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WEB OF CAUSATION BETWEEN DIETARY PATTERNS AND CHILDHOOD OBESITY: APPLYING HILL'S CRITERIA

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Abstract

Since their publication in 1965, the Bradford Hill criteria for causality have been largely used as a framework for causal inference in epidemiology. We aim at employing this classical approach to shed new light onto the web of causation of childhood obesity. Although the fundamental cause of obesity is the long-term imbalance between energetic need and intake, this medical condition is multifactorial in its origin, influenced by genetic, behavioral, socioeconomic, and environmental factors. This imbalance leads to accumulation of excessive adipose tissue. Observational studies tend to mostly quantify association between dietary factors and accumulation of adipose tissue. On the other hand, multivariate analysis proved some of these associations to be spurious, therefore prospective trials are needed to demonstrate causality. Short term experimental studies have been conducted to identify unique dietary pattern changes on specific outcomes, but long term, community-based studies would offer more comprehensive answers on dietary pattern effects. We conducted a literature review on PubMed, Scopus, Web of Science, and Google Scholar. From a total of 323 papers identified at first stage, we further discuss the applicability of Bradford Hill criteria for 31 articles, by examples of dietary patterns and accumulation of excess body fat as exposure-response associations. We also put forward and analyzed the evidence prospective studies would bring, as foundation for future interventions.

key words: *pediatric obesity, diet habits, nutritional quality, fatty tissue, criteria for causation*

Background and aims

There are more than 50 years since Sir Austin Bradford Hill has explained and discussed in the Proceedings of the Royal Society of Medicine his famous nine criteria for finding and proving causation between exposure and disease, useful as a guide, rather than a check list [1]. Kenneth Rothman resumed the criteria and explained the several exceptions in proving causality when employing heuristic methods such as causal criteria, proposing other models, such as the sufficient and component cause models, and multiple causality [2].

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In the field of nutritional epidemiology, the cause is "remote and indirect", except for food borne microbiological diseases, when the cause is "immediate and direct", especially for non-communicable diseases. Public health recommendations for certain dietary patterns are based on well proven causality relations. However, there are timely delays in applying scientific findings in recommendations for communities, too often even when there is a proven a causal relation [3].

Obesity, a non-communicable disease, has prolonged positive imbalance between intake and expenditure of energy as a fundamental cause. As of 2013, the prevalence of overweight and obesity in children and adolescents reached the highest known levels: 23.8% (95%CI 22.9–24.7 %) and 22.6% (95%CI 21.7–23.6 %) for boys and girls, respectively, in developed countries, and 12.9% (95%CI 12.3–13.5 %) and 13.4% (95%CI 13.0–13.9 %) for boys and girls, respectively, in developing countries [4].

In humans, energy intake is associated with food consumption, in the form of macronutrients: protein, carbohydrate, fat and alcohol. Since 1970s increasing time trends of total energy intake have been observed in adults. Besides the increased intake, a shift of macronutrients was reported, with lower shares of protein and fats, and higher contribution of carbohydrates to total intake [5]. For children, the intake increased with an average of 179 kcal/day, with a positive shift of 255 kcal/day between the energy eaten away and the intake at home [6].

Energy expenditure is the sum of resting metabolic rate, the thermic effect of ingested food, and the energy consumed through physical activity (the most variable component). In the second half of the 20th century compared to mid-20th century, drastic reductions in physical activity levels have been recorded, especially in industrialized countries. Major factors in adults were related to: (a) the occupational physical activity, accounting for a reduction of approximatively 142 kcal/day in both men and women [7]; and (b) leisure time physical activity reduction, with increased sedentary activities [8-10]. For children and adolescents, major contributing factors are decreasing levels of active transport, the reduced participation in leisure time sports activities and increased indoor and screen-time activities [11,12].

Obesity is multi-factorial in origin [13], factors such as genetics, diet, physical activity, income, education, environment, microbiological exposure, and social contributors being included as component factors in the sufficient cause model proposed by Rothman and Greenland [2].

Dietary components, e.g. the intake of high-fructose corn syrup (HFCS) as an ingredient found in sugar sweetened beverages (SSBs) and the consumption of high-fat dense calories diets were used for testing causality with accumulation of excess fat or obesity as an outcome, were reviewed and reported in this paper. Applying and discussing each of Hill's criteria, we discuss how some high-risk patterns of diet might influence the causal inference of adipose tissue accumulation.

Methods

Primary literature search was conducted in PubMed, Scopus, Web of Science, and Google Scholar. Figure 1 shows the searching process flowchart, according to the PRISMA recommended methodology (*http://www.prisma-statement.org/*). The Mendeley application (*https://www.mendeley.com/*) was employed for references' management.

Results reported by cohort studies and randomized controlled trials (RCTs) were primarily included, thus providing high quality information. Other types of studies, such as

ecological and cross-sectional studies, were also taken into consideration and discussed when the reported statistical methods compensated possible design issues.

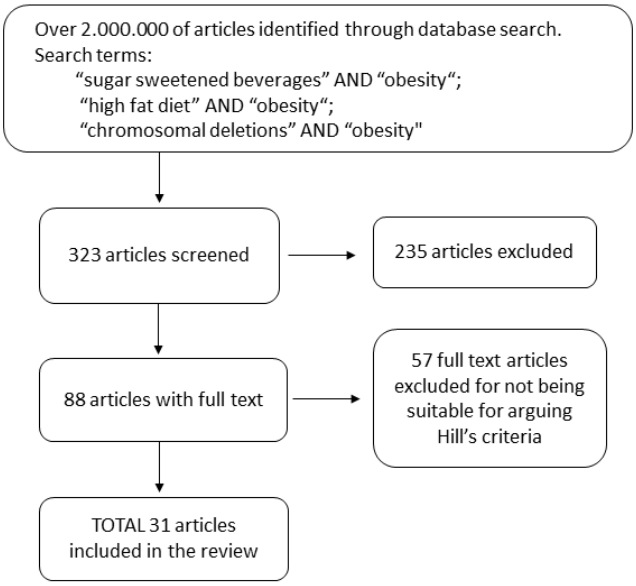

Fig 1. *Flow diagram of study selection.*

Results. Criteria analysis

Criterion 1. Strength of association or effect size

Before 2000s, literature on the effects of regular SSBs consumption on weight gain and obesity risk was scarce. For the first time, Nurses' health study, an observational cohort provided evidence to sustain this association [14]. Later, prospective cohorts and RTCs intensely studied this relation in pediatric and adult populations. Meta-analyses conducted on these high evidence trials further quantified this relation as positive and significant. DeBoyer *et al.* [15] used data from the *Early Childhood Longitudinal Survey - Birth Cohort* to compare infrequent users and non-consumers with children consuming more than one serving of SSBs daily, in unadjusted models at 4 and 5 years of age, with OR= 1.35 (95%CI 1.10–1.66) and OR=1.65 (95%CI 1.29–2.12), respectively. When adjusting the model for gender, race/ethnicity, SES, mother's BMI, television viewing only for children at 5 years the relation remained significant, with OR= 1.43 (95%CI 1.10–1.85) [15].

Using longitudinal data from *UK, Avon Longitudinal Study of Parents and Children*, Johnson *et al.* [16] showed a 36% increase in the risk of obesity, at 9 years for every extra kilojoule/g of dietary energy-dense foods consumed at the age of 7, OR=1.36 (95% CI 1.09-1.69), as a consequence of the lost ability to compensate the extra-calories in energy-dense foods.

Although the strength of association was not as high as Bradford Hill's initial recommendations and examples to sustain this point in proving causality [1], advances in statistical methods related to meta-analysis and controlling of external factors helped scientists to bring medical meaning. Nevertheless, these advances in statistical processing techniques are not error proof, as Malik *et al.* pointed out [17] by stressing the importance of performing the analysis without adjustment for total energy, since the contribution of SSBs to diets consists largely of excess sugar intake.

Criterion 2. Consistency or reproducibility

Exposure to high-fat diet was tied to obesity in a diverse manner, both in prospective and retrospective views, in different populations and locations, although it was not as difficult to investigate as Hill's stated dilemma on this criterion related to smoke exposure and lung cancer [1]. This positive relation between high-fat diet and obesity has been studied in humans, with different study designs, from cross-sectional to RCT [18,19]. In animal models, high fat diet of mothers did not directly affect the offspring's birth weight, but positively influenced the weaning weight, adiposity and the lipaemia and insulinemia [20]. In a novel animal

model for C57BL/6J mouse pups, Ullah *et al.* [21] proved that introducing high fat diet starting in lactation and continuing through weaning induced postnatal obesity and precocious puberty.

The SSBs consumption has been associated with weight gain, both in adults and children. Data from the prospective cohort *Nurses' Health Study* showed that women who increased their SSBs consumption and maintained it at high levels, gained an average of 8 kg on the course of 8 years, while women who decreased the SSBs intake and maintained it low gained an average of 2.8 kg [14]. In children, not all observational studies presented positive associations between intake of SSBs and weight gain [22], but the ORs of being overweight or obese reported by reviews of prospective studies performed in children, after a year follow-up, were 1.55 (95%CI 1.32 - 1.82) for the highest intake compared to lowest intake of SSBs [23].

Criterion 3. Specificity

"If specificity exists, we may be able to draw conclusions without hesitation", argued Hill in favor of the use of specificity, the most disputed criteria of causality. Since in medicine only a few examples of one specific exposure which could cause a unique outcome to exist, in the last decades this criterion benefited from the help of new molecular tools and resources. In recent years, some Mendelian forms of obesity have been reported, one of them, being located on the short arm of chromosome 16, in the distal region in 16p11.2 that includes the *SH2B1* gene. The deletion of the *SH2B1* locus associated with hyperphagia, leptin resistance and obesity [24-25].

Criterion 4. Temporality

Temporality is a logical criterion in the cause – effect relation, in the way that cause must always precede the effect. The answer to Hill's question: "Does a particular diet lead to disease or do the early stages of the disease lead to those peculiar dietetic habits?" could be easily formulated using a prospective or even an RCT design, and avoiding cross-sectional enquiries. Using a prospective design, Cantoral et al [26] followed 227 children from early age to mid-adolescence, measuring SSB intake every 6 months the and found that cumulated SSB intake was a predictor of general and abdominal obesity: compared with children in the lowest tertile of cumulative consumption, children in the highest tertile of consumption were 3 times more likely (95% CI: 1.27, 7.00) to have general obesity and 2.7 more likely (95% CI: 1.03, 7.03) to have abdominal obesity at 8-14 years of age. In a recent RCT, performed in 4-11 years old children, with normal weight, which have received either sugar-free or a SSB for 18 months, BMI z-score has increased statistically significant, with a mean difference of 0.15 +/- 0.42 units in the SSB group as compared to 0.02 +/- 0.41 units in the sugar free group [27,28].

Criterion 5. Biological gradient

The presence of a biological gradient, or a dose-response relationship would consolidate the association between exposure and effect. Using data from EPIC-Potsdam cohort, that included 17369 nonsmoking subjects, Schultz et al [29] have shown that short term large weight gain (>=2 kg) could be predicted by fat-rich diets in a dose-response relationship: for each of 100g/day increment in intake the risk of more than 2 kg body weight was increased by the consumption of: fats with OR = 1.75; 95% CI, 1.01-3.06, sauces with OR = 2.12; 95% CI, 1.17-3.82 and meat with OR = 1.36; 95% CI, 1.04-1.79.

Using data from UK Avon Longitudinal Study of Parents and Children authors [30] have confirmed that for children between 7-15 years, intake of dietary patterns containing energy-

dense, high-fat and low-fiber is prospectively associated adiposity: for each standard deviation unit increase in the dietary pattern z score, OR increased by 13% (OR = 1.13; 95% CI, 1–27%).

For children and adolescents, for each increase of one standard SSB, an increase of 0.06 kg/m^2 (95% CI: 0.02, 0.10) in BMI was observed [31].

Criterion 6. Plausibility

As Hill acknowledged "What is biologically plausible depends upon the biological knowledge of the day". High fructose corn syrup (HFCS) is a sweetener found in "most soft drinks and fruit drinks, candied fruits and canned fruits, dairy desserts and flavored yogurts, most baked goods, many cereals, and jellies". Most HFCS contain 40-60 % fructose, but it has been reported that some products might contain up to 90% fructose. The HFCS consumption is contributing to weight gain through a direct mechanism, when added to diet without accounting the energy balance and through a metabolic consequence of its consumption, outside the add-on on energy balance. Liquid carbohydrate intake, as opposed to solid food containing carbohydrates produces an incomplete reduction in energy intake at subsequent meals due to a decrease in satiety, and produces a positive energy balance and weight gain [32,33].

Due to recent research, it is clear for us now the biological pathway of *de novo* lipogenesis effect of high fructose corn syrup (HFCS) consumption. Once absorbed via the portal vein, fructose is delivered to the liver in much higher concentrations than in any other tissues, due to *Glut5 transporter*, an enzyme present only in the hepatocyte. In hepatic cells it increases the protein levels of all *de novo* lipogenesis enzymes while it is being transformed to triglycerides. In plus, fructose stimulates directly SREBP1c, a transcriptional regulator of *de novo* lipogenesis, upregulated normally by insulin signaling. Through the production of uric acid it additionally shortcuts the insulin regulated pathway to *de novo* lipogenesis [34-36].

Criterion 7. Coherence

About coherence as a criterion for cause – effect relationship, Hill recommended that it should not contradict the natural history and biology of disease. Several authors have linked the increase of incidence of obesity to dietary patterns containing increased intake carbohydrates. Using an ecological analysis, Siervo et al [37] linked the country specific estimates of overweight and obesity with year- and country-specific food and energy availability. The multivariate regression model showed positive connections between physical inactivity and sugar consumption with the prevalence of obesity.

In another approach, a temporal association between the rise of use of high fructose corn syrup (HFCS) in beverages with the increase of prevalence of obesity was reported [34]. Between 1970-1990 an unprecedented rise in the use of HFCS of more than 1000% was observed. Fructose is an inexpensive sweetener, obtained, with glucose as an intermediate, from corn starch using a *glucose-isomerase*. In the last century an increase from ~15 g/d representing 4% of total energy, to 75 g/d representing 12% of total energy was observed for fructose [38]. The HFCS percentage of total sweeteners increased from 16% in 1978 to 42% in 1998 and then stabilized [39]. Similar trends of increase in obesity prevalence in the USA and the trends of HFCS availability were found by Bray [34].

Criterion 8. Experiment

Results of a systematic review [40] of RCTs that have followed children and young adults from 12 months to 2 years, have proved that

reducing the amount of saturated fat in diet, as compared to the group with normal diet was associated with a reduction of 1.50 kg/m^2 (95% CI=-2.45 kg/m^2 ; -0.55 kg/m^2) of BMI. The results of a RCT in a sample of obese Hispanic children with age 7-12 years, followed for 2 years, which compared 2 diets: a low–glycemic load diet from 2.25 (95%CI 2.16, 2.34) to 2.10 (2.02, 2.16) and a low-fat diet from 2.24 (2.17, 2.31) to 2.16 (2.09, 2.22), showed that both diets produced reductions of BMI- z score, but no significant differences between the diets were observed [41].

The influence of low-fat complex vs simple carbohydrates dietary pattern on weight management was analyzed by CARMEN study [42], an intervention of 6 months, which included moderately obese adults. Using a low-fat diet, but with simple carbohydrates in an intervention group and complex carbohydrates on another research group versus a seasonal diet intervention group and versus a non-intervention group, authors have found significant weight loss of - 0.9 +/- 3.6 kg in simple carbs group, of - 1.8 +/- 3.2 kg in complex carbohydrates group and a non-significant weight gain of 0.8 +/- 4.1 kg in the seasonal diet (control) group.

Criterion 9. Analogy

When Hill referred to analogy, he had given us the advice to "accept slighter, but similar evidence to another…", of already proven cause-effect relations. Although fructose has a similar chemical structure with its isomer, glucose, regarding health effects and actions on human body, it resembles ethanol, except the intoxication properties observed in latest [43,44]. Both fructose and alcohol enter the hepatocyte without stimulating insulin secretion, and with the help of *fructokinase* for fructose and *alcohol-dehydrogenase* for alcohol, both are metabolized in several steps to form Acetyl-CoA. In both of them, reactive species of oxygen will be formed, along with de novo lipogenesis. There are some common long-term use consequences of ethanol and fructose exposure, such as: hypertension, dyslipidemia, pancreatitis, obesity, insulin resistance, alcoholic/non-alcoholic fatty liver disease and addiction/habituation [43-45]. Lustig has pointed out several physiological similarities, but he admits that quantitative and mechanistic studies have not proved this qualitative analogy.

Conclusion

Food patterns, such as increased consumption of HFCS in the form of liquid calories or high-fat diets, contribute to higher intake of energy and accumulation of excess adipose tissue, thus leading to obesity when these intake patterns are maintained. This causal relation was found positive in both children and adults. Despite the general awareness of the causal relations and public health messages, risky diet recommendations are still frequent. Therefore, every possible effort should be made to increase public awareness to tackle obesity.

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