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Basic nutritional investigation

Fructose content in popular beverages made with and without high-fructose corn syrup☆

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ABSTRACT

Objective: Excess fructose consumption is hypothesized to be associated with risk for metabolic disease. Actual fructose consumption levels are difficult to estimate because of the unlabeled quantity of fructose in beverages. The aims of this study were threefold: 1) re-examine the fructose content in previously tested beverages using two additional assay methods capable of detecting other sugars, especially maltose, 2) compare data across all methods to determine the actual free fructose-to-glucose ratio in beverages made either with or without high-fructose corn syrup (HFCS), and 3) expand the analysis to determine fructose content in commonly consumed juice products.

Methods: Sugar-sweetened beverages (SSBs) and fruit juice drinks that were either made with or without HFCS were analyzed in separate, independent laboratories via three different methods to determine sugar profiles.

Results: For SSBs, the three independent laboratory methods showed consistent and reproducible results. In SSBs made with HFCS, fructose constituted 60.6% ± 2.7% of sugar content. In juices sweetened with HFCS, fructose accounted for 52.1% ± 5.9% of sugar content, although in some juices made from 100% fruit, fructose concentration reached 65.35 g/L accounting for 67% of sugars.

Conclusion: Our results provide evidence of higher than expected amounts of free fructose in some beverages. Popular beverages made with HFCS have a fructose-to-glucose ratio of approximately 60:40, and thus contain 50% more fructose than glucose. Some pure fruit juices have twice as much fructose as glucose. These findings suggest that beverages made with HFCS and some juices have a sugar profile very different than sucrose, in which amounts of fructose and glucose are equivalent. Current dietary analyses may underestimate actual fructose consumption.

Introduction

Assessment of fructose content in foods and beverages is an important public health issue to consider, as Americans consume more per-capita high-fructose corn syrup (HFCS) than any other nation [1]. Fructose consumption in the U.S. population has doubled over the past 3 decades [2] and the consumption of excess fructose, due primarily to the way in which fructose is specifically metabolized by the liver [3,4], has been linked to fatty liver disease [5], dyslipidemia [6], type 2 diabetes [1], obesity [7], and gout [8]. However, others have posted that fructose is no different than sucrose, without any adverse health effects [9], and that HFCS-55 is roughly equivalent [10] to or similar in composition [11] to sucrose. A growing body of clinical evidence suggests that fructose consumption plays a direct role in the risk for metabolic disease [12,13] and may have adverse effects on central appetite regulation compared with glucose [14]. Despite this evidence, current food-labeling practices do not provide information on fructose content in foods and beverages made with HFCS, fruit juice concentrate or crystalline fructose,

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all of which contain fructose and are being used in increasing amounts as added sugar in the food supply [15]. Because there are currently no disclosures of fructose content in foods and beverages [15], and many nutrition databases only rely on product label information, it is challenging to accurately determine actual fructose consumption levels in nutrition research.

Previous work has shown that the fructose content of sugar-sweetened beverages (SSBs) made with HFCS can be as high as 65% of total sugar content, higher than that suggested by the fructose content of HFCS-55 (55% fructose) [16], potentially contributing to unexpectedly more fructose in the diet. However, this initial study was criticized [17] for not measuring other trace sugars (e.g., maltose) thought to be present in SSBs made with HFCS. Therefore, the aims of the present study were to: 1) re-examine the fructose content in previously tested beverages using two additional assay methods capable of detecting other sugars, especially maltose; 2) compare data across all methods to determine the actual free fructose-to-glucose (F:G) ratio in beverages made either with or without HFCS, and 3) expand the analysis to determine fructose content in commonly consumed juice products.

Methods and procedures

Based on product popularity [18], we selected 10 of the 23 beverages, that were previously tested using liquid chromatography (LC) [16], for follow-up analysis using two alternative methods to determine sugar content: 1) a metabolomics-type (MET) approach based on mass spectrometry (MS) with combined liquid and gas chromatography (GC) and 2) GC. Additionally, we extended the use of GC to analyze a selection of juice products, as described here.

Metabolomics-type approach

Popular SSBs were purchased from retailers in East Los Angeles, California, in 2012. Beverages were selected to replicate a previous study [16], in which the selection of beverages was based on consumption frequencies of children in past studies. Nutrition label information and serving size data were recorded. Immediately after opening bottled/canned beverages, 500 μL samples were aliquoted and transferred to Eppendorf cryotubes. All samples were held under refrigeration and sequentially flash frozen in liquid nitrogen within 1 h of the initial transfer. Samples were stored at −20°C overnight before shipment. Glucose, fructose, sucrose, and maltose standard solutions were created from research grade reagents (Sigma-Aldrich, St. Louis, MO, USA) to serve as controls. Ten grams of the sucrose, fructose, and glucose reagents were added to 100 mL of Millipore water and brought into solution. Two concentrations of maltose were prepared, 10 g/L and 1 g/L. Finally, a 50:50 solution of fructose and sucrose was prepared by combining 5 g of each reagent with 100 mL of water. These sugar standard concentrations were chosen to replicate the approximate sugar-content equivalents found in most sweetened beverages with the two maltose preparations representing the very small amounts of this sugar that may be found in sweetened beverages. For all standards, 500 μL aliquots were taken and prepared as previously described. All samples were shipped overnight packed in dry ice to Metabolon (Research Triangle Park, Durham, NC, USA). Samples were split into equal parts for analysis on the gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) platforms based on previously published methodology [19]. The GC column was 5% phenyl and the temperature ramp was from 40°C to 300°C in a 16-min period. Samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole MS using electron impact ionization. The LC/MS portion of the platform was based on a Waters ACQUITY UPLC and a Thermo-Finnigan LTQ MS, which consisted of an electrospray ionization source and linear ion-trap mass analyzer. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. Identification of known chemical entities was based on comparison to metabolomic library entries of purified standards. The combination of chromatographic properties and mass spectra gave an indication of a match to the specific compound or an isobaric entity. Metabolon was blinded to the source of all samples and standards and samples were analyzed according to previously described methodologies using a metabolomics approach to examine a broad array of simple and complex sugars [19]. Data for sucrose, glucose, fructose and maltose are presented in this manuscript.

Gas chromatography

The 10 SSBs analyzed in the MET analysis were again selected along with 4 additional randomly selected SSBs and 20 other juice products. Online shopping databases for Walmart, SuperValu, and Safeway were accessed to select samples. To control for location and inventory, online store inventories were selected within a defined zip code region (90033). Twenty juices were randomly selected by choosing every 10th product in the retailers' databases until 10 products made with HFCS and 10 products made without HFCS, according to package ingredients labels, were selected. One juice product was omitted from the analysis due to handling error, resulting in 19 products that proceeded to assay. All samples were aliquoted to sterile, sealed containers and sample weights were determined and recorded. Samples were packaged and shipped overnight on dry ice to Covance Laboratories (Madison, WI, USA) for subsequent blinded analysis via GC, against internal standards, according to previously published methods [20–22]. The sugar profile analysis conducted at Covance was applicable to the determination of fructose, galactose, glucose, sucrose, lactose, and maltose in as little as 10 g of food products, syrups, and beverages using GC, as described later. Once received, samples were prepared in accordance with Covance procedures and sugars were extracted from the homogenized sample with water. Aliquots were dried under inert gas and reconstituted with a hydroxylamine hydrochloride solution in pyridine containing phenyl--β-D-glucoside as the internal standard. The resulting oximes were converted to silyl derivatives with hexamethyldisilazane and trifluoroacetic acid treatment and analyzed by GC [20,21] using a flame ionization detector (Agilent 6890 N). An additional 10% of each sample analytical run was tested in duplicate and validated against two internal validated controls. Results underwent quality control comparison with internal validated controls, linearity expectations, and historical data. The limit of quantitation for most matrices is 0.1%. The relative standard deviations, on a cereal matrix, for fructose, glucose, sucrose, and maltose were 4.9%, 7.4%, 3.2%, and 6.4%, respectively. Specific gravity testing was conducted [22] on all liquid samples to allow the reporting of sugar content in appropriate units of measure.

Comparison of laboratory obtained sugar values versus nutritional database values

The Nutrition Data System for Research (NDSR, University of Minnesota, MN, USA) was used to assemble sugar content data for some of the products included in this study. All SSB and juice products listed in the NDSR database were compared against the GC-determined sugar values. The Nutrition Coordinating Center Food and Nutrient Database served as the source of food composition information in NDSR [23]. The U.S. Department of Agriculture Nutrient Data Laboratory was the primary source of nutrient values and nutrient composition. These values were supplemented by food manufacturers' information and data available in the scientific literature [24]. Standardized, published imputation procedures were applied to minimize missing values [25]. Fructose, sucrose, and glucose contents for all SSBs and juice products, with an exact product match in the NDSR database, were assembled for comparison. NDSR product volumes (fl oz.) varied, thus all product volumes were normalized to 12 fl oz. and sugar amounts in grams were calculated based on the NDSR referent volume. These data were compared against the values obtained through GC, as described previously. The mean GC-obtained sugar contents across matched products were compared with the mean NDSR sugar values across matched products, and percent difference was reported.

Data reporting

Examination of sugar composition in 10 beverages across three different methods

A mean with SD (reflecting the differences between analytical methods) and coefficient of variation (CV) for intermethod variability were calculated for fructose, glucose, sucrose, and maltose to assess consistency across the independent methods (SPSS v18 [SPSS Inc, Chicago, IL, USA]). Percent of total sugar (% TS) was calculated for all measured sugars in the SSBs analyzed via the three methodologies.

SSB and juice GC analysis

Data for individual sugars were reported in the following formats; %TS, concentration of each sugar in grams per liter (g/L) and grams per serving (g/s). Free F:G ratios and the concentration of free fructose ($F_{concentration}$) in each product were also assessed. The raw F:G ($F:G_{Raw}$) was adjusted ($F:G_{Adjusted}$) to account for the additional glucose that the disaccharide maltose may contribute to the overall sugar profile of the products. F:G values were reported using the first number, representing fructose, as the referent (e.g., F:G of 60:40; reported as 60). Formulas used to obtain these values are presented in Table 1.