


## Obesity Comorbidity/Etiology and Pathophysiology

# Pathways and mechanisms linking dietary components to cardiometabolic disease: thinking beyond calories

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### Summary

Calories from any food have the potential to increase risk for obesity and cardiometabolic disease because all calories can directly contribute to positive energy balance and fat gain. However, various dietary components or patterns may promote obesity and cardiometabolic disease by additional mechanisms that are not mediated solely by caloric content. Researchers explored this topic at the 2017 CrossFit Foundation Academic Conference 'Diet and Cardiometabolic Health – Beyond Calories', and this paper summarizes the presentations and follow-up discussions. Regarding the health effects of dietary fat, sugar and non-nutritive sweeteners, it is concluded that food-specific saturated fatty acids and sugar-sweetened beverages promote cardiometabolic diseases by mechanisms that are additional to their contribution of calories to positive energy balance and that aspartame does not promote weight gain. The challenges involved in conducting and interpreting clinical nutritional research, which preclude more extensive conclusions, are detailed. Emerging research is presented exploring the possibility that responses to certain dietary components/patterns are influenced by the metabolic status, developmental period or genotype of the individual; by the responsiveness of brain regions associated with reward to food cues; or by the microbiome. More research regarding these potential 'beyond calories' mechanisms may lead to new strategies for attenuating the obesity crisis.

**Keywords:** Cardiometabolic disease, dietary fat, dietary sugar, obesity.

**Abbreviations:** apo, apolipoprotein; BMI, body mass index; BMIZ, BMI z-score; CHD, coronary heart disease; CHO, carbohydrate; CVD, cardiovascular disease; DNL, *de novo* lipogenesis; Ereq, energy requirement; FFA, free fatty acids; fMRI, functional magnetic resonance imaging; FPI, fasting plasma insulin; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HFCS, high-fructose corn syrup; HOMA-IR, homeostatic model assessment insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NHANES, National Health and Nutrition Examination Survey; NIH-AARP, National Institutes of Health–American Association of Retired Persons; NNS, non-nutritive sweetener; *PNPLA3*, patatin-like phospholipase domain-containing protein 3; *P/B*, *Prevotella* spp. to *Bacteroides* spp.; PUFA, polyunsaturated fatty acid; RCT, randomized controlled trial; sdLDL, small dense low-density lipoprotein; SFA, saturated fatty acid; SSB, sugar-sweetened beverages; TG, triglyceride; T2D, type 2 diabetes; USDA, United States Department of Agriculture; VLDL, very-low-density lipoprotein.

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## Introduction: Janet King, Laura Schmidt

With the emergence and global spread of the highly palatable, processed foods that are major components of the Western diet and the rising rates of obesity and cardiometabolic diseases (e.g. cardiovascular disease [CVD] and type 2 diabetes [T2D]), the focus of nutrition science has shifted from concerns about dietary deficiencies to concerns about dietary excesses. Given the well-documented relationship between Western diet and obesity (1–4) and between obesity and cardiometabolic disease (5) (Fig. 1), the dietary excess of greatest concern is caloric consumption. Unquestionably, most Americans are consuming too many calories; 69% of US adults are overweight (6), and new estimates, just released, indicate 39.8% of US adults are obese (7).

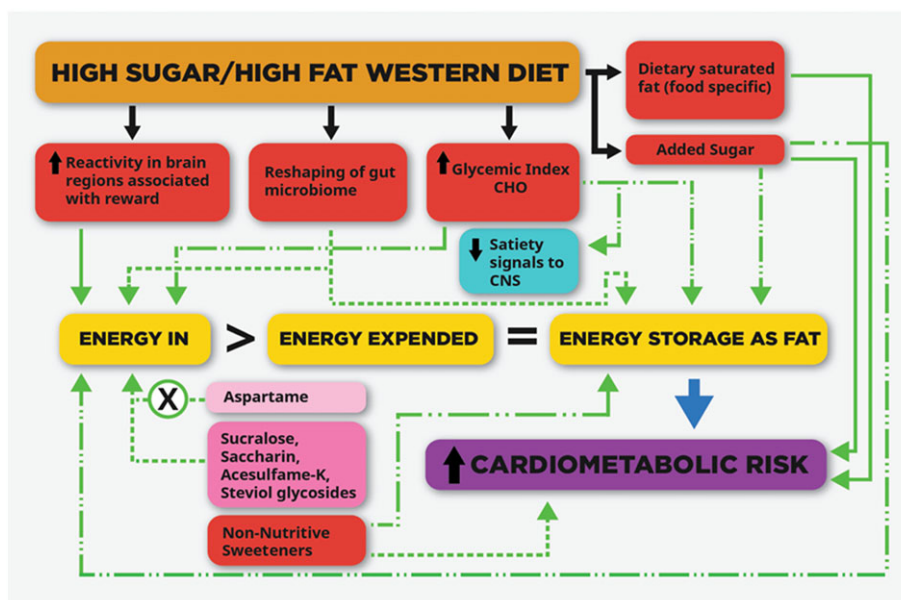
Yet a major question remains: are all calories equal with regard to effects on cardiometabolic disease and obesity? This was the question that was deliberated by national and international researchers at the 2017 CrossFit Foundation Academic Conference 'Diet and Cardiometabolic Health – Beyond Calories' in San Francisco, California.

In one important aspect, the answer to this question is clearly 'yes'. The first law of thermodynamics states that energy can be neither created nor destroyed: energy in = energy out. Nutritionally, this means that once full growth is reached, if the energy consumed from foods and beverages is greater than the energy expended through metabolism, thermogenesis and physical activity, the surfeit will be stored mostly as body fat. Thus, our traditional energy balance paradigm

dictates that excess consumption of calories from any food will drive energy storage and increase the risk of obesity and cardiometabolic disease as defined by

$$\text{Energy in} > \text{energy expended} = \uparrow \text{energy storage as fat.}$$

There is no doubt that positive energy balance, due to excessive caloric consumption and/or inadequate physical activity, is the main driver of the obesity and cardiometabolic epidemics. However, it was the first objective of the Conference to consider whether certain dietary components increase risk for cardiometabolic disease by metabolic effects that are not driven solely by positive energy balance and fat gain. The evidence specific for saturated fat and added sugar, and the challenges pertaining to obtaining such evidence, were presented and discussed. It was the second objective of the conference to consider whether certain dietary patterns or components have the potential to promote fat gain via mechanisms that are in addition to their specific contribution of calories to the 'energy in' side of the energy balance equation. These mechanisms could include promoting excess energy consumption, more efficient extraction of energy from food within the intestine or preferential partitioning of energy towards fat storage. Evidence addressing this question was presented and discussed for long-debated topics such as high carbohydrate (CHO) diets and non-nutritive sweeteners (NNSs). Also, newer, emerging evidence was presented for the effects of caloric and NNSs during critical periods of development and for effects



**Figure 1** The potential links between dietary patterns and components and cardiometabolic risk. The totality of the evidence suggests that added sugar and certain saturated-fat-containing foods increase risk for cardiometabolic disease by metabolic mechanisms that are not mediated solely by positive energy balance and fat gain. There is also evidence that certain dietary patterns or components can increase 'energy in' and/or 'energy storage as fat' via mechanisms that are not explained solely by their specific contribution of calories to the 'energy in' side of the energy balance equation. The strength of the links is indicated by the green lines as follows. *Solid green line*: supported by evidence from animal studies and clinical observational and dietary intervention studies. *Dashed line*: evidence from prospective cohort studies and/or clinical dietary intervention studies suggests heightened risk during critical developmental periods and in persons with compromised glucose tolerance or insulin sensitivity. *Dotted line*: supported mainly by evidence from observational and/or animal studies only. *Dotted line w/X*: evidence from 100% of the clinical dietary intervention studies do not support the evidence from the observational and animal studies. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

of the *Western* diet on reactivity in brain regions associated with reward and on reshaping gut microbiota. This paper summarizes the evidence presented for each topic, the areas of agreement, the topics that require more research and the nutritional approaches that should be emphasized for improving public health.

### Objective 1: do certain dietary components increase risk for cardiometabolic disease by metabolic effects that are not driven solely by positive energy balance and fat gain?

#### Dietary fats: Ronald Krauss

A review, published in 1958, on the relationship of dietary fat to atherosclerotic disease stated:

The evidence now appears to be conclusive that sufficient quantities of polyunsaturated fat in the diet, with proportional decrease in saturated fat, will result in major decrease in blood lipid. Some evidence indicates that such blood lipid lowering produces a desirable effect upon existing atherosclerosis (8).

Fast forward to 2014 and there are at least two reviews on the same topic. One of them concludes that 'data provide

support for current recommendations to replace saturated fat with polyunsaturated fat for primary prevention of coronary heart disease (CHD)' (9). The other concludes 'current evidence does not clearly support CVD guidelines that encourage high consumption of polyunsaturated fatty acids and low consumption of total saturated fats' (10). Clearly, both cannot be correct. Therefore, in addition to reviewing the evidence that supports the conclusion that dietary fats differ in their weight-independent effects on cardiometabolic risk, it is also important to consider the challenges involved in conducting and interpreting nutritional research on the topic of dietary macronutrients. These challenges have contributed to 60 years of sometimes inconsistent and conflicting conclusions regarding the role of dietary fat and other macronutrients in the development and prevention of cardiometabolic disease.

#### Macronutrient subtypes

The major macronutrients, particularly fat and CHO, include subtypes that need to be carefully controlled/monitored and reported when conducting nutrition studies. This may seem obvious, but the results of many nutrition studies are challenging to interpret because the amounts and types of fat or CHO were not monitored and/or reported. One of the many examples is a study designed to compare the cholesterol-lowering and triglyceride

(TG)-lowering effects of four fat-restricted diets (30%, 26%, 22% and 18% of energy as fat), achieved via CHO replacement. The results were reported in the *Journal of the American Medical Association* in 1997 without any description of the type of CHO prescribed or consumed. The words sugar, simple CHO, sucrose, high-fructose corn syrup (HFCS) or other key CHO descriptors do not appear in the article (11). In the case of fats, one must also consider that beyond the major categories of saturated, monounsaturated, *n*-3 and *n*-6 polyunsaturated, and trans fatty acids, there is heterogeneity within each group that may contribute to differing biologic and clinically relevant effects. Failure to distinguish between macronutrient subtypes, which has occurred even in recent reports (12), has likely contributed to conflicting dietary conclusions and to the 'high fat versus high CHO diet' debate that still rages today.

### Food context

The food context within which macronutrients are consumed can also have a significant impact on CVD risk. For example, a 10-year cohort study provided evidence that the consumption of saturated fatty acids (SFA) from dairy foods was associated with decreased risk of CVD, while consumption of the same amount of SFA from meat (including red and processed meat, fish and poultry) was associated with increased CVD risk (13). Yet, even within the same food category, differences in formulations or processing can impact health effects. The fermentation of dairy products provides a notable example. While current evidence does not support an association between intake of dairy products and risk of cardiometabolic disease, fermented dairy products, such as cheese and yogurt, generally show inverse associations (14). Indeed, results from randomized crossover trials show that consumption of SFA in cheese lowers total and/or low-density lipoprotein cholesterol (LDL-C) compared with consumption of SFA in butter (15–18). As another example, a meta-analysis of 17 prospective cohorts and three case-control studies indicated that consumption of processed meat was associated with increased risk of CHD, but consumption of red meat was not (19).

### Meta-analyses

Meta-analyses that combine studies that compared macronutrient A with B and studies that compared A with C or D or E can lead to misleading conclusions (20). Hooper *et al.* (21) illustrated this by pooling randomized controlled trials (RCTs) comparing SFA with polyunsaturated fatty acids (PUFAs; *n*-6 fatty acids), monounsaturated fat, CHO or protein and reporting a 17% reduction in CVD events with replacement of SFA. However, in separate analyses with each of the replacement nutrients, replacement of SFA with *n*-6 fatty acids yielded a significant 27% reduction in CVD events. The separate analyses for replacement of

SFA with monounsaturated fat, CHO or protein all showed non-significant effects on CVD events (21). Differences in the effects of the replacement nutrients in both RCTs and observational cohort studies, and the pooling of these effects, likely help to explain the discrepant results from studies and meta-analyses investigating the effects of dietary SFA and other macronutrients on CVD risk (9,10,22–25).

Conclusions drawn from meta-analyses that include inappropriate studies can also be misleading. For example, the 2014 meta-analysis (10) that challenged the CVD benefit of replacing SFA with *n*-6 fatty acids has been criticized (26) for including potentially confounded data from the Sydney Diet Heart Study (27). The Sydney Diet Heart Study had included margarine with high trans fats in the *n*-6 fatty acids supplementation arm. Exclusion of the Sydney Diet Heart Study resulted in a seven-study meta-analysis with a relative risk estimate of 0.81 (0.68–0.98), thus supporting a CVD benefit of replacing SFA with *n*-6 fatty acids (28). However, concerns have been raised regarding the rationale for exclusion of this trial (29), as well as the inclusion of other trials (the Finnish Mental Hospital (30) and Oslo Diet-Heart (31) studies) that allowed for the continued consumption of trans fats from margarines in the control arms of the studies, thus confounding comparisons with the *n*-6 fatty acid diets (28). Furthermore, during the Oslo Heart Study, the experimental patients and spouses were taught in their homes how to select and prepare study foods, while the control group was not (31). In short, decisions regarding what studies are appropriate to include or exclude from a meta-analysis can vary among investigators and contribute to conflicting conclusions.

### Randomized controlled trials with cardiovascular disease outcomes

Despite the limitations of the Finnish Mental Hospital (30) and Oslo Diet-Heart (31) studies, both of these RCTs, along with two others conducted at Wadsworth Hospital and Veterans Administration Center in Los Angeles (32) and by the British Medical Research Council (33), have been described as providing the highest-quality evidence available regarding the effects of dietary SFA compared with *n*-6 fatty acids (24). The population sizes in these four trials ranged from 400 to 1,200, and maximum diet exposure was 4–8 years, long enough to obtain CVD outcomes. All four trials monitored blood lipid levels and compliance biomarkers, and the two longest and largest trials also provided standardized diets (30,32). These studies were conducted more than 50 years ago, yet they compose the core evidence upon which a 2017 Presidential Advisory from the American Heart Association based the following summary: 'randomized controlled trials that lowered intake of dietary saturated fat and replaced it with polyunsaturated vegetable oil reduced CVD by approximately 30%' (24). Given the challenges and escalating costs associated with conducting

nutritional RCTs with CVD outcomes, these four trials are likely to remain the highest-quality evidence available regarding the effects of dietary SFA compared with *n*-6 fatty acids (24).

Exemplifying the challenge and the expense of RCTs with CVD outcomes is the more recent Women's Health Initiative Dietary Modification Study of ~48,000 women with an estimated cost of over \$400m (34). Women were randomized to receive intensive behaviour modification in group and individual sessions aimed at reducing total fat intake to 20% of calories and increasing intake of vegetables/fruits to five servings per day and grains to at least six servings per day or to the no-diet-modification group that received only diet-related education material. By year 6, the diet-modification group reported a mean increase of 1.1 and 0.5 servings per day of fruit/vegetable and grain, respectively, and a mean 8% reduction in total fat energy. This resulted in a group reduction in fat consumption that was less than anticipated (37% of daily energy down to 28.8%), no significant reduction in CVD outcomes and only a modest reduction in LDL-C of 3.6% during the ~8-year intervention (35). It is not possible to know if the intervention failed to achieve reduction in CVD outcomes because the fat restriction was focused on total fat rather than subtype or because the dietary modifications reported by the participants were too modest or were overestimations of the dietary changes that actually occurred. The two latter limitations, potential non-compliance (36) and inaccurate reporting of food consumption (37) by research participants, are formidable challenges faced by all nutritional research studies, with the exception of those conducted in well-monitored inpatient facilities.

In contrast to the Women's Health Initiative, a recent and smaller multicentre trial conducted in Spain focused on subtypes of fat in specific foods (38). Approximately 7,000 participants with T2D or three CVD risk factors were randomized to *ad libitum* Mediterranean diets supplemented either with extra-virgin olive oil (50 mL d<sup>-1</sup> prescribed for participants, 1 L week<sup>-1</sup> provided for family needs) or with mixed nuts (30 g d<sup>-1</sup> provided for participants, 1000 g/3 months provided for family needs) or to a control diet (non-food gifts provided) that was centred around a Mediterranean diet with advice to reduce dietary fat. After 4 years, the Mediterranean diets supplemented with either extra-virgin olive oil or nuts resulted in a relative risk reduction of approximately 30% for major CVD events (primarily stroke) compared with the control diet (38). These beneficial results were mainly mediated by consumption of the supplemental extra-virgin olive oil and the nuts, as there were few diet differences among the groups that were not due to these foods (38). It is worth noting that despite receiving supplemental calories in the olive oil (~9,000 kcal week<sup>-1</sup>) or nuts (~1,400 kcal week<sup>-1</sup>), neither of the experimental groups nor the control group gained

body weight during the trial (group means for  $\Delta$ body weight at 4.8 years ranged from -0.9 to -0.4 kg) (39). The authors suggest that these results provide evidence that restricting intake of healthy fats is not required for maintenance of body weight (39).

#### *Biomarkers that can demonstrate causality*

Given the high costs and challenges associated with RCTs with CVD outcomes, the next strongest line of research to draw on is prospective cohort studies. However, while these studies increase the feasibility of studying CVD outcomes, they cannot prove causality and are limited by the inaccuracies of self-reported food intake (37) and the challenges related to identifying and adjusting for relevant covariates. Evidence of causality must often rely on RCTs in which outcomes are biomarkers of CVD rather than CVD events. Among modifiable CVD risk biomarkers, LDL-C and blood pressure have been the most strongly validated (40), and they provide the major rationale for therapies aimed at reducing disease risk (28). However, reliance on identifying dietary macronutrient effects on LDL-C may obscure effects on LDL particles, especially small dense LDL (sdLDL). sdLDLs may have more direct and specific effects on the development and progression of CVD than are predicted by the LDL-C measurement (41). Increased atherogenicity of sdLDL may be due in part to a longer residence time in plasma, which exposes the arterial endothelium to proinflammatory and proatherogenic particle components such as apolipoprotein (apo)CIII (28,41,42). sdLDLs have been shown to be reduced with lower CHO intake (43). Further, the prevalence of LDL subclass pattern B, a categorical marker for atherogenic dyslipidaemia defined by the predominance of sdLDL, has been linearly and positively associated with increasing concentrations of dietary CHO (consisting of 50% starch and 50% sugar) in RCTs owing to effects of CHO that can occur in as few as 3 d (43). In contrast to CHO, dietary SFA in the ranges generally consumed appears to mainly increase larger LDL particles, which are less strongly associated with risk of CVD (41). Thus, SFA-induced increases in LDL-C may not signify an increase in CVD risk commensurate with that predicted from the relationship of LDL-C to CVD risk in the population (41). For example, recent results from the large multinational Prospective Urban Rural Epidemiology study found a correlation between SFA intake and LDL-C. However, simulation models indicated that LDL-C provided a poor measure of risk of CVD events and mortality as opposed to the ratio of serum apolipoprotein (apo)B (a measure of the total number of atherogenic lipoprotein particles) to apoAI (the principal high-density lipoprotein [HDL] protein), a ratio that in turn is associated with sdLDL levels (44). More studies are needed regarding whether there are specific pathophysiological properties of particles within the spectrum of sdLDL that merit the use

of standardized assays for their measurement as a more informative biomarker of CVD risk than LDL-C (41). Another candidate CVD biomarker is apoCIII (28). apoCIII has been found consistently to be positively associated with the risk of CVD, likely because of its capacity to retard plasma clearance of atherogenic remnant lipoproteins (45) as well as its direct proinflammatory activity (46). Further, apoCIII in apoB-containing particles is increased with high-CHO diets (47,48). Given our reliance on the totality of the scientific evidence (prospective cohort studies: association between nutrient and disease outcome + RCTs: direct effects of nutrient on disease biomarker), valid biomarkers are essential.

### Nutrient substitutions

Any isocaloric change in one macronutrient requires changes in others, and hence, it is difficult to determine if effects are caused by the increase in macronutrient A or the decrease in macronutrient B. Thus, rather than focus on effects of high versus low consumption of single nutrients on CVD risk, it is more appropriate to statistically evaluate the effects of nutrient or, better yet, food substitutions. The effects of nutrient substitutions for SFA were evaluated in over 127,500 men and women who were followed for 24–30 years (49). Replacing 5% of energy intake from SFA with equivalent energy intake from PUFAs, monounsaturated fatty acids or CHOs from whole grains was associated with a 25%, 15%, and 9% lower risk of CHD, respectively. Replacing SFA with trans fats or CHOs from refined starches/added sugars was not significantly associated with CHD risk (49). These results are consistent with the findings from recent meta-analyses that are not confounded by the challenges discussed above (9,21,24), although the effects of macronutrient heterogeneity and food context, as discussed above, also require consideration.

The evidence concerning omega-3 (*n*-3) PUFAs and cardiometabolic risk remains unclear. The consumption/supplementation of *n*-3 fatty acid is associated with reduced CVD risk in prospective cohort studies (10,50), but randomized controlled supplementation trials have not shown significant beneficial effects (10). Nevertheless, a recent Science Advisory from the American Heart Association (51) stated that treatment with omega-3 PUFA supplements of patients with prevalent CHD such as a recent myocardial infarction is reasonable as even a potential modest reduction in CHD mortality (10%) in this clinical population would justify treatment with a relatively safe therapy.

### Conclusions

Focusing on effects of nutrient substitution rather than high versus low SFA indicates that replacement of SFA with *n*-6 fatty acids is associated with lower CVD risk, while replacement of SFA with refined CHOs (starches and sugars) is

associated with a neutral or adverse effect. However, more research is needed to consider the food and dietary context in which specific fats are consumed and to develop better tools to assess dietary patterns. Given the challenges of large RCTs with CVD disease endpoints, and the limitations of observational studies, identification and validation of additional surrogate biomarkers for RCTs would be of great value.

### Dietary sugars: Kimber Stanhope and Jean-Marc Schwarz

All the challenges involved with conducting and interpreting research on dietary fat are also pertinent to the topic of dietary CHO/sugar. Therefore, similar to dietary fat, recent reviews (52–58) and meta-analyses (59–65) offer very conflicting conclusions concerning the effects of added sugar on cardiometabolic risk.

### Definitions of added sugars and free sugars

As defined by the US Food and Drug Administrations, added sugars include sugars that are either added during the processing of foods or are packaged as such and include sugars (free, monosaccharides and disaccharides), sugars from syrups and honey and sugars from concentrated fruit or vegetable juices that are in excess of what would be expected from the same volume of 100% fruit or vegetable juice of the same type (66). The World Health Organization's definition of free sugars is similar except that it also includes the sugar naturally present in 100% fruit juices and fruit juice concentrates (67).

### Natural sugars

The sugars naturally present in whole fruit are exempt from both definitions, and dietary guidelines emphasize the importance of consuming whole fruits and vegetables. Prospective cohort studies consistently support this with evidence that fruit consumption (68–75) or fruit-plus-vegetable consumption (76–80) is inversely associated with incidence of CVD and T2D (81). The evidence from RCTs is inconclusive (82,83), and this is possibly due to the generally modest changes in fruit and vegetable intake that have been achieved in these studies (84). While dietary intervention studies comparing the consumption of added sugar to isocaloric amounts of sugar in whole fruit are lacking, the results from three RCTs suggest that consumption of naturally sweetened orange juice (85,86) or grape juice (87) decreases risk factors compared with sugar-sweetened beverages (SSB). In support of this, there are population studies that report that incidence/prevalence of metabolic syndrome (88,89), CVD (90) and T2D (91–93) and their risk factors (94–96) are associated with consumption of SSB or fruit juice with added sugar (93), but not with consumption of 100% fruit juice. However, the findings from

several prospective cohort studies suggest that both 100% fruit juice and SSB consumption are positively and comparably associated with metabolic syndrome (97) and T2D (98,99). Therefore, more RCTs comparing SSB and naturally sweetened fruit juice are warranted, as well as RCTs comparing added sugar with the sugar in whole fruit (82).

#### Added sugar

Regarding disagreements among meta-analyses concerning the health effects of added sugar, conclusions differ between those that compare fructose with any CHO, including sucrose and HFCS (60,100), and those that compare high added sugar diets with lower added sugar diets (62). Meta-analyses that include inappropriate studies also provide conflicting conclusions. An example of an inappropriate study, included in at least four meta-analyses reporting little or no detrimental effects of fructose consumption (61,100–102), is one in which the fructose in the high-fructose diet (60 g of fructose per day) was not added sugar but was rather provided by whole fruit (103). Furthermore, both the high-fructose and low-fructose (20 g of fructose per day) diets were weight loss diets; thus, subjects in both interventions lost significant amounts of weight (diet with fruit:  $-4.2 \pm 0.3$  kg; diet without fruit:  $-2.8 \pm 0.3$  kg) and, not unexpectedly, exhibited improvements in cardiometabolic risk factors (103).

#### Prospective cohort studies

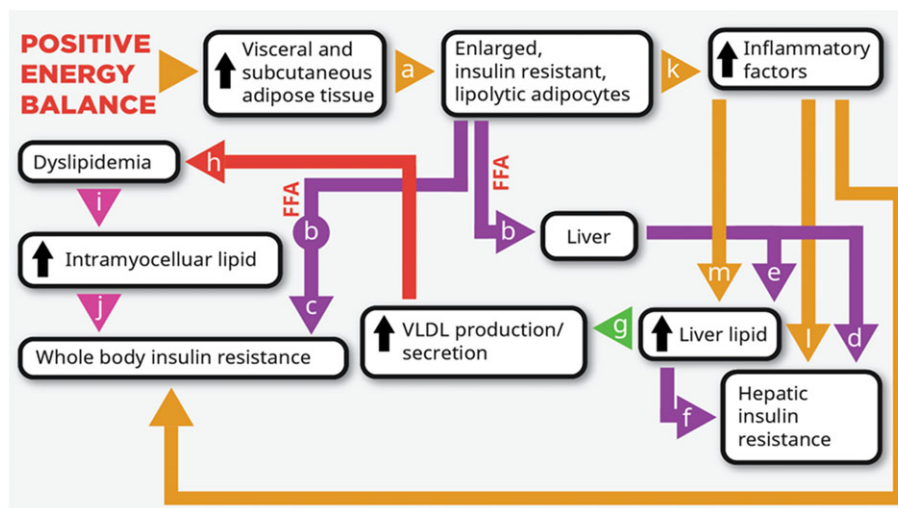
The recent meta-analyses of prospective cohort studies investigating consumption of SSB have consistently shown positive relationships with CVD (59,104,105), T2D (106–110) and hypertension (59,111). The Nutrition and Chronic Diseases Expert Group systematically reviewed the evidence for effects of dietary factors on cardiometabolic diseases, including comprehensively assessing evidence for causality (112). They concluded that evidence from prospective studies suggests a body mass index (BMI)-independent effect of SSB on incidence of T2D and CHD and an additional effect on adiposity (112).

The consumption of added sugar has been less extensively studied than SSB. In 2014, two large prospective cohort studies came to differing conclusions concerning the association between added sugar consumption and CVD mortality. Consumption of added sugar was positively associated with CVD mortality over 15 years in 11,733 National Health and Nutrition Examination Survey (NHANES) participants (age 20 and above) (113), but not over 13 years in 353,751 National Institutes of Health–American Association of Retired Persons (NIH-AARP) Diet and Health Study participants (age 50–71) (114). In the latter study (114), fructose in beverage was positively associated with CVD mortality. Study differences that may have influenced the results include sugar intake: the NHANES participants reported a mean baseline level of added sugar

consumption of ~15.7% of daily energy (113), while the NIH-AARP Diet and Health Study participants reported a mean baseline level of free sugar consumption of ~9.6% of daily energy (114). The definition of free sugars for this study included sugar from dried fruit and applesauce, as well as from 100% fruit juice (114). A 10-year prospective study in 2,379 girls (9–10 years at baseline) showed that consuming <10% of energy as added sugar resulted in increasing concentrations of HDL-C compared with consuming  $\geq 10\%$  of energy as added sugar (115). However, a study that divided added sugar into sugar added to beverage (mean intake: 2.6% of daily energy) and sugar added to solid food (mean intake: 9.4% of daily energy) found that the development of impaired glucose homeostasis and insulin resistance over 2 years in 8- to 10-year-old children ( $n = 564$ ) at risk for obesity was only associated with added sugar in beverages (116).

#### Fructose versus glucose

There is evidence and plausible mechanisms to suggest that there are differences between CHOs with regard to their effects on cardiometabolic risk factors that mainly involve the differential metabolism of fructose and glucose. Even though both pure fructose and pure glucose are generally not used as added sugars, investigations of their specific metabolic effects have provided important mechanistic insights into the effects of sucrose and HFCS, the most commonly used added sugars that contain both fructose and glucose. The effects of fructose and glucose were compared in adults (mean age: 54 years, mean BMI: 29 kg m<sup>-2</sup>) who consumed 25% of their energy requirement (Ereq) as fructose-sweetened or glucose-sweetened beverages for 10 weeks (117). These subjects resided at the clinical research centre and consumed eucaloric diets consisting of 55% Ereq as complex CHO for 2 weeks while baseline procedures were conducted. This was followed by an 8-week outpatient period during which subjects consumed the fructose-sweetened or glucose-sweetened beverages along with their usual *ad libitum* diets. Intervention procedures were conducted during the last 2 weeks of the study while subjects resided at the clinical research centre and consumed eucaloric diets consisting of 30% Ereq complex CHO and 25% Ereq as the assigned beverage. Although both groups gained comparable amounts of body weight (~1.5%) and body fat (~3%), there were marked differences between the effects of the two sugars. Subjects consuming glucose exhibited markedly higher post-meal glucose and insulin responses than those consuming fructose (118). Also, in keeping with the established paradigm by which positive energy balance promotes the development of metabolic syndrome through the increased insulin resistance and lipolytic activity of enlarged adipocytes (119) (Fig. 2), subjects consuming glucose exhibited higher 24-h circulating free fatty acids (FFA) (117). In contrast,



**Figure 2** Established paradigm by which positive energy balance promotes the development of the metabolic syndrome. Positive energy balance leads to fat accumulation and larger and more insulin-resistant adipocytes (a). The insulin resistance increases lipolytic activity and circulating free fatty acids (FFA) (b). While lipolytic activity is higher in visceral adipose tissue than subcutaneous adipose, upper body subcutaneous adipose is a major contributor to the increased levels of FFA (328,329). High levels of FFA can mediate muscle (c) (330) and liver (d) (331) insulin resistance. Hepatic uptake of FFA leads to increased liver lipid (e), which is associated with liver insulin resistance (f) (332) and promotes very-low-density lipoprotein (VLDL) production/secretion (g) and dyslipidaemia (h) (328,333). Increased exposure to circulating triglyceride promotes intramyocellular lipid accumulation (i) (334), which is associated with insulin resistance (j) and type 2 diabetes (332). Inflammatory factors released by insulin-resistant visceral adipose tissue (k) may also promote hepatic insulin resistance (l) and lipid accumulation (m) (119). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

fructose consumption did not affect FFA levels. Yet it was the subjects consuming fructose who exhibited increased *de novo* lipogenesis (DNL), reduced fat oxidation, increased circulating TG (postprandial only), LDL-C, sdLDL-C, oxidized LDL, apoB, apoCIII and uric acid and decreased insulin sensitivity (117,120,121). The essential mechanistic feature that explains these results is that the hepatic uptake and metabolism of glucose is regulated by hepatic energy status, which allows glucose to bypass the energy-replete liver and raise post-meal blood glucose and insulin levels. In contrast, the hepatic uptake and metabolism of fructose is unregulated (122,123); thus, excessive fructose consumption results in a hepatic substrate overload that increases uric acid production (121,123–125) and up-regulates DNL (117,126,127). As illustrated in Fig. 3, the major downstream effects include inhibition of fat oxidation (120,127), increased liver lipid content (127–129), up-regulated secretion of large very-low-density lipoproteins 1 (130), dyslipidaemia (131) and hepatic insulin resistance (132,133). Increased inflammation induced by increases in visceral fat (117,128) or fructose exposure in the intestine (134,135) or liver (136) may also mediate or enhance metabolic dys-regulation.

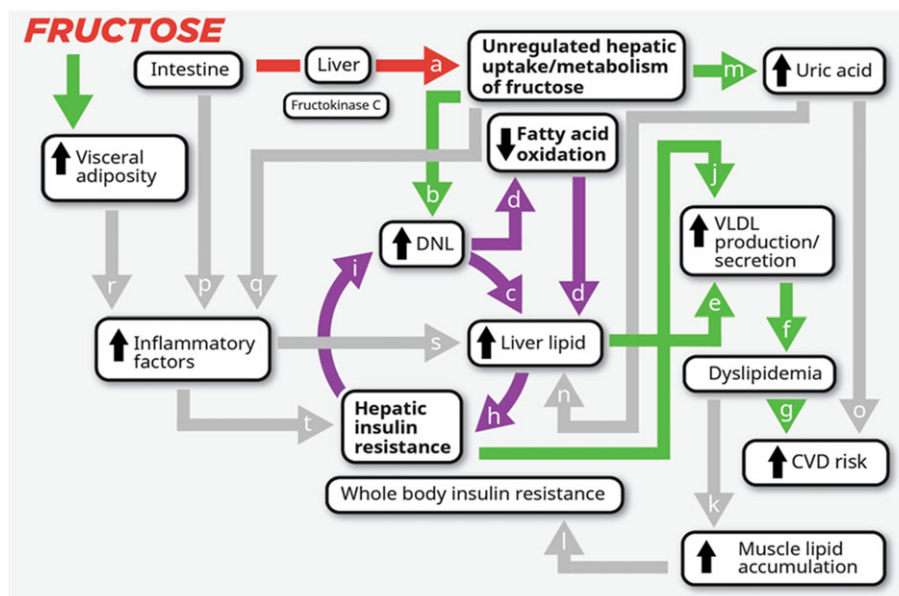
#### High fructose corn syrup versus fructose and glucose

The results from the 10-week intervention comparing fructose and glucose (117) suggest that the hepatic substrate overload induced by excessive consumption of fructose (Fig. 3) is a more rapid pathway to metabolic dys-regulation

than the increased FFA (Fig. 2) and post-meal hyperglycaemia/hyperinsulinaemia induced by excessive consumption of glucose. These studies also suggest that because the commonly consumed sugars HFCS and sucrose contain 50–55% fructose, their overconsumption would have less detrimental effects on risk factors than isocaloric amounts of pure fructose. Data from a 2-week dietary intervention study (125,137), in which adults (mean age: 26 years, mean BMI: 25 kg m<sup>-2</sup>) consumed beverages containing 25% Ereq as fructose, glucose or HFCS along with their usual *ad libitum* diet, showed that circulating postprandial TG, apoCIII and uric acid concentrations were highest during fructose consumption ( $\Delta$ body weight: -0.1 kg), lowest during glucose consumption ( $\Delta$ body weight: 0.4 kg) and intermediate during HFCS consumption ( $\Delta$ body weight: 0.4 kg) (137). However, surprisingly, this was not the pattern for fasting and postprandial concentrations of LDL-C, non-HDL-C and apoB. The levels of these CVD risk factors were highest after HFCS consumption, lowest after glucose consumption and intermediate after fructose consumption (137), suggesting potentially synergistic effects of fructose and glucose consumption on these measures when the two sugars are consumed concurrently.

Utilizing the same 2-week dietary intervention protocol (125,137), adults (mean age: 25 years, mean BMI: 26 kg m<sup>-2</sup>) consuming beverages containing 0% (aspartame-sweetened beverage), 10%, 17.5% or 25% Ereq from HFCS with their usual *ad libitum* diets exhibited dose-dependent increases of fasting and postprandial non-HDL-





**Figure 3** Potential mechanisms by which consumption of fructose promotes the development of metabolic syndrome. The initial phosphorylation of dietary fructose in the liver is largely catalysed by fructokinase C (a), which is not regulated by hepatic energy status (122, 123). This results in unregulated fructose uptake and metabolism by the liver. The excess substrate leads to increased *de novo* lipogenesis (DNL) (b) (117, 127). DNL increases the intra-hepatic lipid supply directly (127–129), via synthesis of fatty acids (c), and indirectly by inhibiting fatty acid oxidation (d) (120, 127). Increased intra-hepatic lipid content promotes very-low-density lipoprotein (VLDL) production and secretion (e) (130). This leads to increased levels of circulating triglyceride (TG) and low-density lipoprotein particles (dyslipidaemia) (f) (131), risk factors for cardiovascular disease (CVD) (g). Increased levels of hepatic lipid may also promote hepatic insulin resistance (132) by increasing levels of diacylglycerol, which may activate novel protein kinase C and lead to serine phosphorylation (serine P) of the insulin receptor and insulin receptor substrate 1 and impaired insulin action (h) (335). Because of selective insulin resistance, DNL is even more strongly activated in the insulin resistant liver (i) (336), which has the potential to generate a vicious cycle (circular arrows) that would be perpetuated by sustained fructose consumption. This cycle would be expected to further exacerbate VLDL production and secretion via increased intra-hepatic lipid supply (130). Hepatic insulin resistance also promotes VLDL production/secretion (j) by increasing apolipoprotein B availability (337, 338) and apolipoprotein CIII synthesis (339) and by up-regulating microsomal TG transfer protein expression (MTP) (336). This exacerbates and sustains exposure to circulating TG, leading to intramyocellular lipid accumulation (k) (334), impaired insulin signalling and whole-body insulin resistance (l) (332). The fructokinase-catalysed phosphorylation of fructose to fructose-1-phosphate, which results in conversion of adenosine triphosphate to adenosine monophosphate and a depletion of inorganic phosphate, leads to uric acid production via the purine degradation pathway (m) (121, 123–125). High levels of uric acid are associated and may contribute to increased risk for development of fatty liver (n) and CVD (o) (340–342). Fructose exposure in the intestine (p) (134, 135) and liver (q) (136) and fructose-induced increases of visceral adipose (r) may promote inflammatory responses (117, 343) that further promote liver lipid accumulation (s) and/or impair hepatic insulin signalling (t) (119). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

C, LDL-C, apoB and uric acid and postprandial apoCIII and TG concentrations (all  $P < 0.001$ ) (125). HFCS consumption also resulted in a dose-dependent increase in body weight ( $\Delta$ body weight: 0%,  $-0.1$  kg; 10%, 0.0 kg; 17.5%, 0.3 kg; 25%, 0.8;  $P < 0.05$ ); however, in adjusted statistical models, the variance attributed to  $\Delta$ body weight was 1–3% and the variance attributed to HFCS dose was 9–29% (125). These results are in contrast to those from a study in which adults consuming beverages containing 8%, 18% or 30% Ereq as sucrose or HFCS with usual *ad libitum* diets for 10 weeks exhibited no differences in cholesterol and LDL-C (138) or 24-h uric acid and TG area under the curve (139) between doses. As previously discussed in detail (58, 125), possible explanations for the conflicting results include differences in the statistical analyses employed, use of three cups per day of low-fat milk as a vehicle for the sugars, lack of a vehicle control group and lack of an objective measure of compliance in the 10-week study (138, 139).

### Sucrose

Data from several older studies suggest that consumption of sucrose also increases total and/or LDL-C (140–144) or postprandial TG (142, 145). More recently, men (mean age: 26 years, mean BMI:  $22 \text{ kg m}^{-2}$ ) consuming beverages containing either  $80 \text{ g d}^{-1}$  sucrose or fructose with their usual *ad libitum* diets for 3 weeks had higher concentrations of total and LDL-C and reduced LDL particle size than when they consumed beverages containing  $80 \text{ g}$  of glucose (146, 147). This study also showed that the fructose-sweetened beverages decreased hepatic insulin sensitivity compared with the glucose-sweetened beverages, even though body weight, body fat and waist circumference were reduced after fructose consumption compared with glucose consumption (146). In the longest of the recent intervention trials, liver fat, and fasting TG, total cholesterol and uric acid concentrations were increased in adults (mean age: 39 years, mean BMI:  $32 \text{ kg m}^{-2}$ ) consuming 1 L of

sucrose-sweetened cola per day with their usual *ad libitum* diets for 6 months compared with subjects consuming isocaloric amounts of low-fat milk or 1 L of aspartame-sweetened cola or water per day (124,128). The changes in body weight ranged from +0.1 (aspartame) to +1.4% (low-fat milk) and did not differ among the four diet groups ( $P = 0.8$ ) (128). A 2-week study that concurrently investigated consumption of 25% Ereq as sucrose-sweetened ( $\Delta$ body weight: 0.5 kg) or HFCS-sweetened beverages ( $\Delta$ body weight: 0.8 kg) with *ad libitum* diets in adults (mean age: 26 years, mean BMI: 25 kg m<sup>-2</sup>) showed that the effects of the two added sugars on LDL-C, apoB, apoCIII, uric acid and postprandial TG were comparable (148).

### Sucrose versus starch

There appears to be only one study that has investigated the effects of replacing starch with sucrose in solid food, as opposed to SSB, at levels less than 30% Ereq. Twenty-four women and men with hyperinsulinaemia (mean age: 36 years, mean BMI: 25 kg m<sup>-2</sup>) consumed three eucaloric (42% Ereq fat, 14% protein and 44% CHO), crossover diets in which the 44% CHO component was provided as 39% starch + 5% sucrose, 26% starch + 18% sucrose or 11% starch + 33% sucrose in solid form for 6 weeks each. Fasting lipids (143), glucose and insulin concentrations, and the glucose and insulin responses to an oral sucrose tolerance test (149) were increased when subjects consumed the 18% and 33% Ereq sucrose diets compared with the higher starch diet containing only 5% Ereq sucrose (143,149). More studies are needed to compare the metabolic effects of sugar consumed in solid and liquid forms with both refined and whole-grain CHO in normoinsulinaemic individuals consuming isocaloric diets.

### Weight-independent effect of fructose/sugar

All of the studies detailed above, with the exception of the investigation on solid-form sucrose versus starch (143,149), provided the intervention sugar as supplements to the participants' usual *ad libitum* diets. While the modest weight gains suggest that most of the subjects partially compensated for the sugar supplements by consuming less of their usual diet, there is still the potential for confounding by excess energy consumption and weight gain. However, studies with dietary protocols that included the provision of eucaloric diets with matched macronutrient distribution between the high-sugar and low-sugar diets have demonstrated that fructose and sucrose consumption can increase risk factors in the absence of positive energy balance and weight gain (127,140,143–145,149–158). These studies include a recent crossover study that provided evidence that all the major effects of fructose overload, detailed in Fig. 3, can occur in the absence of positive energy balance and weight gain. Eight men (mean age: 42 years, mean BMI: 24 kg m<sup>-2</sup>) resided at a clinical research centre and

consumed eucaloric diets in which the CHO component consisted of 25% Ereq fructose-sweetened beverage + 25% complex CHO or 50% Ereq complex CHO for 9 d each (127). Despite weight maintenance, this short-term exposure to fructose-sweetened beverage increased DNL, inhibited fat oxidation, increased liver fat and postprandial plasma TG concentrations and decreased hepatic insulin sensitivity compared with the complex CHO diet. Each of these effects was observed in all eight participants, with the exception that postprandial TG was not increased in one individual (127). It is important to note, however, that these results could have been affected by both the differences between fructose and complex CHO and the differences between beverage and solid food. However, in a much older study, Hallfrisch *et al.* compared the isocaloric substitution of starch with 0%, 7.5% or 15% Ereq as fructose in solid food, utilizing eucaloric crossover diets that were provided for 5 weeks each to 12 hyperinsulinaemic and 12 normoinsulinaemic men (mean age: 40 years, mean BMI: 26 kg m<sup>-2</sup>). Compared with the 0% fructose diet, the 7.5% and 15% fructose diets increased total cholesterol and LDL-C (154) and the 15% fructose diet increased glucose and insulin responses to a 3-h oral sucrose tolerance test in both groups of subjects (153). More recently, a study that provided eucaloric diets including 25% Ereq as fructose-sweetened ( $n = 15$ ) or glucose-sweetened beverages ( $n = 17$ ) for 2 weeks to men (mean age: 34 years, mean BMI: 29 kg m<sup>-2</sup>) did not reveal differences in liver fat, but fasting uric acid concentrations and homeostatic model assessment insulin resistance (HOMA-IR) were increased by fructose compared with glucose consumption (155). Similar results were reported from a crossover study in which men (mean age: 46 years, mean BMI: 32 kg m<sup>-2</sup>) were provided eucaloric low-sucrose (sucrose 5.2%, total sugar 17.1% of daily calories) or high-sucrose (sucrose 14.9%, total sugar 30.2% of daily calories – details about non-sucrose sugar are not provided) diets for 6 weeks (156). While there were no differences in peripheral glucose utilization and suppression of endogenous glucose production during a two-step hyperinsulinaemic euglycaemic clamp, fasting and oral glucose tolerance test areas under the curve were higher for both glucose and insulin after the high-sucrose versus low-sucrose diet (156).

### Sugar restriction

In a study designed to demonstrate weight-independent effects of fructose restriction to reverse the adverse metabolic effects of excessive fructose consumption, eucaloric diets were provided for home consumption to 43 Latino and African-American adolescents (mean age: 13 years) with metabolic syndrome for 9 d (159). The diets contained 10% Ereq as added sugar, considerably less than the average of 27% Ereq recorded for the participants' usual diets (159). In response to the lower-sugar diets, the adolescents

exhibited numerous benefits including reductions of liver fat, visceral adipose tissue and DNL (160), plasma lipids and lipoproteins (161), glucose and insulin excursions during oral glucose tolerance test and diastolic blood pressure (159). Body fat was not affected ( $-0.3$  kg,  $P = 0.17$ ), but body weight was significantly decreased ( $-0.9$  kg,  $P < 0.01$ ). Despite efforts by the investigators to promote body weight maintenance by providing more food, body scales for daily weight monitoring at home, and individualized weight maintenance counselling, 34 of the 43 participants exhibited a decrease in body weight (159). This confounds assessment of the direct metabolic effects of fructose restriction as opposed to those mediated by negative energy balance and weight loss. However, the subgroup of nine subjects who did not lose weight also had statistically significant reductions in most of the outcomes (DNL, liver fat, visceral adipose tissue, fasting glucose, insulin and HOMA-IR) (159–161).

Two more recent studies demonstrate benefits of sugar restriction in children with obesity (162,163). In response to a 6-week dietary intervention consisting of advice to reduce fructose consumption from a usual intake  $>70$  to  $<20$  g d<sup>-1</sup>, 54 children (age range: 6–11 years) exhibited significant decreases in liver fat and fasting TG, but no changes in body weight or BMI (162). A 6-month dietary intervention consisting of advice to reduce consumption of fructose and high glycaemic index foods resulted in lowered systolic blood pressure, alanine aminotransferase, apoB and HOMA-IR in 12 children (age range: 7–18 years) with non-alcoholic fatty liver disease (NAFLD) (163). An important limitation of all three of these studies (159,162,163) is they did not include control groups. However, in a recent RCT, adults (mean BMI: 31 kg m<sup>-2</sup>), whose normal diets contained ~94 g of sugar per day from SSB, were randomized to receive and consume their usual quantity of beverage sweetened with sucrose ( $n = 14$ ) or with an NNS ( $n = 13$ ) for 12 weeks (164). The group consuming NNS exhibited significant decreases in liver fat compared with the group who continued to consume SSB (87.9 g sugar from SSB consumed per day at 12 weeks) (164). However, while the changes in body weight were not significant, the NNS group lost ~1.4 kg and the SSB group gained ~1 kg. More adequately powered RCTs are required to delineate the weight-independent and weight-dependent effects of sugar restriction.

### Conclusions

Consumption of fructose-sweetened, HFCS-sweetened or sucrose-sweetened beverages leads to greater increases in risk factors for cardiometabolic disease than isocaloric amounts of starch. More studies comparing the metabolic effects of SSB with those of added sugar in solid food and comparing added sugar in solid foods with both refined and whole-grain complex CHO are required. The metabolic

dys-regulation induced by excessive consumption of fructose-containing sugar is mainly caused by hepatic fructose overload increasing DNL, which leads to inhibited fat oxidation, increased liver fat and increased very-low-density lipoprotein production/secretion. Risk factors associated with metabolic dys-regulation increase even when fructose-containing sugars are consumed with diets that do not result in positive energy intake and weight gain.

### Objective 2: do certain dietary patterns or components have the potential to promote fat gain via mechanisms that are in addition to their specific contribution of calories to the 'energy in' side of the energy balance equation?

Older and emerging evidence on the high-carbohydrate/high-glycaemic-index diet: Anja Bosy-Westphal

#### *The high-carbohydrate versus high-fat diet debate*

The potential to promote fat gain via mechanisms that are in addition to caloric content has been attributed to both high-CHO and high-fat diets. Thirty years ago, the consensus was that a high-fat diet promotes greater fat gain than a high-CHO diet because dietary fat is converted to body fat much more efficiently than dietary CHO (165). At that time, clinical studies showed that the body does not handle fat ingestion in the same way as CHO or protein ingestion. More specifically, (a) unlike CHO ingestion, which stimulates CHO oxidation and even DNL in extreme cases (166), the ingestion of dietary fat is not reciprocated by an increase in fat oxidation (167–169); (b) similarly, under eucaloric conditions, replacement of CHO by fat in the diet takes several days or weeks to stimulate fat oxidation, even in the presence of increased physical activity (169–172); (c) the thermic effect of dietary fat is lower than that from CHO or protein (173–175); (d) because of the higher energy density of fat and its low satiating effect, fat ingestion leads to food overconsumption (176,177). Together, these results strongly suggested that dietary fat was a key culprit in the western diet leading to enhanced storage of body fat (178). All these studies lead to the development of the 'Flat hypothesis', which proposes that unlike CHO and protein, excess fat intake is not rapidly buffered by increased fat oxidation but requires a significant amount of weight gain to re-establish a new fat balance (179). At this new weight and body composition, the amount of CHO, fat and protein oxidized will match the amount of CHO, fat and protein consumed (i.e. respiratory quotient = food quotient) and weight can be maintained (179).

The consensus that dietary fat is the specific culprit in the obesity crises has waned with the perception that while Americans decreased their intake of dietary fat, the rates of overweight and obesity have risen (180). Whether this

occurred because dietary fat does not promote fat gain more efficiently than CHO or because Americans did not achieve substantial reductions in fat intake or because their fat calories were replaced by even more calories of low-fat/high-CHO/high-sugar foods is not known. However, it increased focus on the possibility that a high-CHO diet has the potential to increase fat gain by affecting both sides of the energy balance equation. Specifically, when meals with a high proportion of glucose-containing CHOs are consumed, higher post-meal glucose excursions lead to larger meal-associated insulin excursions than when isocaloric high-fat/low-CHO meals are consumed (181). This physiologic hyperinsulinaemia has the potential to promote fat gain by driving glucose and FFA into storage forms, which also decreases the availability of circulating metabolic fuels and promotes hunger (182).

#### Energy-restricted weight loss diets

This mechanism serves as the rationale for many popular high-fat weight loss diets. However, it is not supported by a recent 14-day inpatient metabolic balance study examining the effect of selective isocaloric reduction of dietary CHO versus fat on body weight, energy expenditure and fat balance in 19 volunteers with obesity. The results showed that calorie for calorie, the high-CHO weight loss diet led to greater body fat loss than the high-fat diet, despite the fact it was the high-fat diet that led to decreased insulin secretion (183). A 2014 meta-analysis of 19 RCTs lasting 3 months to 2 years with ~3,200 participants showed no significant differences in loss of body weight between participants assigned to consume low-CHO weight loss diets compared with those assigned to consume isocaloric higher-CHO (45–65% of energy) weight loss diets (184). A second meta-analysis also indicated there was no significant difference in weight loss between low-fat and high-fat weight loss interventions when the interventions were concordant for caloric restriction (185). Furthermore, an RCT with a 2 × 2 factorial design comparing four energy-restricted diets with low or high amounts of protein (10% or 20% of energy) and low or normal amounts of CHO (25% or 50% of energy) showed that weight loss after the 12-month intervention was unaffected by CHO content but significantly greater on the high protein diets (186). A recent 12-month weight loss diet study showed comparable weight reduction in subjects consuming a healthy low-fat diet (−5.3 kg) vs. a healthy low-CHO diet (−6.0 kg). Notably, the dietary instructions for both diet groups included the following: (1) maximize vegetable intake; (2) minimize intake of added sugars, refined flours and trans fats; and (3) focus on whole foods that were minimally processed, nutrient dense and prepared at home whenever possible (187). Collectively, these results suggest that hypocaloric high-CHO diets do not impede fat loss compared with

hypocaloric low-CHO diets in the majority of the population.

#### Ad libitum diets

However, because the majority of people do not spend the majority of their time consuming hypocaloric diets, far more important to the obesity epidemic is the question of whether high-CHO/lower-fat diets promote fat gain compared with low-CHO/higher-fat diets when consumed *ad libitum* (188). In the *ad libitum* condition, a differential effect between the two diets to promote weight gain could be mediated by mechanisms related to satiety and energy intake, as well as by differences in their potential to promote fat storage versus fat oxidation. Results from a meta-analysis of 16 studies suggest high-CHO/lower-fat diets do not cause more weight gain than low-CHO/high-fat diets. Instead it was concluded that a reduction in dietary fat to 27% of energy without intentional restriction of energy intake caused weight loss compared with control diets containing 37% of energy as fat (and less CHO) in subjects without obesity (189). A more recent meta-analysis of 30 RCTs comparing participants consuming *ad libitum* lower-fat versus usual or moderate-fat diets also showed a consistent but small effect of low-fat intake to reduce body fat and/or weight (190). However, the majority of the studies included in both of these meta-analyses provided the participants with dietary guidelines rather than diets, and the dietary instructions and counselling time spent with the groups consuming the fat-restricted diets were considerably more extensive than those provided to the groups consuming the higher-fat control diets. A separate analysis with five trials that did equalize attention between both diets groups showed there was still significantly more weight loss in the groups consuming the lower-fat diets (190).

Another recent meta-analysis does not support this conclusion (185). This 17-trial meta-analysis excluded trials shorter than 1 year because initial, maximal weight loss after approximately 6 months is often followed by weight regain (185). It showed that the weight loss exhibited by the low-fat groups was specific to comparisons with groups consuming 'usual diet' and receiving less attention in the form of dietary instruction and/or counselling. Only four of the included trials provided comparable attention to both the low-fat and higher-fat diet groups, and two showed greater weight loss with the low-fat diet while the other two did not. Therefore, the authors of this meta-analysis concluded that evidence from RCTs does not support low-fat diets over other dietary interventions for beneficial effects on energy balance (185).

Thus, the effects of high-CHO/lower-fat diets compared with low-CHO/higher-fat diets on energy balance continues to be a subject of controversy owing to the lack of well-controlled studies of sufficient duration. The challenges involved in filling this gap do not consist of just ensuring

that the attention provided to the intervention and control groups are equal. Study participants often fail to meet the goals of their dietary assignments, especially in the later months of the intervention period, even when equal, and even very intensive (191), dietary instructions and counseling are provided (191,192). This suggests that the better way to compare the effects of *ad libitum* high-CHO/low-fat diets with low-CHO/high-fat diets on energy intake and body weight gain is to provide the participants with ample food and meals formulated to the specification of the assigned diet and prohibit the consumption of any other foods. This is an expensive protocol that will still be limited by potential non-compliance (36) and failure to report the non-compliance (37). Future technological advances may address the compliance and reporting limitations with, for example, wearable food intake monitoring devices (193). However, even if/when perfect compliance and accurate reporting are achieved, there still remains the possibility that provided study diets lead to reductions in *ad libitum* energy intake because of monotony and curtailment of freedom of dietary choice, rather than to the specific dietary manipulations. Unintended weight loss, which could have been related to the lack of dietary choice rather than dietary composition, confounded the results of a recent crossover study that provided subjects with high-CHO meals for 4 weeks and then very-low-CHO meals for 4 weeks (194). The lack of freedom of choice is also a potential confounder for long-term inpatient protocols, especially ones in which the change of energy intake is the primary outcome. Restrictions related to dietary freedom can reduce energy intake, but inpatient confinement can lead to boredom and/or depression and increase energy intake.

Possibly, the protocol that best minimizes the monotony and the freedom of dietary choice issues for studies investigating effects of *ad libitum* diets on energy intake and body weight gain is the 'shop' model utilized in studies conducted in Europe for 6-month interventions (195). This protocol provides free foods that are appropriate to the formulations of the assigned experimental diets in a grocery shop setting. At the check-out stand, the foods selected by the participants are scanned to ensure that the selections in total meet the specifications for the assigned diet and provide at least 100% of daily Ereq. The uneaten foods are returned and re-scanned for calculation of energy and nutrient intake. The feasibility of utilizing this protocol for 1-year interventions is limited by the expense.

In addition to controlling the potential effects that restricted dietary choice and monotony may have on energy intake, the optimal protocol for comparing the effect of high-CHO versus high-fat diets on weight gain must also control for other dietary components that can affect satiety and energy intake. This includes protein, which has been shown to dose-dependently increase postprandial fullness, decrease postprandial hunger and affect homeostatic

hormones involved in the regulation of energy intake (196). There is also evidence to suggest that a high-protein diet reduces reward-driven eating behaviour (197,198). Fibre also affects satiety (199) by mechanisms that may include energy density, decreasing and slowing nutrient absorption from the intestine or triggering signals related to fullness by causing water absorption and distention in the stomach (200). Because whole grains contain more fibre and have a lower energy density than refined grain, it is not surprising that most short-term studies suggest they promote greater satiety (201). It also then would not be surprising if a study comparing a high-fat diet with a high-CHO diet with whole grains yielded different results than a study comparing a high-fat diet with a high-CHO diet with refined grains. The type of fat to be studied may also affect results as there is evidence to suggest that *n*-3 fatty acids increase satiety (202).

Thus, the challenges involved in resolving the high-CHO versus high-fat diet debate are immense. Research effort and funds may be better directed to determining the optimal *ad libitum* diet for promoting satiety and reduced energy intake. This was recently undertaken by Arguin *et al.* (200) who compared an *ad libitum* diet consisting of food components (protein > 20% of energy, whole grain, whole fruit and vegetable, *n*-3 fatty acids, chilli peppers with capsaicinoids (203) and calcium (204)) known to have satiety-enhancing properties (e.g. low-energy density and fibre) with an *ad libitum* control diet based the Canadian Food Guide for Healthy Eating for 16 weeks. Fat mass was significantly decreased, and the satiety quotient for hunger, fullness and prospective food consumption was significantly increased in male participants who consumed the diet with the satiety-enhancing food components compared with those who consumed the control diet. However, an important limitation of the dietary protocol that may have affected subject retention rates (satiety diet: 91.4%; control diet: 55.9%) and study results was the provision of one pre-prepared meal per day to the high-satiety diet group, but none to the control diet group (200). Nevertheless, the advantages of changing focus from diets designed around macronutrient groups to diets designed around high-nutrient, satiating food components that have the palatability to compete with low-nutrient, energy-dense processed foods warrant exploration.

#### *Preventing weight regain in weight-reduced subjects*

Another area that warrants more focus is how to prevent weight regain in subjects who have lost weight on energy-restricted diets. Numerous studies show that the energy homeostatic systems regulating energy intake, energy expenditure, neuroendocrine function and autonomic function in weight-reduced subjects conspire to oppose reduced weight maintenance (205–208), in individuals with or without obesity (207). These adaptations, rather than the

comparative efficacy of various weight-loss diets or treatments, are responsible for the depressingly unsuccessful clinical attempts to reverse the obese or overweight states. Investigations focusing on the leptin signalling pathways may have the most potential to yield strategies to prevent weight regain. Leptin administration, which has little effect on subjects at their usual body weight or on subjects consuming energy-restricted diets, has been shown to, at least partially, reverse many of the metabolic, autonomic, neuroendocrine and behavioural adaptations that lead to weight regain in weight-reduced subjects (209).

The weight regain period following hypocaloric diets may represent a specific scenario during which an *ad libitum* high-CHO diet does promote more weight gain than a low-CHO diet. A recent study showed that weight loss was not different in men consuming low-calorie diets containing either a high glycaemic load (65% of energy as CHO with a high glycaemic index) or lower glycaemic load (50% of energy as CHO with a low glycaemic index) (210), in agreement with the conclusions from the meta-analyses cited above (184,185). However, during the follow-up 3-week overfeeding period, the same men consuming 150% Ereq as the high-CHO/high-glycaemic-load diet gained 1 kg more body weight than the men consuming 150% Ereq as the low-CHO/low-glycaemic-load diet (210). Fat regain among all subjects was inversely associated with fasting fat oxidation (210). Two other investigations in weight-reduced subjects provide evidence that high-CHO/high-glycaemic-load diets promote greater weight regain (211) or lower energy expenditure (212) than lower-CHO/lower-glycaemic-load diets.

Energy restriction and weight reduction are associated with improved insulin sensitivity (213,214). However, with cessation of energy restriction, it may be possible that the augmented insulin secretion caused by high-glycaemic-load diets selectively impairs insulin sensitivity in muscle while maintaining it in white adipose tissue, thus resulting in lowered fat oxidation and increased fat storage. This mechanism, lowered muscle insulin sensitivity and increased adipose sensitivity, has been well documented in rodents exposed to hyperinsulinaemia (215–217), including in a rat model of weight recovery (218). Conversely, it was demonstrated that mice lacking insulin receptors in adipose tissue had normal whole-body glucose metabolism, but the insulin-stimulated glucose uptake in their adipocytes was reduced by 90%. This resulted in mice with reduced adipose tissue mass and increased longevity (219,220).

Other conditions associated with positive energy balance and lowered insulin sensitivity in skeletal muscle relative to adipose tissue could also lead to a disproportional gain in fat mass in response to a high-glycaemic-load diet. One example may be physical inactivity, which specifically lowers muscle insulin sensitivity (221). In support of this,

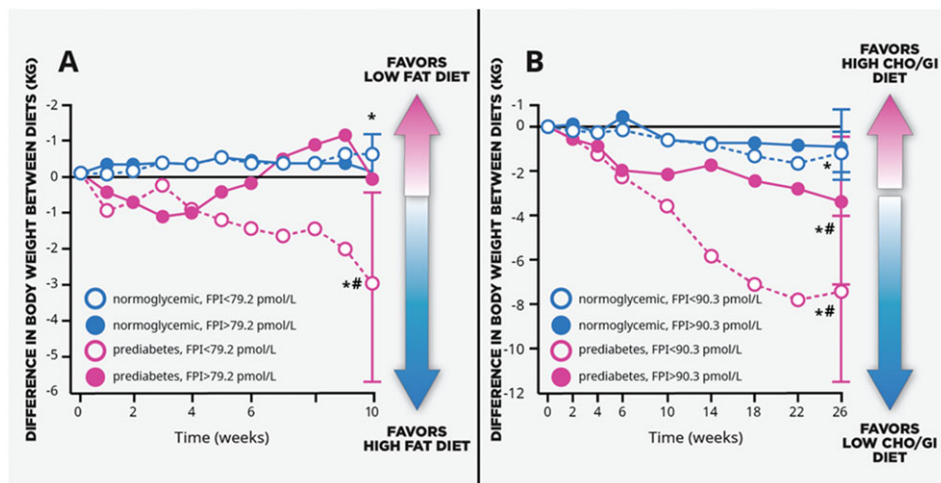
young healthy men displayed lowered whole-body glucose utilization during a hyperinsulinaemic euglycaemic clamp after 10 d of bed rest than before bed rest (222). This suggests decreased insulin sensitivity primarily in their skeletal muscle. In contrast, the glucose uptake in their subcutaneous abdominal adipose tissue was increased after bed rest (222). Lowered insulin sensitivity in skeletal muscle relative to adipose tissue may also occur in metabolically healthy obesity (223), a transient state (224) that may be explained by high adipose insulin sensitivity promoting adipose expansion over ectopic lipid deposition (225).

### Conclusions

At comparable levels of energy restriction, high-CHO/low-fat weight loss diets do not impede fat loss compared with low-CHO/high-fat diets. More well-controlled trials lasting at least 1 year are needed to determine the effects of *ad libitum* high-CHO/low-fat diets compared with *ad libitum* low-CHO/high-fat diets on energy balance. Emerging evidence suggests that, following weight loss on energy-restricted diets, *ad libitum* consumption of a high-CHO/high-glycaemic-load diet may, via increased insulin exposure, decrease insulin sensitivity in muscle and increase insulin sensitivity in adipose, thus increasing susceptibility to weight regain. More clinical studies are needed to test this hypothesis, but also to investigate other strategies that may affect weight regain following otherwise successful non-surgical weight loss.

### High-carbohydrate/high-glycaemic-load diet and metabolic status: Arne Astrup

A recent publication provides evidence to suggest that susceptibility to weight gain on a high-CHO diet may be influenced by the metabolic status of the individual (226). The investigators reanalysed data from three dietary intervention studies in which diets that differed in CHO content (227,228) or glycaemic load (192) were compared for their effects on weight loss or weight regain. The reported effects of diet group on body weight in the total population of individuals with obesity were quite small (weight-reduced subjects on low-glycaemic-load diet regained 1.9 kg less than those on the high-glycaemic-load diet over 6 months (192)) or undetectable (227,228). However, when the subjects were divided into subgroups based on their baseline fasting glucose and insulin levels, consistent and more pronounced effects of the different diets were detected (226). In all three studies, participants with high baseline fasting glucose and low fasting insulin exhibited a greater loss of body weight on the diets with a low CHO content or glycaemic load than participants with the same glucose and insulin profile on diets with higher CHO content (Fig. 4A) or glycaemic load (Fig. 4B). These analyses suggest that insulin-resistant obese individuals, with a low capacity



**Figure 4** (A) Reanalysed results from the NUGENOB Study: subjects with obesity, prediabetes and low fasting insulin lost more weight on a high-fat vs. low-fat diet. (B) Reanalysed results from the DioGenes Study: subjects with obesity, prediabetes and low fasting insulin regained three to four times less weight on a low-carbohydrate (CHO)/low-glycaemic-index (GI) diet than subjects with normal glycaemia and obesity.  $P < 0.05$  from zero;  $\#P < 0.05$  between glycaemic/insulinaemic groups. Fasting plasma insulin (FPI). Modified from Hjorth *et al.* (226). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

to increase insulin secretion and overcome the resistance, achieve more satiety and weight loss on lower-CHO/ lower-glycaemic-load diets.

In another study, 90 participants who consumed the New Nordic Diet (more calories from local and wild countryside plant foods, more seafood and seaweed from local seas and lakes and fewer calories from meat (229)) for 6 months lost 3 kg more body weight than 56 participants who consumed the average Danish diet (230). Subsequent analysis showed that participants with high fasting glucose/low fasting insulin lost 6 kg more body weight consuming the New Nordic Diet than participants with the same glucose and insulin profile consuming the average Danish diet (226). It is important to note that food records suggest that the two groups consumed comparable amounts of available CHO, but the New Nordic diet group consumed  $15 \text{ g d}^{-1}$  more fibre (230) and more whole grain. This suggests that the glycaemic index/load (not assessed in the study, but likely lower in the New Nordic diet due to the fibre and whole grain) rather than CHO content is involved in the body weight differences between the participants with high fasting glucose/low fasting insulin on the two diets. However, a recent report from the study investigators also suggests an influence of gut microbiota on diet response (231). Faecal samples were collected from a subgroup of the subjects before starting the New Nordic or average Danish diets, and the ratio of the relative abundance of *Prevotella* spp. to *Bacteroides* spp. (P/B) was determined. Previous reports have identified *Prevotella* and *Bacteroides* as two typical bacterial clusters present in humans (232), with the relative abundance of *Prevotella* associated positively with host diet (vegetarian and fibre) (233,234) and negatively with cardiometabolic risk factors (234). Among individuals with a high P/B ratio, the New Nordic Diet

resulted in a 3.2 kg larger body fat loss compared with the average Danish diet, while no difference in body fat loss was observed between the Nordic diet and average Danish diet among individuals with low P/B. Among individuals on the New Nordic diet, those with a high P/B ratio lost significantly more body fat than those with a low P/B ratio, while body fat loss was not different between high-P/B and low-P/B groups consuming the average Danish diet (231). Recently, dietary-fibre-induced improvements in postprandial blood glucose and insulin were found to be positively associated with the abundance of *Prevotella* (235). Therefore, the importance of baseline fasting glucose and insulin (i.e. insulin sensitivity) for diet response with regard to weight loss may be linked to gut microbiota and dietary fibre content (231).

Grouping subjects by a combination of fasting glucose and fasting insulin was found to be superior to other various indices of insulin resistance, such as the 30-min response to a glucose dose, for detecting group-by-diet interactions. A good prediction is also achieved when using fasting glucose alone. Patients with T2D or with three or more CVD risk factors were assigned to consume a low-fat Mediterranean diet or a Mediterranean diet with extra-virgin olive oil, and after 5 years, the weight loss difference between the two diet groups was less than 0.5 kg (39). However, when regrouped by fasting glucose, the subjects with higher glucose levels on the Mediterranean diet lost  $\sim 2$  kg more body weight than the subjects with higher glucose levels on the lower-fat diet.

### Conclusions

Emerging evidence suggests that high-CHO diets, or more specifically, high-glycaemic-load/low-fibre diets may promote weight gain or impede weight loss in subjects with

impaired glucose metabolism/insulin resistance. Pretreatment fasting glucose and insulin measurements could be useful for identifying individuals who would benefit most from a low-glycaemic-load/high-fibre diet that may improve their weight loss and/or weight maintenance success.

### Non-nutritive sweeteners: Allison Sylvetsky

Because NNSs such as aspartame, sucralose, saccharin, acesulfame K and steviol glycosides such as rebaudioside A (extracted from the stevia plant) contain zero or negligible calories, they do not directly contribute to the energy intake side of the energy balance equation. However, it has been suggested that NNS consumption, through several potential mechanisms, may indirectly affect energy balance (236–238). A meta-analysis of nine prospective cohort studies of adults and children demonstrated a positive association between NNS consumption and BMI ( $0.03 \text{ kg m}^{-2}$ , 95% confidence interval: 0.01, 0.06) (239). Epidemiological studies have also shown positive associations between NNS and metabolic syndrome (240–242) and T2D (241,243–248). These studies do not demonstrate causation and are limited by the possibility of residual confounding and reverse causality. Specifically, individuals with higher BMI who are concerned about their weight or patients with T2D may then choose to consume NNS instead of caloric sweeteners. However, studies in which rodents gained more weight (249,250) or exhibited inflammation (251) or glucose intolerance (252) consuming NNS compared with rodents consuming glucose (249) or sucrose (250) provide support for potential cause and effect relationships.

Mechanisms such as sweet taste receptor activation, disturbance of the expected relationship between sweetness and calories, changes in taste preferences and alteration of gut microbiota may explain these associations (236–238). It is also possible that caloric compensation occurs, negating calories ‘saved’ by using NNS. This compensation could be psychological, whereby one’s knowledge of consuming a lower-calorie NNS-containing alternative may lead to giving oneself permission for greater calorie ingestion at subsequent meals. Compensation could be physiological, in which consumption of lower-calorie NNS-containing alternatives promotes heightened hunger and subsequently higher calorie intake.

However, it has been questioned whether any of the above mechanisms occur in humans and whether they occur consistently in rodents. Based on a systematic review, it was reported that in 62 of 90 animal studies, NNS did not increase body weight (253), and a more recent meta-analysis of 12 prospective cohort studies did not support an association between NNS consumption and BMI ( $-0.002 \text{ kg m}^{-2}$ ; 95% confidence interval:  $-0.009$ ,  $0.005$ ) (253). Furthermore, two separate meta-analyses consisting of 10 (239)

and eight (253) RCTs both indicated that substituting NNS for sugar resulted in a modest weight loss in adults. Findings also favoured weight loss when NNS was compared with water, but this meta-analysis only included three trials (253). Because the benefits of substituting NNS for sugar is a very different question than the benefits of substituting NNS for water, more studies comparing NNS and water are warranted. This is especially true for the sucralose, saccharin, acesulfame K and steviol glycosides, which have been much less studied than aspartame. Aspartame was utilized in nine of the studies included in the meta-analyses (239,253), and three of these nine studies administered aspartame in commercially available beverages, consistent with the manner in which aspartame is frequently consumed (254).

It does not appear that any of these RCTs revealed adverse effects of NNS consumption on risk factors for cardiometabolic disease (255). Therefore, NNS may be a useful tool for increasing adherence to behavioural weight loss regimens. The longest intervention study conducted to date included 163 obese women who were randomly assigned to consume or to abstain from aspartame-sweetened foods and beverages during 16 weeks of a 19-week weight reduction programme (active weight loss), a 1-year maintenance programme and a 2-year follow-up period. The aspartame group lost significantly more weight overall and regained significantly less weight during the 1-year maintenance and the 2-year follow-up than the no-aspartame group (256). During a more recent study in which 303 men and women who habitually consumed diet (NNS) beverages participated in a weight loss programme that included a randomized assignment to either stop drinking NNS beverages and consume 24 oz (710 mL) of water or to continue drinking NNS beverages daily for 1 year (257), those in the NNS beverage group lost more weight. These results should be interpreted with caution as those randomized to NNS beverages were essentially continuing their usual beverage intake, while those who were assigned to switch to water were required to implement and sustain a behaviour change. Furthermore, consuming NNS in the context of an intensive weight loss programme focused on calorie reduction may promote more weight loss than NNS use in the general population. Nevertheless, several studies that were not conducted as part of weight loss programmes also showed no effects of NNS to increase body weight (125,128,258,259). This includes the 6-month intervention cited above in which overweight/obese subjects consumed 1 L of sucrose-sweetened cola, isocaloric amounts of low-fat milk or 1 L of aspartame-sweetened cola or water per day. There were no significant differences between the effects of aspartame-sweetened cola and water on body weight, visceral adiposity, liver fat and metabolic risk factors including blood TGs, HDL, total cholesterol, fasting plasma glucose, fasting plasma insulin, HOMA-IR and uric



acid (124,128). Indeed, there are no clinical intervention studies involving chronic NNS exposure in which NNS induced a weight increase relative to sugar, water or habitual diet (253).

While studies investigating effects of NNS on weight in children are limited, the largest study to date included 641 normal-weight children, aged 4–11 years, who were randomized to groups consuming 8 oz of sucralose-sweetened beverage or SSB daily for 18 months (260). Compared with SSB consumption, consumption of sucralose-sweetened beverage reduced weight gain and fat accumulation. However, this study did not include an unsweetened control. Because children gain weight due to growth over an 18-month period, without a water or attention control group, it cannot be determined whether the sucralose-sweetened beverage had no effect on body weight or just less effect than the SSB.

### Conclusions

Randomized controlled trials consistently demonstrate that consumption of NNS may promote decreased energy intake, particularly when used as part of comprehensive weight loss programmes. Additional well-controlled RCTs are warranted, most specifically for saccharin, acesulfame K and steviol glycosides, which have been studied less frequently than aspartame and for periods no longer than 16 weeks. More studies in children and more studies assessing metabolic and health outcomes beyond body weight, such as effects on glucose tolerance and inflammation, are needed.

### Responses in brain regions associated with reward to the palatable Western diet: Eric Stice

Palatable, high-sugar and/or high-fat foods, major components of the typical Western diet, can indirectly affect the energy-in side of the energy balance equation through activation of brain regions associated with reward that promotes overeating. Importantly, it is not the greater reward region response to the tastes of palatable food that predicts future weight gain, but rather the greater reward region response to images of palatable foods (including advertisements) and visual cues (eg. Golden Arches™) that signal impending tastes of palatable foods. Both animal (261,262) and human (263) experiments indicate that after repeated pairings of cues (cartoon picture of chocolate milkshake (263)) that predict palatable food tastes and actual palatable food tastes, dopamine signalling increases in response to predictive cues but decreases in response to actual food tastes. Therefore, excessive caloric intake that results in weight gain is associated with reduced caudate (one of the brain structures that compose the reward system) response to tastes of high-sugar/high-fat foods (264) and an increase in reward region response to visual cues that suggest impending tastes of these foods (265). An apparent result of this process is that exposure to food cues prompts eating in the absence of hunger,

which contributes to excessive weight gain. Indeed, prospective studies show that elevated reward responses to television advertisements depicting palatable foods that are high in dietary fats and sugars (266) or to food cues (267) predicted weight gain in adolescent girls and boys (266–268), young women (269) and adults (270) over periods ranging from 6 months to 3 years. Furthermore, interventions that reduce reward region responses to food cues, either through food response training (271,272) or blockage of sweet taste receptors (272,273), promoted weight loss or reduced intake of high-sugar foods.

Functional magnetic resonance imaging (fMRI) is considered to be one of the best tools currently available for the detection of regional brain activations to specific cues or tasks (274). However, correlations from studies utilizing fMRI have been faulted for being artificially inflated mainly due to small sample sizes (275). More recent studies, though, have confirmed the predictive relationship between increases in reward region response to food cues and energy intake or future weight gain using prospective data that establish temporal precedence (265), split-half replication (265,267) and sample sizes of ~150 (265,267,276). Furthermore, in these larger studies (265,267,276), healthy body weight (BMI > 18 and <25 kg m<sup>-2</sup>) was an inclusion criteria, thus ensuring that prior overeating or obesity did not contribute to the reward region response measured by fMRI at baseline (267,276). Collectively, these studies indicate the potential value of fMRI as a tool to study vulnerabilities to weight gain and also highlight food cues for their likely importance in the obesity epidemic (277).

### Conclusions

Elevated reactivity in brain regions associated with reward in response to food cues predicts future weight gain. Regular intake of palatable foods that are high in fat and sugar increases responses to food cues in brain regions associated with reward, further increasing overeating. Strategies that reduce responses to food cues in brain regions associated with reward may be effective for promoting weight loss and preventing weight gain. This is an evolving area with high potential to increase knowledge regarding the development of obesity and perhaps help to curb the obesity epidemic.

### Emerging evidence about sugar consumption during critical periods and in vulnerable populations:

#### Michael Goran

Elevated reactivity in brain regions associated with reward in response to high-sugar-food cues may explain the positive associations between added sugar and SSB consumption and weight gain in both prospective cohort trials (278–283) and RCTs (281,283). However, it does not explain the emerging evidence that suggests effects of direct and indirect sugar exposure on body composition in very young children.

### *Vulnerability to second-hand sugar exposure during gestation*

Gestation is a critical developmental period during which second-hand sugar exposure may increase susceptibility to obesity in the offspring. Gilman *et al.* recently reported that in 1,078 mother–child pairs, maternal SSB consumption during the second trimester of pregnancy was positively associated with several indices of obesity in 7-year-old children, including fat mass measured by dual-energy X-ray absorptiometry (284). The associations were not attenuated when adjusted for maternal energy intake or for the number of SSB servings per day consumed by the children. The results also showed no association between maternal intake of NNS beverages and child BMI/adiposity. Both findings are in conflict with the results of a recent study by Zhu *et al.* who reported a positive association between child BMI z-score at age 7 and maternal intake of NNS beverages during gestation, but no association with maternal intake of SSB (918 mother–child pairs) (285). Study differences that may have contributed to the discordant results include less accurate and objective outcomes (child height and weight reported by the mother (285) versus height, weight and body fat via dual-energy X-ray absorptiometry measured by study staff (284)) and less precise adjustment increments for the child's intake of SSB and NNS beverages at age 7 (<1 or ≥1 serving per week (285) versus servings per day (284)) in the latter study (285). It is also possible that the association between maternal consumption of NNS beverages and child BMI z-score represented reverse causation. In the Zhu *et al.* study, pre-pregnancy BMI was higher (27.6 kg m<sup>-2</sup>) (285) than in the Gilman study (24.6 kg m<sup>-2</sup>) (284), and it was positively associated with NNS beverage consumption ( $P < 0.001$ ) (285). However, another recent study also reported positive associations between maternal consumption of NNS during pregnancy and risk of overweight of the offspring during infancy and no such association with maternal consumption of SSB (3,033 pairs) (286). Further analyses from this study showed the positive associations between maternal consumption of NNS during pregnancy and risk of overweight were specific to male offspring and to infants who were not breastfed for at least 6 months (286). Also, *post hoc* analyses revealed positive associations between maternal SSB, fruit drinks and fruit juice consumption and risk of overweight in the offspring (287). As recently reviewed, the evidence concerning the long-term effects of NNS exposure during gestation, infancy and childhood is limited and inconsistent, and more investigation is needed (288).

### *Vulnerability to second-hand sugar exposure during lactation*

Lactation is also a critical developmental period during which second-hand sugar exposure may increase susceptibility to obesity in the offspring. A recent study of 25 mother–child pairs provided evidence that the fructose

concentration in breast milk is associated with infant body composition, including fat mass (289). Breast milk samples collected at 1 and 6 months post-partum contained fructose, but at much lower concentrations than glucose and lactose (means = 0.0037, 0.14, and 421 mmol L<sup>-1</sup>, respectively). However, the concentrations of fructose were quite variable, and it was the only component of breast milk that was positively associated with infant body weight, fat mass, lean mass and bone mineral content at 6 months of age (289). While this level of fructose has been shown to enhance transcription factors involved in adipocyte differentiation (peroxisome proliferator-activated receptor  $\gamma$  and CCAAT/enhancer-binding protein  $\alpha$ ) and increase glucose transporter 4 expression in preadipocyte culture (290), more research will be needed to determine whether it has *in vivo* effects in breastfed infants.

### *Vulnerability to first-hand sugar exposure during infancy and early childhood*

If infants are susceptible to the effects of second-hand fructose exposure during gestation and lactation, then it follows that they are also susceptible to direct effects of fructose consumption. In support of this, the prevalence of obesity in a study of 1,189 6-year-old children was twice as high in those who consumed SSB during infancy (0–12 months of age) compared with those who consumed none (17.0% vs. 8.6%) (291). Adjustment for SSB intake at age 6 did not eliminate this association (291). Importantly, consumption of any SSB during infancy also doubled the risk that these children would be consuming at least one SSB per day at age 6 (292).

Added sugar consumption may contribute to the susceptibility of Hispanic children to early-onset obesity within the first few years of life. The prevalence of obesity in 1,483 Hispanic children, 2–4 years of age, from the Los Angeles County Women, Infants and Child programme, was 15% (293) compared with the average of 10% in 2–5-year-old NHANES children born in the same years (294). Based on caregiver reports, 18% of the Hispanic children consumed two or more servings of SSB per day (serving = 12 oz), 25% consumed one serving and 57% consumed zero SSB serving per day. Overall, this a 50% higher level of SSB consumption than that reported for 2- to 3-year-old children in NHANES 2003–2004 (295). SSB consumption by the young Hispanic children was associated with BMI, with the prevalence of obesity exceeding 20% among children who consumed two or more SSB servings per day (293). On the positive side, it was also observed that the prevalence of obesity was reduced to 10% among the children consuming two or more SSB servings per day who had been breastfed for ≥1 year, resulting in a significant interaction between SSB intake and breastfeeding ( $P = 0.005$ ) (293). More study of young Hispanic children will be needed to determine the contributions of added sugar consumption,

socio-economic status, cultural factors and genetics to their high prevalence of obesity and to examine the mechanisms underlying the protective effects of longer-term breastfeeding.

#### *Sugar consumption in vulnerable populations*

Sugar exposure may have unique effects in certain populations. Hispanics for example may be particularly vulnerable to the adverse effects of sugar exposure because they are disproportionate carriers of the variant allele of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene. The *PNPLA3* variant allele has a frequency rate of about 50% in Hispanics compared with <10% in other populations (296). The *PNPLA3* variant predisposes to NAFLD (297) and is associated with increased liver fat (296,298), elevated plasma alanine transaminase levels (299,300) and cirrhosis/advanced fibrosis (301) in the Hispanic population. Hispanic children with obesity, ranging in age from 8 to 18 years, also exhibited significantly increased liver fat as a function of *PNPLA3* risk allele number, with 64% of the homozygous carriers having a liver fat content greater than 5.5% (302) (>5% liver fat = NAFLD).

The effects of the *PNPLA3* variant allele may be exacerbated in the context of a high-sugar diet (303). *PNPLA3* variant allele knock-in mice had normal levels of hepatic fat on a chow diet, but when challenged with a high-sucrose diet, their liver fat levels increased twofold to threefold compared with wild-type littermates consuming a high-sucrose diet (297). Obese Hispanic children with two copies of the *PNPLA3* variant were particularly susceptible to increased liver fat when consuming a diet high in added sugar (302). Conversely, when adults homozygous for the variant allele were placed on a low-calorie, low-CHO diet, they lost 3 kg of body weight and reduced liver fat by 45% in only 6 d. Control subjects, who were homozygous for the wild-type gene and matched for BMI and liver fat, lost the same amount of weight, but their reduction in liver fat (18%) was significantly lower (304). More studies are needed to confirm a gene \* diet interaction between the *PNPLA3* gene variant and dietary sugar intake.

#### *Conclusions*

Added sugar consumption in early life is associated with higher obesity in childhood. More research is needed to determine if first-hand and second-hand sugar exposure during critical developmental periods, specifically gestation, lactation and infancy, is associated with higher risk of obesity. The greater frequency of the *PNPLA3* gene variant in Hispanics and its potential interaction with dietary sugar may make Hispanic children and adults particularly susceptible to the negative health effects of high-sugar diets.

#### Contributions of the gut microbiome to diet-induced obesity: Peter Turnbaugh

Consumption of a high-fat, high-sugar Western diet may affect one or both sides of the energy balance equation by shifting the types of microbes found within the gastrointestinal tract (the gut microbiome) and their metabolic activity. Consumption of the *Western* diet results in a significant change to the gut microbiome (often increased Firmicutes and decreased Bacteroidetes) (305), a microbial signature that is remarkably reproducible across inbred and outbred mice of diverse genotypes and mice with defects in immunity and metabolism (306). Gut microbes from mice with Western-diet-induced obesity or from lean mice consuming a low-fat, plant-polysaccharide-rich diet were transplanted into germ-free (GF) recipients. The GF mice receiving the microbiome from mice with Western-diet-induced obesity gained more body fat than the mice receiving the microbiome from the lean donors (305), demonstrating that differences in the mouse gut microbiome can affect host energy balance.

Human studies also support causal links between diet, the gut microbiome and host energy balance (307–309). Short-term dietary interventions revealed that consumption of a high-fat diet rich in animal products reshapes the human gut microbiome within a single day (310). Transplantation of the stool microbiome from human twins discordant for obesity demonstrated that the mice receiving the microbiomes of the twins with obesity gained more body fat than mice colonized with the microbiomes of lean twins (308).

Multiple mechanisms have been proposed through which changes in the structure and function of the gut microbiome influence host adiposity, encompassing both sides of the energy balance equation (311). One possible mechanism by which gut bacteria may increase 'energy in' is by liberating additional calories from the diet and/or altering the intestinal absorption of nutrients. Compared with lean controls, the distal gut microbiomes from obese mice were enriched for genes that encode enzymes that break down substrates such as plant polysaccharides that cannot be metabolized by human enzymes (312). Consistent with studies of the Western-diet-associated microbiome (305), transplantation of the distal gut microbiome from obese mice resulted in a significant increase in host adiposity (312). Other groups have identified single bacterial strains and even specific proteins that are sufficient to ameliorate diet-induced obesity. Colonization with the gut Verrucomicrobium *Akkermansia muciniphila* led to increased stool calories (consistent with altered intestinal metabolism and/or absorption), normalization of plasma lipopolysaccharide concentration and decreased insulin resistance and triglyceridaemia in high-fat-fed mice (313). These effects appeared to be partly mediated by activation of toll-like receptor 2 by a specific protein present on the outer membrane of *A. muciniphila* (313,314). Recent studies have also suggested that gut

bacterial metabolites may increase appetite through the activation of the parasympathetic nervous system (315).

On the other hand, there is emerging evidence to suggest that gut bacteria can also affect energy output (316). An example of one potential mechanism involves microbiota-induced changes in bile acid composition, which can alter signalling to the bile acid nuclear receptor, farnesoid X receptor (FXR) (317–319). A recent study in conventionally raised (CONVR) or GF mice that were wild type (FXR<sup>+/+</sup>) or FXR knockout (FXR<sup>-/-</sup>) suggested that the gut microbiota impacts diet-induced obesity via FXR signalling. While consuming a high-fat diet for 10 weeks, CONVR FXR<sup>+/+</sup> mice gained more body weight and had higher levels of liver fat than CONVR FXR<sup>-/-</sup> mice, GF FXR<sup>+/+</sup> mice or GF FXR<sup>-/-</sup> mice (320)). Previous studies in mice treated with an FXR inhibitor have shown that the decreases in liver fat were accompanied by unaffected energy intake and increased energy expenditure (321).

### Conclusions

Consumption of a processed high-fat, high-sugar diet alters the structure and function of the mouse gut microbiome (322). Data from animal models suggest that the gut microbiome can shape both sides of the energy balance equation, altering energy intake and expenditure. More research is needed to better understand the mechanisms responsible, the role of diet-induced changes to the gut microbiome in the pathophysiology of cardiometabolic disease and the translational relevance of these findings for the treatment of human obesity.

### Overall conclusions

The overall purpose of the 2017 CrossFit Foundation Academic Conference was to address the following question: are all calories equal with regard to effects on cardiometabolic disease and obesity? It was the first objective of the conference to consider whether certain dietary components, specifically fat and sugar, increase risk for cardiometabolic disease by metabolic mechanisms that are not mediated solely by positive energy balance and fat gain. Although this is a complex area involving the need to account for many inter-related factors, including the challenges related to manipulating and/or documenting dietary intake in human research subjects, the authors agree on the following points:

- Evidence suggests that consumption of *n*-6 fatty acids results in lower cardiometabolic risk factors/risk compared with isocaloric amounts of SFA. However, differences exist between individual SFA, and the food matrix needs to be considered; e.g. dairy foods such as cheese and yogurts are associated with reduced cardiometabolic risk. More research is needed to clarify

the differences among the individual SFA and SFA-containing foods.

- Evidence strongly suggests that consumption of fructose-sweetened, HFCS-sweetened or sucrose-sweetened beverages increases cardiometabolic risk factors/risk compared with isocaloric amounts of starch. More research is needed comparing the metabolic effects of SSB versus sugar in solid food and sugar in solid food versus refined or whole grain starch.

Under the second objective of the conference, it was considered whether all calories were equal with regard to their potential to promote fat gain and obesity. More specifically, evidence was presented suggesting that certain dietary patterns or components have the potential to promote fat gain via mechanisms that are in addition to their specific contribution of calories to the 'energy in' side of the energy balance equation. The authors agree on the following points:

- There is currently insufficient evidence that a high-CHO diet affects weight gain or weight loss to a different extent than a high-fat diet. Susceptibility to weight gain when consuming diets high in refined CHO/glycaemic load may be affected by the metabolic status of the individual (i.e. glucose tolerance/insulin sensitivity). More studies focused on strategies to prevent weight regain in weight-reduced subjects are needed.
- RCTs ranging from 4 weeks to 3 years in duration demonstrate that consumption of aspartame does not promote body weight gain in adults. Well-controlled and long-term RCTs in adults are warranted to assess the effects of saccharin, acesulfame K and steviol glycosides on body weight and other health outcomes. More studies to assess the effects of all types of NNSs in children are needed.
- Continued research on the following topics could provide important insights and strategies for slowing the obesity epidemic.
  - The high-sugar, high-fat palatable *Western* diet could be perturbing both sides of the energy balance equation through effects on brain regions associated with reward and/or on the gut microbiome.
  - Susceptibility to weight gain may be affected by exposure to sugar and/or NNS during critical periods of development from pre-conception to adult life.

### Nutrition and public health

Rates of obesity and cardiometabolic disease continue to climb globally (7,323–326). This is clearly a multifactorial problem that involves genetic, metabolic, behavioural and environmental factors, and progress towards a resolution will require consideration of all these factors (327). Regarding nutrition, the speakers and delegates at this conference

agree that strategies to solve the complex problems associated with obesity should include consideration of the above conclusions regarding added sugar and specific high-SFA foods. Also, we need more research on the effects of diet in vulnerable populations and during critical periods of development, and the ways that responses to diet may be mediated by the genotype or metabolic status of the individual, by the responsiveness of brain regions associated with reward to food cues or by the microbiome. Such research may provide new strategies for attenuating the obesity crisis by developing more individual-based/precision-based nutrition approaches. Therefore, the search for solutions must include support for research to promote the potential of these new strategies and to fill knowledge gaps. And, finally, they also agree that the scientific evidence related to any single food or macronutrient or mechanism is less likely to impact the global epidemics of obesity and cardiometabolic disease as significantly as solutions related to the following:

- *Prevention*: The food environment needs to be improved. Food policies that will improve the availability and reduce the costs of healthy foods compared with high-calorie, palatable foods are needed. Lowering the plethora of food cues that may promote overeating of high-sugar, high-fat palatable foods could also be an effective strategy.
- *Healthy dietary patterns*: Strategies that emphasize the health benefits and palatability of dietary patterns consisting of whole grains, fruits, vegetables and healthy fats, rather than the negative consequences of a single food or food group, are likely a better approach to prevention.
- *Personalized nutrition*: Individual variability needs to be considered when defining the healthy diet. For example, the optimal diet composition for weight control may depend on the individual's glucose metabolism. In addition, genetics, the microbiome, life stages (pregnancy, infancy and early childhood), culture, dietary preferences and weaknesses (i.e. 'Is the brain reward region more activated by sweet candy bars or salty potato chips?') should all be considered when determining the optimal diet pattern for an individual or susceptible segment of the population.

### Conflict of interest statement

The following authors have no conflicts of interest to report: Drs Schmidt, Turnbaugh and Bray.

The following authors acknowledge these conflicts of interest.

Dr Stanhope received honoraria from the CrossFit Foundation for serving as a Conference Academic Organizer and for writing the manuscript.

Dr Goran received honorarium from the CrossFit Foundation for serving on the Advisory Committee and travel reimbursement from the CrossFit Foundation for attending the conference in July 2017.

Drs Bosy-Westphal, King, Schwarz, Sylvetsky, Gardner, Mason, Rosenbaum and Allister-Price and Ms Sigala received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017.

Dr Malik received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017. She was paid for consulting services by the City of San Francisco for litigation related to health warning labels of soda and is on a pro bono retainer to The Center for Science in the Public Interest for expert support in litigation related to sugar-sweetened beverages.

Dr Havel received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017. He has received research grants from Bristol Myers Squibb and Arrowhead Pharmaceuticals.

Dr Stice received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017. His institution has received research funding from Crave Crush, and he has received reimbursement for travel from Crave Crush.

Dr Ravussin received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017. He serves on the Scientific Advisory Board to the Nutrilite Health Institute with Amway and for the Institute of Cardiometabolism and Nutrition in Paris, France; has a consultant contract with Janssen and with Nutrilite Health Institute with Amway; gives lectures at the Open Academy in Venice; and is a lecturer/advisor for the Center for Medical Weight Loss. He has received research grants or unrestricted gifts from Amway, Nestle, the Nutrition Science Initiative, Novartis, Sanofi-Aventis, Weight Watchers and Ethicon Surgery. He has a patent for 'Night Moderate Hypoxia to Treat Insulin Resistance and Cardiometabolic Syndrome'.

Dr Welsh received travel reimbursement from the CrossFit Foundation for attending the conference in July 2017. She received payment from the Sugar Foundation for an analysis and presentation on sugar consumption in toddlers.

Dr Greenwood received honorarium for serving as Chairperson of the manuscript planning meeting and travel reimbursement from the CrossFit Foundation for attending the conference in July 2017.

Dr Astrup received honorarium from CrossFit Foundation for serving on the Advisory Committee and travel reimbursement from CrossFit Foundation for attending the conference in July 2017. He reports personal fees from Dutch Beer Institute, NL; Feast Kitchen A/S, Denmark; Groupe Éthique et Santé, France; McCain Foods Limited, USA; Nestlé Research Center, Switzerland; Weight

Watchers, USA; BioCare Copenhagen, Zaluvida, Switzerland; Basic Research, USA; Beachbody, USA; Danish Agriculture & Food Council, Novo Nordisk, Denmark; Pfizer, Germany; Saniona, Denmark; Sanofi-Aventis, Germany; S-Biotek, Denmark; Scandinavian Airlines System, Denmark; and Tetra Pak, Sweden; personal fees and other from Gelesis, USA; grants from Arla Foods, DK; Danish Dairy Research Council; and Gelesis, USA outside the submitted work. In addition, Dr Astrup has a patents pending to the University of Copenhagen 'Methods of inducing weight loss, treating obesity and preventing weight gain' (licensee Gelesis, USA) and 'Biomarkers for predicting degree of weight loss' (licensee Nestec SA, CH), and he is a co-inventor of a number of other patents owned by the University in accordance with Danish law. Astrup receives royalties for the books *Verdens Bedste Kur*, Politikens Forlag, Denmark, 2012 (subsequently published in English as *World's Best Diet*, Penguin, Australia, and *The Nordic Way*, Random House, USA), and *Spis dig slank efter dit blodsukker (Eat according to your blood sugar and be slim)*, Politikens Forlag, Denmark, 2017. He is a co-author of several books in the pipeline about personalized nutrition for weight loss; a co-owner and member of the board of the consultancy company Dentacom Aps, Denmark, co-founder; and a co-owner of UCPH spin-outs Mobile Fitness A/S & Flaxslim ApS (where he is also a member of the board, 2015–present) and Personalized Weight Management Research Consortium ApS (Gluco-diet.dk/2017–present).

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