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REVIEW

Clinical Research Strategies for Fructose Metabolism[1,2]

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ABSTRACT

Fructose and simple sugars are a substantial part of the western diet, and their influence on human health remains controversial. Clinical studies in fructose nutrition have proven very difficult to conduct and interpret. NIH and USDA sponsored a workshop on 13–14 November 2012, "Research Strategies for Fructose Metabolism," to identify important scientific questions and parameters to be considered while designing clinical studies. Research is needed to ascertain whether there is an obesogenic role for fructose-containing sugars via effects on eating behavior and energy balance and whether there is a dose threshold beyond which these sugars promote progression toward diabetes and liver and cardiovascular disease, especially in susceptible populations. Studies tend to fall into 2 categories, and design criteria for each are described. Mechanistic studies are meant to validate observations made in animals or to elucidate the pathways of fructose metabolism in humans. These highly controlled studies often compare the pure monosaccharides glucose and fructose. Other studies are focused on clinically significant disease outcomes or health behaviors attributable to amounts of fructose-containing sugars typically found in the American diet. These are designed to test hypotheses generated from short-term mechanistic or epidemiologic studies and provide data for health policy. Discussion brought out the opinion that, although many mechanistic questions concerning the metabolism of monosaccharide sugars in humans remain to be addressed experimentally in small highly controlled studies, health outcomes research meant to inform health policy should use large, long-term studies using combinations of sugars found in the typical American diet rather than pure fructose or glucose. *Adv. Nutr. 5: 248–259, 2014.*

Introduction

In response to public, industry, and academic interest in the health effects of fructose and other simple sugars, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Heart Lung and Blood Institute, and the USDA supported a workshop on 13–14 November 2012 entitled "Clinical Research Strategies for Fructose Metabolism" (1). Despite substantial ongoing research, it has been difficult to reach consensus regarding the roles played by fructose and other simple sugars in the recent rise in obesity and related metabolic diseases. Therefore, the main objective of this workshop was to identify the most important unanswered questions concerning dietary fructose and other sugars and explore optimal designs in clinical research to answer these questions. The workshop was chaired by Drs. John Bantle, Peter Havel, and Elizabeth Parks and was attended by >100 participants from academia, government, advocacy groups, and industry.

Fructose is a simple sugar that exists as a natural constituent of foods, in either free form or a 1:1 combination with glucose as the disaccharide sucrose. Fructose-containing sugars (FCSs)[8] include sucrose, high fructose corn syrup (HFCS; 42–55% fructose), honey, fruit juice concentrates, agave nectar, and crystalline fructose. Starting in the 1970s, there was both an increase in overall sugar consumption in the United States and a number of other countries and a replacement of sucrose with HFCS in beverages and

[1] On behalf of the organizers and participants in a 2012 National Institutes of Health and USDA-sponsored workshop, "Clinical Research Strategies for Fructose Metabolism." Funding for the workshop was from the National Institute of Diabetes and Digestive and Kidney Diseases, the National Heart Lung and Blood Institute, and the USDA.

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[8] Abbreviations used: FCS, fructose-containing sugars; HFCS, high fructose corn syrup; NAFLD, non-alcoholic fatty liver disease.

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other processed foods (2). Although the consumption of HFCS rose at approximately the same time as the increase in obesity and diabetes (3), no clear association between consumption of fructose in any form and metabolic consequences has been demonstrated conclusively in the absence of overeating and weight gain (4–6). In fact, the per capita availability of total added sugars in the United States had declined 13.7% by 2011 from a peak in 1999, and ~80% of that decline was from reduced HFCS (7), whereas rates of overweight among adults aged ≥20 y have continued to increase from 65.2% in 1999–2002 to 68.5% in 2007–2010, and diabetes rose from 9.8% to 11.4% in the same timeframe (8).

Studies of the fate of fructose in cells and animal models have also drawn attention to this molecule as a possible trigger for obesity and metabolic syndrome (9,10), and the ultimate goal of the clinical studies that use a fructose-rich diet intervention is to determine whether the metabolic pathways and health effects noted in animal models also occur in humans. Typically, however, animals are fed high doses of pure fructose or other sugars to elicit health-related outcomes [for instance, 60% of calories are derived from fructose for the fructose-fed rat model (10)]. Much of the mechanistic research performed on humans also compares substantial doses (for example, 25% of estimated total caloric need) of pure fructose with glucose. Humans normally ingest FCS rather than pure fructose, in variable doses and in combination with a variety of other foods. Therefore, research is still critically needed to understand whether fructose, in the doses and forms typically consumed, poses health risks to the American people.

Although similar to glucose in caloric yield, fructose is metabolized quite differently. Glucose is obtained from simple sugars and complex carbohydrates in the diet, is also produced endogenously, and is metabolized by virtually all cells and organs in the body. There are a multitude of regulatory systems that function to maintain blood glucose within tight limits, because both hyperglycemia and hypoglycemia have pathologic consequences. Therefore, extra glucose in the diet may provide unneeded calories but is likely metabolized along its normal highly regulated pathways. Fructose is almost exclusively derived from the diet. It is quickly removed from the circulation primarily by the liver in which a sizable fraction is converted to glucose. It promotes the synthesis and storage of glycogen and TG in the fed state (9). Animals fed large doses of fructose [rats at 60% of energy (10), monkeys supplemented with 75 g/d fructose (11)] gained considerable adipose tissue and exhibit many of the hallmarks of metabolic syndrome (12). Fructose, unlike glucose, is not associated with the postprandial rise in glucose and insulin secretion or diurnal leptin production that is regulated by insulin and glucose and that together contribute to short-term satiety and the long-term regulation of energy homeostasis (13). This raises the question of whether dietary fructose promotes overeating. These and similar observations form the basis for a focus on the human health effects of fructose and the fructose component of FCS.

The workshop was organized in 4 scientific sessions with ample time for discussion: *1)* an overview of fructose metabolism; *2)* its effect on energy balance; *3)* the effect of fructose feeding studies in humans and nonhuman primates; and *4)* the effect of fructose consumption on renal and hepatic metabolism and function. During a final session, hypothetical clinical study designs were presented for discussion. This exercise allowed participants to discuss best practices in protocol design for interventional clinical studies of the health and metabolic effects of fructose and simple sugars. The following brief description of the presentations and discussions is organized by science rather than by session. There are outstanding recent reviews of fructose metabolism and its role in human disease (14–16), and the current report will not attempt to improve on these. Rather, the emphasis here will be on novel information and dominant themes from the workshop presentations and discussions that inform clinical trial design.

Workshop Summary

Fructose and its metabolism

In 2004, sugar consumption in the United States was ~18% of energy in the typical U.S. diet. A total of 42% of this sugar was consumed as HFCS, which is a mixture of the monosaccharides glucose, fructose, and other sugars, 44% was in the form of sucrose, and other sugars, such as lactose and the free fructose and glucose in fruit, made up the balance (2). The contribution made by HFCS has been falling since approximately 1999, and the most recent data compiled by the USDA for 2011 show that HFCS now comprises ~36% of total added sugars in the food supply, whereas sucrose contributes ~51% (7). Regardless of the form, approximately half of all dietary sugar is fructose, and it is never consumed as the only sugar in a normal diet.

Bernadette Marriott analyzed 1999–2004 NHANES data to estimate mean fructose consumption, which varies by gender and age. On average, Americans consumed 49 g/d fructose (187 kcal/d), which is 9.4% of a 2000 kcal/d diet. On the low end, women aged >51 y consumed 32 g/d (8.0% of energy intake), whereas boys aged 15–22 y were the highest consumers at 75 g/d. American boys and girls this age thus consume on average 11–12% of their energy as fructose, and those at the 95th percentile of mean intake get 16.0–17.9% of their daily energy from fructose (2). Because fructose makes up approximately half of all sugars, these numbers must be doubled to describe U.S. sugar consumption. This is well above the AHA recommendation limiting all added sugars to 100 kcal/d (26.2 g/d) for women and 150 (39.3 g/d) for men (17), or the Health and Human Services/USDA Dietary Guidelines for Americans 2010, which, rather than addressing dietary sugars in isolation, recommend a combined limit for solid fats and added sugars of 5–15% of total calories (18).

The metabolic pathways and extremes of the phenotypes associated with high fructose diets have been investigated in animal models. Andrew Bremer presented the results of studies in which healthy middle-aged rhesus macaques

were fed, in addition to an ad libitum diet, a large daily dose of fructose (75 g/d, ~30% of ingested calories) for 1 y. Although total daily calorie intake was increased by only ~3% during fructose feeding, all monkeys exhibited increases of body weight and adiposity, insulin resistance, dyslipidemia, and increased circulating inflammatory markers, and 4 of 29 animals developed overt type 2 diabetes (11). Richard Johnson presented data on the metabolic pathways of acute and chronic fructose metabolism in rodent and cell models. Fructose rapidly enters the liver, one-third to one-half is converted to glucose, and it facilitates glucose uptake, as well. The sugars are oxidized, released as lactate, stored as glycogen, or converted into lipid to be stored in adipose or liver. Fructose is metabolized so quickly that, in rats fed 60% of calories as fructose, it can cause elevated plasma uric acid, possibly via a transient reduction in liver ATP and subsequent additional breakdown of adenine nucleotides. Uric acid has lipogenic effects at high concentrations (10,12,19,20). Chronic fructose or sucrose feeding induces proteins associated with fructose transport (glucose transporter 5) and metabolism (fructokinase, carbohydrate response element binding protein, sterol regulatory element binding protein-1c, peroxisome proliferator-activated receptor γ coactivator-1, and FA synthase) in rodents, further stimulating fructose disposal and potential side effects of obesity, inflammation, fatty liver, insulin resistance, hypertriglyceridemia, hypertension, and kidney disease (12,14,21).

These and similar observations in animal models and cells exposed to substantial doses of fructose underlie concern about the health effects of the typical amounts of dietary fructose in humans, but clinical studies have been difficult to conduct and interpret, and experimental evidence for these effects are controversial. John Sievenpiper reported the results of a series of meta-analyses of >50 small, short clinical studies of high daily fructose ingestion that used a variety of protocols on different populations. Fructose doses ranged from well below the typical U.S. diet (22.5 g/d, or 4.5% of required energy) to well above (300 g/d, equal to 55% of required energy) (4). Together, they show no consistent effect of fructose on important health outcomes, such as weight gain, hyperlipidemia, hypertension, hyperglycemia, and liver fat when individuals were constrained to a defined weight-maintenance diet. However, if sugar-sweetened beverages are added to an ad libitum diet, individuals often, but not always, fail to reduce food intake to compensate for the excess calories in the sweetened beverages, and they gain weight. Under these circumstances, many of the markers of metabolic syndrome (fatty liver, insulin resistance, TGs, uric acid) are clearly elevated with high fructose diets (4–6). It is not clear which effects are caused by fructose per se, the state of positive energy balance, or as a consequence of synergy between the 2. This is a fundamentally important question regarding the metabolic effects of dietary FCS that can only be addressed with new carefully controlled clinical studies. Furthermore, the measured outcomes of fructose feeding studies are highly dependent on study design. Meta-analysis is useful in that it can identify strong signals from a series of small studies but can also suppress the real outcomes of individual studies. Data interpretation is further complicated by the highly variable ability of individuals to absorb dietary fructose from the gut, which is rarely measured but which is affected by previous history of fructose ingestion, ethnicity, gender, obesity, and other dietary components (22,23). Therefore, these parameters likely affect the outcome of clinical research focused on the health effects of fructose. John Sievenpiper repeatedly called for additional larger, longer, well-designed studies.

Fructose effects on energy balance and ingestive behavior

Animals tend to accumulate fat on high fructose diets (10,11), and FCS consumption in the United States correlated with obesity over time until approximately 2000 in human population studies (7,8). But does fructose per se contribute to weight gain? If so, it must either reduce energy expenditure or increase food intake. Luc Tappy, Nancy Keim, Jonathan Purnell, and Sonia Caprio addressed this question by presenting data from studies of energy balance in individuals consuming fructose and by monitoring ingestive behavior or using brain imaging approaches after a fructose challenge. Ten weeks of fructose ingestion at 25% of the total energy requirement established at study baseline reduced resting metabolic rate relative to a diet with the same glucose dose in obese, insulin-resistant middle-aged individuals, but a similar amount of fructose for 4 wk (18% of total energy requirements) did not alter energy expenditure in lean individuals (24,25). In fact, a fructose meal increased postprandial thermogenesis relative to glucose in lean and obese individuals and those with diabetes, indicating that weight gain in response to fructose is not likely initiated through reduced energy expenditure (26–28). Do fructose calories go undetected by the brain and fail to curb hunger? There are currently only limited data available that can address this question. Intracerebroventricular infusion of 400 μg of glucose into mice increases ATP in the hypothalamus and reduces food intake on the following day, but a similar load of fructose reduces hypothalamic ATP and actually doubles subsequent food intake (29,30). How these results relate to ingestion of fructose, which is rapidly cleared by the liver, remains speculative. After a meal, hunger is curbed through the combined action of many neural and circulating signals. However, fructose ingestion (30% of total estimated energy needs) did not elicit the large postprandial excursion of the satiety hormone insulin seen in normal-weight women after a glucose meal, the typical diurnal rise in plasma leptin was reduced, and the orexogenic hormone ghrelin was less suppressed (13). Therefore, a fructose preload may result in the subsequent ingestion of more calories than a similar glucose preload as observed by some researchers (31). However, others observed that individuals eat less after a fructose than glucose challenge (32), and still others reported similar weight gain on high fructose and

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glucose diets (33). It must be noted that studies of food intake in humans are notoriously difficult to conduct. Individuals do not always eat naturally when under observation in the laboratory environment, other meal components, such as starch, may affect hunger, and studies in free living individuals rely on inadequate measures (questionnaires, food diaries) to detect small changes in a complex behavior.

Despite conflicting physiologic and behavioral data regarding the energy balance effects of fructose and glucose, imaging studies show differences in the response to i.v. infusion of 0.3 mg/kg of these 2 sugars in brain fMRI activation patterns (34) and in cerebral blood flow after ingestion of 75 g of sugar (35). It is difficult to control these studies for all the factors that affect the brain response to food, such as sensory perception (taste, smell, mouth feel, visual cues), circulating nutrients and hormones, memories, and emotional responses. Therefore, imaging data cannot always be interpreted in a straightforward way. However difficult it has been to demonstrate a single mechanism whereby fructose directly causes weight gain in humans, there do appear to be detectable phenomena in the brain and periphery that may be involved, and new tools and experiments are needed for additional investigation to better understand the relevance of brain activity to eating behaviors. These observations should inform design of future metabolic studies, as well; overfeeding studies that add sugar supplements to an ad libitum diet often result in a hypercaloric diet, and a positive energy status clearly conveys adverse health effects in the absence of high sugars. However, if humans routinely fail to compensate for added FCS as a result of fructose effects on satiety pathways, this experimental paradigm more faithfully represents the normal U.S. diet than those constrained to energy balance. A more complete understanding of the role of dietary sugars in brain responses and eating behavior will require the use of complex meals that contain both glucose and fructose.

Fructose effects on lipid metabolism

Elizabeth Parks reported that fructose, especially when absorbed quickly in liquid form, stimulates the de novo synthesis of lipid in the human liver. Stimulation of de novo lipogenesis has both quantitative and qualitative implications—upregulation of this pathway elevates intracellular malonyl-CoA, which increases the re-esterification of dietary fat (36,37), the FAs synthesized significantly contribute to liver TG stores (38) and to VLDL TG synthesis (39–41), and the primary products of de novo lipogenesis are saturated FAs that can have negative effects on cellular metabolic functions, such as insulin signaling (42). Fructose and glucose are highly synergistic; glucose facilitates intestinal absorption of fructose (22), and fructose stimulates glucose uptake into the liver (43,44). Inclusion of fructose with an oral glucose load stimulates lipogenesis 4-fold relative to glucose alone (36). In the presence of obesity, non-alcoholic fatty liver disease (NAFLD), and insulin resistance, lipogenesis is further elevated with a loss of circadian modulation that results in continuous lipid production (38). TG clearance and lipid oxidation are suppressed (23,45), leading to a substantial increase in total and saturated LDL TGs over that seen with glucose or complex carbohydrates (46,47).

After 10 wk of ad libitum diet including 25% of energy from a single monosaccharide sugar, Kimber Stanhope observed that obese insulin-resistant adults fed fructose had significantly increased de novo lipogenesis, increased visceral adiposity, reduced insulin sensitivity, elevated postprandial TGs, increases of lipid risk factors, including LDL-cholesterol, small-dense LDL, and remnant lipoproteins, reduced fat oxidation, and increased carbohydrate oxidation relative to those fed the same amount of glucose, despite similarly increased calorie intake and weight gain (23,33). The same group showed that effects can occur very quickly and be independent of positive energy balance. LDL-cholesterol and apo-B were increased after only 2 wk of either HFCS or pure fructose consumed at 25% of energy requirements in a group of individuals that included both normal-weight and overweight/obese young men and women but who did not experience significant weight gain (47). Jean-Marc Schwarz showed preliminary data in which individuals fed a controlled diet for only 1 wk with 25% of energy requirements derived from fructose appeared to have an impaired metabolic phenotype relative to those on an isocaloric complex carbohydrate diet (personal communication from Jean-Marc Schwarz, Touro University, Vallejo, CA).

Chronic hyperlipidemia (particularly in which saturated lipids are elevated), visceral adiposity, and fatty liver are all known risk factors for inflammation, insulin resistance, and cardiovascular disease. Although still an important open question that requires additional research, the presentations discussed above (see Fructose effects on lipid metabolism) raised the issue of whether long-term habitual ingestion of FCSs, even in the absence of weight gain, can lead to frank metabolic disease. Does the fructose-induced dyslipidemia experienced by obese insulin-resistant individuals make them more susceptible to diabetes and cardiovascular disease? What are the threshold doses and modulating factors? For instance, a protective role of physical activity was demonstrated recently (48).

Increased intrahepatic TG in children

Miriam Vos and Michael Goran presented studies focused on the role of fructose in obese children and adolescents with NAFLD. In 2010, 10.7% of adolescents in the United States had fatty liver, including almost half of all obese boys, who are particularly susceptible (49). Fructose feeding (33% of required energy for all 3 meals of 1 d) increased 24-h circulating TGs in all adolescents by almost 2-fold, but plasma TG concentrations were higher and more variable in those with fatty liver (1421 vs. 622 mg/dL) (50). Excess weight gain can be reduced by avoiding sugar-sweetened beverages for 18 mo in normal-weight children aged 5–12 y (51). Michael Goran discussed the influence of ethnicity and race. It was observed recently that, after 2 y, an intervention to reduce intake of sugar-sweetened beverages in

overweight and obese adolescents showed no differences in weight gain overall, but analysis of the data by ethnicity showed 10 kg less weight gain in Hispanic children compared with untreated Hispanic children (52). Hispanic children are more likely to have fatty liver and insulin resistance, in part because of a high incidence of the patatin-like phospholipase domain-containing protein 3 susceptibility gene variant. African-American children rarely have this mutation and have a much lower incidence of fatty liver, but if they do develop fatty liver they are also more likely to be insulin resistant and have diabetes, as well. It is interesting to note that ~71% of African Americans experience fructose malabsorption relative to only 43% of Hispanics (23). It is not known whether this helps protect African Americans from the metabolic consequences of a high FCS diet, especially because malabsorption may not be appreciable except when fructose is ingested in the absence of other sugars and nutrients (53,54).

These studies reveal that genetics, ethnicity, and gender all likely interact with diet to produce the current high amounts of obesity, NAFLD, and insulin resistance. Restriction of sweetened drinks can have a measureable positive effect, although the relative contributions from reduced fructose, altered satiety, or simple calorie restriction have not been investigated thoroughly. Because very early metabolic disease is associated with lifelong health risks, it is important to identify and remediate the triggers of childhood obesity and attendant complications.

Influence of fructose on adipose inflammation and liver and kidney disease

This portion of the conference explored some of the mechanisms that may mediate the effects of fructose metabolism on disease and organ dysfunction. Inflammation is recognized to contribute to insulin resistance and is associated with increased deposition of visceral adipose tissue, NAFLD, and elevated saturated lipids, all of which are seen with both high amounts of ingested fructose and obesity. To tease these apart, Mario Kratz showed preliminary data from a crossover study in which individuals on an ad libitum diet drank beverages providing 25% of their daily energy requirement from glucose, fructose, or with the noncaloric sweetener aspartame for 8 d. Treatment phases were separated by washout periods. Glucose and fructose drinks resulted in similar levels of overeating (15–19%), but circulating amounts of the inflammatory markers C-reactive protein and interleukin-6 were elevated only in individuals consuming fructose (55) (personal communication from Mario Kratz, Fred Hutchinson Cancer Research Center, Seattle, WA). A recent paper found no increase in inflammatory markers in lean young individuals with 4 wk of 150 g/d (575 kcal) fructose or glucose consumption as part of a hypercaloric diet (56).

Manal Abdelmalek presented preliminary data indicating that a fructose challenge might serve as a biomarker for liver disease. The transient dip in liver ATP seen with ^{31}P magnetic resonance spectroscopy during infusion of 250 mg/kg body weight fructose is blunted in non-alcoholic steatohepatitis, and recovery of the ATP signal may be delayed as liver disease progresses and mitochondrial function is compromised (57). Perhaps secondary to this disturbance in liver ATP, ingestion of high amounts of fructose is associated with elevated uric acid in rodents (58) and humans (19). Miguel Lanaspa reported the results of studies in rodents and cultured cells showing how fructose, in part via the generation of uric acid, provokes endothelial inflammation, hypertension, and altered glomerular hemodynamics that may ultimately underlie chronic kidney disease (59). He also showed data that support the idea that fructose can be generated endogenously in the polyol pathway through the action of aldose reductase and sorbitol dehydrogenase. Hepatic and plasma fructose were elevated by 30–50% in mice fed an unpurified diet after 14 wk, during which drinking water was replaced with 10% glucose solution, and these changes, as well as elevated liver TG, were abolished in mice that lacked aldose reductase (60). It is not known whether fructose is produced endogenously in individuals eating a typical diet.

In summary, the presentations and discussion at the workshop indicated that high-quality studies to investigate the effects of dietary fructose and FCS are still needed to understand whether the disease mechanisms postulated from animal experiments are operational in humans. Many of the studies designed to elucidate health and metabolic effects used pure fructose at 25% or more of required energy as an intervention, which rarely occurs in a normal diet. Therefore, larger studies in susceptible populations using real-world dietary interventions (for instance, sucrose or HFCS within the range typically consumed in the United States) would clarify whether dietary sugars play a role in disease progression and inform the public concerning safe amounts of dietary disaccharides or combinations of monosaccharides.

Panel Discussion: Commentary on Design of Clinical Studies for Investigation of Fructose Metabolism and the Health Effect of Sugars in the Diet

Workshop participants submitted responses to the following questions, and the answers are summarized in **Tables 1–3**. In addition, 3 groups of attendees were asked to present hypothetical study protocols in a last session led by Meredith Hawkins. These hypothetical studies focused on at-risk populations, such as obese children and adults with prediabetes, and on the outstanding questions in Table 1. Important points taken from discussion of these protocols are found in Table 2: *1)* what are the important outstanding questions concerning fructose and human health that remain to be answered? (Table 1); *2)* what are important experimental parameters and other design considerations for clinical studies? (Table 2); and *3)* what are important outcomes variables for clinical studies? (Table 3).

A few words are needed about some questions that were raised but after discussion were considered inappropriate for inclusion in Table 1. Although differences of opinion exist (61), overall there was a sense that the considerable body

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TABLE 1 Important questions regarding the metabolism and health effects of fructose-containing sugars[1]

Does fructose in the diet uniquely alter ingestive behavior or energy balance and therefore initiate or worsen obesity?
Does fructose affect satiety after a meal differently than other nutrients?
Does the state of obesity alter the influence of fructose on satiety and energy homeostasis?
Are brain chemistry and function changed by chronic consumption of FCS? Which regions and pathways are involved—the reward systems, regions that control energy homeostasis?
Is the energy of FCS consumed in beverages sensed differently by the body than sweetened solid food, and does that affect satiety and ingestive behavior?
Does fructose promote fat deposition and obesity more than other nutrients with similar calories?
Does a diet high in FCS promote diabetes or liver, cardiovascular, or kidney disease beyond that caused by excess calories?
Does chronic FCS consumption contribute to the development of insulin resistance in muscle, liver, and adipose tissue? Can consumption of FCS influence β-cell function and thereby predispose to type II diabetes?
Does chronic FCS consumption contribute to the onset of diabetes?
Does habitual FCS consumption contribute to fatty liver, hypertension, or cardiovascular and kidney diseases?
Is there an effect of FCS on the gut microbiota and intestinal permeability, which play a role in many diseases?
Does a diet high in FCS differently affect some populations?
Are there ethnic or racial populations that are more susceptible to fatty liver disease or other metabolic complications when exposed to a diet high in FCS?
Are children who ingest high amounts of fructose at increased risk of future metabolic complications?
Are individuals with chronic metabolic disease (obesity, diabetes, cardiovascular disease) more adversely affected by a diet rich in FCS?
Are there maternal diet effects of FCS on offspring with respect to infant growth, learning, and future metabolic disease?
Do chronic over-nutrition and physical activity modulate the effects of a high FCS diet?
Does chronic ingestion of FCS worsen the health effect of a hypercaloric diet?
Does chronic ingestion of FCS worsen the health effect of a sedentary lifestyle? Can physical activity counteract health effects of a diet high in FCS?
If fructose in the diet affects health,
What is the dose relation between dietary fructose and adverse metabolic effects, and to what extent does variation in fructose absorption play a role?
Do the non-sugar components of fruit (vitamins, polyphenols, anthocyanins, fiber) mitigate any adverse effects of dietary fructose?

[1] FCS, Fructose-containing sugars.

of research comparing health effects of HFCS (45–55% fructose) with sucrose (50% fructose) had not shown large differences between these 2 sources of simple sugars. There was also discussion regarding the usefulness of comparing diets rich in pure fructose and glucose; because these pure monosaccharides are not found in normal diets, studies focused on the health effects of sugar-rich diets should likely use sucrose, HFCS, or a combination of glucose and fructose in other forms (i.e., fruit). However, it remains important to understand differences in how the human body handles the monosaccharides, and such studies are included herein in the category of "mechanistic studies" (Table 2). It was noted that glucose is abundant in blood and produced rapidly from stored glycogen or ingested carbohydrates and proteins and that the ratio of glucose/fructose in a typical diet is >3:1 (62). Therefore, it is unlikely that fructose, even when present as the only simple sugar in the diet, is ever metabolized in the absence of glucose.

Table 3 lists the outcomes measures that workshop participants judged to be most informative, divided into acute measures of sugar and lipid metabolism, eating behavior or brain activity, and behaviors and disease biomarkers that may be altered by chronic changes in dietary sugar ingestion. Although the techniques for making these measurements will not be discussed in any detail, it is important to note that, to be compared among groups and interpreted, some of them may require a short preparatory period lasting ≥1 d, during which diet and activity are controlled [such measures include de novo lipogenesis and insulin resistance (33,36)]. The hydrogen breath test, although not entirely reliable, indicates that 30–80% of healthy individuals experience malabsorption, but not necessarily symptoms, after large doses of ingested fructose (25–50 g) in the absence of other nutrients (53,54). This must be kept in mind. It should also be noted that the acute response to a sugar challenge can be used to study a particular pathway (such as the appearance of fructose carbons in newly synthesized FAs) but can also serve as a disease biomarker [such as the oral glucose tolerance test or the recovery rate of liver ATP after fructose infusion (57)], and so care should be taken to distinguish among the reasons for including outcomes measures in the study design and to use only those that can be interpreted in light of the hypothesis and design.

In general, designs for interventional clinical studies of dietary sugars tend to fall into 2 categories: *1)* small short mechanistic studies designed to validate observations made in animals, to elucidate the pathways of fructose metabolism or influence of fructose on other pathways in humans; and *2)* larger, longer studies focused on clinically significant disease outcomes or health behavior resulting from chronically consumed diets, designed to test hypotheses generated from short-term mechanistic studies in humans or epidemiologic studies and to provide data for dietary recommendations and health policy. The design criteria can be quite different for these 2 types of studies. However, depending on the specific questions being posed, study designs can mix elements of the 2, and care should be taken to ensure that outcomes are interpretable in terms of either biologic mechanism or the realm of public health.

Mechanistic Studies

Hypotheses

The hypothesis for small, short studies should be narrowly framed and may be highly mechanistic (Table 1) but should

TABLE 2 Protocol design considerations[2]

Variable	Mechanistic studies	Disease outcomes or health behaviors studies	Notes
Participant characteristics	Gender		Men may be more susceptible to hypertriglyceridemia (46,67) and visceral fat gain with high sugar diets (33).
	Age		Children and adolescents have a very high fructose diet (2). Older individuals may be at risk of metabolic complications (33).
	Pregnancy or menopausal status		Pregnancy reduces fat oxidation, and postmenopausal women may be more susceptible to changes in metabolic parameters (68).
	Fitness level, exercise		Fitness and exercise may modify metabolic response to a high sugar diet (48,69).
	Patient populations		Individuals at risk of metabolic disease may be appropriate if health effects are reversible and do not put individuals at risk (45,70).
	Fructose intolerance, absorption		A substantial fraction of healthy individuals may be "fructose intolerant" in the absence of other nutrients (23,53,71). Coingestion of glucose increases fructose absorption (53). For acute response to pure fructose, absorption can be measured to ensure proper dose (54).
	Participant's habitual intake of sugars		A high fructose diet can induce the enzymes in the fructose pathways (12,14,21)
	Baseline biochemical values		The higher the fasting blood TGs, the greater the response to fructose (72).
	Control groups		Several control groups may be needed.
Group size	Small number of well-defined participants	Large heterogeneous participant groups	Large studies can be powered to investigate the effect of race, gender, age, disease risk factors, etc. Small studies may require homogeneous populations with similar habitual sugar intake.
Study baseline diet	Same dietary baseline imposed in all test groups before intervention	For long dietary studies in which groups are to be compared at completion, a common dietary baseline may not be needed	Habitual diet may contain more sugar than intervention, which may affect outcomes especially in short studies. If habitual diet is similar among participants, a common baseline diet imposed before the study may be less important.
Dietary sugars	Pure monosaccharides (glucose, fructose) or FCS (HFCS, sucrose)	Use typical foods and beverages sweetened with sucrose, HFCS, etc.	Dissolved sucrose can be unstable and hydrolyze to free glucose and fructose, so sucrose, fructose, and glucose should be measured in test liquids before and after study (61).
	Sugar dose can be higher than the typical U.S. diet to elicit measureable responses in a short period of time	Sugar dose should not greatly exceed typically ingested amounts	Especially for at-risk populations or those already consuming a high FCS diet, a reduced-sugar intervention to look for evidence of disease biomarker improvement may be appropriate. Refer to the study by Marriott et al. (2) for 2004 U.S. fructose ingestion rates.
	Form of sugar	Form of sugar	Sugar in liquids is more lipogenic (73) and may affect satiety differently than the same sugar in solids (63).
	Test sugar provided as supplement or distributed throughout all foods in diet	Sugar often provided as supplement but can be distributed throughout all foods in diet	Supplements (often beverages) are easy to use and naturalistic but may represent calories added to the diet. It may be difficult to control ingestion of sugars from the rest of the diet.
	Dietary control	Dietary control	Control comparator of highest concern (starch, artificial sweetener, fruit juice, glucose, low FCS dose). Diets should be matched when possible for taste, nutrient content, etc.
	Study success depends on full compliance with diet	Study success will depend on close compliance with diet	Compliance to study diet can be monitored, potentially with several indirect measures if no direct measure is available [*p*-aminobenzoic acid (65),carbon isotope ratio (66), riboflavin (23)].

(Continued)

TABLE 2 (*Continued*)

Variable	Mechanistic studies	Disease outcomes or health behaviors studies	Notes
Non-sugar nutrients and calories	Eucaloric, hypercaloric, or ad libitum diets can be used, diets can be highly artificial	Ad libitum diets should be highly naturalistic and generalizable to questions concerning human health and/or behavior	Eucaloric or hypercaloric diets can be provided to participants. Compliance should be monitored. Ad libitum diets (often in addition to a sugar-sweetened supplement) are used to study ingestive behavior or to allow for naturalistic eating behavior. Ad libitum diet can be a participant's normal diet, very low sugar diet, or foods made with the test sugar. Food ingestion can be measured, and weight may be a useful surrogate.
	Control diet	Control diet	The type of dietary control depends on the research question, but for mechanistic studies, the control diet should be matched to test diet for energy and other characteristics (fiber, fat type, cholesterol content) as much as possible.
Duration	Can be of short duration (hours to weeks), long enough to test hypothesis	Long duration (weeks to months) to account for habituation and caloric compensation and to achieve health-related outcomes	The duration should be specific for the metabolic phenomena to be studied. For example, alterations in lipoprotein synthesis and turnover may take 6 wk (72), but measureable changes in fat distribution may take 8 wk (33).
	Free living or inpatient	Free living	Free living studies generate data that are more generalizable but may need a larger sample size to accommodate greater variability.
Participant preparation before tests	Metabolic measurements (insulin resistance, de novo lipogenesis)		Recent exercise (69), alcohol intake (74,75), weight stability, or sleep (76) can all affect metabolic outcomes. A short inpatient period may be needed to normalize diet/activity before certain metabolic measurements.

[2] FCS, fructose-containing sugars; HFCS, high fructose corn syrup.

still address human health. The experiment can be designed to answer only 1 such question or a small number of related questions. Outcomes to be measured can be focused on biologic pathways, keeping in mind that an outcome can be statistically significant without being clinically relevant. Demonstration of both is needed.

Study and control populations

Because mechanistic studies must be highly controlled to ensure compliance to the protocol and tend to use difficult complex measurements, they can be expensive and demanding. Depending on the hypothesis to be tested, the study population can therefore be very narrowly defined and the number of participants and duration of the study quite limited.

More than 1 control group may be needed to interpret the observed effects. For instance, if the study group is obese insulin-resistant children treated with a low FCS diet to monitor changes in liver fat, controls may include similar children on a high FCS diet and obese insulin-sensitive children on a low FCS diet. An alternative to multiple control groups might be a very narrowly defined hypothesis.

Study diets and duration

Baseline diet. An individual's habitual sugar ingestion before participating in the study may be an inclusion/exclusion criterion, because chronic high FCS ingestion can upregulate the pathways involved in fructose metabolism and alter the metabolic response to the intervention diet (14). Diet may also affect the health outcomes to be measured in response to an intervention, such as liver fat or rates of de novo lipogenesis. Alternatively, all participants enrolled in a short mechanistic study could be treated with a common lead-in diet for some period of time before the intervention. If there is no normalizing baseline period, care should be taken when comparing changes from pre-intervention to post-intervention among groups.

Defined diets. The intervention diet can be highly artificial and controlled, and inpatient studies conducted in clinical research centers can markedly increase compliance with dietary regimens. At 1 extreme would be a multi-week inpatient stay in which participants consume only prepared meals that had been made either with pure fructose as a sweetener or without fructose (i.e., sweetened only with glucose). Defined diets and/or inpatient experiments may be necessary to investigate whether fructose affects health outside of the weight gain caused by a hypercaloric diet, to understand the protective role of exercise, or when food intake needs to be carefully monitored to understand whether fructose causes humans to overeat.

Ad libitum diets. It has been observed that, when sugar-sweetened beverages are added to the diet, individuals sometimes, but not always, fail to compensate by reducing the intake of other food and therefore consume more calories overall (31–33). However, it remains an open question

TABLE 3 Outcomes measures

Acute measures of fructose metabolism
De novo TG production
Plasma lipids measured over the course of 24 h
Gluconeogenesis, hepatic glucose production
Glucose uptake (muscle, liver, etc.)
Hunger/satiety questionnaire, hunger/satiety hormones, subsequent food intake
Functional brain activity
Liver ATP transient
Serum uric acid
Postprandial plasma glucose and insulin, leptin, etc.
Flux through metabolic pathways used by and influenced by fructose
Chronic effects of fructose consumption
Weight, BMI
Mood, fatigue, sleep patterns
Daily energy intake
Daily physical activity, spontaneous or intentional
Energy balance
Taste perception, food choice, thirst
Fat mass and distribution, body composition
Protein synthesis
Liver and muscle fat
Liver and kidney function
Bone health
Blood pressure, plasma lipids
Risk factors for chronic diseases
Whole-body and liver insulin resistance
Glucose tolerance, fasting plasma glucose, hemoglobin-A1c, diabetes onset
Fasting plasma nutrients, metabolites, hormones, adipokines
Inflammation markers
Metabolomic analysis
Gene expression

whether there are physiologic mechanisms that mandate overeating as an appropriate biologic response to sugar consumption. There is also concern that the energy in sugars ingested in beverages may be sensed differently than the same sugars eaten in solid food (63). The effects of sugars on eating behavior can be studied using a sugar-sweetened supplement added to a diet in which the participants are otherwise allowed to eat normally (ad libitum diet). Beverage supplements have frequently been used—they are a relatively easy route to deliver a measured dose of sugar, and they are naturalistic and easy to comply with because individuals are used to drinking them. Sugar patties or other candy-like supplements added to an ad libitum low sugar diet could also be used to investigate the effect of solid vs. liquid sugars on eating behavior or metabolic pathway intermediates. Alternatively, a study focused on the role of fructose in development of NAFLD might be better done using a completely controlled diet made with pure glucose vs. those made with FCS.

Special consideration is needed when an ad libitum diet is used in a mechanistic study. The intervention needs to be applied for a long enough duration to achieve normalistic behavior before outcomes are measured, because short-term ingestive behavior could be altered by, for example, the knowledge that one is being observed, by unfamiliar foods in the test diet, and by dose and type of sugar tested. The palatability of the supplement or intervention diet is important, because many sensory inputs (taste, smell, texture, visual cues, other nutrients, etc.) affect the sensation of hunger and eating behavior (and therefore calorie intake).

Control diets. The choice of diet for a control group must be chosen in light of the specific mechanistic question to be addressed, with attention paid to the likelihood of compliance. For highly mechanistic studies, non-sugar nutrients and palatability should be matched as closely as possible to the study diet. For studies meant to inform on the influence of typical dietary sugars, a diet low in FCS (5% of needed energy) or one in which all sugars are replaced by complex carbohydrates, may be the appropriate control for a high FCS (25–30% of energy) diet. For studies focused on fructose metabolic pathways or mechanisms by which fructose could affect eating behavior, replacement of a fructose-rich diet with a glucose-rich, fructose-free diet may be more informative. More than 1 control diet may be needed to ensure that outcomes can be interpreted.

Duration. The study duration need not be long for mechanistic questions. For extremely acute measures (brain imaging, postprandial satiety, metabolic pathways), the duration may be a few hours. Measureable changes in liver fat or insulin resistance in response to a high sugar hypercaloric diet may require a single week (64), whereas alterations in weight, body composition, and eating behavior may take a longer period of time and different endpoints will require varying durations to achieve steady state. Pilot studies may be useful to determine optimum duration.

Disease Outcomes or Health Behavior Studies

Hypotheses

Disease outcomes or health behavior research is designed to inform health policy and dietary guidelines. Studies tend to be of long duration and have enough participants that the results can be generalizable. It can be difficult to control all the variables, and therefore the outcomes must be of significant magnitude to be able to overcome large inter-individual variability.

The hypothesis may be quite broad and encompass a range of health-related questions (Table 1). Interventions must be fairly easy to perform and reflective of normal behaviors. It is unlikely that highly specific mechanistic questions can be answered with a very naturalistic intervention because of the difficulty of monitoring compliance with the test diet and accurate measurement of calorie intake. Care should be taken to distinguish between the hypothesis that ingestion of a particular diet modifies a health outcome, and the hypothesis that advice to eat a particular diet modifies the health outcome.

Study and control populations

For dietary health outcomes studies, large diverse groups of individuals yield the most generalizable data. If a more narrowly defined population, such as children or teenagers, is to be studied, it should still be representative of a substantial group

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of typical individuals. The choice of a control population is important. When interventional diets are to be compared, the control and study groups are likely as closely matched as possible. For studies comparing groups of individuals, such as those focused on health disparities, groups should be matched as closely as possible for remaining traits, such as age, BMI, gender, and disease state. Researchers should consider that the habitual diets of different ethnic groups may contain very different amounts and types of sugars, which may affect study outcomes and present issues for compliance.

There are particular ethical considerations when studying at-risk populations in long-term protocols. An intervention designed to improve health outcomes could be used, such as a diet without sugar-sweetened beverages for 12 mo to see whether liver fat is reduced or fasting blood sugar is less impaired. Such an intervention tests a realistic strategy to improve health in an at-risk population and could inform health policy. For participants who enter the study with a low-quality diet, they may benefit from knowledge and experience at the conclusion of the trial that could help them achieve better eating habits.

Study diets and duration

For disease outcomes and health behaviors research, the intervention should be as naturalistic as possible to obtain generalizable data. Sugars in the study and control diets should be in the form and amounts typically found in the U.S. diet. It may be too expensive to supply the entire diet for an extended period of time to a large group of study participants, and therefore a design that relies on a dietary supplement or dietary advice may be necessary. Unless the study design allows for careful monitoring of calorie intake, it may be impossible to know whether differences in health outcomes (or even weight or fat mass) among groups are attributable to the metabolism of the sugar in the test diet or differences in caloric intake. The hypothesis should be framed in light of these considerations.

The study should be long enough, with a large enough population, to be able to detect meaningful differences in health-related biomarkers between study and control groups. Although it would be unethical to use an intervention to induce real disease endpoints (such as diabetes or a cardiovascular event), any biomarkers used as outcomes should be widely accepted as meaningful indicators of disease risk or progression.

On the importance of compliance

Studies of diet and nutrition are notoriously difficult because of the complexity of human eating behavior and the lack of tools to control and measure it accurately, yet the success and interpretability of a dietary study, particularly one focused on biologic mechanisms, is completely dependent on having the baseline, intervention, and control diets imposed as designed. Compliance should be monitored, and, when no direct measures are available [such as direct observation of test beverage ingestion or a specific blood or urine test for biomarkers included in the test diets (33,65,66)], it may be prudent to use multiple independent indirect measures (i.e., return of uneaten test diet, dietary recall questionnaires, interviews, participant weight). Large studies may need to be piloted in a small group of individuals to determine whether the intervention can be accomplished and compliance can be monitored. Realistic projections about compliance over the long term are essential to achieve adequate statistical power to test the primary hypothesis.

Conclusions

The workshop served to highlight the challenges faced when designing clinical trials to monitor the health effects of the current FCS-rich U.S. diets or to identify the mechanisms underlying the metabolic effects of dietary fructose in humans. It is clear that, although any one design may answer only a subset of the important questions surrounding health and eating behavior, a variety of well-designed, carefully conducted studies when taken together will provide the experimental evidence needed for the formulation of dietary recommendations and health policy. Discussion brought out the opinion that, although many mechanistic questions concerning the metabolism of monosaccharide sugars in humans remain to be addressed experimentally in small highly controlled studies, health outcomes research meant to inform health policy should use large, long-term studies using combinations of sugars and intake amounts found in the typical U.S. diet rather than pure fructose or glucose. For these studies, HFCS and sucrose are likely to be very similar. The most important outstanding questions include whether there is an obesogenic role for FCS via effects on eating behavior and energy balance, and whether a high FCS diet promotes metabolic changes that lead to type 2 diabetes, liver, and cardiovascular disease. If so, it is important to determine the doses of sugar that alter health outcomes and identify any special populations that are particularly susceptible or protected from the adverse health effects of a high sugar diet.

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