**?

- NEVER (GO TO QUESTION 68)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

67a. Each time you ate **roast beef** or **steak IN SANDWICHES, SUBS or WRAPS**, how much did you usually eat?

- Less than 1 slice or less than 2 ounces
 1 to 2 slices or 2 to 4 ounces
 More than 2 slices or more than 4 ounces

Over the past 12 months...

68. How often did you eat **turkey or chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami), including those used in sandwiches, subs or wraps? (*DO NOT include turkey or chicken cold cuts made with soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 69)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

68a. Each time you ate **turkey or chicken COLD CUTS**, how much did you usually eat?

- Less than 1 slice
 1 to 3 slices
 More than 3 slices

69. How often did you eat **luncheon or deli-style ham** including luncheon or deli-style ham used in sandwiches, subs or wraps? (*DO NOT include luncheon or deli-style ham made with soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 70)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

69a. Each time you ate **luncheon or deli-style ham**, how much did you usually eat?

- Less than 1 slice
 1 to 3 slices
 More than 3 slices

69b. How often was the luncheon or deli-style ham you ate **light, low-fat, or fat-free**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

70. How often did you eat **other cold cuts or luncheon meats** (such as beef bologna, corned beef, pastrami, or others, including low-fat)? Include other cold cuts and luncheon meats used in sandwiches, subs or wraps. (*DO NOT include ham, turkey, salami, chicken cold cuts or cold cuts made with soy or vegetable protein.*)

- NEVER (GO TO QUESTION 71)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

70a. Each time you ate **other cold cuts or luncheon meats**, how much did you usually eat?

- Less than 1 slice
 1 to 3 slices
 More than 3 slices

70b. How often were the other cold cuts or luncheon meats you ate **light, low-fat, or fat-free cold cuts or luncheon meats**? (*Please do not include ham, turkey, or chicken cold cuts.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Please continue on next page.

71. How often did you eat **canned tuna** (including in salads, sandwiches, or casseroles)?

- NEVER (GO TO QUESTION 72)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

71a. Each time you ate **canned tuna**, how much did you usually eat?

- Less than ¼ cup or less than 2 ounces
 ¼ to ½ cup or 2 to 3 ounces
 More than ½ cup or more than 3 ounces

71b. How often was the canned tuna you ate **water-packed tuna**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Over the past 12 months...

71c. How often was the canned tuna you ate **prepared with mayonnaise or other dressing** (including low-fat)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

72. How often did you eat **GROUND chicken or turkey**? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 73)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

72a. Each time you ate **GROUND chicken or turkey**, how much did you usually eat?

- Less than 2 ounces or less than ½ cup
 2 to 4 ounces or ½ to 1 cup
 More than 4 ounces or more than 1 cup

73. How often did you eat **beef hamburgers or cheeseburgers**? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 74)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

73a. Each time you ate **beef hamburgers or cheeseburgers**, how much did you usually eat?

- Less than 1 patty or less than 2 ounces
 1 patty or 2 to 4 ounces
 More than 1 patty or more than 4 ounces

73b. How often were the beef hamburgers or cheeseburgers you ate made with **lean ground beef**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

74. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, or meatloaf)? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 75)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

74a. Each time you ate **ground beef in mixtures**, how much did you usually eat?

- Less than 3 ounces or less than ½ cup
 3 to 8 ounces or ½ to 1 cup
 More than 8 ounces or more than 1 cup

75. How often did you eat **hot dogs** or **frankfurters**?
(DO NOT include sausages or vegetarian hot dogs.)

- NEVER (GO TO QUESTION 76)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

75a. Each time you ate **hot dogs** or **frankfurters**, how many did you usually eat?

- Less than 1 hot dog
 1 to 2 hot dogs
 More than 2 hot dogs

75b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Over the past 12 months...

76. How often did you eat beef mixtures such as **beef stew**, **beef pot pie**, **beef and noodles**, or **beef and vegetables**? (DO NOT include products made with soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 77)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

76a. Each time you ate **beef stew**, **beef pot pie**, **beef and noodles**, or **beef and vegetables**, how much did you usually eat?

- Less than 1 cup
 1 to 2 cups
 More than 2 cups

77. How often did you eat **roast beef** or **pot roast**?
(DO NOT include roast beef or pot roast in sandwiches, subs or wraps. DO NOT include soy or vegetable protein substitutes. We will ask about these later.)

- NEVER (GO TO QUESTION 78)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

77a. Each time you ate **roast beef** or **pot roast** (including in mixtures), how much did you usually eat?

- Less than 2 ounces
 2 to 5 ounces
 More than 5 ounces

78. How often did you eat **steak** (beef)? (DO NOT include steak in sandwiches, subs or wraps.)

- NEVER (GO TO QUESTION 79)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

78a. Each time you ate **steak** (beef), how much did you usually eat?

- Less than 3 ounces
 3 to 7 ounces
 More than 7 ounces

78b. How often was the steak you ate **lean steak**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

79. How often did you eat **pork** or **beef spareribs**?

- NEVER (GO TO QUESTION 80)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

79a. Each time you ate **pork** or **beef spareribs**, how much did you usually eat?

- Less than 4 ribs
 4 to 12 ribs
 More than 12 ribs

80. How often did you eat **roast turkey, turkey cutlets, or turkey nuggets** (including in sandwiches)? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 81)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

80a. Each time you ate **roast turkey, turkey cutlets, or turkey nuggets**, how much did you usually eat? (*Please note: 4 to 8 turkey nuggets = 3 ounces.*)

- Less than 2 ounces
 2 to 4 ounces
 More than 4 ounces

81. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews, or other mixtures**? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 82)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

81a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews, or other mixtures**, how much did you usually eat?

- Less than 1/2 cup
 1/2 to 1 1/2 cups
 More than 1 1/2 cups

82. How often did you eat **baked, broiled, roasted, stewed, or fried chicken** (including nuggets)? (*DO NOT include chicken in mixtures or products made with soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 83)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

82a. Each time you ate **baked, broiled, roasted, stewed, or fried chicken** (including nuggets), how much did you usually eat?

- Less than 2 drumsticks or wings, less than 1 breast or thigh, or less than 4 nuggets
 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
 More than 2 drumsticks or wings, more than 1 breast or thigh, or more than 8 nuggets

82b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

- Almost never or never
 About 1/4 of the time
 About 1/2 of the time
 About 3/4 of the time
 Almost always or always

82c. How often was the chicken you ate **WHITE meat**?

- Almost never or never
 About 1/4 of the time
 About 1/2 of the time
 About 3/4 of the time
 Almost always or always

82d. How often did you eat chicken **WITH skin**?

- Almost never or never
 About 1/4 of the time
 About 1/2 of the time
 About 3/4 of the time
 Almost always or always

83. How often did you eat **baked ham** or **ham steak**?
- NEVER (GO TO QUESTION 84)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |
- 83a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?
- Less than 1 ounce
 1 to 3 ounces
 More than 3 ounces
84. How often did you eat **pork** (including chops, roasts, sausage and in mixed dishes)? (*DO NOT include ham, or ham steak.*)
- NEVER (GO TO QUESTION 85)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |
- 84a. Each time you ate **pork**, how much did you usually eat?
- Less than 2 ounces or less than 1 chop
 2 to 5 ounces or 1 chop
 More than 5 ounces or more than 1 chop
85. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc.?
- NEVER (GO TO QUESTION 86)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |
- 85a. Each time you ate **gravy** on meat, chicken, potatoes, rice, etc., how much did you usually eat?
- Less than 1/8 cup
 1/8 to 1/2 cup
 More than 1/2 cup

- Over the past 12 months...**
86. How often did you eat **liver** (all kinds) or **liverwurst**?
- NEVER (GO TO QUESTION 87)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |
- 86a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?
- Less than 1 ounce
 1 to 4 ounces
 More than 4 ounces
87. How often did you eat **bacon** (including low-fat but **not** imitation)? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)
- NEVER (GO TO QUESTION 88)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |
- 87a. Each time you ate **bacon**, how much did you usually eat?
- Fewer than 2 slices
 2 to 3 slices
 More than 3 slices
- 87b. How often was the bacon you ate **light, low-fat, or lean bacon**?
- Almost never or never
 About 1/4 of the time
 About 1/2 of the time
 About 3/4 of the time
 Almost always or always
- Please continue on next page.**

88. How often did you eat **beef sausage** (including low-fat)? (*DO NOT include soy or vegetable protein substitutes. We will ask about these later.*)

- NEVER (GO TO QUESTION 89)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

88a. Each time you ate **beef sausage**, how much did you usually eat?

- Less than 1 patty or 2 links
 1 to 3 patties or 2 to 5 links
 More than 3 patties or 5 links

88b. How often was the beef sausage you ate **light, low-fat, or lean beef sausage**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

89. How often did you eat **fish sticks, fried fish, or fried seafood** (not including clams, shrimp, or other shellfish or soy or vegetable protein fish substitutes)?

- NEVER (GO TO QUESTION 89b)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

89a. Each time you ate **fish sticks, fried fish, or fried seafood** how much did you usually eat?

- Less than 2 ounces or less than 1 fillet
 2 to 7 ounces or 1 fillet
 More than 7 ounces or more than 1 fillet

89b. How often did you eat **clams, mussels, oysters, or scallops**?

- NEVER (GO TO QUESTION 89d)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

89c. Each time you ate **clams, mussels, oysters, or scallops** how much did you usually eat?

- Less than 2 ounces
 2 to 7 ounces
 More than 7 ounces

89d. How often did you eat **shrimp, crab, or lobster**? (*DO NOT include imitation products or products made with soy or vegetable protein substitutes.*)

- NEVER (GO TO QUESTION 90)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

89e. Each time you ate **shrimp, crab, or lobster** how much did you usually eat?

- Less than 2 ounces or less than 1 fillet
 2 to 7 ounces or 1 fillet
 More than 7 ounces or more than 1 fillet

90. How often did you eat **fish or seafood** (not including clams, shrimp, or other shellfish or soy or vegetable protein fish substitutes) that was **NOT FRIED**? (Include intake from **fish sushi**.)

- NEVER (GO TO QUESTION 90b)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

90a. Each time you ate **fish or seafood that was NOT FRIED**, how much did you usually eat?

- Less than 2 ounces or less than 1 fillet
 2 to 5 ounces or 1 fillet
 More than 5 ounces or more than 1 fillet

90b. How often did you eat **shellfish** (all kinds) that was **NOT FRIED**?

- NEVER (GO TO QUESTION 91)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

90c. Each time you ate **shellfish** (all kinds) that was **NOT FRIED** how much did you usually eat?

- Less than 2 ounces
- 2 to 7 ounces
- More than 7 ounces

Over the past 12 months...

Now think about all the meat, poultry, and fish you ate in the past 12 months and how they were prepared.

91. How often was **oil, butter, margarine, or other fat** used to **FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

- NEVER (GO TO QUESTION 92)
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

91a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? *(Mark all that apply.)*

- Margarine (including low-fat)
- Butter (including low-fat)
- Lard, fatback, or bacon fat
- Olive oil
- Corn oil
- Canola or rapeseed oil
- Oil spray, such as Pam or others
- Other kinds of oils
- None of the above

92. How often did you eat **tofu**?

- NEVER (GO TO QUESTION 93)
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

92a. Each time you ate **tofu**, how much did you usually eat?

- Less than 1/4 cup or less than 2 ounces
- 1/4 to 1/2 cup or 2 to 4 ounces
- More than 1/2 cup or more than 4 ounces

93. **Over the past 12 months**, did you eat **soups**?

- NO (GO TO QUESTION 94)
- YES

93a. How often did you eat **soup DURING THE WINTER**?

- NEVER
- 1-6 times per winter
- 7-11 times per winter
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

93b. How often did you eat **soup DURING THE REST OF THE YEAR**?

- NEVER
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

93c. Each time you ate **soup**, how much did you usually eat?

- Less than 1 cup
- 1 to 2 cups
- More than 2 cups

93d. How often were the soups you ate **bean soups**?

- Almost never or never
- About 1/4 of the time
- About 1/2 of the time
- About 3/4 of the time
- Almost always or always

93e. How often were the soups you ate **cream soups** (including chowders)?

- Almost never or never
- About 1/4 of the time
- About 1/2 of the time
- About 3/4 of the time
- Almost always or always

Over the past 12 months...

93f. How often were the soups you ate **tomato** or **vegetable soups**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

93g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

94. How often did you eat **pizza**?

- NEVER (GO TO QUESTION 95)
 1–6 times per year 2 times per week
 7–11 times per year 3–4 times per week
 1 time per month 5–6 times per week
 2–3 times per month 1 time per day
 1 time per week 2 or more times per day

94a. Each time you ate **pizza**, how much did you usually eat?

- Less than 1 slice or less than 1 mini pizza
 1 to 3 slices or 1 mini pizza
 More than 3 slices or more than 1 mini pizza

94b. How often did you eat pizza with **pepperoni**, **sausage**, or **meat other than beef**? (*DO NOT include pizza made with soy or vegetable protein substitutes. We will ask about these later.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

94c. How often did you eat pizza with **ground beef** or **beef meatballs**? (*DO NOT include pizza made with soy or vegetable protein substitutes. We will ask about these later.*)

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

95. How often did you eat **crackers**?

- NEVER (GO TO QUESTION 96)
 1–6 times per year 2 times per week
 7–11 times per year 3–4 times per week
 1 time per month 5–6 times per week
 2–3 times per month 1 time per day
 1 time per week 2 or more times per day

Please continue on next page.

95a. Each time you ate **crackers**, how many did you usually eat?

- Fewer than 4 crackers
 4 to 10 crackers
 More than 10 crackers

96. How often did you eat **corn bread** or **corn muffins**?

- NEVER (GO TO QUESTION 97)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

96a. Each time you ate **corn bread** or **corn muffins**, how much did you usually eat?

- Less than 1 piece or muffin
 1 to 2 pieces or muffins
 More than 2 pieces or muffins

97. How often did you eat **biscuits**?

- NEVER (GO TO QUESTION 98)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

97a. Each time you ate **biscuits**, how many did you usually eat?

- Fewer than 1 biscuit
 1 to 2 biscuits
 More than 2 biscuits

98. How often did you eat **potato chips, tortilla chips, or corn chips** (including low-fat, fat-free, or low-salt)?

- NEVER (GO TO QUESTION 99)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the past 12 months...

98a. Each time you ate **potato chips, tortilla chips, or corn chips**, how much did you usually eat?

- Fewer than 10 chips or less than 1 cup
 10 to 25 chips or 1 to 2 cups
 More than 25 chips or more than 2 cups

98b. How often were the chips you ate **Wow chips** or other **chips made with fat substitute** (Olean or Olestra)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

98c. How often were the chips you ate other **low-fat** or **fat-free chips**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

99. How often did you eat **popcorn** (including low-fat)?

- NEVER (GO TO QUESTION 100)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

99a. Each time you ate **popcorn**, how much did you usually eat?

- Less than 2 cups, popped
 2 to 5 cups, popped
 More than 5 cups, popped

100. How often did you eat **pretzels**?

- NEVER (GO TO QUESTION 101)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

100a. Each time you ate **pretzels**, how many did you usually eat?

- Fewer than 5 average twists
 5 to 20 average twists
 More than 20 average twists

101. How often did you eat **peanuts, walnuts, seeds, or other nuts**?

- NEVER (GO TO QUESTION 102)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

101a. Each time you ate **peanuts, walnuts, seeds, or other nuts**, how much did you usually eat?

- Less than ¼ cup
 ¼ to ½ cup
 More than ½ cup

102. How often did you eat an **energy, high-protein, or breakfast bar**?

- NEVER (GO TO QUESTION 103)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

102a. Which type of **energy, high-protein or breakfast bar** did you usually eat? (Mark as many that apply.)

- Nutri-Grain Bar/Kellogg's Granola Bar
 Power Bar
 Power Bar Performance/Luna Bar
 Power Bar Protein Plus
 Power Bar Harvest
 Power Bar Pria
 Balance Bar
 Slimfast Bar
 Zone Bar
 Other

102b. Each time you ate an **energy, high-protein, or breakfast bar**, how much did you usually eat?

- Less than 1 bar
 1 bar
 More than 1 bar

103. How often did you eat **yogurt** (NOT including frozen yogurt)?

- NEVER (GO TO QUESTION 104)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

Over the **past 12 months...**

103a. Each time you ate **yogurt**, how much did you usually eat?

- Less than ½ cup or less than 1 container
 ½ to 1 cup or 1 container
 More than 1 cup or more than 1 container

104. How often did you eat **cottage cheese** (including low-fat)?

- NEVER (GO TO QUESTION 105)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

104a. Each time you ate **cottage cheese**, how much did you usually eat?

- Less than ¼ cup
 ¼ to 1 cup
 More than 1 cup

105. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches, subs or wraps)? (*DO NOT include cheese made from soy or vegetable protein.*)

- NEVER (GO TO QUESTION 106)
- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

105a. Each time you ate **cheese**, how much did you usually eat?

- Less than ½ ounce or less than 1 slice
 ½ to 1½ ounces or 1 slice
 More than 1½ ounces or more than 1 slice

105b. How often was the cheese you ate **light** or **low-fat cheese**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

105c. How often was the cheese you ate **fat-free cheese**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

106. How often did you eat **frozen yogurt, or sherbet** (including low-fat or fat-free)? **Please do not include Tofutti.**

- NEVER (GO TO QUESTION 107)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

106a. Each time you ate **frozen yogurt, or sherbet**, how much did you usually eat?

- Less than ½ cup or less than 1 scoop
- ½ to 1 cup or 1 to 2 scoops
- More than 1 cup or more than 2 scoops

107. How often did you eat **ice cream or ice cream bars**, (including low-fat or fat-free)?

- NEVER (GO TO QUESTION 108)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

107a. Each time you ate **ice cream or ice cream bars**, how much did you usually eat?

- Less than ½ cup or less than 1 scoop
- ½ to 1½ cups or 1 to 2 scoops
- More than 1½ cups or more than 2 scoops

107b. How often was the ice cream you ate **light, low-fat, or fat-free ice cream**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

Over the past 12 months...

108. How often did you eat **cake** (including low-fat or fat-free)?

- NEVER (GO TO QUESTION 109)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

108a. Each time you ate **cake**, how much did you usually eat?

- Less than 1 medium piece
- 1 medium piece
- More than 1 medium piece

108b. How often was the cake you ate **light, low-fat, or fat-free cake**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

109. How often did you eat **cookies or brownies** (including low-fat or fat-free)?

- NEVER (GO TO QUESTION 110)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

109a. Each time you ate **cookies or brownies**, how much did you usually eat?

- Less than 2 cookies or 1 small brownie
- 2 to 4 cookies or 1 medium brownie
- More than 4 cookies or 1 large brownie

109b. How often were the cookies or brownies you ate **light, low-fat, or fat-free cookies or brownies**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

110. How often did you eat **doughnuts, sweet rolls, Danish, or pop-tarts**?

- NEVER (GO TO QUESTION 111)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

110a. Each time you ate **doughnuts, sweet rolls, Danish, or pop-tarts**, how much did you usually eat?

- Less than 1 piece
- 1 to 2 pieces
- More than 2 pieces

111. How often did you eat **sweet muffins or dessert breads** (including low-fat or fat-free)?

- NEVER (GO TO QUESTION 112)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

111a. Each time you ate **sweet muffins or dessert breads**, how much did you usually eat?

- Less than 1 medium piece
- 1 medium piece
- More than 1 medium piece

111b. How often were the sweet muffins or dessert breads you ate **light, low-fat, or fat-free sweet muffins or dessert breads**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

112. How often did you eat **fruit crisp, cobbler, or strudel**?

- NEVER (GO TO QUESTION 113)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

Over the **past 12 months...**

112a. Each time you ate **fruit crisp, cobbler, or strudel**, how much did you usually eat?

- Less than ½ cup
- ½ to 1 cup
- More than 1 cup

113. How often did you eat **pie**?

- NEVER (GO TO QUESTION 114)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

113a. Each time you ate **pie**, how much did you usually eat?

- Less than 1/8 of a pie
- About 1/8 of a pie
- More than 1/8 of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

113b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

113c. How often were the pies you ate **cream, pudding, custard, key lime or meringue pie**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

113d. How often were the pies you ate **pumpkin** or **sweet potato pie**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

113e. How often were the pies you ate **pecan pie**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

114. How often did you eat **chocolate candy**?

- NEVER (GO TO QUESTION 115)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

114a. Each time you ate **chocolate candy**, how much did you usually eat?

- Less than 1 average bar or less than 1 ounce
- 1 average bar or 1 to 2 ounces
- More than 1 average bar or more than 2 ounces

115. How often did you eat **other candy**?

- NEVER (GO TO QUESTION 116)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

115a. Each time you ate **other candy**, how much did you usually eat?

- Fewer than 2 pieces
- 2 to 9 pieces
- More than 9 pieces

116. How often did you eat **eggs** or **egg whites**, (NOT counting **egg substitutes** or eggs in baked goods and desserts)? (Please include eggs in salads, quiche, and soufflés.)

- NEVER (GO TO QUESTION 116b)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

Over the **past 12 months...**

116a. Each time you ate **eggs**, how many did you usually eat?

- 1 egg
- 2 eggs
- 3 or more eggs

116b. Over the past 12 months, how often did you eat an **egg substitute product** (NOT counting egg substitute product used in baked goods and desserts)? (Please include egg substitute used in quiche, soufflés, and egg dishes.)

- NEVER (GO TO QUESTION 116e)
- 1–6 times per year
- 7–11 times per year
- 1 time per month
- 2–3 times per month
- 1 time per week
- 2 times per week
- 3–4 times per week
- 5–6 times per week
- 1 time per day
- 2 or more times per day

116c. Which of the following brands do you eat most often (mark as many that apply)?

- Morningstar Farms Scrambles
- Morningstar Farms Better n' Eggs
- Eggbeaters
- Second Nature
- Other

116d. Each time you ate **egg substitute product**, how much did you usually eat?

- Less than ½ cup
- ½ to 1 cup
- More than 1 cup

116e. How often were the eggs or egg substitute product you ate **cooked in oil, butter, or margarine**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

116f. How often were the eggs you ate part of **egg salad**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

117. How many cups of **coffee**, caffeinated or decaffeinated, did you drink?

- NEVER (GO TO QUESTION 118)
- Less than 1 cup per month
- 1–3 cups per month
- 1 cup per week
- 2–4 cups per week
- 5–6 cups per week
- 1 cup per day
- 2–3 cups per day
- 4–5 cups per day
- 6 or more cups per day

117a. How often was the coffee you drank **decaffeinated**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

118. How many glasses of **ICED tea**, caffeinated or decaffeinated, did you drink?

- NEVER (GO TO QUESTION 119)
- Less than 1 cup per month
- 1–3 cups per month
- 1 cup per week
- 2–4 cups per week
- 5–6 cups per week
- 1 cup per day
- 2–3 cups per day
- 4–5 cups per day
- 6 or more cups per day

119a. How often was the iced tea you drank **decaffeinated or herbal tea**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

119. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

- NEVER (GO TO QUESTION 120)
- Less than 1 cup per month
- 1–3 cups per month
- 1 cup per week
- 2–4 cups per week
- 5–6 cups per week
- 1 cup per day
- 2–3 cups per day
- 4–5 cups per day
- 6 or more cups per day

119a. How often was the hot tea you drank **decaffeinated or herbal tea**?

- Almost never or never
- About ¼ of the time
- About ½ of the time
- About ¾ of the time
- Almost always or always

Over the past 12 months...

120. How often did you add **sugar or honey** to your coffee or tea?

- NEVER (GO TO QUESTION 121)
- Less than 1 time per month
- 1–3 times per month
- 1 time per week
- 2–4 times per week
- 5–6 times per week
- 1 time per day
- 2–3 times per day
- 4–5 times per day
- 6 or more times per day

120a. Each time **sugar or honey** was added to your coffee or tea, how much was usually added?

- Less than 1 teaspoon
- 1 to 3 teaspoons
- More than 3 teaspoons

121. How often did you add **artificial sweetener** to your coffee or tea?

- NEVER (GO TO QUESTION 122)
- Less than 1 time per month
- 1–3 times per month
- 1 time per week
- 2–4 times per week
- 5–6 times per week
- 1 time per day
- 2–3 times per day
- 4–5 times per day
- 6 or more times per day

- 121a. What kind of **artificial sweetener** did you usually use?
- Equal or aspartame
 Sweet N Low or saccharin
122. How often was **non-dairy creamer** added to your coffee or tea?
- NEVER (GO TO QUESTION 123)
- Less than 1 time per month
 1–3 times per month
 1 time per week
 2–4 times per week
- 5–6 times per week
 1 time per day
 2–3 times per day
 4–5 times per day
 6 or more times per day
- 122a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually used?
- Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons
- 122b. What kind of **non-dairy creamer** did you usually use?
- Regular powdered
 Low-fat or fat-free powdered
 Regular liquid
 Low-fat or fat-free liquid
123. How often was **cream** or **half and half** added to your coffee or tea?
- NEVER (GO TO QUESTION 124)
- Less than 1 time per month
 1–3 times per month
 1 time per week
 2–4 times per week
- 5–6 times per week
 1 time per day
 2–3 times per day
 4–5 times per day
 6 or more times per day
- 123a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?
- Less than 1 tablespoon
 1 to 2 tablespoons
 More than 2 tablespoons
124. How often was **milk** added to your coffee or tea?
- NEVER (GO TO QUESTION 125)
- Less than 1 time per month
 1–3 times per month
 1 time per week
 2–4 times per week
- 5–6 times per week
 1 time per day
 2–3 times per day
 4–5 times per day
 6 or more times per day
- 124a. Each time **milk** was added to your coffee or tea, how much was usually added?
- Less than 1 tablespoon
 1 to 3 tablespoons
 More than 3 tablespoons
- 124b. What kind of **milk** was usually added to your coffee or tea?
- Whole milk (including lactose-free variety)
 2% milk (including lactose-free variety)
 1% milk (including lactose-free variety)
 Skim, nonfat, or ½% milk (including lactose-free variety)
 Evaporated or condensed (canned) milk
 Soy milk
 Rice milk
 Other
- Over the past 12 months...**
125. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods.)
- NEVER (GO TO INTRODUCTION TO QUESTION 126)
- 1–6 times per year
 7–11 times per year
 1 time per month
 2–3 times per month
 1 time per week
- 2 times per week
 3–4 times per week
 5–6 times per week
 1 time per day
 2 or more times per day
- 125a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?
- Less than 1 teaspoon
 1 to 3 teaspoons
 More than 3 teaspoons

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

126. Over the past 12 months, did you eat **margarine**?

- NO (GO TO QUESTION 127)
 YES

126a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

126b. How often was the margarine you ate **light** or **low-fat margarine** (stick or tub)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

126c. How often was the margarine you ate **fat-free margarine**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

127. Over the past 12 months, did you eat **butter**?

- NO (GO TO QUESTION 128)
 YES

127a. How often was the butter you ate **light** or **low-fat butter**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

128. Over the past 12 months, did you eat **mayonnaise** or **mayonnaise-type dressing**?

- NO (GO TO QUESTION 129)
 YES

128a. How often was the mayonnaise you ate **regular-fat mayonnaise**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

128b. How often was the mayonnaise you ate **light** or **low-fat mayonnaise**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

Over the past 12 months...

128c. How often was the mayonnaise you ate **fat-free mayonnaise**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

129. Over the past 12 months, did you eat **sour cream**?

- NO (GO TO QUESTION 130)
 YES

129a. How often was the sour cream you ate **regular-fat sour cream**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

129b. How often was the sour cream you ate **light, low-fat, or fat-free sour cream**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

130. Over the past 12 months, did you eat **cream cheese**?

NO (GO TO QUESTION 131)

YES

130a. How often was the cream cheese you ate **regular-fat cream cheese**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

130b. How often was the cream cheese you ate **light, low-fat, or fat-free cream cheese**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

131. Over the past 12 months, did you eat **salad dressing**?

NO (GO TO INTRODUCTION TO QUESTION 132)

YES

131a. How often was the salad dressing you ate **regular-fat salad dressing** (including oil and vinegar dressing)?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

131b. How often was the salad dressing you ate **light or low-fat salad dressing**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

131c. How often was the salad dressing you ate **fat-free salad dressing**?

- Almost never or never
 About ¼ of the time
 About ½ of the time
 About ¾ of the time
 Almost always or always

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

132. Over the past 12 months, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

- | | |
|---|--|
| <input type="checkbox"/> Less than 1 per week | <input type="checkbox"/> 2 per day |
| <input type="checkbox"/> 1–2 per week | <input type="checkbox"/> 3 per day |
| <input type="checkbox"/> 3–4 per week | <input type="checkbox"/> 4 per day |
| <input type="checkbox"/> 5–6 per week | <input type="checkbox"/> 5 or more per day |
| <input type="checkbox"/> 1 per day | |

Over the past 12 months...

133. Over the past 12 months, how many servings of **fruit** (not including juices) did you eat per week or per day?

- | | |
|---|--|
| <input type="checkbox"/> Less than 1 per week | <input type="checkbox"/> 2 per day |
| <input type="checkbox"/> 1–2 per week | <input type="checkbox"/> 3 per day |
| <input type="checkbox"/> 3–4 per week | <input type="checkbox"/> 4 per day |
| <input type="checkbox"/> 5–6 per week | <input type="checkbox"/> 5 or more per day |
| <input type="checkbox"/> 1 per day | |

134. Over the past month, which of the following foods did you eat **AT LEAST THREE TIMES**? (Mark all that apply.)

- | | |
|---|---|
| <input type="checkbox"/> Avocado, guacamole | <input type="checkbox"/> Olives |
| <input type="checkbox"/> Cheesecake | <input type="checkbox"/> Oysters |
| <input type="checkbox"/> Chocolate, fudge, or butterscotch toppings or syrups | <input type="checkbox"/> Pickles or pickled vegetables or fruit |
| <input type="checkbox"/> Chow mein noodles | <input type="checkbox"/> Plantains |
| <input type="checkbox"/> Croissants | <input type="checkbox"/> Pork neckbones, hock, head, feet |
| <input type="checkbox"/> Dried apricots | <input type="checkbox"/> Pudding or custard |
| <input type="checkbox"/> Egg rolls | <input type="checkbox"/> Veal, venison, lamb |
| <input type="checkbox"/> Granola bars | <input type="checkbox"/> Whipped cream, regular |
| <input type="checkbox"/> Hot peppers | <input type="checkbox"/> Whipped cream, substitute |
| <input type="checkbox"/> Jello, gelatin | <input type="checkbox"/> NONE |
| <input type="checkbox"/> Milkshakes or ice-cream sodas | |

Please continue on next page.

135. For **ALL** of the past 12 months, have you followed any type of **vegetarian diet**?

NO (GO TO INTRODUCTION TO QUESTION 136)

YES

135a. Which of the following foods did you **TOTALLY EXCLUDE** from your diet? **(Mark all that apply.)**

- Beef, veal
 Pork, lamb
 Poultry (chicken, turkey, duck)
 Fish and seafood
 Shellfish
 Eggs (*please do not include egg substitutes*)
 Dairy products (milk, cheese, etc.) (*please do not include milk/dairy substitutes*)

136. Over the past 12 months, did you eat any **meat substitute** products **made with soy or vegetable protein**?

- NO **Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:**
- **Did not skip any pages**
 - **Crossed out the incorrect answer and circled the correct answer if you made any changes**

YES (GO TO QUESTION 137)

137. How often did you eat **breakfast patties or breakfast links made with soy or vegetable protein**?

NEVER (GO TO QUESTION 138)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

137a. Which of the following brands do you eat most often (mark as many that apply)?

- Morningstar Farms
 Worthington
 Loma Linda
 Other (such as Boca, Quorn, etc.)

137b. Each time you ate a **breakfast patty or breakfast link made with soy or vegetable protein**, how much did you usually eat?

- Less than 1 patty or 2 links
 1 to 3 patties or 2 to 5 links
 More than 3 patties or 5 links

138. How often did you eat **breakfast strips (imitation bacon) made with soy or vegetable protein**?

NEVER (GO TO QUESTION 139)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

138a. Which of the following brands do you eat most often? (Mark as many that apply.)

- Morningstar Farms
 Worthington
 Loma Linda
 Other (such as Boca, Quorn, etc.)

138b. Each time you ate **breakfast strips (imitation bacon) made with soy or vegetable protein**, how much did you usually eat?

- Fewer than 2 slices
 2 to 3 slices
 More than 3 slices

139. How often did you eat **burgers made with soy or vegetable protein**?

NEVER (GO TO QUESTION 140)

- | | |
|--|--|
| <input type="checkbox"/> 1–6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7–11 times per year | <input type="checkbox"/> 3–4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5–6 times per week |
| <input type="checkbox"/> 2–3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

139a. Which of the following brands do you eat most often? (Mark as many that apply.)

- Morningstar Farms
- Worthington
- Loma Linda
- Other (such as Boca, Quorn, etc.)

139b. Each time you ate a **burger made with soy or vegetable protein**, how much did you usually eat?

- Less than 1 patty or less than 2 ounces
- 1 patty or 2 to 4 ounces
- More than 1 patty or more than 4 ounces

140. How often did you eat **imitation meat dinner entrees (not including imitation chicken or fish) made with soy or vegetable protein?**

- NEVER (GO TO QUESTION 141)
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

140a. Which of the following brands do you eat most often (mark as many that apply)?

- Morningstar Farms
- Worthington
- Loma Linda
- Other (such as Boca, Quorn, etc.)

140b. Each time you ate an **imitation meat dinner entree (not including imitation chicken or fish) made with soy or vegetable protein**, how much did you usually eat?

- Less than 1 portion or 1/2 cup
- One portion or 1 cup
- More than one portion or more than 1 cup

141. How often did you eat **imitation chicken/turkey patties, nuggets, wings or other imitation chicken/turkey product made with soy or vegetable protein?**

- NEVER (GO TO QUESTION 142)
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

141a. Which of the following brands do you eat most often? (Mark as many that apply.)

- Morningstar Farms
- Worthington
- Loma Linda
- Other (such as Boca, Quorn, etc.)

141b. Each time you ate an **imitation chicken/turkey patties, nuggets, wings or other imitation chicken/turkey product made with soy or vegetable protein**, how much did you usually eat?

- Less than 1 patty or less than 2 ounces
- 1 patty or 2 to 4 ounces
- More than 1 patty or more than 4 ounces

142. How often did you eat entrees like chili, Mexican foods (tacos, burritos, tostados, enchiladas, etc) lasagna, manicotti, ravioli, stuffed shells, tortellini spaghetti with meat sauce, meatballs or casserole **made with soy or vegetable protein products like Burger Crumbles?**

- NEVER (GO TO QUESTION 143)
- 1-6 times per year
- 7-11 times per year
- 1 time per month
- 2-3 times per month
- 1 time per week
- 2 times per week
- 3-4 times per week
- 5-6 times per week
- 1 time per day
- 2 or more times per day

142a. Which of the following brands do you eat most often? (Mark as many that apply.)

- Morningstar Farms
- Worthington
- Loma Linda
- Other (such as Boca, Quorn, etc.)

142b. Each time you ate an entree like chili, Mexican foods (tacos, burritos, tostados, enchiladas, etc) lasagna, manicotti, ravioli, stuffed shells, tortellini spaghetti with meat sauce, meatballs or casserole **made with soy or vegetable protein products like Burger Crumbles**, how much did you usually eat?

- Less than 1/2 cup
- 1/2 to 1 cup
- More than 1 cup

143. How often did you eat **cold cut substitutes (such as meatless salami, meatless bologna, meatless chicken roll, meatless smoked turkey, meatless corned beef) made with soy or vegetable protein?**

- NEVER (GO TO END)
- | | |
|--|--|
| <input type="checkbox"/> 1-6 times per year | <input type="checkbox"/> 2 times per week |
| <input type="checkbox"/> 7-11 times per year | <input type="checkbox"/> 3-4 times per week |
| <input type="checkbox"/> 1 time per month | <input type="checkbox"/> 5-6 times per week |
| <input type="checkbox"/> 2-3 times per month | <input type="checkbox"/> 1 time per day |
| <input type="checkbox"/> 1 time per week | <input type="checkbox"/> 2 or more times per day |

143a. Which of the following brands do you eat most often? (Mark as many that apply.)

- Morningstar Farms
 Worthington
 Loma Linda
 Other (such as Boca, Quorn, etc.)

143b. Each time you ate **cold cut substitutes (such as meatless salami, meatless bologna, meatless chicken roll, meatless smoked turkey, meatless corned beef) made with soy or vegetable protein**, how much did you usually eat?

- Less than 3 slices or less than 2 ounces
 3 to 6 slices or 2 to 4 ounces
 More than 6 slices or more than 4 ounces

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- Did not skip any pages
- Crossed out the incorrect answer and circled the correct answer if you made any changes

APPENDIX C
SCRIPT FOR DIET HISTORY QUESTIONNAIRE INSTRUCTIONS

Diet History Questionnaire:

Thank you for participating in our study. We are going to spend about 15 minutes reviewing the Diet History Questionnaire, going over the instructions and answering any questions that you might have. I would like to explain what is in your packet. You have been given a copy of each of the informed consents that you signed this morning. These are for you to review when you get home. They are your copies and do not need to be sent back. You have also been given a copy of the Diet History Questionnaire. Everyone's is exactly the same, there are no differences and they were printed at the same time. They are all white in color and double sided. Before filling out the questionnaire make sure you answer the 4 questions on the front. There is also a "bar code" or "subject id" label on the front. **(Show participants where this is located.)** This has already been filled in for you. It is the same number you received today. So, when you mail it back to us we only know that subject #250 has turned theirs in, but we do not know the name. Lastly, you have been given a return envelope. Once you complete the questionnaire, you will mail it to us using the envelope. The only thing we need back in your packet is the questionnaire. You can keep everything else. The envelope has been pre-addressed to us and we have already paid for it. Do not put any postage on the envelope.

The purpose of the Diet History Questionnaire is to give us an idea of the type of foods you have been eating over the past year. This is a very accurate questionnaire and has been tested against other questionnaires that have been used in research studies. As long as the person completing the questionnaire is honest and accurate, the information that we learn from this questionnaire is very true to their actual food intake. The information will tell us a lot about your nutritional status. If you are interested, sometime in the future we can share this information with you.

The questionnaire will only ask you about food items. It will not ask about any thing additional that you might take, such as vitamins, minerals, multivitamin, protein powder, or even nutritional yeast. If you do take anything additional, even once a month, that is fine, we just need to know about it. We record the information and when we get the results from the questionnaire, we add that information to the final results to give us an overall idea of your total intake.

(If they take a supplement/nutrition yeast have them record it on colored paper and put into file. If they do not remember, give them the paper to take home and return with the questionnaire. Record their subject number and put a note in their file (on colored paper) that says, "Subject will return supplement paper with questionnaire." If they take

nutritional yeast make sure you distinguish whether it is made by RED STAR or if it is from bulk (like Ward's). Red Star adds vitamins/minerals especially B12, bulk does not.)

We have given you two sets of instructions. It is important to read both instructions before you begin. The General Instructions are located on the first page of the Diet History Questionnaire and the Additional Instructions are included in your envelope. The General Instructions combined with the Additional Instructions will guide you while completing the questionnaire.

Next, we will review the Additional Instructions (**Read each instruction out-loud**)

Instruction #1:

- **When answering each question, think about your diet over the past year and NOT the past few weeks.**

We would like to get an idea of the type of food you have eaten for the past year. Therefore, it is important that you focus on your average intake over the past year and not your intake over the past few weeks. Sometimes we tend to eat the same thing day-to-day or even week-to-week. If we were to fill out the questionnaire based on one day or one week, it wouldn't be an accurate representation of your overall food intake.

Instruction #2:

- **Several questions refer you to additional handouts that have been provided in your packet. Please be sure to use the handouts when you get to these questions.**

We have provided you a chart that gives you the question number and the name of the handout to use when answering these questions. There is also a reminder next to these questions on the Diet History Questionnaire reminding you to refer to the handouts. These are what the three handouts look like. (**Show participants the three handouts**)

Explain Seasonal Handout

When you reach the questions that ask you how often you eat a fruit or vegetable when it is "in season". You want to first look at the handout called Seasonal Fruits and Vegetables. This handout gives you a list of when these fruits and vegetables are in season. So, as an example let's look at question #19a on page 6. This question asks you, how often you eat fresh peaches, nectarines, or plums when in season. If you look at the Seasonal Fruit and Vegetable handout, it states that peaches are in season from June to September, nectarines are in season from July to September, and plums are in season from June to October. Therefore, you want to answer question 19a based on how often you eat these fruits within those months. Question 19b asks you how often you eat these fruits the rest of the year. So, you would think about how often you eat these fruits

during the months that they are not considered “in season”. Please use our handout as a guide for when fruits are in season. Please do not use other materials or your own knowledge about when fruits and vegetables are in season.

As a reminder, if a question like question #19a, asks you about multiple food items, you want to make sure you think of each time you eat those foods individually and add those times together. For example, I eat a fresh peach once a week, a nectarine once a week, and a plum twice a week, when in season. Therefore, I eat these fruits a total of 4 times p/wk. I would mark 3-4 times per week as my answer for question #19a.

Does anyone have any questions about how to use the handouts?

Instruction #3:

- **If you have any questions while completing the questionnaire, please contact Amanda Brown at 352-392-1991 ext 246. Please leave a voice message with your name, contact number, and best time or way to reach you.**

Next, we will review the General Instructions located on the first page of the Diet History Questionnaire.

Instruction #1:

- **Answer each question as well as you can. Estimate if you are not sure. A guess is better than leaving a blank.**

For some questions, it may be helpful if you measure out how much you usually eat or drink of a certain food or beverage by using measuring cups and spoons. You may also want to review food labels of the foods you eat because we do not want you to under-estimate or over-estimate the amount of a certain food you are eating. For example, I occasionally have a bottle of orange juice for breakfast. Since one bottle is not provided as an answer choice on the Diet History Questionnaire, referring to the label on the orange juice bottle will help me determine the amount of cups or ounces that I am drinking. When I look at the juice bottle, I find that each time I drink the entire juice bottle; I am drinking 15 fl. oz (roughly 2 cups) of orange juice. Therefore, I can mark 15 fluid ounces or 2 cups as my answer.

Instruction #2

- **Use only a black ballpoint pen. Do not use a pencil or felt-tip pen. Do not fold, staple, or tear the pages.**

(Hold up an example of a black ballpoint pen) We have provided you with a black ball-point pen that you can use to fill out the questionnaire. Please do not fold the questionnaire when you send it back to us. The envelope that is in your packet is large enough that you can slip the questionnaire right into the envelope and you do not have to fold it. As a reminder, you only have to send the Diet History Questionnaire back. You can keep the rest of the material that is in your packet.

Instruction #3 & 4

- **When selecting an answer, put an X in the box next to your answer.**
- **If you make any changes, cross out the incorrect answer and put an X in the box next to the correct answer. Also draw a circle around the correct answer.**

Please look at the handout entitled “Directions on How to Change your Answer Choice”.

The question asks, “Over the past 12 months, how often did you drink tomato or vegetable juice?” Over the past 12 months, you decided that you drink vegetable juice or tomato juice less than one time per month. You select “a” as your first answer, but then you remember you actually drink it much more frequently. So, the next step would be to cross out answer “a” and to mark an “X” in your new answer. Then you would want to circle your new answer, so it is clear which is your final answer.

Instruction #5

- **If you mark NEVER, NO, or DON’T KNOW for a question, please follow any arrows or instructions that direct you to the next question.**

Open your questionnaire to page 13 question #48g. This question asks “how often do you add cheese or cheese sauce to your potatoes”. Notice that you have the option to select “Never”. If you never add cheese or cheese sauce to your potatoes you would mark an “X” in the “never” box. Please notice that next to the “never” choice it also states the question number you should go to next. In this example, next to the “never” choice it says go to question 49. Does everyone see this? **(Pause)** There is also an arrow that directs you to the next page. It is important to be aware of the proper question to go to. The arrows will direct you, but make sure you go to the question that is stated next to the “never” choice. In this example, you are directed to question 49. If you follow the arrow and turn the page, it seems that you should answer question 48h. Although, this is wrong. You want to go to question 49. **(Pause)** Does anyone have any questions about marking never and how to know what question to answer next?

Additional Information to Discuss:

I want to go through some additional questions to make sure you understand how to answer these questions. Each question asks you how often you eat a specific food and the amount you eat. Please approach each question thoughtfully. It is very important that we get the most accurate information about your diet. Open the questionnaire to page 2 question #1. This question asks you, “How often do you drink tomato juice or vegetable juice?” Think about how many times a day or a week you drink tomato or vegetable juice and then mark the appropriate answer. If you drink tomato or vegetable juice you would continue to the second part of the question: “1a”. This question asks about the serving amount each time you drank tomato or vegetable juice. Once completing question “1a”

you can continue to question 2, and so forth. If you never drink tomato or vegetable juice, select never and follow the directions to the next appropriate question. In this example, you would go to question number 2.

Remember when answering these questions, we want you to think about your diet over the past year and NOT the past few weeks. I want to go over a few examples, which you may find helpful when completing the questionnaire. We have provided you with written examples of what I am about to cover.

1. "Please look at page 16 question #57. This question asks, "How often did you eat pancakes, waffles, or French toast?" I go to IHOP for breakfast on the first Sunday of every month of the year. I always order one medium size Belgian waffle. I never make waffles, pancakes, or French toast at home. I only eat waffles at IHOP. Since there are 12 months in a year, and I go on the first Sunday of each month, that is 12 times a year. 12 months divided into 12 times, gives you an average of 1 time per month. Which makes sense because there is only 1 first Sunday of every month, so I would only eat waffles 1 time per month. If you look at question #57, I would mark an "X" for 1 time per month. For question #57a, I would answer 1 to 3 medium pieces."

Here is another example:

2. Please look at page 31 question #107. This question asks, "How often did you eat ice cream or ice cream bars, (including low-fat or fat-free)?" I only eat ice cream during the summer months of June, July, and August. During that time, I eat 1 cup of ice cream twice a week. That means I eat ice cream 8 times during each of those months. So that adds up to 24 times. Since I do not eat ice cream during the other months, I can say that I only have ice cream 24 times a year. If I remember that there are 12 months in a year that means, on average, I only have ice cream twice a month. If you look at question #107 on page 31, I would mark an "X" for 2-3 times a month. For question #107a, I would mark $\frac{1}{2}$ to $1\frac{1}{2}$ cups or 1 to 2 scoops.

Do you have any questions about these two examples? **(Pause)** All instructions have been written down, so please re-read each direction before starting the Diet History Questionnaire.

If you have any questions please let me know. Remember the questionnaire you have been given in your packet has questions on the front and back of each page. Please make sure you answer each question as directed by the arrows. Again, it is very important you take your time filling out the questionnaire and be as thorough as possible. The information we gather is very important. You have my number if you have any questions while filling out the questionnaire. Once you return the questionnaire we can process your payment. We will not begin the processing of your payment until we receive your Diet History Questionnaire. You have two weeks to complete this questionnaire. Please

return only the Diet History Questionnaire by _____. If you cannot return the questionnaire within 2 weeks, please contact Amanda Brown to find a date by which you can return it. We prefer to have to questionnaire returned at some point rather than never having it returned at all.

This completes the session for today. **(Make sure you collect their clipboard, consent form, and payment form. Restock clipboards when needed)**

APPENDIX D
DIRECTIONS AND SURVEY FOR DIET HISTORY QUESTIONNAIRE PRETEST

Thank you for agreeing to complete the Diet History Questionnaire. Please follow the General Instructions located on the first page of the questionnaire and the Additional Instructions attached to the front of the Diet History Questionnaire. The General Instructions combined with the Additional Instructions will guide you while completing the questionnaire. Upon completion of the questionnaire, please answer the following questions. Be specific with your responses. Please record the time at which you started and completed the questionnaire.

Time started:

Time completed:

If you have any questions while completing the questionnaire, please email **Amanda Brown** at **abrown14@ufl.edu** or phone at **352-392-1991 ext 246**. Please leave a voice message with your name, contact number, and best time or way to reach you.

Please return the questionnaire by Tuesday, September 28, 2004.

Thank you in advance!

For bonus credit please print name: _____
Your response will be kept confidential

Please answer the following questions after completing the Diet History Questionnaire.

1. How long did it take for you to complete the Diet History Questionnaire?

2. How clear were the General Instructions for the Diet History Questionnaire? Please explain.

3. Were there any questions on the Diet History Questionnaire you did not understand? If yes, please list the question number and explain why you did not understand the question.

4. Were there any questions on the Diet History Questionnaire you could not answer? If yes, please list the question number and explain why it was difficult to answer.

5. Were there any questions on the Diet History Questionnaire for which the response you would have given was not an option? If yes, please provide the question number, the response you would have given, and why you would have given that response.

6. Is there any food item you eat frequently that was not listed on the Diet History Questionnaire?

7. Please provide additional comments in the space provided below.

APPENDIX E
ADDITIONAL INSTRUCTIONS PACKET

Please read these instructions prior to beginning the Diet History Questionnaire. These additional instructions combined with the “General Instructions” (found on the first page of the Diet History Questionnaire) will guide you while completing the questionnaire.

ADDITIONAL INSTRUCTIONS

Question Number	Handout
8e	Caffeinated versus Non-caffeinated Beverages
13b	Fortified Cereal
19a, 21a, 22a, 23a, 24a, 25a, 34a, 42a	Seasonal Fruits and Vegetables

1. When answering each question, think about your diet over the past year and NOT the past few weeks.
2. Several questions refer you to additional handouts that have been provided in your packet. Please be sure to use these handouts when you get to these questions.

If you have any questions while completing the questionnaire, please contact **Amanda Brown at 352-392-1991 ext 246**. Please leave a voice message with your name, contact number, and best time or way to reach you.

Remember when answering these questions, we want you to think about your diet over the past year and NOT the past few weeks. Here are a few examples, that you may find helpful when completing the questionnaire.

1. Please look at question #57 on page 16. I go to IHOP for breakfast on the first Sunday of every month of the year. I always order one medium size Belgian waffle. I never make waffles, pancakes, or French toast at home. I only eat waffles at IHOP. Since there are 12 months in a year, I eat 12 waffles a year or 1 waffle a month. If you look at question #57, I would mark an "X" for 1 time per month. For question #57a, I would answer 1 to 3 medium pieces.
2. Please look at question #107 on page 31. I only eat ice cream during the summer months of June, July, and August. During that time, I eat 1 cup of ice cream twice a week. That means I eat ice cream 8 times during each of those months. So that adds up to 24 times. Since I do not eat ice cream during the other months, I can say that I only have ice cream 24 times a year. If I remember that there are 12 months in a year that means, on average, I only have ice cream twice a month. If you look at question #107 on page 31, I would mark an "X" for 2-3 times a month. For question #107a, I would mark $\frac{1}{2}$ to $1\frac{1}{2}$ cups or 1 to 2 scoops.

Directions on How to Change Your Answer Choice

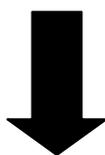
Over the past 12 months, how often did you drink **tomato juice** or **vegetable juice**?

- a. 1 time per month or less
- b. 2-3 times per month
- c. 1-2 times per week
- d. 3-4 times per week
- e. 5-6 times per week

You select “a” as your first answer, but then you change your mind.

- a. ~~1 time per month or less~~
- b. 2-3 times per month
- c. 1-2 times per week
- d. 3-4 times per week
- e. 5-6 times per week

Step #1
Cross out answer “a”



- a. ~~1 time per month or less~~
- b. 2-3 times per month
- c. 1-2 times per week
- d. 3-4 times per week
- e. 5-6 times per week

Step #2

Mark an “X” in the box of your new choice and circle that answer too.

Please use this list for Question 8e

Caffeinated versus non-caffeinated beverages

Caffeinated

A & W Creme Soda
 Barq's Root Beer
 Canada Dry Cola
 Coffee
 Diet A & W Creme Soda
 Diet Coke with Lemon
 Diet Cherry Coke
 Cherry Coke
 Cherry Pepsi
 Coke
 Diet Coke
 Diet Barq's Root Beer
 Diet Dr. Pepper
 Diet Mountain Dew
 Diet Pepsi
 Diet RC Cola
 Diet Shasta Cola
 Diet Snapple Flavored teas
 Diet Sunkist Orange
 Dr. Pepper
 Hot Chocolate Mix
 Jolt
 Lipton Brisk
 Mellow Yellow
 Mountain Dew
 Mountain Dew Code Red
 Nestea Sweetened & Unsweetened Iced Teas
 Pepsi
 Pepsi One
 Pepsi Twist
 RC Cola
 Shasta Cola
 Snapple Flavored teas
 Sunkist Orange
 Surge
 Tea: Iced, Brewed, Instant, Green

Non-caffeinated

7-Up
 A & W Root Beer
 Diet 7-Up
 Diet A & W Root Beer
 Diet Sprite
 Minute Maid Orange
 Sprite



Please use this list for Question 13b

Fortified Cereals

All-Bran – Buds
All-Bran – Extra Fiber
All-Bran – Original
Cheerios – Multigrain
Complete Oat Bran Flakes
Complete Wheat Bran Flakes
Just Right Fruit and Nut
KASHI Heart to Heart
Kellogg's Low Fat Granola no raisins
Kellogg's Low Fat Granola with raisins
Mueslix
Product 19
Smart Start
Smart Start Soy Protein
Special K
Total – Original
Total - Whole Grain
Total Brown Sugar and Oat
Total Corn Flakes
Total Raisin Bran



Please use this list for Questions
19a, 21a, 22a, 23a, 24a, 25a, 34a, 42a

Seasonal Fruits and Vegetables

Fruit or Vegetable	Months When In Season
Cantaloupe	March to July
Corn	August to June
Grapefruit	September to June
Honeydew Melon	June to October
Nectarines	July to September
Oranges	October to June
Peaches	June to September
Plums	June to October
Strawberries	October to June
Tangelos	November to February
Tomatoes	September to June



LIST OF REFERENCES

1. Banerjee RV, Frasca V, Ballou DP, Matthews RG. Participation of cob(I)alamin in the reaction catalyzed by methionine synthase from *Escherichia coli*: a steady-state and rapid reaction kinetic analysis. *Biochemistry* 1990;29:11101-9.
2. Beck W. Cobalamin (Vitamin B12). 3rd ed. In: Rucker RB, Suttie JW, McCormick DB, Machlin LJ, eds. *Handbook of Vitamins*. New York, NY: Marcel Dekker, Inc., 2001:463-512.
3. Seetharam B, Alpers DH. Absorption and transport of cobalamin (vitamin B12). *Annu Rev Nutr* 1982;2:343-69.
4. US Department of Agriculture Agricultural Research Services. USDA National Nutrient Database for Standard Reference Release 17. Version current 29 August 2005. Internet: <http://www.nal.usda.gov/fnic/foodcomp/Data/SR17/sr17.html> (accessed 15 May 2005).
5. Herrmann W, Geisel J. Vegetarian lifestyle and monitoring of vitamin B-12 status. *Clin Chim Acta* 2002;326:47-59.
6. Mezzano D, Kosiel K, Martinez C, Cuevas A, Panes O, Aranda E, Strobel P, Perez DD, Pereira J, Rozowski J, Leighton F. Cardiovascular Risk Factors in Vegetarians: Normalization of Hyperhomocysteinemia with Vitamin B12 and Reduction of Platelet Aggregation with n-3 Fatty Acids. *Thrombosis Research* 2000;100:153-160.
7. Pittock SJ, Payne TA, Harper CM. Reversible myelopathy in a 34-year-old man with vitamin B12 deficiency. *Mayo Clin Proc* 2002;77:291-4.
8. Groenen PMW, van Rooij IALM, Peer PGM, Gooskens RH, Zielhuis GA, Steegers-Theunissen RPM. Marginal maternal vitamin B12 status increases the risk of offspring with spina bifida. *Am J Obstet Gynecol* 2004;191:11-17.
9. Miller DR, Specker BL, Ho ML, Norman EJ. Vitamin B-12 status in a macrobiotic community. *Am J Clin Nutr* 1991;53:524-9.
10. Rauma AL, Torronen R, Hanninen O, Mykkanen H. Vitamin B-12 status of long-term adherents of a strict uncooked vegan diet ("living food diet") is compromised. *J Nutr* 1995;125:2511-5.

11. Leblanc JC, Yoon H, Kombadjian A, Verger P. Nutritional intakes of vegetarian populations in France. *Eur J Clin Nutr* 2000;54:443-9.
12. Martens JH, Barg H, Warren MJ, Jahn D. Microbial production of vitamin B12. *Appl Microbiol Biotechnol* 2002;58:275-85.
13. Hodgkin DC, Kamper J, Mackay M, Pickworth J, Trueblood KN, White JG. Structure of vitamin B12. *Nature* 1956;179:64-6.
14. Davis R. Clinical chemistry of vitamin B12. *Adv Clin Chem* 1984;24:163-216.
15. Raux E, Schubert HL, Warren MJ. Biosynthesis of cobalamin (vitamin B12): a bacterial conundrum. *Cell Mol Life Sci* 2000;57:1880-93.
16. Farquharson J, Adams JF. The forms of vitamin B12 in foods. *Br J Nutr* 1976;36:127-36.
17. Meister K. *The Role of Beef in the American Diet*. New York: American Council on Science and Health, 2003.
18. National Oceanic and Atmospheric Administration. US Department of Commerce. 2004 Seafood Consumption Statistics. Version current 2005. Internet: http://www.nmfs.noaa.gov/mediacenter/archive_2005.html (accessed 15 January 2006).
19. National Oceanic and Atmospheric Administration. US Department of Commerce. Fisheries of the United States 2004. National Marine Fisheries Service Office of Science and Technology. Maryland: Fisheries Statistics Division, 2005.
20. Institute of Medicine. Vitamin B12. Dietary Reference Intakes. Washington, D.C.: National Academy Press, 1998:306-356.
21. Scott JM. Folate and vitamin B12. *Proc Nutr Soc* 1999;58:441-8.
22. Chanarin I. *The Absorption of Vitamin B12. The Megaloblastic Anaemias*. 2nd ed. St. Louis: Blackwell Scientific Publications, 1979:93-125.
23. Carmel R. Measuring and interpreting holo-transcobalamin (holo-transcobalamin II). *Clin Chem* 2002;48:407-9.
24. Chanarin I. *Vitamin B12 Binding Proteins. The Megaloblastic Anaemias*. 2nd ed. St. Louis: Blackwell Scientific Publications, 1979:59-75.
25. el Kholty S, Gueant JL, Bressler L, Djalali M. Portal and biliary phases of enterohepatic circulation of corrinoids in humans. *Gastroenterology* 1991;101:1399-408.

26. Castle WB. Vitamin B12. 2nd ed. In: Combs G, ed. *The Vitamins Fundamental Aspects in Nutrition and Health*. San Diego, CA: Academic Press Limited, 1992:377-394.
27. Ellenbogen L, Cooper BA. Vitamin B12. 2nd ed. In: Machlin LJ, ed. *Handbook of Vitamins*. New York, NY: Marcel Dekker, Inc., 1991:491-536.
28. Amin S, Spinks T, Ranicar A, Short MD, Hoffbrand AV. Long-term clearance of [57Co]cyanocobalamin in vegans and pernicious anaemia. *Clin Sci (Lond)* 1980;58:101-3.
29. Stabler SP. Vitamin B12. In: Bowman BA, Russell RM, eds. *Present Knowledge in Nutrition*. Washington, D.C.: ILSI Press, 2001:230-240.
30. Smulders YM, Smith DE, Kok RM, Teerlink T, Swinkels DW, Stehouwer CD, Jakobs C. Cellular folate vitamers distribution during and after correction of vitamin B deficiency: a case for the methylfolate trap. *Br J Haematol* 2006;132:623-9.
31. Horster F, Hoffmann GF. Pathophysiology, diagnosis, and treatment of methylmalonic aciduria-recent advances and new challenges. *Pediatr Nephrol* 2004;19:1071-4.
32. Ervin RB, Wright JD, Wang CY, Kennedy-Stephenson J. Dietary intake of selected vitamins for the United States population: 1999-2000. *Adv Data* 2004:1-4.
33. Martin DC, Francis J, Protetch J, Huff FJ. Time dependency of cognitive recovery with cobalamin replacement: report of a pilot study. *J Am Geriatr Soc* 1992;40:168-72.
34. Valuck RJ, Ruscin JM. A case-control study on adverse effects: H2 blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. *J Clin Epidemiol* 2004;57:422-8.
35. Drummond JT, Matthews RG. Nitrous oxide inactivation of cobalamin-dependent methionine synthase from *Escherichia coli*: characterization of the damage to the enzyme and prosthetic group. *Biochemistry* 1994;33:3742-50.
36. Wulffele MG, Kooy A, Lehert P, Bets D, Ogterop JC, Borger van der Burg B, Donker AJ, Stehouwer CD. Effects of short-term treatment with metformin on serum concentrations of homocysteine, folate and vitamin B12 in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *J Intern Med* 2003;254:455-63.
37. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch Intern Med* 1996;156:1097-100.
38. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: a guide for the primary care physician. *Arch Intern Med* 1999;159:1289-98.

39. Carmel R. Cobalamin (Vitamin B12). 10th ed. In: Shils ME, Shike M, Ross AC, Cabeallero B, Cousins RJ, eds. *Modern Nutrition in Health and Disease*. Baltimore, MD: Lippincott Williams & Wilkins, 2006:482-497.
40. Minot GR, Murphy WP. Landmark article (JAMA 1926). Treatment of pernicious anemia by a special diet. By George R. Minot and William P. Murphy. *JAMA* 1983;250:3328-35.
41. Kuzminski AM, Del Giacco EJ, Allen RH, Stabler SP, Lindenbaum J. Effective treatment of cobalamin deficiency with oral cobalamin. *Blood* 1998;92:1191-1198.
42. Campbell AK, Miller JW, Green R, Haan MN, Allen LH. Plasma vitamin B-12 concentrations in an elderly latino population are predicted by serum gastrin concentrations and crystalline vitamin B-12 intake. *J Nutr* 2003;133:2770-6.
43. Lindgren A, Bagge E, Cederblad A, Nilsson O, Persson H, Kilander AF. Schilling and protein-bound cobalamin absorption tests are poor instruments for diagnosing cobalamin malabsorption. *J Intern Med* 1997;241:477-84.
44. Green TJ, Venn BJ, Skeaff CM, Williams SM. Serum vitamin B12 concentrations and atrophic gastritis in older New Zealanders. *Eur J Clin Nutr* 2005;59:205-10.
45. Ho C, Kauwell GP, Bailey LB. Practitioners' guide to meeting the vitamin B-12 recommended dietary allowance for people aged 51 years and older. *J Am Diet Assoc* 1999;99:725-7.
46. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *New Eng J Med* 1988;318:1720-1728.
47. Afman LA, Van Der Put NMJ, Thomas CMG, Trijbels JMF, Blom HJ. Reduced vitamin B12 binding by transcobalamin II increases the risk of neural tube defects. *QJM* 2001;94:159-166.
48. van Rooij IA, Swinkels DW, Blom HJ, Merkus HM, Steegers-Theunissen RP. Vitamin and homocysteine status of mothers and infants and the risk of nonsyndromic orofacial clefts. *Am J Obstet Gynecol* 2003;189:1155-60.
49. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *Q J Med* 1993;86:703-8.
50. Louwman MW, van Dusseldorp M, van de Vijver FJ, Thomas CM, Schneede J, Uleland PM, Refsum H, van Staveren WA. Signs of impaired cognitive function in adolescents with marginal cobalamin status. *Am J Clin Nutr* 2000;72:762-9.
51. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. *J Pediatr* 1992;121:710-4.

52. Grattan-Smith PJ, Wilcken B, Procopis PG, Wise GA. The neurological syndrome of infantile cobalamin deficiency: developmental regression and involuntary movements. *Mov Disord* 1997;12:39-46.
53. von Schenck U, Bender-Gotze C, Koletzko B. Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. *Arch Dis Child* 1997;77:137-9.
54. Renault F, Verstichel P, Ploussard JP, Costil J. Neuropathy in two cobalamin-deficient breast-fed infants of vegetarian mothers. *Muscle Nerve* 1999;22:252-4.
55. Weiss R, Fogelman Y, Bennett M. Severe vitamin B12 deficiency in an infant associated with a maternal deficiency and a strict vegetarian diet. *J Pediatr Hematol Oncol* 2004;26:270-1.
56. Jadhav M, Webb JK, Vaishnava S, Baker SJ. Vitamin B12 deficiency in Indian infants. A clinical syndrome. *Lancet* 1962;2:903-7.
57. Ueland PM, Refsum H, Beresford SA, Vollset SE. The controversy over homocysteine and cardiovascular risk. *Am J Clin Nutr* 2000;72:324-32.
58. Splaver A, Lamas GA, Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. *Am Heart J* 2004;148:34-40.
59. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. *Annu Rev Nutr* 2004;24:299-326.
60. Selhub J, Jacques PF, Rosenberg IH, Rogers G, Bowman BA, Gunter EW, Wright JD, Johnson CL. Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. *Ann Intern Med* 1999;131:331-9.
61. Gao X, Yao M, McCrory MA, Ma G, Li Y, Roberts SB, Tucker KL. Dietary pattern is associated with homocysteine and B vitamin status in an urban Chinese population. *J Nutr* 2003;133:3636-42.
62. Obeid R, Geisel J, Schorr H, Hubner U, Herrmann W. The impact of vegetarianism on some haematological parameters. *Eur J Haematol* 2002;69:275-9.
63. Herrmann W, Schorr H, Obeid R, Geisel J. Vitamin B-12 status, particularly holotranscobalamin II and methylmalonic acid concentrations, and hyperhomocysteinemia in vegetarians. *Am J Clin Nutr* 2003;78:131-136.
64. Krajcovicova-Kudlackova M, Blazicek P, Kopcova J, Bederova A, Babinska K. Homocysteine levels in vegetarians versus omnivores. *Ann Nutr Metab* 2000;44:135-8.

65. Bor MV, Lydeking-Olsen E, Moller J, Nexø E. A daily intake of approximately 6 microg vitamin B-12 appears to saturate all the vitamin B-12-related variables in Danish postmenopausal women. *Am J Clin Nutr* 2006;83:52-8.
66. Lindenbaum J, Allen R. Clinical spectrum and diagnosis of folate deficiency. In: Bailey L, ed. *Folate in Health and Disease*. New York, NY: Marcel Dekker, Inc., 1995:43-73.
67. Lindenbaum J, Savage DG, Stabler SP, Allen RH. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol* 1990;34:99-107.
68. Bolann BJ, Solli JD, Schneede J, Grottum KA, Loraas A, Stokkeland M, Stallemo A, Schjøth A, Bie RB, Refsum H, Uleland PM. Evaluation of indicators of cobalamin deficiency defined as cobalamin-induced reduction in increased serum methylmalonic acid. *Clin Chem* 2000;46:1744-50.
69. Bates CJ, Schneede J, Mishra G, Prentice A, Mansoor MA. Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. *Eur J Clin Nutr* 2003;57:349-57.
70. Gultepe M, Ozcan O, Avsar K, Cetin M, Ozdemir AS, Gok M. Urine methylmalonic acid measurements for the assessment of cobalamin deficiency related to neuropsychiatric disorders. *Clin Biochem* 2003;36:275-82.
71. Herrmann W, Obeid R, Schorr H, Geisel J. Functional vitamin B12 deficiency and determination of holotranscobalamin in populations at risk. *Clin Chem Lab Med* 2003;41:1478-88.
72. von Castel-Dunwoody KM, Kauwell GP, Shelnutt KP, Vaughn JD, Griffin ER, Maneval DR, Theriaque DW, Bailey LB. Transcobalamin 776C→G polymorphism negatively affects vitamin B-12 metabolism. *Am J Clin Nutr* 2005;81:1436-41.
73. Stabler SP, Lindenbaum J, Allen RH. The use of homocysteine and other metabolites in the specific diagnosis of vitamin B-12 deficiency. *J Nutr* 1996;126:1266S-72S.
74. Slimani N, Ferrari P, Ocke M, Welch A, Boeing H, Liere M, Pala V, Amiano P, Lagiou A, Mattisson I, Stripp C, Engeset D, Charrondiere R, Buzzard M, Staveren W, Riboli E. Standardization of the 24-hour diet recall calibration method used in the European prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr* 2000;54:900-17.
75. Raper N, Perloff B, Ingwersen L, Steinfeldt L, Anand J. An overview of USDA's Dietary Intake Data System. *Journal of Food Composition and Analysis* 2004;17:545-555.

76. McBride J. Was it a slab, a slice, or a sliver? *Agricultural Research* 2001;49:4-7.
77. Moshfegh A, Goldman J, LaComb R, Perloff B, Cleveland L. Research results using the new USDA Automated Multiple Pass Method. *FASEB Journal* 2001;15:A278.
78. Kolar AS, Patterson RE, White E, Neuhouser ML, Frank LL, Standley J, Potter JD, Kristal AR. A practical method for collecting 3-day food records in a large cohort. *Epidemiology* 2005;16:579-83.
79. Morin P, Herrmann F, Ammann P, Uebelhart B, Rizzoli R. A rapid self-administered food frequency questionnaire for the evaluation of dietary protein intake. *Clin Nutr* 2005;24:768-74.
80. Craig MR, Kristal AR, Cheney CL, Shattuck AL. The prevalence and impact of 'atypical' days in 4-day food records. *J Am Diet Assoc* 2000;100:421-7.
81. Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, McNutt S, McIntosh A, Rosenfeld S. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. *Am J Epidemiol* 2001;154:1089-99.
82. Subar AF, Thompson FE, Smith AF, Jobe JB, Ziegler RG, Potischman N, Schatzkin A, Hartman A, Swanson C, Kruse L, Hayes RB, Lewis D, Harlan LC. Improving food frequency questionnaires: a qualitative approach using cognitive interviewing. *J Am Diet Assoc* 1995;95:781-8.
83. Subar AF, Midthune D, Kulldorff M, Brown CC, Thompson FE, Kipnis V, Schatzkin A. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. *Am J Epidemiol* 2000;152:279-86.
84. Haddad EH, Tanzman JS. What do vegetarians in the United States eat? *Am J Clin Nutr* 2003;78:626S-632.
85. Janelle KC, Barr SI. Nutrient intakes and eating behavior scores of vegetarian and nonvegetarian women. *J Am Diet Assoc* 1995;95:180-6, 189.
86. Barr SI, Broughton TM. Relative Weight, Weight Loss Efforts and Nutrient Intakes among Health-Conscious Vegetarian, Past Vegetarian and Nonvegetarian Women Ages 18 to 50. *J Am Coll Nutr* 2000;19:781-788.
87. Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. *Am J Clin Nutr* 1999;70:586S-593S.
88. Larsson CL, Johansson GK. Dietary intake and nutritional status of young vegans and omnivores in Sweden. *Am J Clin Nutr* 2002;76:100-6.

89. Larsson CL, Johansson GK. Young Swedish vegans have different sources of nutrients than young omnivores. *J Am Diet Assoc* 2005;105:1438-41.
90. Dunn-Emke SR, Weidner G, Pettengill EB, Marlin RO, Chi C, Ornish DM. Nutrient adequacy of a very low-fat vegan diet. *J Am Diet Assoc* 2005;105:1442-6.
91. Wright JD, Bialostosky K, Gunter EW, Carroll MD, Najjar MF, Bowman BA, Johnson CL. Blood folate and vitamin B12: United States, 1988-94. *Vital Health Stat* 11 1998:1-78.
92. Carmel R, Mallidi PV, Vinarskiy S, Brar S, Frouhar Z. Hyperhomocysteinemia and cobalamin deficiency in young Asian Indians in the United States. *Am J Hematol* 2002;70:107-14.
93. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Orning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A, Uleland PM. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr* 2001;74:233-241.
94. Janda S, Trocchia PJ. Vegetarianism: Toward a greater understanding. *Psychology & Marketing* 2001;18:1205-40.
95. The Vegetarian Resource Group. How many vegetarians are there? Version current 15 September 2003. Internet: <http://www.vrg.org/press/2003poll.htm> (accessed 13 May 2005).
96. Kim EH, Schroeder KM, Houser RF, Jr., Dwyer JT. Two small surveys, 25 years apart, investigating motivations of dietary choice in 2 groups of vegetarians in the Boston area. *J Am Diet Assoc* 1999;99:598-601.
97. Davey GK, Spencer EA, Appleby PN, Allen NE, Knox KH, Key TJ. EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr* 2003;6:259-69.
98. American Dietetic Association and Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. *J Am Diet Assoc* 2003;103:748-65.
99. White R, Frank E. Health effects and prevalence of vegetarianism. *West J Med* 1994;160:465-70.
100. Food Marketing Institute. Food and Nutrition. Version current 2001. Internet: http://www.fmi.org/facts_figs/superfact.htm (accessed 15 January 2006).
101. Fraser GE. Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. *Am J Clin Nutr* 1999;70:532S-538S.

102. Slattery ML, Jacobs DR, Jr., Hilner JE, Caan BJ, Van Horn L, Bragg C, Manolio TA, Kushi LH, Liu KA. Meat consumption and its associations with other diet and health factors in young adults: the CARDIA study. *Am J Clin Nutr* 1991;54:930-5.
103. Nicklas TA, Farris RP, Myers L, Berenson GS. Impact of meat consumption on nutritional quality and cardiovascular risk factors in young adults: the Bogalusa Heart Study. *J Am Diet Assoc* 1995;95:887-92.
104. O'Dea K, Traianedes K, Chisholm K, Leyden H, Sinclair AJ. Cholesterol-lowering effect of a low-fat diet containing lean beef is reversed by the addition of beef fat. *Am J Clin Nutr* 1990;52:491-4.
105. Beauchesne-Rondeau E, Gascon A, Bergeron J, Jacques H. Plasma lipids and lipoproteins in hypercholesterolemic men fed a lipid-lowering diet containing lean beef, lean fish, or poultry. *Am J Clin Nutr* 2003;77:587-93.
106. Snetselaar L, Stumbo P, Chenard C, Ahrens L, Smith K, Zimmerman B. Adolescents eating diets rich in either lean beef or lean poultry and fish reduced fat and saturated fat intake and those eating beef maintained serum ferritin status. *J Am Diet Assoc* 2004;104:424-8.
107. Enns C, Goldman J, Cook A. Trends in Food and Nutrient Intakes by Adults: NFCS 1977-78, CSFII 1989-91, and CSFII 1994-95. *Family Economics and Nutrition Review* 1997;10:2-15.
108. Food and Nutrition Information Center. US Department of Agriculture. Dietary Guidelines for Americans 2005. Version current 8 December 2005. Internet: <http://www.nal.usda.gov/fnic/dga/> (accessed 15 January 2006).
109. Yoshino K, Inagawa M, Oshima M, Yakota K, Umesawa M, Endo M, Yamagishi K, Tanigawa T, Sato S, Shimamoto T, Iso H. Trends in dietary intake of folate, vitamins B6, and B12 among Japanese adults in two rural communities from 1974 through 2001. *J Epidemiol* 2005;15:29-37.

BIOGRAPHICAL SKETCH

Amanda was born in Herndon, Virginia. She received her undergraduate degree in human nutrition, foods, and exercise from Virginia Tech in Blacksburg, Virginia, in 2001. After graduation she was accepted into The National Institutes of Health (NIH) Dietetic Internship in Bethesda, Maryland. In 2003, she passed the Registration Exam for Dietitians and practiced as a Registered Dietitian before enrolling in graduate school at the University of Florida. As a Registered Dietitian she worked with low-income women, infants, and children teaching basic nutrition messages. In 2005, she was awarded the Presidential Recognition Award at the University of Florida. In 2006, Amanda received the North American College and Teachers of Agriculture (NACTA) Graduate Student Teaching Award. Her hobbies include playing soccer at a competitive level and singing in an a cappella group. Upon completion of her graduate degree, she plans to return to practice as a Registered Dietitian.