

## Qualitative and/or Quantitative Drinking Water Recommendations for Pediatric Obesity Treatment

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### Abstract

**Objective:** The qualitative recommendation to 'drink water instead of caloric beverages' may facilitate pediatric obesity treatment by lowering total energy intake. The quantitative recommendation to 'drink enough water to dilute urine' might further facilitate weight loss by increasing fat oxidation via cell hydration-mediated changes in insulin.

**Methods:** This 8 week randomized intervention tested whether both Qualitative-plus-Quantitative (QQ) drinking water recommendations result in more weight loss than the Qualitative recommendation alone (Q) in 25 children (9-12 y) with body mass index at or above the 85<sup>th</sup> percentile, given a reduced glycemic diet and usual physical activity. Random urine osmolality, saliva insulin, and body weight were assessed weekly. Mixed models explored if insulin mediated an effect of urine osmolality on weight loss.

**Results:** In intention-to-treat analyses, QQ and Q participants did not differ significantly with respect to level of urine osmolality, saliva insulin, or weight loss. Only 4 out of 16 QQ participants complied with instruction to drink enough water to dilute urine, however. In completers analyses, the compliant QQ participants, who diluted urine osmolality from  $910 \pm 161$  mmol/kg at baseline to below 500 mmol/kg over time (8 week mean  $\pm$  SE:  $450 \pm 67$  mmol/kg), had significantly lower saliva insulin over time (8 week mean  $\pm$  SE:  $13 \pm 8$  pmol/l vs.  $22 \pm 4$  pmol/l) and greater weight loss (mean  $\pm$  SE:  $-3.3 \pm 0.7$  kg vs.  $-2.0 \pm 0.5$  kg) than compliant Q participants (7 out of 9 participants) who maintained elevated urine osmolality over time (8-week mean  $\pm$  SE:  $888 \pm 41$  mmol/kg). Urine osmolality below 500 mmol/kg was significantly associated with weight loss. Change in saliva insulin partially explained the association.

**Conclusions:** QQ recommendations may increase weight loss for those able to dilute urine. Work is warranted to pursue cell hydration effects of drinking water for pediatric obesity treatment.

**Keywords:** Drinking water; Urine osmolality; Weight loss; Saliva insulin; Children; Adolescents

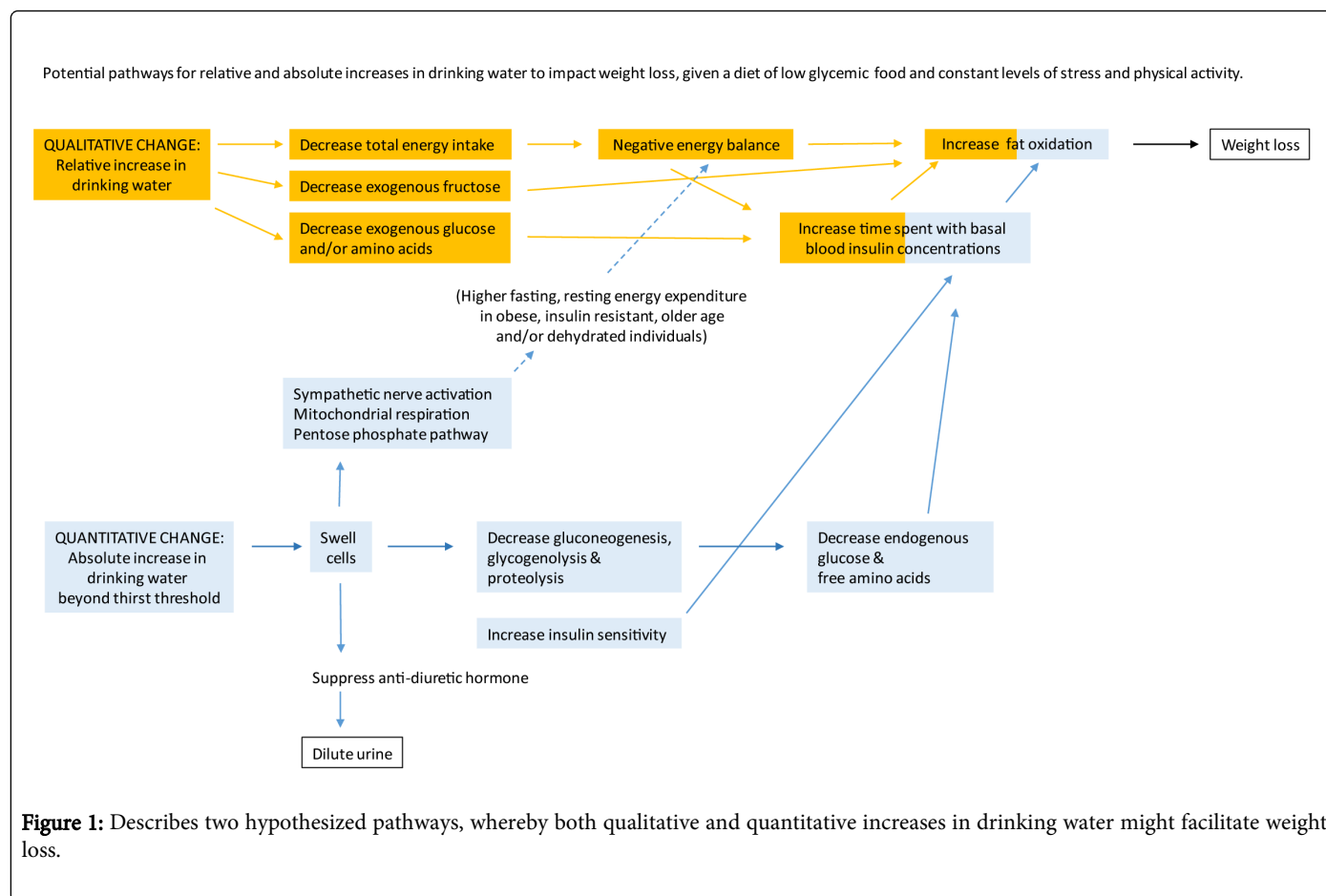
### Introduction

Drinking water instead of caloric beverages is recommended for pediatric obesity treatment [1,2]. This qualitative change in beverage intake is recommended because the type of beverage paired with a meal significantly impacts total energy intake. Drinking water results in lower total energy intake than the same volume of caloric beverage because individuals do not completely compensate for beverage calories by eating less food [3].

Beyond changing the type of beverage consumed to lower total energy intake, it might be possible to further impact pediatric obesity by also increasing the absolute amount of drinking water consumed to increase fat oxidation. Figure 1 describes two hypothesized pathways,

whereby both qualitative and quantitative increases in drinking water might facilitate weight loss. Independent of change in total energy intake, an absolute increase in water intake may facilitate fat oxidation by swelling cells.

In healthy adults, cell swelling favors fat oxidation by limiting blood carbohydrate and insulin concentrations [4,5]. Cell swelling can lower endogenous blood glucose and free amino acid concentrations, by down-regulating gluconeogenesis, glycogenolysis, and proteolysis [6-9]. At the same time, cell swelling may accelerate glucose clearance by improving insulin sensitivity [8,9]. Elevated carbohydrate and insulin concentrations prioritize carbohydrate oxidation over fat oxidation, i.e. increase the respiratory quotient, by inhibiting the rate-limiting enzymes (hormone sensitive lipase, acylcarnitine transferase, and pyruvate carboxylase) for triglyceride breakdown, free fatty acid transport into the mitochondria, and free fatty acid oxidation by the tricarboxylic acid/Krebs cycle [10-13].



Cell swelling may also increase the overall amount of fat oxidized by increasing energy expenditure, without changing the respiratory quotient. Cell swelling can increase sympathetic nerve activity [14], stimulate the mitochondrial respiratory chain [15], shift the mitochondrial and cytosolic NADH systems to a less reduced state [16], and/or enhance flux through the pentose phosphate shunt [17].

In daily life, overnight water restriction, solute intake at meals, and insensible (via skin and lungs) and obligatory (via urine) water loss routinely create osmotic stress that draws water out of cells [18-21]. In free-living children aged 9-11 y, hyperosmotic cell shrinkage is prevalent [22] and associated with not drinking water [22]. Given that obesity is characterized by an increased Extracellular relative to Intracellular Fluid (ECF/ICF) ratio [23], there is reason to expect that obese children and adolescents may not optimally rehydrate or swell cells daily.

In overweight or obese adults, intake of 500 ml drinking water after an overnight fast increases resting fat oxidation, via hypoosmotic stimulation [24]. Similarly, in overweight or obese children, a 10 ml/kg body weight bolus of drinking water increases resting fat oxidation after overnight water restriction [25]. In both overweight or obese adults and children, the short term increases in fat oxidation after drinking water appear to be mediated by increased resting energy expenditure with a stable respiratory quotient [24,25].

In overweight dieting, pre-menopausal [26], and older adults [27], drinking water is associated with greater weight loss over 8 to 12 weeks, independent of change in total energy intake and physical

activity. Cell hydration pathways conceivably mediate the extra weight loss because the effect is associated with intake of drinking water in excess of 1 L/d [26] and/or significant urine dilution [27] (specific gravity <1.01, i.e. urine osmolality <300 mmol/kg [28]). Urine dilution is a sensitive indicator of hypo-osmotic cell swelling [21,29] (Figure 1). The effect may, furthermore, reflect cell hydration-mediated changes in carbohydrate and insulin metabolism because the magnitude of extra weight loss appears to depend on the carbohydrate content of the background diet. Drinking 1+L/d water is associated with 4.7 kg additional weight loss for women on the Atkins diet, but only 2.0-3.0 kg additional weight loss for women on higher carbohydrate diets [26].

In children aged 8-15 y, drinking water interventions evaluate effects of qualitative drinking water recommendations on weight gain or obesity prevention in study populations that include normal weight or non-dieting children [30-43]. It remains to be determined if absolute increases in drinking water and cell hydration are associated with weight loss in overweight or obese children seeking obesity treatment.

The aim of this randomized intervention study was to determine if instruction to 'drink water instead of caloric beverages' plus instruction to 'drink enough water to dilute urine' (QQ: Qualitative-plus-Quantitative recommendation) results in greater weight loss over 8 weeks in overweight or obese children than the Qualitative instruction alone (Q), controlling for background diet, under conditions of usual physical activity.

The 8 week time frame was chosen, given detectable effects of drinking 1+L/d over 8 weeks in adults aged 25 to 50 y [26]. The study focused on drinking enough to dilute urine osmolality, as opposed to a specific absolute volume of water, because water requirements to swell cells are not defined for overweight or obese children. Dilute urine osmolality flags the exposure of interest-cell swelling [21], without need to know the absolute water requirement. A secondary aim of this study was to explore if insulin concentration might mediate or modify weight change effects of drinking enough water to dilute urine.

## Methods

### Study participants

Boys and girls (9-12 y) who attended information sessions about pediatric obesity treatment at Children's Hospital Oakland, CA, between 9/2010 and 2/2011, were invited to participate in this study. Inclusion criteria included Body Mass Index (BMI) percentile at or above 85%, motivation to lose weight, daily intake of caloric beverages, less than 2L/d drinking water, ability to attend weekly clinic visits during the study period, and willingness to consume the study foods. Exclusion criteria included inability to speak, read, and write English or Spanish, renal disease, congestive heart failure, adrenal insufficiency, syndrome of inappropriate anti-diuretic hormone, chronic pain, psychogenic polydipsia, bleeding disorders, recent cancer chemotherapy, medication use, acute illness, and weight loss in the previous 2 months. The protocol was reviewed and approved by the Children's Hospital & Research Center Oakland, Institutional Review Board. Participants and guardians signed assent, consent, and privacy forms. Participants were compensated \$10 per clinic visit.

### Study protocol

Following enrollment, baseline measurements, treatment assignment, and one-on-one orientation (Week 1), each participant had 8 weekly clinic visits, where each received ready-to-eat foods, diet and activity log forms, and encouragement to adhere to the protocol. Urine osmolality, saliva insulin, and body weight status were measured weekly. Home delivery of bottled water was provided for 8 weeks.

### Baseline assessment

At the first clinic visit, study staff collected baseline process and outcome measures, administered a urine dilution test, and interviewed each participant about his or her previous day diet and physical activity. The urine dilution test involved collecting an initial urine sample, providing 500 ml drinking water, collecting a second urine sample 60 min later, and calculating the pre-post bolus change in urine osmolality.

Multiple-pass recall methods were used to prompt each participant to report the timing, type, preparation, and quantity of all foods and drinks consumed on the previous day, and all physical activity. Each participant was asked about participation in organized sports, including school PE, team sports, and/or after school physical activity. The groups mean daily nutrient intakes were estimated from the 24-hour diet recalls using Nutrition Data Systems for Research software [Version 2010, NCC]. The average of one-day 24-hour diet recalls provides a valid estimate for group intake [44].

### Study foods, bottled water delivery, and physical activity

All participants (with parent) received one-on-one instruction to consume foods with a glycemic index below 50 or a glycemic load below 11 [45], and avoid higher glycemic starches and caloric beverages, to limit blood carbohydrate and insulin concentrations, which might mask drinking water effects. Low glycemic diets are feasible and effective for weight loss in overweight children [46-48]. To insure availability of low glycemic foods and facilitate weekday meals at school, participants were given free, commercially-prepared, ready-to-eat foods, which included 1oz protein and 2C fruit for breakfast, 2oz protein, 1C vegetables or legumes and 1C fruit for lunch, and 100-200 kcal of dried fruit and/or nuts for snack. The breakfast, lunch, and snack foods provided about 1000 kcal/d, 45 g/d fat, 55 g/d protein, 130 g/d carbohydrate, a glycemic load of 75, and 900 g food water. The participants were encouraged to take the study foods with them to school for lunch and snack. For dinner, participants were instructed to eat the same protein (4-6oz) and vegetables (1-2C) as their families, but substitute salad instead of high glycemic starches. The instructions were aligned with recommendations for fruit, vegetables and protein intake for children [49].

All participants received their choice of plain, non-carbonated or carbonated bottled water, in large or small bottles, home-delivered for 8 weeks. Enough water was delivered to share with family members. Study participants were encouraged to take the bottled water with them to school.

### Physical activity

No changes in physical activity were prescribed, given the hypothesized effects of cell swelling on resting fat oxidation [24,25]. The participants were instructed to maintain their usual physical activity routine.

### Random group assignment

Each participant was randomly assigned to receive either the standard of care, Qualitative recommendation (Q) or the Qualitative-plus-Quantitative recommendation (QQ), on a first-come, first-serve basis, in a single-blind manner using computer-generated random numbers ([www.randomizer.org](http://www.randomizer.org)). Both groups were instructed to follow the study diet and drink water instead of other beverages. The QQ group was further instructed to drink water to have diluted urine at least once each day, using light urine color to gauge how much water to drink. Urine concentration correlates strongly with urine color [50]. Checking for light-straw colored urine is a recognized way for children to self-monitor their hydration status [51,52]. QQ participants were told that first morning urine is normally concentrated and yellow, after not drinking water all night, but becomes light colored within two hours, if enough water has been consumed.

### Weekly process measures

Participants recorded the types of foods consumed at each meal on a daily log form. The log form included a list of free, ready-to-eat study foods for breakfast, lunch, and snack, as well as spaces for participants to write in other food types for each meal. The log form included spaces for participants to write in the type of foods selected for dinner, and the total number of 16.9 fl. oz (500 ml) bottles of water consumed that day. To minimize participant burden, the log forms did not

request details about brand, cooking method, additives, or portion size.

The daily logs had an open space for participants to record the amount of time spent doing Moderate or Vigorous Physical Activity (MVPA) [53], moving enough to sweat (e.g. fast walking) or get out of breath (e.g. running).

Each week, the participants had a 10 min drop-in clinic visit between 10:30 am and 4 pm to turn in completed diet and activity logs, pick up new log forms and study foods for the following week, rate thirst, and collect a random urine and saliva sample.

Each week, the study coordinator reviewed the log forms, and estimated the proportion of meals consumed without a high glycemic item. Participants who did not consume a high glycemic food at more than 75% of meals (equivalent to 3 out of 4 meals per day or breakfast, lunch, and snack each day) were considered compliant with respect to the study diet. Each week, participants were encouraged to avoid high glycemic foods and caloric beverages.

The log data were used to estimate the weekly mean daily bottled water intake. Thirst was monitored as an index of hyperosmotic cell shrinkage and driver of water intake [21,54]. Participants rated their thirst on a visual analog scale from “not at all thirsty” to “extremely thirsty” [55].

The urine sample was stored on ice until processed for urine osmolality by freezing point depression osmometer (Advanced Instruments, Norwood, MA). The principal investigator reviewed the previous week’s urine osmolality with each QQ participant, and encouraged those with a urine osmolality over 500 mmol/kg to drink more water to dilute below that threshold. Midday random urine osmolality below normal (<500 mmol/kg) [56] was targeted to induce hypo-osmolality, cell swelling, and anti-diuretic hormone suppression, as opposed to iso-osmolality (no change in cell volume or antidiuretic hormone).

Unstimulated saliva was collected and stored at -80°C until assayed for insulin by ELISA (IBL International Corp., Toronto, Ontario, Canada). Saliva insulin, which varies with plasma insulin in healthy children and adults [57], was monitored instead of plasma insulin to avoid participant drop-out related to weekly blood sampling.

Personnel responsible for clinical measurements and specimen collection were unaware of the treatment assignments.

### Weekly outcome measures

Body weight was measured in duplicate using a calibrated clinical scale (Scale-Tronix, Carol Stream, Illinois, USA) after the urine collection. Standing height was measured in duplicate using a standard wall-mounted stadiometer. Anthropometric data were expressed as BMI percentile relative to CDC growth curves [58,59]. Absolute weight loss was assessed, because the BMI percentile is insensitive to 10 lb weight loss in very obese children. Over 8 weeks, for example, while height increased by 0.1 cm, a weight loss of 6.4 kg resulted in a shift in BMI percentile from 99.5% to 99.3%.

### Statistics

Statistical analyses were done using Stata software [Version 9.2, 2006, StataCorp, College Station, TX].

### Intention-to-treat analyses

Intention to treat analyses compared the QQ and Q groups, excluding data for 3 participants who dropped out of the study in the first week after randomization, and only contributed baseline data. Including data from the 3 participants who dropped out in the intention-to-treat comparisons, by carrying forward the baseline value over 8 weeks, did not alter the results (data not shown).

T-tests were used to evaluate baseline differences in the intervention process and outcome measures. Mixed models that included a main effect of time, group indicator variable, and the group-by-time interaction term were used to test for significant change relative to baseline. Outcome data were available for all time points for 23 out of 25 participants. Missing values were not imputed, and assumed missing at random. P-values were considered statistically significant at the two-tailed 0.05 level.

### Completer analyses

Completer analyses compared 4 groups defined based on adherence to the protocol: DILUTE URINE, NOT DILUTE URINE, REPLACE, AND HIGH GLYCEMIC (HG) DIET. The DILUTE URINE group included participants who were initially assigned to the QQ group, adhered to the study diet, and had urine osmolality below 500 mmol/kg at 4 or more weekly visits. The NOT DILUTE URINE group included participants who were initially assigned to the QQ group, who met the diet condition, but did not dilute urine osmolality below 500 mmol/kg at 4 or more clinic visits. The REPLACE group included participants who were initially assigned to the Q group, who adhered to the study diet. The HG DIET group included QQ or Q participants who reported High Glycemic (HG) foods at more than 25% of meals.

Linear regression models that controlled for demographic variables and sports participation were used to test for baseline differences in process and outcome measures between the 4 completer groups. Mixed models that included baseline status, main effects for time and the group indicator variables, the group-by-time interaction terms, and the demographic and sports covariates were used to test for significant change over time. The DILUTE URINE group was treated as reference in these models.

### Check for effect mediation by insulin

A mixed model tested for a significant association between change in urine osmolality and change in saliva insulin, controlling for baseline saliva insulin, demographic variables and sports participation. Two nested multivariable mixed models were used to estimate the extent to which saliva insulin concentrations explained the effect of urine dilution on weight change. The first model estimated the magnitude of effect of having urine osmolality <500 vs. ≥500 mmol/kg, controlling for initial status, demographic variables, and sports participation. The second model estimated the same effect, with additional control for saliva insulin (<15 vs. ≥15 pmol/l). The change in the estimated magnitude of effect of urine dilution before and after control for saliva insulin was calculated. To limit the influence of outlier saliva insulin values, saliva insulin was treated as a dichotomous variable in the nested models, using the median value for this study sample as cutoff. Although 90% of the saliva insulin measurements ranged between 5 and 60 pmol/l, saliva insulin ranged from 1-185 pmol/l.

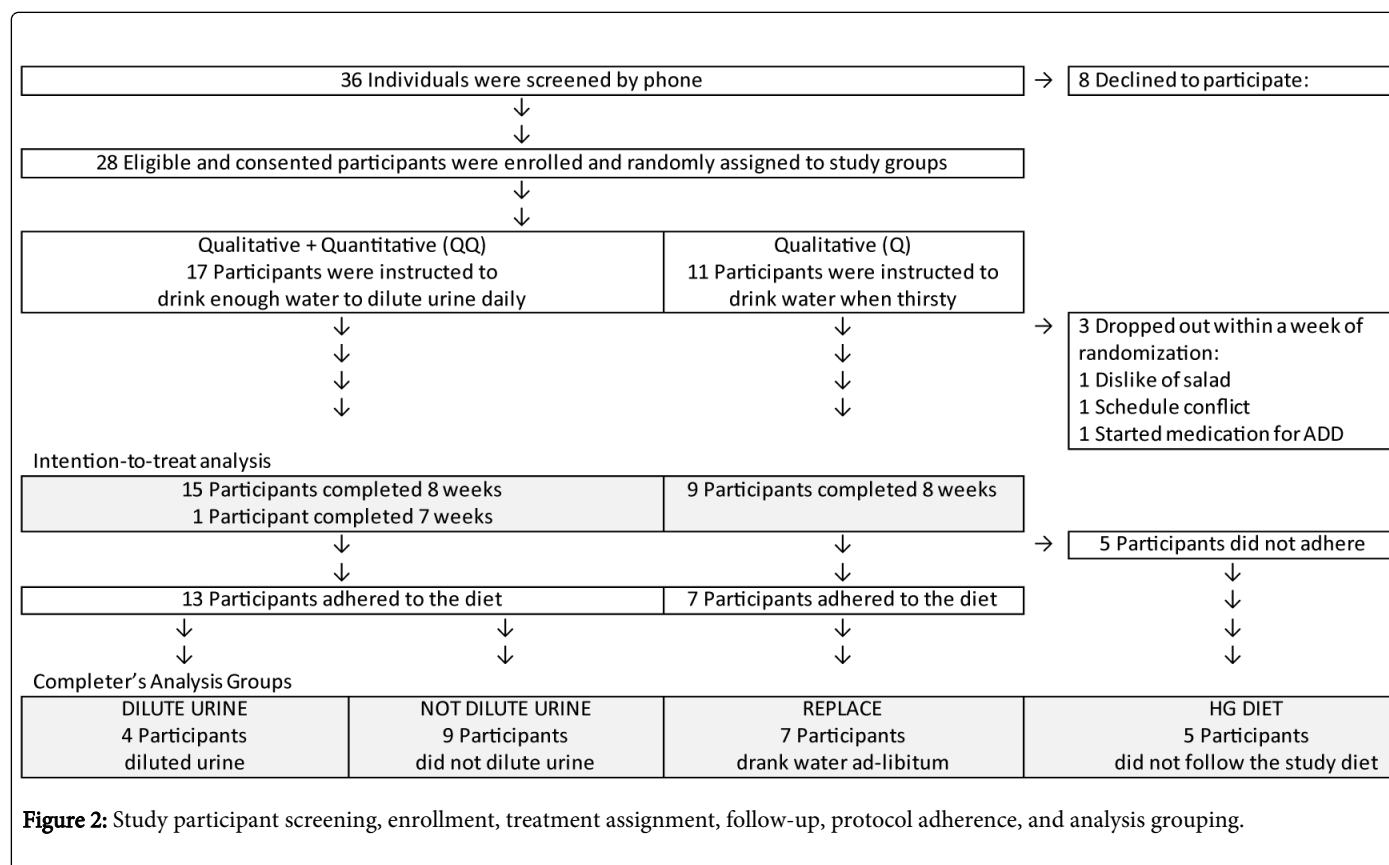
### Check for effect modification by insulin

A last multivariable mixed model explored whether the effect of dilute urine depended on the saliva insulin concentration. This model included dichotomous variables representing dilute urine and saliva insulin, and their interaction term, as well as baseline weight, demographic variables, and sports participation.

### Results

#### Study group characteristics at baseline

Figure 2 describes the study participant screening, enrollment, treatment assignment, adherence, and analysis groups. The majority of participants (22 out of 25) were obese (BMI >95<sup>th</sup> percentile). The study groups did not differ by age or sex.



### Intention-to-treat analyses

Table 1 describes the process measures of the intention-to-treat groups at baseline. The QQ and Q groups did not differ with respect to mean daily intake of total energy, macronutrients, total water, or glycemic load. Collectively, the mean daily intake was 1585 ± 120 kcal, 62 ± 5 g fat, 65 ± 6 g protein, 198 ± 18 g carbohydrate, and 1566 ± 135 g total water, including 256 ± 72 g sweetened caloric beverages. The participants reported a mean daily glycemic load of 153 ± 16. The intention-to-treat groups did not differ with respect to time spent doing moderate or vigorous physical activity, intake of drinking water, thirst, random midday urine osmolality, urine dilution response to 500 ml drinking water, or random saliva insulin. The QQ group was significantly less likely to participate in organized team or afterschool sports than the Q group (5 out of 16 vs. 4 out of 9 participants).

Table 2 describes the weight status of the intention-to-treat groups at baseline. Absolute body weight did not differ between groups. The QQ group had a significantly higher BMI percentile at baseline.

Table 1 describes changes in the weekly process measures over time for the intention-to-treat groups. After orientation, the proportion of

low glycemic meals increased similarly in both the QQ and Q groups, from under 15% to over 80% of meals. The reported time spent doing moderate or vigorous physical activity did not change significantly relative to baseline for either study group. In both groups, intake of drinking water increased significantly. The change in absolute volume of drinking water did not differ by group. On average, the QQ group diluted urine to a significantly greater extent than the Q group, but only 4 out of the 17 (24%) participants assigned to the QQ group had a urine osmolality below 500 mmol/kg at half or more weekly visits. Mean urine osmolality remained over 800 mmol/kg (i.e. elevated [29]) over time for the Q group. One out of the 11 (9%) participants assigned to the Q group had urine osmolality below 500 mmol/kg at 4 visits. Saliva insulin decreased significantly relative to baseline in the QQ group.

A trend towards more weight loss in the QQ group was not statistically significant (Table 2). Change in BMI percentile was significantly greater in the Q group, with and without adjustment for the difference in initial BMI percentile.

| Intervention process measure                   | Baseline   |           | Weeks 2-9 |         | Change from baseline within group |         | Difference in change between groups |         |
|--|------------|-----------|-----------|---------|-----------------------------------|---------|-------------------------------------|---------|
|  | Mean ± SE  | Mean ± SE | Mean ± SE | p-value | Mean ± SE                         | p-value | Mean ± SE                           | p-value |
| <b>Low glycemic meals, % of all meals</b>      |            |           |           |         |                                   |         |                                     |         |
| QQ   | 12 ± 6     | 83 ± 3    | +70 ± 6   | <0.001  |                                   |         |                                     |         |
| Q  | 8 ± 8      | 81 ± 4    | +73 ± 7   | <0.001  | 3 ± 9                             |         | 0.78                                |         |
| <b>MVPA, min/d</b>                             |            |           |           |         |                                   |         |                                     |         |
| QQ   | 56 ± 9     | 44 ± 7    | -12 ± 7   | 0.09    |                                   |         |                                     |         |
| Q  | 46 ± 9     | 40 ± 7    | -6 ± 7    | 0.34    | 6 ± 10                            |         | 0.59                                |         |
| <b>Intake of drinking water, bottles/d</b>     |            |           |           |         |                                   |         |                                     |         |
| QQ   | 2 ± 0.3    | 3 ± 0.2   | +2 ± 0.3  | <0.001  |                                   |         |                                     |         |
| Q  | 1 ± 0.4    | 3 ± 0.3   | +2 ± 0.4  | <0.001  | 0.2 ± 0.4                         |         | 0.65                                |         |
| <b>Thirst, mm/100</b>                          |            |           |           |         |                                   |         |                                     |         |
| QQ   | 50 ± 7     | 41 ± 5    | -9 ± 5    | 0.08    |                                   |         |                                     |         |
| Q  | 29 ± 7     | 32 ± 5    | +3 ± 6    | 0.61    | 12 ± 8                            |         | 0.14                                |         |
| <b>Random urine osmolality, mmol/kg</b>        |            |           |           |         |                                   |         |                                     |         |
| QQ   | 874 ± 81   | 726 ± 47  | -148 ± 76 | 0.05    |                                   |         |                                     |         |
| Q  | 691 ± 84   | 840 ± 56  | +149 ± 74 | 0.05    | 296 ± 115                         |         | 0.01                                |         |
| <b>Urine dilution test osmolality, mmol/kg</b> |            |           |           |         |                                   |         |                                     |         |
| QQ   | -542 ± 44  |           |           |         |                                   |         |                                     |         |
| Q  | -543 ± 148 |           |           |         |                                   |         |                                     |         |
| <b>Saliva insulin, pmol/l</b>                  |            |           |           |         |                                   |         |                                     |         |
| QQ   | 37 ± 6     | 20 ± 3    | -17 ± 7   | 0.01    |                                   |         |                                     |         |
| Q  | 23 ± 6     | 21 ± 4    | -2 ± 6    | 0.79    | 15 ± 10                           |         | 0.12                                |         |

**Table 1:** Mean ± SE status at baseline and over 8 weeks of follow-up by intention-to-treat group. The mean ± SE status and change estimates were calculated using mixed models without adjustment for covariates. The Weeks 2-9 estimates represent the status sustained over the follow-up period. The p-values for changes relative to baseline within each study group test the hypothesis that the mean change was zero. The p-values for differences in change between the study groups test the hypothesis that the mean changes in each group were the same. The Qualitative-plus-Quantitative (QQ) group (n=16) was instructed to consume meals with low glycemic foods, maintain usual activity, drink water instead of caloric beverages, and drink enough water to dilute urine. The Qualitative only (Q) group (n=9) was instructed to consume meals with low glycemic foods, maintain usual activity, and drink water instead of caloric beverages. MVPA: Self-reported time spent doing Moderate or Vigorous Physical Activity (MVPA).

### Completer analyses

Table 3 describes the baseline status of completer groups defined by protocol adherence. At baseline, the completer groups were similar with respect to intakes of total energy, protein, carbohydrate, total water, sweetened caloric beverages, and glycemic load. The DILUTE URINE group differed from the NOT DILUTE URINE group with respect to fat intake (44 ± 3 g/d vs. 78 ± 7 g/d) and saliva insulin at baseline. Saliva insulin exceeded 15 pmol/L for all 4 participants in the

DILUTE URINE group vs. 4 out of 7 participants in the REPLACE group.

Table 3 describes the intervention process measures over 8 weeks by completer group. The DILUTE URINE and REPLACE groups completed the study as instructed. The DILUTE URINE and REPLACE groups complied with the study diet. For 8 weeks, 3 out of 4 meals did not include a high glycemic food. Neither group increased the time spent doing moderate or vigorous physical activity.

| Intervention outcome measure | Baseline   | Week 9     | Change from baseline within group |         | Difference in change/wk between groups |         |
|------------------------------|------------|------------|-----------------------------------|---------|--|---------|
|                              |            |            | Mean ± SE                         | p-value | Mean ± SE                              | p-value |
| <b>Body weight, kg</b>       |            |            |                                   |         |  |         |
| QQ                           | 70.4 ± 4.5 | 68.5 ± 4.5 | -2.0 ± 0.3                        | <0.001  |  |         |
| Q                            | 62.7 ± 5.7 | 61.2 ± 5.7 | -1.6 ± 0.4                        | <0.001  | 0.03 ± 0.06                            | 0.56    |
| <b>BMI percentile, %</b>     |            |            |                                   |         |  |         |
| QQ                           | 98.4 ± 0.4 | 98.0 ± 0.4 | -0.5 ± 0.1                        | 0.001   |  |         |
| Q                            | 96.3 ± 1.1 | 95.5 ± 1.1 | -0.8 ± 0.3                        | 0.01    | -0.08 ± 0.03                           | 0.009   |

**Table 2:** Mean ± SE weight status at baseline and after 8 weeks of follow-up by intention-to-treat group. The mean ± SE weight status at baseline and Week 9 and change estimates were calculated using mixed models without adjustment for covariates. The p-values for changes relative to baseline within each study group test the hypothesis that the mean change was zero. The p-values for differences in change between the study groups test the hypothesis that the mean changes in each group were the same. The Qualitative-plus-Quantitative (QQ) group (n=16) was instructed to consume meals with low glycemic foods, maintain usual activity, drink water instead of caloric beverages and drink enough water to dilute urine. The Qualitative only (Q) group (n=9) was instructed to consume meals with low glycemic foods, maintain usual activity, and drink water instead of caloric beverages. Adjustment for initial weight status did not change the magnitude or significance of the change estimates. The BMI percentile differed significantly between groups at baseline by t-test.

| Intervention process measure               | Baseline | Weeks 2-9 | Change from baseline within group |         | Difference in change between groups |         |
|--|----------|-----------|-----------------------------------|---------|-------------------------------------|---------|
|  |          |           | Mean ± SE                         | p-value | Mean ± SE                           | p-value |
| <b>Completer group</b>                     |          |           |                                   |         |                                     |         |
| <b>Low glycemic meals, % of all meals</b>  |          |           |                                   |         |                                     |         |
| DILUTE URINE                               | 18 ± 8   | 87 ± 4    | +69 ± 8                           | <0.001  |                                     |         |
| NOT DILUTE                                 | 3 ± 4    | 90 ± 2    | +87 ± 5                           | <0.001  | +18 ± 12                            | 0.14    |
| REPLACE                                    | 7 ± 6    | 88 ± 3    | +81 ± 6                           | <0.001  | +11 ± 12                            | 0.36    |
| HG DIET                                    | 23 ± 14  | 54 ± 7    | +31 ± 16                          | 0.05    | -39 ± 13                            | 0.004   |
| <b>MVPA, min/d</b>                         |          |           |                                   |         |                                     |         |
| DILUTE URINE                               | 67 ± 10  | 26 ± 4    | -41 ± 11                          | <0.001  |                                     |         |
| NOT DILUTE                                 | 50 ± 15  | 53 ± 13   | +3 ± 13                           | 0.81    | 45 ± 14                             | 0.002   |
| REPLACE                                    | 40 ± 7   | 38 ± 6    | -2 ± 6                            | 0.73    | 39 ± 14                             | 0.004   |
| HG DIET                                    | 61 ± 16  | 56 ± 14   | -5 ± 8                            | 0.53    | 36 ± 15                             | 0.015   |
| <b>Intake of drinking water, bottles/d</b> |          |           |                                   |         |                                     |         |
| DILUTE URINE                               | 2 ± 0.4  | 4 ± 0.3   | +2 ± 0.3                          | <0.001  |                                     |         |
| NOT DILUTE                                 | 2 ± 0.5  | 4 ± 0.3   | +2 ± 0.4                          | <0.001  | 0.1 ± 0.6                           | 0.91    |
| REPLACE                                    | 1 ± 0.5  | 3 ± 0.4   | +2 ± 0.4                          | <0.001  | 0.4 ± 0.7                           | 0.58    |
| HG DIET                                    | 1 ± 0.6  | 3 ± 0.3   | +2 ± 0.7                          | 0.009   | -0.2 ± 0.7                          | 0.78    |
| <b>Thirst rating, mm/100</b>               |          |           |                                   |         |                                     |         |
| DILUTE URINE                               | 38 ± 13  | 25 ± 8    | -13 ± 12                          | 0.28    |                                     |         |
| NOT DILUTE                                 | 58 ± 9   | 46 ± 7    | -11 ± 7                           | 0.09    | +1 ± 12                             | 0.91    |
| REPLACE                                    | 31 ± 9   | 35 ± 3    | +4 ± 8                            | 0.64    | +16 ± 13                            | 0.20    |

|  |            |           |            |        |           |        |
|--|------------|-----------|------------|--------|-----------|--------|
| HG DIET  | 34 ± 11    | 36 ± 9    | +3 ± 7     | 0.72   | +15 ± 140 | 0.26   |
| <b>Random urine osmolality on arrival at clinic, mmol/kg</b>           |            |           |            |        |           |        |
| DILUTE URINE   | 910 ± 161  | 450 ± 67  | -459 ± 170 | 0.007  |           |        |
| NOT DILUTE   | 839 ± 99   | 772 ± 39  | -67 ± 105  | 0.53   | 392 ± 162 | 0.016  |
| REPLACE  | 642 ± 78   | 888 ± 41  | +247 ± 76  | 0.001  | 705 ± 169 | <0.001 |
| HG DIET  | 903 ± 126  | 835 ± 101 | -68 ± 87   | 0.47   | 391 ± 181 | 0.03   |
| <b>Change in urine osmolality after 500 ml drinking water, mmol/kg</b> |            |           |            |        |           |        |
| DILUTE URINE   | -493 ± 55  |           |            |        |           |        |
| NOT DILUTE   | -528 ± 53  |           |            |        |           |        |
| REPLACE  | -542 ± 209 |           |            |        |           |        |
| HG DIET  | -635 ± 120 |           |            |        |           |        |
| <b>Saliva insulin, pmol/l</b>  |            |           |            |        |           |        |
| DILUTE URINE   | 70 ± 14    | 13 ± 8    | -58 ± 14   | <0.001 |           |        |
| NOT DILUTE   | 29 ± 6     | 20 ± 3    | -9 ± 6     | 0.15   | 49 ± 13   | <0.001 |
| REPLACE  | 18 ± 7     | 22 ± 4    | +3 ± 6     | 0.58   | 61 ± 14   | <0.001 |
| HG DIET  | 24 ± 13    | 28 ± 8    | +3 ± 13    | 0.81   | 61 ± 15   | <0.001 |

**Table 3:** Mean ± SE status at baseline and over 8 weeks of follow-up by completer group. The mean ± SE status and within-group change estimates were calculated using mixed models without adjustment for covariates. The Weeks 2-9 estimates represent the status sustained over the follow-up period. The p-values for changes relative to baseline within each study group test the hypothesis that the mean change was zero. The p-values for differences in change between the study groups test the hypothesis that the mean changes in each group were the same, controlling for baseline status, age, sex, race-ethnicity, and sports participation. The DILUTE URINE group (n=4) was initially assigned to the QQ group, consumed 3 out of 4 meals without high glycemic foods, and had urine osmolality below 500 mmol/kg at 4 or more clinic visits. The NOT DILUTE group (n=9) was initially assigned to the QQ group, consumed 3 out of 4 meals without high glycemic foods, but did not dilute urine osmolality below 500 mmol/kg over time. The REPLACE group (n=7) was initially assigned to the Q group and consumed 3 out 4 meals without high glycemic foods. The REPLACE group drank water ad-libitum. The HG DIET group (n=5) included participants who consumed high glycemic foods at more than 1 out of 4 meals over the 8 week study period. MVPA: Self-reported time spent doing Moderate or Vigorous Physical Activity (MVPA); Low glycemic meals: The proportion of meals that did not include a high glycemic food.

The DILUTE URINE group significantly decreased urine osmolality to below 500 mmol/kg over time. All 4 participants in the DILUTE URINE group had urine osmolality below 500 mmol/kg, in weeks 4, 5, 6, 8, and 9. The REPLACE group did not decrease urine osmolality relative to baseline.

Saliva insulin decreased significantly by 81% in the DILUTE URINE group. Saliva insulin was below 15 pmol/L in weeks 6, 7, and 8 for all 4 participants in the DILUTE URINE group. Saliva insulin was above this value over time in the REPLACE group.

Absolute weight loss over 8 weeks was significantly greater in the DILUTE URINE group than the REPLACE group (Table 4). The completer groups did not differ significantly with respect to change in BMI percentile.

#### Effect mediation and/or modification by saliva insulin

A decrease in urine osmolality to below 500 mmol/kg was significantly associated with a mean ± SE decrease in saliva insulin of 9.9 ± 4.9 pmol/l (p=0.04).

Table 5 describes the estimated weight loss attributable to urine dilution before and after control for saliva insulin. Diluting urine osmolality to below 500 mmol/kg was associated with an average ± SE 0.6 ± 0.3 kg more weight loss than having urine osmolality above that concentration, controlling for baseline weight, demographic variables, and sports participation. Addition of saliva insulin to the multivariable model reduced the estimated magnitude of effect by 17%, from 0.6 ± 0.3 kg (in Model 1) to 0.5 ± 0.2kg (in Model 2).

Saliva insulin significantly modified the association between urine osmolality and weight change (Table 5, Model 3). For individuals with a saliva insulin of 15 pmol/l or higher, having a urine osmolality below 500 mmol/kg was not associated with significant additional weight loss (0.05 ± 0.4 kg, p=0.89). For individuals with a saliva insulin below 15 pmol/l, having a urine osmolality below 500 mmol/kg was associated with 0.9 ± 0.5 kg additional weight loss (p<0.05).



| Intervention outcome measure | Baseline    | Week 9      | 8 week change within group |         | Difference in change/wk between groups |         |
|------------------------------|-------------|-------------|----------------------------|---------|--|---------|
|                              | Mean ± SE   | Mean ± SE   | Mean ± SE                  | p-value | Mean ± SE                              | p-value |
| <b>Body weight, kg</b>       |             |             |                            |         |  |         |
| DILUTE URINE                 | 64.5 ± 9.6  | 61.2 ± 9.6  | -3.3 ± 0.7                 | <0.001  |  |         |
| NOT DILUTE                   | 70.8 ± 5.6  | 68.9 ± 5.6  | -1.9 ± 0.3                 | <0.001  | 0.3 ± 0.08                             | <0.001  |
| REPLACE                      | 67.2 ± 6.1  | 65.2 ± 6.0  | -2.0 ± 0.5                 | <0.001  | 0.2 ± 0.08                             | 0.006   |
| HG DIET                      | 65.2 ± 11.0 | 64.9 ± 11.1 | -0.3 ± 0.4                 | 0.41    | 0.5 ± 0.08                             | <0.001  |
| <b>BMI percentile, %</b>     |             |             |                            |         |  |         |
| DILUTED URINE                | 98.1 ± 1.3  | 97.1 ± 1.3  | -1.0 ± 0.5                 | 0.04    |  |         |
| NOT DILUTE                   | 98.8 ± 0.3  | 98.5 ± 0.3  | -0.2 ± 0.1                 | <0.001  | 0.1 ± 0.04                             | 0.001   |
| REPLACE                      | 96.8 ± 1.1  | 95.6 ± 1.1  | -1.2 ± 0.3                 | 0.001   | -0.04 ± 0.04                           | 0.36    |
| HG DIET                      | 96.5 ± 1.7  | 96.5 ± 1.7  | -0.04 ± 0.3                | 0.89    | 0.1 ± 0.05                             | 0.003   |

**Table 4:** Mean ± SE weight status at baseline and after 8 weeks of follow-up by completer group. Study participants who were randomly assigned to the QQ group were instructed to consume the study diet, maintain usual activity, drink water instead of caloric beverages and drink enough water to dilute urine. Study participants who were randomly assigned to the Q group were instructed to consume the study diet, maintain usual activity, and drink water instead of caloric beverages. The mean ± SE weight status at baseline and Week 9 and change estimates were calculated using mixed models without adjustment for covariates. The p-values for changes relative to baseline within each study group test the hypothesis that the mean change was zero. The p-values for differences in change between the study groups test the hypothesis that the mean changes in each group were the same. Adjustment for initial status did not change the magnitude or significance of the change estimates (not shown). The BMI percentile differed significantly between groups at baseline by t-test.

| Dichotomous independent variable   | Estimated mean difference in weight change associated with the independent variable, kg |       |            |        |            |       |
|--|---|-------|------------|--------|------------|-------|
|  | Model 1   |       | Model 2    |        | Model 3    |       |
|  | β ± SE  | p     | β ± SE     | p      | β ± SE     | p     |
| Urine osmolality <500 mmol/kg  | -0.6 ± 0.3  | 0.012 | -0.5 ± 0.2 | 0.029  | 0.05 ± 0.4 | 0.89  |
| Saliva insulin <15 pmol/l  |   |       | -0.7 ± 0.2 | <0.001 | -0.6 ± 0.2 | 0.002 |
| Urine osmolality <500 mmol/kg x Saliva insulin <15 pmol/l interaction term |   |       |            |        | -0.9 ± 0.5 | 0.048 |

**Table 5:** Estimated weight change associated with having urine osmolality below 500 mmol/kg and saliva insulin below 15 pmol/l. Mixed models predicting 8 week weight (kg) change were used to check whether saliva insulin might mediate or modify an effect of dilute urine on weight loss. All models included 25 participants and controlled for initial status, age, sex, race/ethnicity, and sports participation. Model 1 estimated the magnitude of effect (β ± SE) of having urine osmolality below 500 mmol/kg vs. 500 mmol/kg or higher over time. Model 2 estimated the same magnitude of effect as Model 1, adding control for saliva insulin concentration (<15 pmol/l vs. 15 pmol). Model 3 tested for a significant interaction between urine osmolality below 500 mmol/kg and saliva insulin below 15 pmol/l. Effect mediation occurs when a variable is intermediate on the pathway between the exposure and outcome. Control for an intermediate reduces the estimated magnitude of effect of the exposure (Model 2 β < Model 1 β). Effect modification occurs when the relationship between exposure and outcome depends on a covariate, i.e. when the effects of the exposure and the covariate interact.

## Discussion

The results of this study suggest that the qualitative recommendation to drink water instead of other beverages might be more effective for pediatric obesity treatment if it is combined with quantitative guidance to drink water to dilute urine, at least for individuals who are able to sustain a diet of low glycemic foods and drink enough water to dilute urine. The results, furthermore, suggest

that insulin concentration and/or a correlate of insulin concentration may mediate and modify effects of drinking water to dilute urine on weight loss.

Participants who were randomly assigned to the Qualitative-plus-Quantitative (QQ) group and complied (i.e. the DILUTE URINE completer group) lost significantly more weight over 8 weeks than participants who were randomly assigned to the Q group and complied (the REPLACE completer group). Given, however, that only

4 out of the 16 participants instructed to dilute urine complied, the QQ recommendation may only benefit select individuals. Weight loss did not differ significantly by intention-to-treat group. Of note, the QQ recommendation doubled the odds of drinking water to dilute urine compared to the Q recommendation.

The intention-to-treat results indicate that one-on-one orientation, free home-delivery of bottled water, and weekly feedback and encouragement to dilute urine below 500 mmol/kg were not enough for most QQ participants to meet the target drinking water condition. The failure to dilute urine cannot be attributed to impaired kidney ability to dilute urine because, when presented with a bolus of 500 ml drinking water during the baseline assessment, all participants diluted urine below 500 mmol/kg. Participants in this study reported lack of thirst and desire to avoid school bathrooms as barriers to drinking more water. Physiologic signals, such as suppressed thirst and involuntary dehydration, and environmental or social cues that discourage drinking water are known barriers to drinking water [60-63]. Participants in the NOT DILUTE URINE group reported significantly lower thirst over weeks 2-9 relative to baseline, unlike the DILUTE URINE group, which reported transiently reduced thirst in Week 3 followed by increases in thirst over weeks 4-9. The transient decrease in thirst in the DILUTE URINE group suggests adjustment to an amount of water that was initially more than thirst indicated.

The results suggest that barriers to drinking water and elevated urine osmolality may be the default or usual condition for the target population. At baseline, 88% of the study participants had a random midday urine osmolality above 500 mmol/kg; 68% of the participants had a urine osmolality above 800 mmol/kg. Without guidance about how much water to drink only 1 (<10%) of the Q participants spontaneously diluted urine osmolality below 500 mmol/kg. The mean urine osmolality above 800 mmol/kg for Weeks 2-9 for the Q group suggests chronic hyperosmotic stress and cell shrinkage.

The results of this study are consistent with insulin mediating effects of urine dilution on weight loss. Change in urine osmolality was significantly associated with change in both saliva insulin and body weight. Compared to the other groups, the DILUTE URINE group had significantly lower saliva insulin over time. The effect of having a urine osmolality below 500 mmol/kg was partially explained by having a saliva insulin below 15 pmol/l.

Saliva insulin concentrations significantly modified the effect of drinking water to dilute urine on weight loss. Saliva insulin below 15 pmol/l magnified the association between urine osmolality and weight change. Urine osmolality below 500 mmol/kg was not associated with weight loss for individuals who maintained saliva insulin above 15 pmol/l. The observed effect modification is consistent with insulin suppression of fat oxidation, as well as previous reports of greater weight loss associated with drinking water when the background diet is lower vs. higher in carbohydrate [26]. The finding implies that recommendations to drink water to dilute urine may be ineffective for weight loss without concomitant attention to background insulin concentrations.

The DILUTE URINE group included 2 participants with high baseline saliva insulin (152 pmol/l and 185 pmol/l), who might be considered insulin hyper-responders and/or more metabolically at-risk [64]. For these participants, the study diet, which limited high glycemic foods, may have been an important condition for effect.

The results warrant studies to elucidate the intermediates and pathway(s) that explain drinking water effects on fat oxidation and

weight loss. Potential intermediates may include gluconeogenesis, glycogen breakdown, insulin sensitivity, mitochondrial function, ATP and NADPH generation, and sympathetic activation [6-9,14-17] (Figure 1). Reduced mitochondrial ATP production is associated with both hyperosmotic conditions and insulin resistance [15,65].

To limit participant burden, changes in total energy intake and energy expenditure were not measured. Although total energy intake at baseline did not vary by study group, and each participant was given the same study foods, it is not possible to rule out group differences in change in total energy intake as an explanation for differences in weight loss. It is unknown if the differences in weight loss reflect change in resting energy expenditure. Considering that the DILUTE URINE group reported less time doing moderate or vigorous physical activity than the other completer groups, it is unlikely that physical activity explains the greater weight loss in this group. Weight loss in this study may reflect the background 30-60 min/d of MVPA.

The small sample size limits interpretation of the results. Further work is needed to rule out bias as explanation for completer group differences, confirm the etiologic mechanism(s), and determine generalizability of the results.

Although drinking water was significantly associated with absolute weight loss, parallel relationships were not observed for change in BMI percentile. The discrepancy in results may reflect insensitivity of the BMI percentile to 10 lb weight change in children with a BMI percentile above 99%. As absolute weight loss may reflect loss of lean mass, studies involving specific measures of body fat mass and distribution are needed to confirm effects of drinking water on body fat oxidation.

Pediatric obesity treatment is notoriously resource intensive [66]. To maximize benefit for effort, further work is warranted to determine if and how multiple etiologic pathways might be leveraged to optimize drinking water effects for pediatric obesity treatment. Adding quantitative guidance about how much water to drink to qualitative advice to drink water instead of other beverages may be one way to leverage multiple pathways. Barriers to drinking water, such as lack of thirst and/or clean bathrooms, remain to be addressed.

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