1. SPECIFIC AIMS

Advice to increase water consumption as part of a weight reducing diet – typically to 8 cups (64 fl oz, ~2 L) per day – is almost ubiquitously recommended by healthcare professionals. Many believe that water promotes weight loss through numerous physiological mechanisms. However, only limited observational data and virtually no experimental data exist regarding the effects of water consumption on body weight. In this study, we proposed a randomized-controlled pilot study in which two groups of overweight adolescents received a standard weight loss regimen, either with (experimental intervention) or without (control intervention) additional advice and support to increase water consumption. We utilized individual sessions, an innovative text messaging protocol, and motivational telephone calls to promote adherence to the interventions. The purpose of this pilot study was to evaluate feasibility and obtain preliminary efficacy data, to inform design of a future, definitive study.

Specific Aim #1. To examine the effects of increasing water consumption to 8 cups per day on body mass index (BMI) z-score in overweight adolescents during a 6-month weight loss regimen.
Hypothesis**:** Increasing water consumption would improve the efficacy of a standard weight loss diet.
Primary endpoint**:** 6-month change in BMI z-score.
Secondary endpoints**:** Body fat percentage; body circumferences (waist, hip, midarm, thigh and calf).

Specific Aim #2. To examine the effects of increasing water consumption to 8 cups per day on diet in overweight adolescents during a 6-month weight loss regimen.
Hypothesis: Increasing water consumption would lead to *1)* decreased consumption of energy-containing beverages, *2)* decreased total energy intake, and *3)* improved diet quality.
Secondary endpoints: Beverage consumption; total energy intake; other measures of diet quality; hunger; satiety; palatability.

Specific Aim #3: To examine the effects of increasing water consumption to 8 cups of per day on overall health including immune status, and cardiovascular and diabetes risk factors.
Hypothesis: Increasing water consumption would improve immune status, and cardiovascular and diabetes risk factors.
Secondary endpoints: immune status (T/B cell subset and lymphocyte proliferation); complete blood count (CBC); serum levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides; C-reactive protein; glucose; insulin; blood pressure.

Specific Aim #4. To evaluate the feasibility of increasing water consumption to 8 cups per day in overweight adolescents during a 6-month weight loss regimen.
Hypothesis**:** A simple behavioral intervention would be feasible and would significantly increase water consumption among subjects in the experimental vs. control groups.
Process/Impact measures: Reported water intake; hydration status (by urine specific gravity); reported satisfaction with intervention.

Specific Aim #5. To evaluate changes in quality of life in response to increasing water consumption to 8 cups per day on diet in overweight adolescents during a 6-month weight loss regimen.
Hypothesis: Increasing water consumption would improve health-related quality of life scores.
Secondary endpoints: Health-related quality of life scores, assessed by the Child Health Questionnaire–Version CF87 (CHQ–CF87).

2. RESEARCH DESIGN AND METHODS

Study Overview

We conducted a 6-month randomized controlled trial using a parallel design, as outlined in **Figure 1**. After the initial baseline screening and assessments, overweight adolescents were randomly assigned (19 subjects per group) to a standard weight-reducing diet with the advice to consume 8 cups of water per day (experimental group) or the same standard weight reducing diet without additional advice (control group). The interventions consisted of nutrition education and behavioral counseling by a registered dietitian, during individual sessions and telephone calls. Mobile text messaging was used to reinforce information presented at the individual sessions and thereby foster adherence to dietary advice. Body mass index, body fat percentage, body circumferences, immune status, cardiovascular and diabetes risk factors, hunger, and satiety were assessed at baseline and 6 months. Urine specific gravity was assessed at the in-person visits. Dietary and physical activity recall interviews were conducted at baseline and 6 months. Satisfaction with the intervention was assessed at 6 months. Study design considerations are summarized in the appropriate sections throughout the protocol.

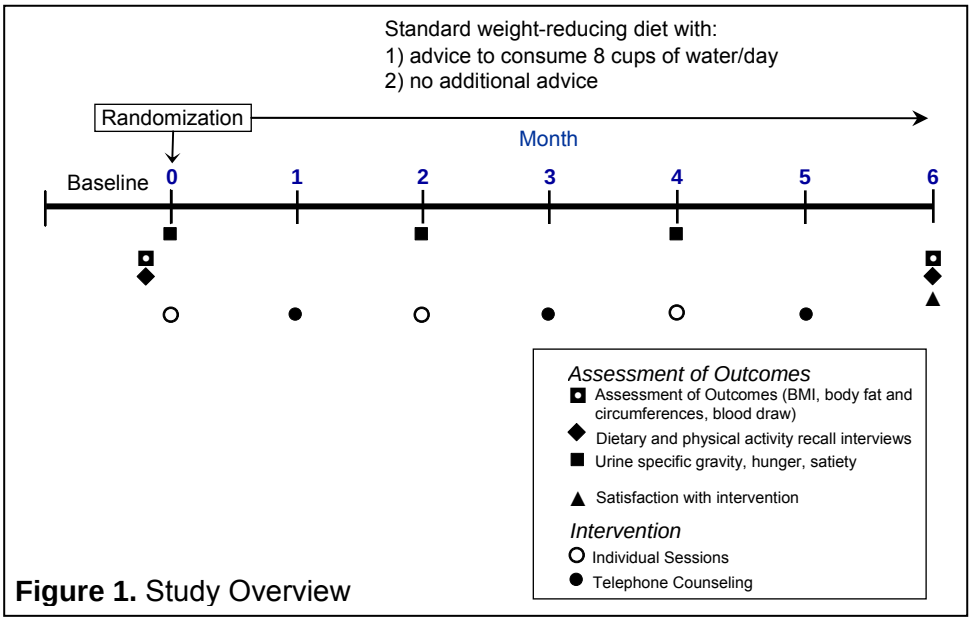

Figure 1. Study Overview

Subjects

Inclusion Criteria

- 12 to 17 years of age (and living at home with a parent).
- BMI ≥ 85th percentile for sex and age according to CDC growth charts[1]
- Access to a working telephone and cell phone
- At least one parent willing and able to participate in the intervention with the subject
- Residing in predominately one household (no more than one weekend every two weeks in a secondary household)
- Medical clearance from a primary care provider or treating physician to rule out any major medical illness, disability, or disorder (e.g., liver disease, renal failure, cancer)

Considerations. We chose the specified age range because the adequate intake level of water, according to the Dietary Reference Intakes,[2] is the same for this age group and is greater than the ~2L (”8x8”) of water advice. A BMI ≥ 85th percentile for sex and age was used to define overweight in adolescents. Access to a working telephone and cell phone were stipulated given the need to contact subjects for counseling, text messaging and dietary assessments. According to recent estimates, nearly every household in the US has a cell phone, with use especially high among teens and young adults.[3] Clearance from the treating physician or primary care provider was necessary to rule out pre-existing medical conditions that could compromise the safety of respective subjects or confound overall interpretation of results. Parental support was important considering the influence exerted by the home environment and parental modeling on adolescent eating behaviors.

Exclusion Criteria

- Intake of more than 4 cups (250 mL per cup) of water per day
- BMI ≥ 40 kg/m^2
- Currently smoking (> 1 cigarette in the past week)
- Major surgery within the previous 6 months
- Does not have a primary care provider
- Physician diagnosis of a major medical illness (e.g. diabetes)
- Previous diagnosis of an eating disorder
- Physical, mental, or cognitive handicaps that prevent participation
- Chronic use of medications that may affect study outcomes (e.g. stimulants or diuretics)
- Another member of the family (first degree relative) or household participating in the study
- A friend, classmate or coworker participating in the study with whom they have contact with one or more times per week
- Girls who are pregnant, planning to become pregnant in the next 6 months, lactating, or within 6 months postpartum

Considerations. We excluded individuals who drank more than 4 cups of water per day for this pilot study, as they would likely achieve the smallest benefits from further increases in consumption. We did not believe that this exclusion would have a major impact on generalizability, as the majority of obese individuals (at least as seen in one recent, major clinical trial) fall into this lower category of consumption.[4] Moreover, we explored effect modification by baseline water consumption among the individuals involved in the intervention across the relatively wide range of 0 to 4 cups per day. Individuals who smoked were not eligible for the study because smoking may affect body weight. We did not enroll members of the same family or household, and friends, classmates or coworkers who interact with each other one or more times per week given the necessity to make independent random assignments, conduct an uncontaminated intervention, and obtain statistically independent data. We did not enroll adolescent girls who were pregnant, planning to become pregnant, lactating, or within 6 months postpartum given the well-documented effects of pregnancy and lactation on body weight.

Recruitment

Screening and Enrollment

We recruited subjects using a variety of strategies that described the study as an opportunity for weight loss for overweight adolescents.

Subjects were recruited in collaboration with physicians at Boston Children's Hospital (BCH) (e.g., Adolescent Medicine Clinic), implementing a multi-step screening and enrollment process as outlined below:

- Flagging. Research assistants flagged potentially eligible patients who were scheduled for a clinic visit. This "flagging" was based on age and BMI percentile.
- Communication with Clinic Provider. When appropriate, a physician or other clinic provider presented the weight loss study to those who have been "flagged" as potentially eligible for the study.
- The provider asked the patient for a telephone number, which was given to study staff. A recruitment brochure also was available for dissemination, should the patient want information for contacting study staff. (Recruitment brochures were available in racks in clinics and in examination rooms.)
- Telephone Conversation. Study staff provided an overview of the study and conducted a telephone screening with the parent of each subject who expressed interest in the study to his/her clinic provider.
- Informational Visit. Dr. Wong, or designated study personnel, met with each subject who was provisionally eligible based on the telephone screening. This person explained the protocol, by reviewing the consent/assent form, and answered questions. Additional screening questions were also asked to assess eligibility criteria. This visit took place in a private room at 1 Autumn Street.
- Medical Clearance. Medical clearance was obtained in writing from the treating physician or primary care provider (with verbal and written permission from the provisionally eligible subject's parent to make this request).
- Baseline Assessment. Written informed consent (from parent and subject) and assent (from subject) was obtained in a private room at the Clinical and Translational Study Unit (CTSU) at BCH.

A similar multi-step screening and enrollment process as outlined above was used for subject recruitment through the primary-care pediatric practices that were members of The Pediatric Physicians' Organization at Children's (PPOC).

- Communication with Clinic Provider. Primary care providers (PCPs) from PPOC practices were asked to identify potentially eligible patients in the course of their routine clinical practice throughout the study's enrollment period. When appropriate, the PCP presented the weight loss study to those who were potentially eligible for the study.
  - The PCP obtained HIPAA Authorization from the parents of potentially eligible patients who expressed interest in the study. This information was sent to the study staff for possible screening and enrollment. The physicians were compensated a nominal fee of $20 per valid HIPPAA Authorization returned to the study office. This was for the time involved in obtaining the necessary HIPPA authorization. A recruitment brochure was also available for dissemination, should the patient and parent want information for contacting study staff.
  - The remaining steps of the multi-step screening and enrollment process were identical to that outlined above [i.e. Telephone Conversation, Informational Visit, Medical Clearance, Baseline Assessment].

A similar multi-step screening and enrolment process as outlined above was used for subject recruitment through the local medical community.

- Communication with Clinic Provider. Providers within the local medical community were asked to identify potentially eligible patients in the course of their routine clinical practice if their patient was within the age range (12-17 years) and overweight (BMI ≥85th percentile). When appropriate, the provider presented the weight loss study to those who were potentially eligible.
- For those patients who expressed interest, a recruitment brochure was also available for dissemination, should the patient and/or parent want information for contacting the study staff. The provider also obtained verbal consent for him/her to forward the patient's contact information via e-mail, mail or fax to study personnel using a standard template.
- The remaining steps of the multi-step screening and enrollment process were identical to that outlined above [i.e. Telephone Conversation, Informational Visit, Medical Clearance, Baseline Assessment].

We also recruited subjects using posted fliers (print and electronic versions), newspaper advertisements, and internal and external Internet links. A similar multi-step screening and enrollment process as outlined above was utilized, except that those who responded to these recruitment strategies were self-identified.

Randomization

A blocked randomization design was employed to ensure balance between the 2 treatment arms at any given time in study enrollment. The master randomization assignments with sequential randomization numbers were prepared in advance by the study statistician. Individual group assignments were specified in a sequence of sealed envelopes, labeled with the same randomization numbers. The appropriate envelope was opened for each enrolled subject the day of the first in-person session with the dietitian.

The diet assignments within each stratum were randomly permuted within blocks of 2 and 4, and the blocks themselves were randomly permuted. The sequence of assignments was thus unpredictable, preventing any deliberate or inadvertent bias on the part of those conducting randomization. Dr. Feldman supervised the entire process and, with assistance from the data manager, performed quality control checks on the randomization assignments. The dietitian providing the dietary counseling cannot be masked to the randomization. However, to avoid any potential bias, personnel conducting the assessments were masked to group assignments.

Retention

Following randomization, maintaining contact with subjects was crucial for the success of the study. At baseline, we asked subjects to provide contact information (home address, day and evening telephone numbers, cell phone numbers, e-mail addresses, name of parent(s) and/or a close relative). We used this information to contact subjects as necessary (e.g., if they do not report for scheduled appointments). As part of our retention plan, we provided $120 to subjects who completed the study. We have had success with a similar protocol in a previous study of young adults, with a retention rate of 90% at 6 months.[5]

Intervention

Diet prescriptions differed only in regard to the specificity of recommendations regarding water consumption, with careful attention to treatment fidelity. Subjects were masked to the specific aims of the study in order to maximize adherence to the randomized intervention. Physical activity recommendations did not

differ between groups. We used a patient-centered approach to communicating with subjects. Interventions were delivered during individual sessions and telephone calls, consistent with our previous work.[5]

Diet Prescriptions

Experimental Group: Standard Diet + Advice to Consume 8 Cups of Water per Day. We counseled subjects to follow a standard weight-reducing diet, including consumption of *1)* ample vegetables, fruits, and legumes; *2)* whole rather than refined grains; and *3)* high-quality proteins at most meals and snacks. Moreover, we recommended limiting intake of added fats and sugars and avoiding juices and sugar-sweetened beverages (per standard practice).

We also counseled subjects in this treatment group to increase their water intake to 8 cups per day, consistent with the popular "8 × 8" recommendation (eight 8-oz glasses of water).[6] Water was defined as tap water and plain bottled water. We instructed subjects not to include non-caloric beverages (e.g., plain tea, coffee) and drinks with nutritive or non-nutritive sweeteners when tallying daily water consumption.

Considerations. We opted for a narrow definition of water (plain bottled water, tap water) for several reasons. *First*, this definition was consistent with recommendations included in the context of popular diets, contributing to the practical significance of the proposed study. *Second*, some non-nutritive sweeteners have been hypothesized to cause an increase in appetite and food intake, thereby compromising weight loss.[7,8] *Third*, caffeine may influence body weight.[9] *Fourth*, consumption of beverages with a characteristic taste or smell may promote hedonic hunger, a phenomenon characterized by consumption for pleasure rather than in response to signals related to metabolic homeostasis.[10]

Control Group: Standard Diet Only. We counseled subjects on the same standard weight-reducing diet, as described above, with no specific advice regarding water consumption. Furthermore, no specific dietary recommendations were provided on altering fluid/beverage intake, other than to decrease calorie-containing beverages as noted above. When subjects asked for a recommendation regarding water intake, we explained that drinking plain water was the best way to satisfy thirst and instructed them to drink when thirsty.

Considerations. Based on considerable data that sugar-sweetened beverages promote a positive energy balance, we feel it was unethical not to provide any recommendation on this topic. We recognized that this recommendation may lead to an increase in water consumption in the control group. However, based on clinical experience and available evidence on prevailing levels of consumption, we were confident that the two groups would differ significantly in water consumption. Indeed, in a secondary analysis of 173 individuals following various popular diets and consuming less than 1 liter of water per day at baseline (similar to the inclusion criteria for our study), water intake increased from a mean of 505 mL per day to only 792 mL/day at 6 months,[4] less than half of the 1920 mL/day target for the experimental group.

Overarching Approach to Communicating with Subjects

We communicated with subjects using a patient-centered approach that involved the subject in decision-making but was also directive in guiding discussion towards exploring and resolving ambivalence regarding dietary change.[11] As described in an article co-authored by Dr. Ebbeling,[11] patient-centered counseling is a practical approach for operationalizing principles of cognitive behavioral theory. Using patient-centered counseling, we respectfully considered patient perspectives, core values, current circumstances, and available resources. One might contend that this is simply good healthcare practice, requiring only basic respect for the patient. However, the process of changing behaviors is highly complex, and even respectful clinicians often resort to giving advice and hoping that patients will comply with their recommendations, without fully considering patient perspectives and ambivalence.[11] Drs. Ebbeling and Wong trained and monitored the study dietitian in this approach to communication.

Intervention Components

A registered dietitian communicated with each subject 1 times per month, either during an individual session or by telephone, as depicted in **Figure 1**.

Individual Sessions. Study subjects attended individual sessions where the primary objective was to foster the necessary knowledge and skills (e.g., meal planning) to follow diet prescriptions. This was achieved through the use of educational materials adapted from our previous work, a cookbook with health guides and recipes written specifically for adolescents to reinforce the translate key messages and foster adherence, and the U.S. Department of Agriculture MyPyramid[12] and MyPlate[13] food guidance systems. All materials consisted of well-defined key messages that were consistent with national dietary guidelines. Each subject also received

a Corelle® plate with appropriate divisions to convey reasonable portion sizes, facilitate meal assembly, and thereby translate knowledge to behavior. The dietitian asked subjects to keep food diaries (~1 day per week) prior to each session during the first two months of intervention, as a strategy for self-monitoring.[11] Thereafter, she encouraged subjects to continue self-monitoring dietary intake with food diaries, but with decreasing frequency. Based on process data collected in our previous study,[5] study participants are reluctant to keep food diaries after the first few months of intervention.

Each session was 60 minutes in duration, with 45 minutes involving the subject and parent interacting with the dietitian and the remaining 15 minutes devoted to one-on-one discussion between the subject and dietitian. The sessions differed between the experimental and control groups only in regard to the discussion devoted to beverage choices. For the experimental group, we provided each subject with a water pitcher with replacement filters and a stainless steel water bottle. The dietitian explained how these behavioral supports could be used to increase adherence to the "8 × 8" recommendation regarding water consumption. We anticipated that these supports would promote availability of water throughout the day and also provide a visual cue to increase water consumption. For the control group, we directed attention towards distinguishing between energy-containing vs. non-caloric beverages, recommending consumption of water in response to thirst rather than giving a specific target amount.

Mobile Text Messaging. Daily mobile text messages were sent to study subjects using a client-based messaging software (HipLink™, Semotus Solutions, Inc.). The text messages consisted of standard dietary advice, reinforcing topics covered in the monthly group sessions, and motivating and encouraging phrases generated by their peers (Bodimojo) to provide ongoing support. The frequency and content of text messages were consistent between the experimental and control groups, with the exception that the experimental group received an additional phrase with each message to encourage the "8 x8" water recommendation. We gave each subject $20 to defray the cost of the text messaging (as part of the total $120 provided to subjects who completed the study).

Considerations. Mobile technology is a novel means of direct communication with study subjects in clinical trials to provide ongoing encouragement for enhancing motivation and adherence, without increasing subject burden. Text messaging technology has been successfully used in previous studies to promote self-efficacy in changing behaviors and thereby improve health outcomes.[14] A recent study of 65 overweight or obese adults showed that those who received multiple daily text messages for 16 weeks lost significantly more weight compared to those who received print materials (difference: -1.97kg).[15] Furthermore, those who received text messages stated that they would recommend the intervention to friends and family. Consistent with this previous work, we also used daily text messaging to maximize the interaction with study subjects and the potential treatment effect. In the situation where subjects have issues with their cell phone during the intervention period (i.e. discontinued service, lost their cell phone, etc.), the daily text messages were sent to their e-mail addresses.

Telephone Calls. The dietitian contacted each subject at a prearranged time 3 times during the study period (**Figure 1**). The purpose was to enhance motivation for translating knowledge and skills to changes in dietary behaviors. Practical application of the patient-centered counseling model relied on four steps during the calls: *1)* assess, *2)* advise, *3)* assist, and *4)* arrange for follow-up. The first step included assessment of dietary intake to evaluate compliance and inform discussion. The dietitian assessed diet as a basis for providing appropriate advice to the subject; this assessment was completely separate from the dietary recall interviews that provided the basis for evaluating dietary outcomes. Each telephone call was 30 minutes in duration.

Physical Activity

Physical activity is an important element of a healthful lifestyle and, thus, was a component of treatment irrespective of group assignment. The dietitian gave the same physical activity advice to subjects in both groups. In brief, we prescribed moderate-intensity exercise, such as brisk walking, for at least 150 minutes per week (30 minutes per day, on most days of the week). This recommendation was consistent with the prescription used in the Diabetes Prevention Program[16] and guidelines issued by the American College of Sports Medicine.[17]

Considerations. The American Heart Association[18] recommends at least 60 minutes per day of physical activity for weight control. Nevertheless, data suggest that 150 minutes of moderate-intensity physical activity per week (approximately 30 minutes per day on most days of the week) is adequate for achieving other health benefits.[17] We recommended 150 minutes of physical activity per week as a minimum and included a measure of physical activity. Because changing dietary behaviors was the focus of the proposed study, we did not want

to overwhelm subjects by recommending potentially unattainable levels of physical activity, particularly given that time constraints are a frequently cited barrier to adopting healthful lifestyles.

Treatment Fidelity

Dietitian adherence to the intervention protocols is conceptualized as treatment fidelity, a term encompassing integrity and differentiation.[19] Integrity is the degree to which treatment is implemented according to established procedures, and differentiation is the extent to which interventions are distinct from one another. We used several strategies to maximize treatment fidelity. *First*, scripts and written education materials were developed for presenting topics during the dietary counseling sessions to ensure *1)* consistent delivery of well-defined nutrition messages regarding the weight-reducing diet and *2)* clear differences in messages with respect to beverage consumption. Concerning the latter point, we scripted the message to increase water consumption to 8 cups per day for the experimental group. In contrast, we scripted only standard advice with respect to decreasing calorie-containing beverages, such as juices and sugar-sweetened beverages, for the control group. We also instructed dietitians not to provide any additional advice regarding water consumption to the control group. Distinct messages, in combination with the aforementioned behavioral supports provided to the experimental group, promoted differentiation between the two dietary interventions. *Second*, telephone calls followed a structured format to foster dietitian adherence to a patient-centered counseling model, with adequate flexibility to address specific issues unique to each individual. Open-ended questions were included throughout the telephone calls to enhance dialogue. The dietitian digitally recorded telephone calls, such that Dr. Wong could review a 33% random sample of the calls to monitor deviations from the protocol and provide feedback as necessary. We also monitored the duration of each individual visit and telephone call. *Third*, the dietitian wrote a progress note following each interaction. *Fourth*, weekly staff meetings provided an opportunity for continued discussion on intervention delivery, particularly strategies for assisting individual subjects without compromising differentiation between treatments.

Assessment of Study Outcomes

Figure 2 provides an overview of the conceptual framework for assessment of study outcomes. In brief, the experimental vs. control intervention was designed to promote increased water consumption (process/impact measure), thereby eliciting decreased total energy intake (secondary endpoints) and improved diet quality (secondary endpoint). According to study hypotheses, these dietary changes would cause a decrease in BMI z-score (primary endpoint) and body fat percentage and body circumferences (secondary endpoints), possibly due to decreased hunger and increased satiety (secondary endpoint). Furthermore, improvements in immune status and cardiovascular and diabetes risk factors (secondary outcomes) would be observed. Urine specific gravity was used to assess hydration status (process/impact measure), an indicator of compliance. Covariates would include demographic data determined by self-report and physical activity assessed by 24-hour recall interviews.

Study endpoints were collected by personnel who are masked to group assignment. BMI z-score, body circumferences, body fat percentage and blood measures were assessed under fasting conditions (at least a 12-hour fast) at baseline and 6-month follow-up. Anthropometric measurements were conducted in a private room. Subjects were also asked to recall their overall hunger and satiety for a 1-week referent period. Hydration status was assessed under post-absorptive conditions immediately prior to individual in-person sessions. Dietary outcomes were assessed by telephone-administered 24-hour recall interviews.

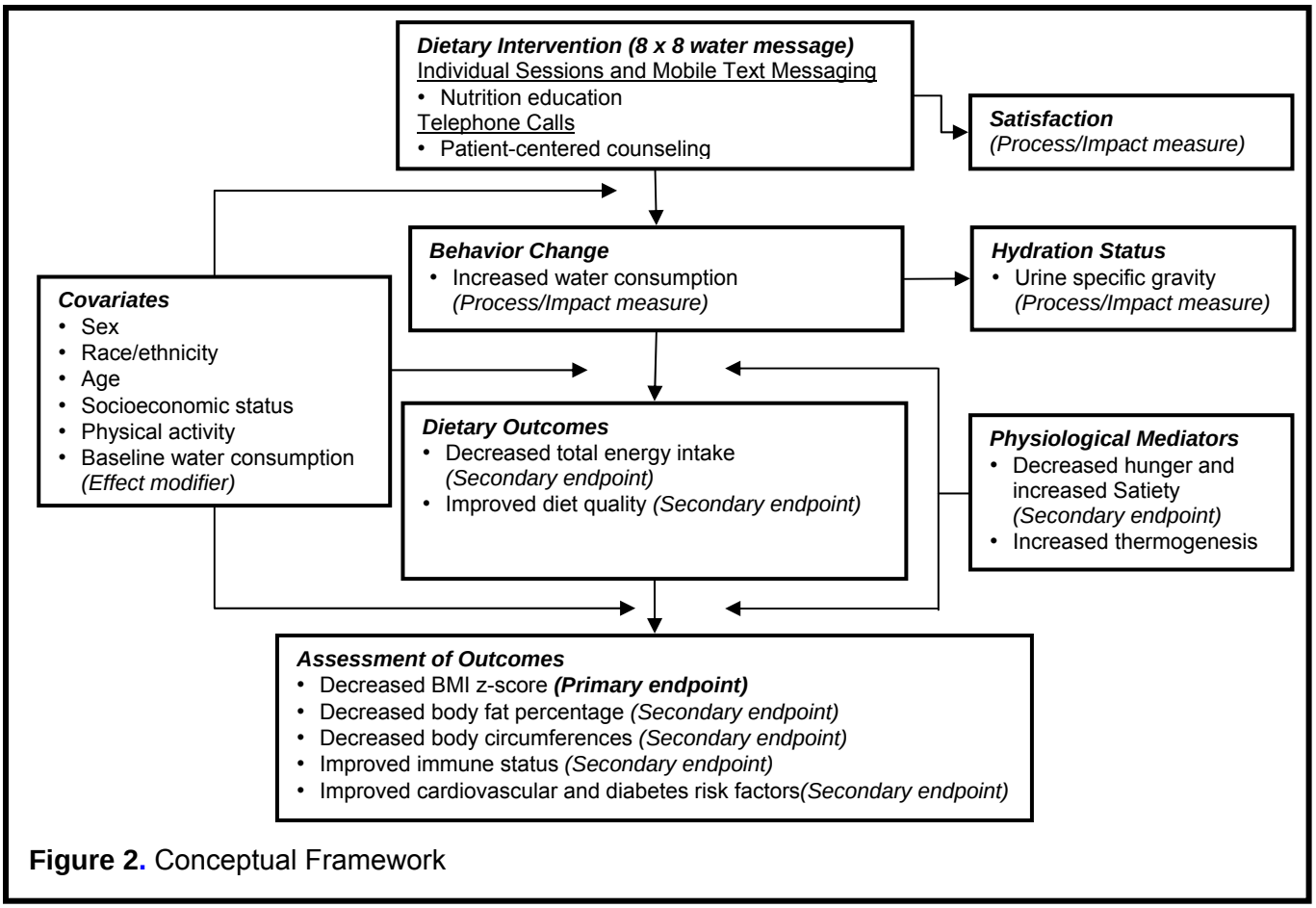

Figure 2. Conceptual Framework

Endpoints

BMI z-score. We calculated BMI z-score using norms for sex and age.[1]

BMI. We measured body weight to the nearest 0.1 kg using an electronic scale (Scale-Tronix, White Plains, NY). Subjects were instructed to remove shoes and heavy clothing and to empty their pockets prior to measurement. BMI was calculated as total mass (kilograms) divided by height (meters) squared. *Quality Control.* The electronic scales at BCH were calibrated annually. In addition, the calibration was checked and documented daily, with re-calibration when readings deviate by more than 0.1 kg from certified calibration weights.

Body Circumferences. We measured body circumferences directly over the skin (except for hip) using a Gulick measuring tape to the nearest 0.1 cm according to the *NHANES Anthropometry Procedures Manual*[20,21] and *Anthropometric Standardization Reference Manual*.[22] The measurer had an assistant to ensure that the tape was positioned correctly at each site. *Waist and Hip.* The subject was asked to stand erect, with feet together and arms at the sides. Waist measurements were taken with the tape in the horizontal plane at the uppermost lateral border of the right ilium. Hip measurements were taken with the tape in the horizontal plane at the widest point over the greater trochanter of the femoral bone. *Midarm*. The subject was asked to position their arms in a relaxed position at the sides. Midarm measurements were taken with the tape positioned perpendicular to the long axis of the right humerus at the point equidistant between the acromion process of the right scapula and the olecranon process of the right ulna. *Thigh and Calf.* The subject was asked to stand erect with weight evenly distributed between the feet. Thigh measurements were taken with the tape measure positioned perpendicular to the long axis of the right femur at the level midway between the inguinal crease and proximal border of the patella. Calf measurements were taken at the level of maximum girth of the right calf by positioning the tape measure horizontally around the calf and moving in a series of up and down measurements to locate the maximum circumference. *Quality Control.* Body circumferences were measured two times. A third measurement was obtained if the two measurements were not within 1 cm.

Body Fat Percentage. We assessed body composition by bioelectrical impedance (BIA) (Quantum II, RJL Systems, Clinton Township, MI), using established prediction equations to calculate percentage body fat.[23]

Immune Status. *T/B Cell subsets*. We measured by flow cytometry the percentage and absolute numbers of total T-helper/inducer, suppressor/cytotoxic, B-lymphocytes and natural killer cells (NK-cells) in whole peripheral blood with two 6-color monoclonal antibody (MAB) combinations (Core Lab, BCH). *Lymphocyte Proliferation.* We measured lymphocyte proliferation by stimulating lymphocytes *in vitro* with the mitogen phytohemagglutinin (PHA) (Core Lab, BCH).

Complete Blood Count (CBC) with Differential. We measured CBC with differential.

Cardiovascular Risk. *Blood Lipids and C-reactive Protein*. We measured serum concentrations of HDL cholesterol, LDL cholesterol, triglycerides, and C-reactive protein. *Blood Pressure.* Consistent with current guidelines,[24,25] we measured blood pressure by auscultation at the right arm using a sphygmomanometer (ADC System 5, American Diagnostic Corporation, Hauppauge, NY) with the cuff bladder encircling 80% of the arm. The subject was seated comfortably (back supported, feet flat on the floor, midpoint of the right arm supported at the level of the heart). Prior to measurement, we asked the subject to remain quiet for 5 minutes (i.e., no conversation or television). Listening for Korotkoff sounds using a stethoscope, we measured systolic (i.e., point of first sound, phase I) and diastolic (i.e., point of the last sound, phase 5) blood pressure 3 times at each assessment visit. The first measurement obtained at each visit was not used in data analysis.

Diabetes Risk. Plasma glucose and insulin were measured.

Diet Quality. We conducted telephone-administered 24-hour recall interviews (2 weekdays and 1 weekend day) at baseline and 6 months to assess diet. We averaged data across 3 days at each time point. The telephone calls for assessing diet were conducted by an interviewer who was masked to group assignment and was completely separate from the telephone calls that were part of the intervention. The recall interviews were unannounced so that the subject did not know the exact dates of the telephone calls in advance. Prior to the first interview, we trained subjects on how to estimate food and beverage portion sizes.

The interviewer implemented a multiple-pass dietary assessment method using the Nutrition Data System for Research Software (NDS-R, Nutrition Coordinating Center, University of Minnesota, Minneapolis). In brief, the interviewer prompted the subject to list in sequence the foods and beverages consumed during the previous day, identify omissions in the initial list, and then provide details (e.g., portion sizes, brand names) concerning each reported item. Intake was reviewed and confirmed at the end of the interview.

Considerations. We acknowledged that self-report methodology for assessing diet was a limitation of the proposed study. Underreporting of dietary intake is a well-recognized phenomenon, common to all studies that aim to collect dietary data under free-living conditions, although adjusting other dietary variables for energy intake may partially correct for underreporting.[26,27]

Hunger and Satiety. Adapted from a previous study,[28] we used the following question to assess hunger and satiety: "On average over the past week, how did you feel before your main meal of the day?" and "How did you feel after your main meal of the day?", respectively. We asked the subject to respond using a 9-point bipolar semantic scale, where −4 is "starved", 0 is "neutral", and +4 is "uncomfortably full". Hunger and satiety were assessed before each individual session throughout the study for both groups, allowing for an unconfounded comparison.

Process/Impact Measures

Water consumption. When evaluating the impact of the intervention, we calculated consumption of tap water and bottled spring water using data from the 24-hour dietary recall interviews, in light of recommendations that were given to the experimental group. Hydration status. We assessed urine specific gravity as a biological measure of hydration status and thus compliance. We collected spot urine samples for determining urine specific gravity, using the MultiStix 10 SG reagent strips (Siemens). Urine samples were collected at the beginning of each in-person visit with the dietitian. Analysis of urine specific gravity was done immediately following collections. Using the proposed methodology, this evaluation was based on the apparent pKa change of certain pre-treated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, colors range from deep blue-green in urine of low ionic concentration through green and yellow-green in urines of increasing ionic concentration. Specific gravity can be determined within a range between 1.000 and 1.030. For individuals consuming 2 L of water per day, we anticipated total beverage intake to exceed 3 L per day, when taking into account coffee, tea, juice and other drinks (not considering water present in foods). In contrast, Stookey et al.[4] found total beverage intake among individuals consuming less than 1 L water per day (consistent with our inclusion criteria) to be 1637 ± 31 mL per day. In view of this potential difference of about 1.5 L per day, we expected specific gravity to be below 1.010 for most individuals in the experimental group, and above 1.010 for most individuals in the control group.

Satisfaction with intervention. We asked subjects to respond to questions regarding satisfaction, using 10-cm visual analog scales with appropriate verbal anchors. Questions addressed satisfaction with the overall program, specific dietary recommendations (including those for beverage intake), and mobile text messaging. We also evaluated difficulties and/or obstacles encountered. We have used this approach to successfully collect process data in previous studies.[3,5]

Covariates

Sociodemographic Data. At baseline, we used a questionnaire to collect demographic data. These data included sex, race/ethnicity, date of birth (to calculate age), and socioeconomic status (household income for the family).

Physical Activity. During the unannounced telephone-administered 24-hour recall interviews described above, we assessed physical activity following the dietary recall. The interviewer prompted the subject to recall physical activity and inactivity using a protocol modeled after established methodology[29] used in our previous studies.[3,5] In brief, the interviewer asked the subject to specify the activity performed most during respective 15-minute time blocks throughout the preceding day (12:00 am–11:59 pm) and then to rate the relative intensity of each reported activity. A metabolic equivalent (MET level) was assigned to each activity to calculate a physical activity factor.[30] Prior to the first interview, we trained subjects on how to estimate duration and intensity of activity.

Baseline water consumption. We analyzed baseline water consumption as an effect modifier, given that increased water consumption as part of the experimental intervention would likely to have the greatest effect among those who drink the smallest volume of water at baseline.

Data Management

Essential data management activities included design and development of the study case report forms (CRFs); design, development, and maintenance of the web-based clinical data management system (CDMS); data entry and verification; and preparation of datasets.

Case Report Forms

All data with regard to primary and secondary outcomes, process/impact measures, and covariates were recorded on CRFs, with the exception of data from the dietary and physical activity recall interviews. The CRFs were designed to minimize data entry errors and to ensure efficient, consistent, and unambiguous data abstraction from source documents. The CRF design included simple introductions for each section, clear directives to data collectors, obvious skip patterns, and standard coding and formatting conventions. A standard operating procedure describing how to complete each CRF was included in the Manual of Operations.

Subject confidentiality was maintained using unique study identification codes. The data manager created a study ID assignment log in MS Excel format. The log was used to assign ID numbers and to record the identifiers for contacting and tracking subjects, consistent with our retention plan. Moreover, the log served as the only link between unique study ID numbers and identifiable contact information. A hard copy of the log was kept in a locked file accessible only to study staff for the purposes of enrolling and tracking subjects, and an electronic copy was kept locally in a computer folder with restricted access. Cell phone numbers entered into the HIPPA compliant client-based messaging software for the purposes of text messaging, as part of the intervention, did not contain any identifiers (i.e. subject name). Subjects' cell phone numbers were only linked to their unique ID numbers.

Clinical Data Management System

Data collected on CRFs were entered into REDCap (Research Electronic Data Capture) and readily exported to statistical software packages (e.g. SAS). The appearance of the data capture screens closely resembled the CRFs to enhance accuracy.

REDCap (http://project-redcap.org) is a secure, web-based application designed to support data capture for research studies developed by a multi-institutional consortium initiated at Vanderbilt University. Features of REDCap included web-based CRFs, real-time data entry validation (e.g. for data types and range checks), audit trails, and designation of different access levels for each research team member. REDCap also complies with HIPAA regulations.

Statistical Analysis

Analysis Plan

All analyses followed the intention-to-treat principle, attributing to each subject his or her assigned diet regardless of compliance. Baseline equivalence of the two trial arms was confirmed by appropriate parametric and non-parametric comparisons. The primary endpoint, 6-month change in BMI z-score, was analyzed by a general linear model, adjusting for baseline covariates that were potentially correlated with the outcome (e.g. demographics, socioeconomic status, anthropometrics, physical activity, and beverage consumption). To adjust for variations in BMI based on age and sex of adolescents, BMI was transformed to BMI z-score (i.e. adjusting for sex and age based on reference data). The null hypothesis was that the pattern of the 6-month change in BMI z-score would not differ between trial arms. We tested for effect modification by covariates, with particular attention to the possibility that higher baseline water consumption might reduce the magnitude of intervention effect. Secondary outcomes were analyzed similarly. Two-sided $P<0.05$ was taken as the criterion for statistical significance. SAS software was used for all computations.

Sample size, power, and detectable effects

An appropriate scalar contrast for assessing statistical power was the 6-month change in weight. Our prior work[5] yielded a 5-kg standard deviation (SD) for 6-month weight loss in young adults counseled to follow a low-glycemic load diet. Assuming a similar SD, the present trial with 30 subjects per arm would have 80% power to detect a 3.7-kg difference between arms for attained weight loss and a 0.122 difference in BMI z-score at 6 months. For secondary endpoints, letting s_d denote the SD of 6-month change, the detectable effect would be $0.74s_d$, conventionally considered a “moderate” effect size.

Manual of Operations

Drs. Ebbeling and Wong oversaw preparation of a comprehensive Manual of Operations during the planning phase of the study. All quality control procedures – with regard to intervention, assessment, data management, and statistical analysis – were described in detail in the Manual.

References

1. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. *Advance data.* Jun 8 2000(314):1-27.
2. *Panel on Dietary Reference Intakes for Electrolytes and Water, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate.* The National Academies Press; 2004.
3. Ebbeling CB, Feldman HA, Osganian SK, Chomitz VR, Ellenbogen SJ, Ludwig DS. Effects of decreasing sugar-sweetened beverage consumption on body weight in adolescents: a randomized, controlled pilot study. *Pediatrics.* Mar 2006;117(3):673-680.
4. Stookey JD, Constant F, Popkin BM, Gardner CD. Drinking water is associated with weight loss in overweight dieting women independent of diet and activity. *Obesity (Silver Spring).* Nov 2008;16(11):2481-2488.
5. Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA.* May 16 2007;297(19):2092-2102.
6. Valtin H. "Drink at least eight glasses of water a day." Really? Is there scientific evidence for "8 x 8"? *American journal of physiology.* Nov 2002;283(5):R993-1004.
7. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *Am J Clin Nutr.* Jan 2009;89(1):1-14.
8. Hampton T. Sugar substitutes linked to weight gain. *Jama.* May 14 2008;299(18):2137-2138.
9. Greenberg JA, Boozer CN, Geliebter A. Coffee, diabetes, and weight control. *Am J Clin Nutr.* Oct 2006;84(4):682-693.
10. Lowe MR, Butryn ML. Hedonic hunger: a new dimension of appetite? *Physiol Behav.* Jul 24 2007;91(4):432-439.
11. Rosal MC, Ebbeling CB, Lofgren I, Ockene JK, Ockene IS, Hebert JR. Facilitating dietary change: the patient-centered counseling model. *J Am Diet Assoc.* Mar 2001;101(3):332-341.
12. Center for Nutrition Policy and Promotion U.S. Department of Agriculture. MyPyramid Food Guidance System. http://www.cnpp.usda.gov/default.htm. Accessed August 19, 2009.
13. Center for Nutrition Policy and Promotion U.S. Department of Agriculture. MyPlate. Accessed June 8, 2011.
14. Krishna S, Boren SA, Balas EA. Healthcare via cell phones: a systematic review. *Telemed J E Health.* Apr 2009;15(3):231-240.
15. Patrick K, Raab F, Adams MA, et al. A text message-based intervention for weight loss: randomized controlled trial. *J Med Internet Res.* 2009;11(1):e1.
16. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* Feb 7 2002;346(6):393-403.
17. Jakicic JM, Clark K, Coleman E, et al. American College of Sports Medicine position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc.* Dec 2001;33(12):2145-2156.
18. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation.* Jul 4 2006;114(1):82-96.
19. Moncher FJ, Prinz RJ. Treatment fidelity in outcome studies. *Clin Psychol Rev.* 1991;11:247-266.
20. Centers for Disease Control and Prevention. NHANES Anthropometry Procedures Manual. 2004.
21. Centers for Disease Control and Prevention. NHANES Anthropometry Procedures Manual. 2009.
22. Lohman TG, Roche AF, Martorell R, eds. *Anthropometric Standardization Reference Manual.* Champaign, IL: Human Kinetics Books; 1988.
23. Sun SS, Chumlea WC, Heymsfield SB, et al. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. *Am J Clin Nutr.* Feb 2003;77(2):331-340.
24. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA.* May 21 2003;289(19):2560-2572.
25. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals

from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension.* Jan 2005;45(1):142-161.
26. de Castro JM. Varying levels of food energy self-reporting are associated with between-group, but not within-subject, differences in food intake. *J Nutr.* May 2006;136(5):1382-1388.
27. Lau C, Toft U, Tetens I, et al. Association between dietary glycemic index, glycemic load, and body mass index in the Inter99 study: is underreporting a problem? *Am J Clin Nutr.* Sep 2006;84(3):641-645.
28. Jenkins DJ, Wong JM, Kendall CW, et al. The effect of a plant-based low-carbohydrate ("Eco-Atkins") diet on body weight and blood lipid concentrations in hyperlipidemic subjects. *Arch Intern Med.* Jun 8 2009;169(11):1046-1054.
29. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. *Am J Clin Nutr.* Mar 1983;37(3):461-467.
30. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* Sep 2000;32(9 Suppl):S498-504.