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SYMPOSIUM

A Strategy for Analyzing Gene–Nutrient Interactions in Type 2 Diabetes

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Abstract

Type 2 diabetes mellitus (T2DM), like all chronic diseases, results from interactions between multiple genes and multiple environmental factors. Nevertheless, many research studies focus on either nutrition or genetic factors independently of each other. The challenges of analyzing gene–nutrient interactions in T2DM are the (i) genetic heterogeneity in humans, (ii) complexity of environmental factors, particularly dietary chemicals, and (iii) diverse physiologies that produce the same apparent disease. Many of these variables are not accounted for in the design or study of T2DM or, indeed, most chronic diseases, although exceptions are noteworthy. Establishing experimental paradigms to analyze the complexity of these interactions and physiologies is challenging, but possible. This article provides a strategy to extend nutrigenomic experimental strategies to include early environmental influences that may promote adult-onset disease.

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Introduction

Type 2 diabetes mellitus (T2DM) was once considered a disease of the old, but is increasingly occurring at earlier ages with the mean age of diagnosis decreasing from 52.0 years in 1976–1980 to 46.0 years in 1999–2000.[1] Although obesity is increasing in all age groups, the incidence of T2DM in children is less than 1% based on results from the 2001 multicenter study Search for Diabetes in Youth Study.[2] This population-based study of 6379 newly diagnosed children and teens from a population of ~3.5 million in six geographic sites included European, African, Native, Latino, and Asian and Pacific Island Americans children under 18 years of age.[3–9] The Center for Disease Control reports that 12.4% of children aged 2–5 and 17% of children aged 6–17 are at or above the 95th percentile for sex- and age-specific body mass index (**Table 1**; see http://www.cdc.gov/nccdphp/dnpa/obesity/childhood/prevalence.htm and Ogden and associates[10]).

Obesity increases one of the hallmarks of diabetes, insulin resistance, which has been found to occur in children.[11] The incidence of obesity in children portends an increase in T2DM in individuals under the age of 18 in the near future.

Personalizing Nutrigenomic Research

Typical nutritional studies analyzing the response of an intervention group to controls provided the same diet lacking a specific nutrients or nutrients. Simple examples

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Abbreviations: (ADP) adenosine diphosphate, (DRI) dietary reference intake, (GWAS) genome-wide association studies, (NAD) nicotinamide adenine dinucleotide, (NHANES) National Health and Nutrition Examination Survey, (QTL) quantitative trait loci, (SNP) single nucleotide polymorphism, (T2DM) type 2 diabetes mellitus

Keywords: gene - nutrient interactions, Metacore, micronutrients, obesity, quantitative trait loci, Type 2 diabetes

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analyzed serum lipid changes in response to a high fat vs control diet or determined differences in nutrient intakes between groups of individuals who have a disease (cases) versus those that do not (i.e., controls). Results of such studies are averages of all members of the control and all members of the intervention group. While population attributable risk yields useful guidelines, it does not provide information for individual members of the group nor can it be confidently applied to individuals not in the study.[12]

Table 1.
2003–2006 NHANES Survey of Obese and Overweight Children[a]

Ancestral group	BMI for age	Percentage (%)[b]
All	> 95% = obese	16.3
Girls, non-Hispanic White	> 97% = obese	8.5
Girls, African American	> 97% = obese	18.0
Boys, non-Hispanic White	> 97% = obese	10.6
Boys, Mexican American	> 97% = obese	17.7
Girls, non-Hispanic White	> 85% = overweight	29.5
Girls, African American	> 85% = overweight	39.2
Boys, non-Hispanic White	> 85% = overweight	31.2
Boys, Mexican American	> 85% = overweight	40.8

[a] Ages 2 through 19, from www.cdc.gov and Ogden and colleagues.[10]
[b] Percentage in BMI for age.

Developing risk factors for individuals will require novel experimental designs. Kaput[12] and others[13,14] have proposed that analyzing the main statistical effect of gene–nutrient interactions rather than just genes or just nutrient intakes is necessary in understanding human phenotypes. Such studies may be done by comparing gene–nutrient interactions in genetically and culturally different individuals to develop the range of human responses to food. Because many population groups were genetically isolated as they adapted to differing food environments in different parts of the world over millennium, individuals of differing ancestral backgrounds have large chromosomal regions that differ in frequencies of single nucleotide polymorphisms.[15] Genes within these regions contribute to skeletal development, skin pigmentation, and carbohydrate metabolism. Developing one optimum diet for each ancestral group is not possible because each population will have a wide range of allele frequencies,[16–20] that is, while the average genotype in one ancestral population (i.e., Asian) may differ from that of another (e.g., Europe), individuals with similar allele frequencies will be found in both populations. Nevertheless, we suggested that individuals with similar gene–environment interactions can be clustered or binned. Dreon and co-workers[21] identified three phenotypic responses to low and medium fat diets, suggesting the presence of fat–metabolism genetic groups.

To begin the identification of these metabolic groups, we proposed that the range of human responses to food intakes must be analyzed. This can be done by comparing gene–environment interactions across many different individuals with subsequent sorting into metabolic groups. Because homeostatic measures of metabolites have wide ranges of normal,[22,23] we analyzed the response of each individual to a known dietary intervention.

The Hypothesis

A person's genetic makeup provides the range of probabilities for developing chronic diseases. The health status within that genetic range is determined by the individual's responses to the nutritional and physical environment.[24] Obesity and related pathologies are influenced by total energy intakes, but suboptimal intakes of other nutrients may also be factors in the initiation and development of these diseases. We are testing whether deficiencies of key vitamins may also contribute to the initiation, severity, and complications of chronic diseases in individuals. A growing body of literature demonstrates that nutrition during pregnancy and early childhood influences susceptibility to chronic diseases and T2DM.[25–30] The focus of much of this published research is on folate and B vitamins, as these nutrients and metabolites influence DNA methylation and epigenetic mechanisms.[28,31] However, other micro- and macronutrients contribute to disease susceptibility or incidence. A vitamin D deficiency, for example, is associated with an increased risk of bone diseases, common cancers, autoimmune diseases, hypertension, and infectious diseases.[32] Low intakes of vitamin D have also been associated with an incidence and pathogenicity of T2DM.[33–38] A suboptimal vitamin B_6 status is associated with certain late-onset diseases initiated in early life but also afflicts the elderly population—impaired cognitive function, Alzheimer's disease, cardiovascular disease, and different types of cancer.[39] Although improved nutrition could ameliorate some of the effects of pre-, peri-, and early postnatal undernutrition, catch-up growth caused by "normal" nutrient intakes has been linked to ongoing insulin resistance and susceptibility to the metabolic syndrome.[40] Low birth weight and associated

abnormalities are often associated with developing countries, but eating unbalanced diets produces malnourishment in individuals in developed countries, including the United States.[41]

The susceptibility of any individual to these diseases is influenced by individual genetic variation. That is, certain individuals (i.e., genetic makeups) who lack key vitamins may be more susceptible to chronic diseases than individuals with genetic makeups that compensate for low vitamin levels. To test this hypothesis, we are analyzing the response to increased levels of a vitamin in an individual and subsequently correlating the response to genes involved in metabolism of the vitamin. We propose analyzing the sequence of genes that interact with, are regulated by, or metabolize micronutrients mapping to quantitative trait loci involved in obesity or diabetes. Genes mapping within such regions are more likely to contribute to the initiation, progression, or severity of the disease,[42–44] which can be assessed by association analyses. Resequencing is necessary to identify previously unidentified alleles within the candidate genes.

The Initial Population

We have initiated dietary intervention studies to examine how an individual child (ages 6–14) will respond to improved nutrient intakes. Although basic in design, our approach employs replacement meals of known composition measured as accurately as possible, analyses of changes in serum metabolites before and after intervention, candidate gene analyses, and whole genome scans to assess potential epistatic interactions. Each individual serves as their own control, as we analyze serum metabolites before and after the intervention, and genomic analyses (i.e., whole genome scans with 600K genotyping arrays) are used for sorting individuals of similar genetic makeups.

The individuals who are collaborating with us and the U.S. Department of Agriculture–Agricultural Research Service Obesity Prevention Research Unit are members of the summer day camp of the Marvell (AR) Boys, Girls, and Adults Community Development Center. The 2003–2006 National Health and Nutrition Examination Survey (NHANES) of children and adolescents (aged 2–19) showed a stunning incidence of obesity among children in the past 20 years (**Table 1**). About 40% of girls and boys in the middle school ages in the Marvell school district are at or above the 85% in age- and sex-adjusted body mass index (BMI).[45] Nutrient intake surveys demonstrated that European and African American children[46] in this rural population are deficient in vitamin E [~20% meeting dietary reference intake (DRI)], calcium (~25% DRI), linoleic (~40% DRI), vitamin A (~50% DRI), folate (~65% DRI), vitamin C (~70% DRI), and vitamin B_6 (~75% DRI). Dietary intake deficiencies also occurred with selected other nutrients and total and fat energy. While the average weight of children in this population is increasing, they are malnourished and specifically deficient (as measured by intake surveys) in vitamins and minerals. In a similar study of homeless youths in Minnesota,[41] Smith and Richards found that food insecurity resulted in an increased intake of food that was calorically dense but nutrient poor. While not homeless, the population of the Delta of Arkansas also suffers from food insecurities.[47,48] Analyses of menus in a rural Mississippi school district suggest that school lunches may contribute to unbalanced nutrient intakes.[49]

The experimental design was based on community-based participatory research methods.[50,51] Our approach provided two more nutritious replacement meals plus a healthy snack per day to children attending a summer day camp. Analyses of metabolites in serum in each child were done before, after 4 weeks of camp, and 1 month following camp. The change in selected resting serum metabolite concentrations measures the individual's response. Whole chromosome scans were also performed, as they provide a measure of genetic ancestry and are eventually used to analyze epistatic interactions. Candidate genes involved in vitamin metabolism were also analyzed. Because the current knowledge base of single nucleotide polymorphism (SNP) frequencies was developed for mapping purposes and used 270 individuals from the Yoruba Africa tribe, Centre d'Etude du Polymorphisme Humain European panels, Han Chinese, and Tokyo-based Japanese, candidate genes were resequenced to analyze alleles of candidate genes. This approach is consistent with the Human Variome Project, which seeks to develop locus-specific databases characterizing each mutation and polymorphism within the human population.[52] One example should suffice: A recent report resequenced methylene tetrahydrofolate reductase in 564 samples from the Coriell Human Diversity Panel and found 14 nonsynonymous changes, including 11 alleles with frequencies <1%, along with the common alleles A222V, E429A, and R594Q. Several of these alleles had altered enzyme activity levels that could be "rescued" by increasing the concentration of substrates and cofactors, suggesting a means to intervene nutritionally in individuals with certain alleles.

Selecting the candidate genes is problematic, as many gene–phenotype association studies have not included diet

or environment as a variable—the approach of these studies was to analyze the statistical main effect of the gene or the genetic makeup (e.g., see Yu and colleagues[53]). While a small group of genes has been identified for each phenotype studied, the genes identified for one phenotype combined typically account for only ~2–10% of disease risk. For example, the 18 gene variants associated with T2DM improve the disease score of estimating disease risk by about 4%. The disease score is composed of family history, clinical measures, and male vs female and was 0.534 without genotype analyses and 0.581 with genotype analyses.[54] Because the genome-wide association studies (GWAS) were not designed to discover gene–nutrient interactions and because our goal was to discover genes that contribute to individual risk factors, we modified a previously published design[42–44] for selecting candidate genes to resequence. The steps in this protocol are based on analyzing candidate genes that metabolize vitamins that map to genetic loci contributing to obesity.

1. Select a nutrient: for this study, 13 vitamins or metabolites (**Table 2**) were selected based on an analytical method (*high-performance liquid chromatography* column in this case) but the strategy applies to any nutrient or analytic technology.

2. Search metabolic and regulator pathways: to identify genes regulated by or involved in vitamin metabolism (**Figure 1**). The pathway for each gene or metabolite is searched individually.

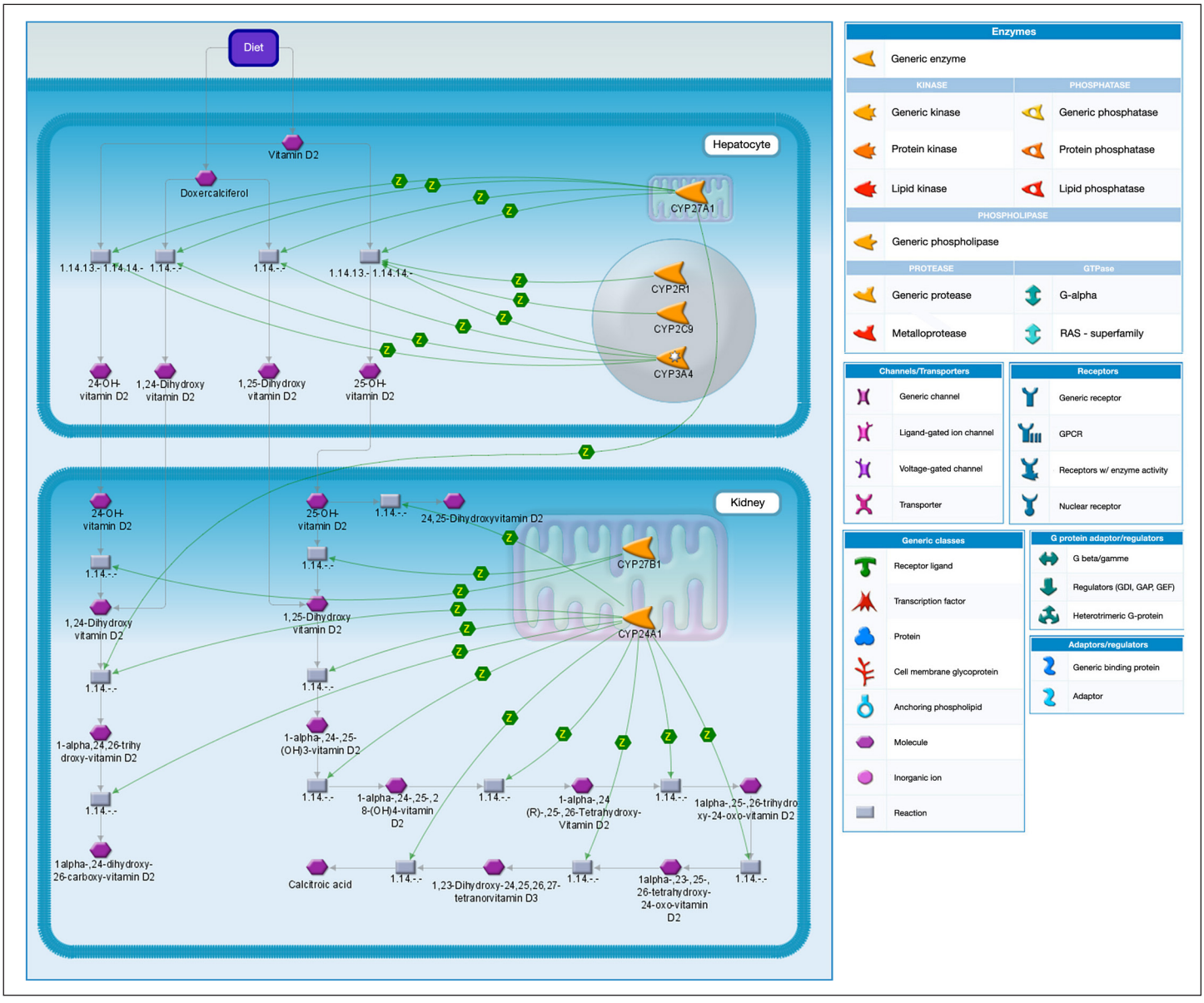

Figure 1. Metabolic pathway map for vitamin D_2 using GeneGo MetaCore™. The subset of genes encoding these proteins that map to QTL is listed in **Table 3**, which also lists candidate gene for micronutrients listed in **Table 2**. Symbols are explained in the legend.

Table 2.
Vitamins and Metabolites Analyzed in Population Study

Vitamin or metabolite	Common name	Food source	Bodily function	Normal blood level[a]	# metabolic genes (Genego)
Vitamin A	Retinol	Mango, broccoli, butternut squash, carrots, tomato juice, sweet potatoes, pumpkin, beef liver,[b] milk, egg yolk, dark green leafy vegetables and yellow fruits are high in vitamin A or β-carotene. The precursor form, β-carotene, is found in plants. Sources of β-carotene are carrots, pumpkin, sweet potatoes, winter squashes, cantaloupe, pink grapefruit, apricots, broccoli, spinach, and most dark green, leafy vegetables, cheese, and margarine[c]	Supports vision, skin, bone and tooth growth, immunity and reproduction.[b] Morphogenesis, vision, immune function, reproduction neural development, antioxidant, prevention of heart disease and cancer. Vitamin A helps form and maintain healthy teeth, skeletal and soft tissue, mucous membranes, and skin. It is also known as retinol because it generates pigments in the retina. Vitamin A promotes good vision, especially in dim light. It may also be required for reproduction and breast-feeding. β-Carotene, which has antioxidant properties, is a precursor to vitamin A. Antioxidants quench free radicals, which are unstable substances that can react with and damage cells, tissues, and organs; fat soluble so a certain amount is necessary, but too much is toxic.[d] Growth, protects the linings of the digestive, urinary, and respiratory tracts.[c]	30–80 μg/dl	32
Vitamin B_1	Thiamine	Spinach, green peas, tomato juice, watermelon, sunflower seeds, lean ham, lean pork chops, soy milk,[b] nuts, bread, cereals, yeast extract[c]	Supports energy metabolism and nerve function.[b] Needed for muscles and nervous system to function. Aids digestion.[c]	9–44 nmol/liter	3
Vitamin B_2	Riboflavin	Spinach, broccoli, mushrooms, eggs, milk, liver, oysters, clams,[b] yogurt, meats, nuts, green leafy vegatables, whole grains, lentils[c]	Supports energy metabolism, normal vision and skin health.[b] Aids hormone production, keeps eyes, skin, and nerves healthy.[c]	4–24 μg/dl	8
Vitamin B_6	Pyridoxine	Bananas, watermelon, tomato juice, broccoli, spinach, acorn squash, potatoes, white rice, chicken breast,[b] fish, brown rice, whole grains[c]	Amino acid and fatty acid metabolism, red blood cell production.[b] Helps formation of red blood cells and making of proteins, fights infection.[c]	5–30 ng/ml	5
Vitamin B_{11}	Folic acid	Tomato juice, green beans, broccoli, spinach, asparagus, okra, black-eyed peas, lentils, navy, pinto and garbanzo beans[b]	Supports DNA synthesis and new cell formation.[b]	3.0–20.0 ng/ml in serum, 140-3-628 ng/ml in red blood cells	15
Vitamin C	l-Ascorbic acid	Spinach, broccoli, red bell peppers, snow peas, tomato juice, kiwi, mango, orange, grapefruit juice, strawberries,[b] citrus fruits, green vegetables, fortified cereals, potatoes[c]	Collagen synthesis, amino acid metabolism, helps iron absorption, immunity, antioxidant.[b] Needed for healthy skin, fights cell damage, particularly during stress and illness, antioxidant.[c] In humans, an exogenous source of ascorbic acid is required for collagen formation and tissue repair by acting as a cofactor in the post-translational formation of 4-hydroxyproline in -Xaa-Pro-Gly- sequences in collagens and other proteins. Ascorbic acid is reversibly oxidized to dehydroascorbic acid in the body. These two forms of the vitamin are believed to be important in oxidation–reduction reactions. The vitamin is involved in tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrate metabolism, synthesis of lipids and proteins, iron metabolism, resistance to infections, and cellular respiration.[e]	0.4–1.5 mg/dl	21

Table 2. Continued

Vitamin or metabolite	Common name	Food source	Bodily function	Normal blood level[a]	# metabolic genes (Genego)
25-OH-vitamin D		Sunlight, fatty fish, organ meats[d]	Increase absorption of calcium in bones.[d] Modulation of immune response, and regulation of cell differentiation, proliferation, and apoptosis[55]	14–60 ng/ml 50 nmol/liter[55]	
Vitamin D_2	Ergocalciferol	Fortified milk	Vitamin D_2 is the form of vitamin D added most commonly to foods and nutritional supplements. Vitamin D_2 must be transformed (hydroxylated) into one of two active forms via the liver or kidney. Once transformed, it binds to the vitamin D receptor, which then leads to a variety of regulatory roles. Vitamin D plays an important role in maintaining calcium balance and in the regulation of the parathyroid hormone. It promotes renal reabsorption of calcium, increases intestinal absorption of calcium and phosphorus, and increases calcium and phosphorus mobilization from bone to plasma. Vitamin D_2 and its analogs appear to promote intestinal absorption of calcium through binding to a specific receptor in the mucosal cytoplasm of the intestine. Subsequently, calcium is absorbed through formation of a calcium-binding protein. Lack of Vitamin D may cause rickets or hypoparathyroidism.[e]		7
Vitamin E	Tocopherol	Polyunsaturated plant oils (soybean, corn, and canola oils), wheat germ, sunflower seeds, tofu, avocado, sweet potatoes, shrimp, cod,[b] vegetable oils, nuts, seeds, wheat germ, green leafy vegetables.[c]	Antioxidant, prevention of heart disease and cancer.[d] Antioxidant, regulation of oxidation reactions, supports cell membrane stabilization.[d] Helps form blood cells.[c]	0.5–1.8 mg/dl[a]	17
Niacin	Nicotinic acid	Meats and fish,[d] spinach, potatoes, tomato juice, lean ground beef, chicken, liver, shrimp,[b] whole grains, nuts.[c]	Water soluble and plays a role in turning food into energy, as well as in the metabolism of fats and carbohydrates. Niacin can also act as an antioxidant within cells, which means it can destroy cell-damaging free radicals. In conjunction with riboflavin and pyridoxine, it helps keep the skin, intestinal tract, and nervous system functioning smoothly; a serious deficiency of niacin causes a condition called pellagra.[a] Supports energy metabolism, normal vision, and skin health.[b] Needed for production of some hormones, forming red blood cells, converting food to energy.[c]		1
S-Adenosyl-l-methionine	SAM-e	See section on folate	DNA methylation		18
S-Adenosyl-l-homocysteine		See section on folate	Product of DNA methylation		18
Homocysteine		See section on folate	Product of DNA methylation		18

[a] http://www.enotes.com/nursing-encyclopedia/vitamins-tests (adults).
[b] http://www.healthchecksystems.com/vitamins.htm.
[c] http://www.online-vitamins-guide.com/vitamins/vitamin-guide.htm.
[d] http://www.yourhealthportal.com/how-get-all-your-vitamins-from-food.html.
[e] http://www.genego.com

3. Obtain a gene list for each vitamin or metabolite (**Table 3**).

4. Map each gene to obesity quantitative trait loci (QTL)(**Table 3**). The chromosomal position of each gene is found using mouse and human mapping information and is compared to chromosomal regions (QTL or regions found in GWAS) identified as contributing to obesity, type 2 diabetes, body weight, or phenotypes that contribute to those diseases (e.g., insulin or glucose level QTL). Vitamin-metabolizing genes that overlap mouse and human QTL involved in the same chronic diseases are candidate genes that may contribute to that disease. Candidate genes will be resequenced to associate alleles with their response to different amounts of vitamin intake.

Table 3.
Micronutrient Genes Mapping to Quantitative Trait Loci[a]

Nutrient	Gene	Protein	Human chromosome	Mouse chromosome	Mouse (cM)	MGI #	Mouse QTL	Mouse QTL chromosome	Mouse QTL (cM)	MGI #
Vitamin E	*ACAA1*	3-Ketoacyl-CoA thiolase, peroxisomal precursor	3p22.2	9	71	2148491	Obq18	9	65	2656144
Vitamin E	*ACAA1*	3-Ketoacyl-CoA thiolase, peroxisomal precursor	3p22.2	9	71	2148491	Lcho1	9	67	2137322
Vitamin E	*ACOT8*	Acyl-coenzyme A thioesterase 8	20q13.12	2	~85.0	2158201	Mob5	2	95.5	1890667
Vitamin E	*ACOX3*	Acyl-coenzyme A oxidase 3, peroxisomal	4p16.1	5	20	1933156	Obq11	5	10	2150700
Riboflavin	*ACP2*	Lysosomal acid phosphatase precursor	11p11.2	2	52	87882	Mob5	2	95.5	1890667
Riboflavin	*ACP2*	Lysosomal acid phosphatase precursor	11p11.2	2	52	87882	T2dm2sa	2		3622822
Riboflavin	*ACP2*	Lysosomal acid phosphatase precursor	11p11.2	2	52	87882	Obq3	2	53	1100506
Riboflavin	*ACP5*	Tartrate-resistant acid phosphatase type 5 precursor	19p13.2	9	6	87883	Obq5	9	19	1349407
Vitamin E	*ACSL1*	Long-chain fatty acid–CoA ligase 1	4q35.1	8	~30	102797	Adip4	8	32	2149044
Vitamin E	*ACSL3*	Long-chain fatty acid–CoA ligase 3	2q36.1	1	24.1	1921455	Obq7	1	28.7	2150696
Vitamin E	*ACSL3*	Long-chain fatty acid–CoA ligase 3	2q36.1	1	24.1	1921455	Insq6	1	37	2148493
Vitamin E	*ACSL5*	Long-chain fatty acid–CoA ligase 5	10q25.2[b]	19	53	1919129	Obwq5	19	52	3531520
Vitamin E	*ACSL6*	Long-chain fatty acid–CoA ligase 6	5q31.1	11	29.35	894291	Fina1	11	~33	3707782
Vitamin E	*ACSL6*	Long-chain fatty acid–CoA ligase 6	5q31.1	11	29.35	894291	Nidd1n	11	31	1355320
Ascorbic	*ADH7*	Alcohol dehydrogenase class 4 μ/σ chain	4q23	3	71.2	87926	Mors2	3	58.8	2149823
SAM	*AHCY*	Adenosylhomocysteinase	20q11.22	2	89	87968	Mob5	2	95.5	1890667
Methionine	*AHCYL2*	Putative adenosylhomocysteinase 3	7q32.1	6	7	1921590	Mob2	6	3.05	99506
Retinol	*ALDH1A2*	Retinal dehydrogenase 2	15q22.1	9	42	107928	Mob8	9	42	1890670
Folic	*ALDH1L1*	10-Formyltetrahydrofolate dehydrogenase	3q21.2	6	~38.0	1340024	Obq14	6	43.5	2150703

continued →

Table 3. Continued

Nutrient	Gene	Protein	Human chromosome	Mouse chromosome	Mouse (cM)	MGI #	Mouse QTL	Mouse QTL chromosome	Mouse QTL (cM)	MGI #
Folic	*ALDH1L1*	10-Formyltetrahydrofolate dehydrogenase	3q21.2	6	~38.0	1340024	Obwq3	6	42	3531518
Retinol	*ALDH2*	Aldehyde dehydrogenase, mitochondrial precursor	12q24.12	5	~68	99600	Hdlq1	5	70	2387134
NAD	*ALPL*	Alkaline phosphatase, tissue-nonspecific isozyme precursor	1p36.12	4	70.2	87983	Adip12	4	70	3531537
Folic	*AMT*	Aminomethyltransferase, mitochondrial precursor	3p21.31	9	~60	3646700	Dob2	9	60	99950
Folic	*ALDH1L1*	10-Formyltetrahydrofolate dehydrogenase	3q21.2	6	~38.0	1340024	Obwq3	6	42	3531518
Retinol	*ALDH2*	Aldehyde dehydrogenase, mitochondrial precursor	12q24.12	5	~68	99600	Hdlq1	5	70	2387134
NAD	*ALPL*	Alkaline phosphatase, tissue-nonspecific isozyme precursor	1p36.12	4	70.2	87983	Adip12	4	70	3531537
Folic	*AMT*	Aminomethyltransferase, mitochondrial precursor	3p21.31	9	~60	3646700	Dob2	9	60	99950
Folic	*AMT*	Aminomethyltransferase, mitochondrial precursor	3p21.31	9	~60	3646700	Obq18	9	65	2656144
NAD	*AOX1*	Aldehyde oxidase	2q33.1	1	23.2	88035	Obq7	1	28.7	2150696
NAD	*ART1*	GPI-linked NAD(P)(+)–arginine ADP-ribosyltransferase 1 precursor	11p15.4[c]	7	50	107511	Idd27	7	47	3608893
NAD	*ART1*	GPI-linked NAD(P)(+)–arginine ADP-ribosyltransferase 1 precursor	11p15.4[c]	7	50	107511	Adip3	7	46.6	2149043
NAD	*ART3*	Ecto-ADP-ribosyltransferase 3 precursor	4q21.1	5	52	1202729	Obwq2	5	44	3531517
NAD	*ART5*	Ecto-ADP-ribosyltransferase 5 precursor	11p15.4[c]	7	50	107948	Idd27	7	47	3608893
NAD	*ART5*	Ecto-ADP-ribosyltransferase 5 precursor	11p15.4[c]	7	50	107948	Adip3	7	46.6	2149043
Folic	*ATIC*	Bifunctional purine biosynthesis protein PURH	2q35	1	~36	1351352	Obq7	1	28.7	2150696
Retinol	*BCMO1*	β-Carotene 15,15'-monooxygenase	16q23.2	8	~56	1926923	Bwq3	8	56	1890413
NAD	*BST1*	ADP-ribosyl cyclase 2 precursor	4p15.3	5	25	105370	Obq11	5	10	2150700
Retinol	*CRABP1*	Cellular retinoic acid-binding protein 1	15q25.1	9	31	88490	Obq5	9	19	1349407
Methionine	*CTH*	Cystathionine γ-lyase	1p31.1[d]	3	~86	1339968	Mors2	3	58.8	2149823
Retinol	*CYP1A1*	Cytochrome P450 1A1	15q24.1	9	31	88588	Neogq1	9	42	3640530
Retinol	*CYP1A2*	Cytochrome P450 1A2	15q24.1	9	31	88589	Obq5	9	19	1349407

continued →

Table 3. Continued

Nutrient	Gene	Protein	Human chromosome	Mouse chromosome	Mouse (cM)	MGI #	Mouse QTL	Mouse QTL chromosome	Mouse QTL (cM)	MGI #
Vitamin D_2	*CYP24A1*	Cytochrome P450 24A1, mitochondrial precursor	20q13.2	2	99	88593	Bglu1	2	87	2149372
Vitamin D_2	*CYP24A1*	Cytochrome P450 24A1, mitochondrial precursor	20q13.2	2	99	88593	Mob5	2	95.5	1890667
Retinol	*CYP2E1*	Cytochrome P450 2E1	10q26.3	7	68.4	88607	Mob1	7	62	99505
Vitamin D_2	*CYP2R1*	Cytochrome P450 2R1	11p15.2	7	~52	2449771	Idd27	7	47	3608893
Vitamin D_2	*CYP2R1*	Cytochrome P450 2R1	11p15.2	7	~52	2449771	Hdlcl1	7	53	3528033
Vitamin D_2	*CYP2R1*	Cytochrome P450 2R1	11p15.2	7	~52	2449771	Obq15	7	51.4	2150704
NAD	*ENPP2*	Ectonucleotide pyrophosphatase/ phosphodiesterase family member 2 precursor	8q24.12[e]	15	27.5	1321390	Bsbob5	15	20.2	3051761
NAD	*ENPP2*	Ectonucleotide pyrophosphatase/ phosphodiesterase family member 2 precursor	8q24.12[e]	15	27.5	1321390	Dob3	15	22.8	99958
SAM	*GCLC*	Glutamate--cysteine ligase catalytic subunit	6p12.1	9	42	104990	Mob8	9	42	1890670
SAM	*GOT1*	Aspartate aminotransferase, cytoplasmic	10q24.2	19	37	95791	Obwq5	19	37	3531520
SAM	*GOT2*	Aspartate aminotransferase, mitochondrial precursor	16q21	8	46	95792	Obq16	8	46	2656142
Sulfur	*GOT2*	Aspartate aminotransferase, mitochondrial precursor	16q21	8	46	95792	Obq16	8	46	2656142
Sulfur	*GOT2*	Aspartate aminotransferase, mitochondrial precursor	16q21	8	46	95792	Hdlq16	8	44	2653462
NAD	*ITGB1BP3*	Nicotinamide riboside kinase 2	19p13.3	10	~43.0	1916814	Insq9	10	44	2148496
Methionine	*MAT1A*	S-Adenosylmethionine synthetase isoform type-1	10q23.1[f]	14		88017	Mors3	14	25	2149824
Methionine	*MTFMT*	Methionyl-tRNA formyltransferase, mitochondrial precursor	15q22.31	9	~35.0	1916856	Mob8	9	42	1890670
Folic	*MTHFR*	Methylenetetrahydrofolate reductase	1p36.22	4	76.4	106639	Adip12	4	70	3531537
Folic	*MTHFS*	5-Formyltetrahydrofolate cyclo-ligase	15q25.1	9	49	1340032	Mob8	9	42	1890670
NAD	*NADK*	NAD kinase	1p36.33	4	82	2183149	Adip12	4	70	3531537
NAD	*NNMT*	Nicotinamide N-methyltransferase	11q23.2	9	29	1099443	Obq5	9	19	1349407
NAD	*NT5C*	5'(3')-deoxyribonucleotidase, cytosolic type	17q25.1	11	~72.0	1354954	Nidd4	11	68	1890953
NAD	*NT5C1A*	Cytosolic 5'-nucleotidase 1A	1p34.3	4	~54.0	2155700	Adip12	4	70	3531537

continued →

Table 3. Continued

Nutrient	Gene	Protein	Human chromosome	Mouse chromosome	Mouse (cM)	MGI #	Mouse QTL	Mouse QTL chromosome	Mouse QTL (cM)	MGI #
NAD	*NT5C1B*	Cytosolic 5'-nucleotidase 1B	2p24.2	12	~5.0	1918131	Adip6	12	45	2149047
NAD	*NT5C1B*	Cytosolic 5'-nucleotidase 1B	2p24.2	12	~5.0	1918131	Chldq7	12	12	3711850
NAD	*NT5C2*	Cytosolic purine 5'-nucleotidase	10q24.33[f]	19	~46.0	2178563	Obwq5	19	52	3531520
NAD	*NT5E*	5'-Nucleotidase precursor	6q14.3	9	~43	99782	Neogq1	9	42	3640530
NAD	*NT5E*	5'-Nucleotidase precursor	6q14.3	9	~43	99782	Adip5	9	42	2149045
NAD	*NT5M*	5'(3')-Deoxyribonucleotidase, mitochondrial precursor	17p11.2	11	~32.0	1917127	Fina1	11		3707782
NAD	*PARP3*	Poly(ADP-ribose) polymerase 3	3p21.1	9	~60.0	1891258	Dob2	9	60	99950
NAD	*PARP3*	Poly(ADP-ribose) polymerase 3	3p21.1	9	~60.0	1891258	Obq18	9	65	2656144
Folic	*SHMT1*	Serine hydroxymethyltransferase, cytosolic	17p11.2	11	34	98299	Fina1	11	~33	3707782
NAD	*SIRT3*	NAD-dependent deacetylase sirtuin-3, mitochondrial precursor	11p15.5[c]	7	~69.0	1927665	Pbwg3	7	72	3035959
NAD	*SIRT3*	NAD-dependent deacetylase sirtuin-3, mitochondrial precursor	11p15.5[c]	7	~69.0	1927665	Mob1	7	62	99505
NAD	*SIRT6*	Mono-ADP-ribosyltransferase sirtuin-6	19p13.3	10	43	1354161	Adip15	10	52	3531540
Vitamin E	*SLC27A2*	Very long-chain acyl-CoA synthetase	15q21.2	2	~66.0	1347099	Obq10	2	58.1	2150699
Thiamine	*TPK1*	Thiamin pyrophosphokinase 1	7q35	6	~25	1352500	Bsbob3	6	26.5	3051759
Thiamine	*TPK1*	Thiamin pyrophosphokinase 1	7q35	6	~25	1352500	Wtlr1	6	26.5	3689539

[a] The micronutrient pathway is where the gene was found. The human map sequence is from Ensembl (http://www.ensembl.org). Human map positions (in red with superscript) overlap loci identified by genome-wide association studies. Mouse genomic information was obtained from Jackson Laboratory (http://www.jax.org). Some mouse QTL were determined based on physical location on the chromosome.
[b] Near SNP rs13266634 at 8q24.11 and candidate gene *SLC30A8*.[54]
[c] Near SNP rs1111875 at 10q23.3 and candidate gene *HHEX*.[54]
[d] Near SNP rs7903146 at 10q225.3 and candidate gene *TCF7L2*.[54]
[e] Near SNP rs689 at 11p15.5 and candidate gene *INS*.[54]
[f] Near SNP rs2815752 at 1p31.1 and candidate gene NEGR1.[56]

Results

Initial analyses identified 54 candidate "micronutrient" genes that mapped to obesity, adiposity, and body weight QTL (**Table 3**). Several genes found by the scheme in **Figure 1** are near loci identified by GWAS for T2DM[54] or obesity[56] (**Table 3**). These were long-chain fatty acid CoA ligase 1 (*ACSL1*), which is part of the vitamin E pathway, glycosylphosphatidylinositol-linked nicotinamide adenine dinucleotide (NAD)(P)(+)-arginine adenosine diphosphate (ADP)-ribosyltransferase 1 precursor (*ART1*)(niacin), ecto-ADP-ribosyltransferase 5 precursor (*ART5*)(niacin), cystathionine γ-lyase (*CTH*)(methionine metabolism), ectonucleotide pyrophosphatase/phosphodiesterase family member 2 precursor (*ENPP2*)(niacin), *S*-adenosylmethionine synthetase isoform type-1 (*MAT1A*) (methionine pathway), cytosolic purine 5'-nucleotidase (*NT5C2*)(niacin), and NAD-dependent deacetylase sirtuin-3,

mitochondrial precursor (*SIRT3*)(niacin). This strategy provides a priority list for resequencing. Lifestyle, metabolite concentrations before, during, and after intervention, activity levels, and whole genome scans for genetic ancestry are analyzed using dimensionality reduction and classification algorithms. The genetic history of different chromosomal regions can be identified using novel "sliding window" algorithms and known mapping SNPs.[57] Such data will be used to analyze epistatic interactions with novel algorithms (in development). The goal of our program is to develop a path for identifying and creating metabolic groups based on similar gene–nutrient interactions.

Disclaimer:

The views presented in this article do not necessarily reflect those of the U.S. Food and Drug Administration.

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