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Abstract:

Obesity is a major public health problem because it is a risk factor for metabolic disorders including type 2 diabetes and cardiovascular disorders. Notably, food pollutants have been charged to contribute to the etiology of obesity and type 2 diabetes. Chemicals are either persistent or short-lived but in either case they have invaded our lives, and diet is the main route of exposure. However, the current threshold model used in risk assessment focused on single chemicals and does not take into account potential effects from mixtures containing pollutants at environmental doses, e.g. the real life exposure. Furthermore, recent data suggest that the deleterious metabolic impact of pollutants may be aggravated in a context of obesity. These findings stress the importance of better delineating the metabolic consequences of life-long consumption of food pollutants, notably when considering vulnerable populations including fetuses and young children as well as populations at risk for metabolic disorders such as overweight and obese people. With this in mind, we recently demonstrated that a mixture of food pollutants at doses lower than the accepted ineffective concentrations does indeed have metabolic and health consequences in chronically exposed obese mice. This proof-of-concept-study highlights the needs for breakthrough of ideas to strengthen public health action for a better protection.

Obesity is a major public health problem because it is a risk factor for metabolic disorders including type 2 diabetes and cardiovascular disorders. Its evolution is pandemic with more than 1.4 billion adults overweight in 2008, and the WHO predicts that by 2015 about 700 million people will be obese. Nowadays, an estimate of 347 million people worldwide has diabetes, and diabetes is projected to be the seventh leading cause of death in 2030 [1]. In addition, diabetes has a heavy cost on the economy and quality of life. For example, the total estimated cost of diagnosed diabetes in 2012 in the USA was $245 billion[2]. Type 2 diabetes results from the body's ineffective use of insulin, a hypoglycaemic hormone secreted by the pancreas that plays a major metabolic role in maintaining blood glucose levels in the normal range. The first step leading to diabetes is insulin resistance that develops in the metabolically active tissues (i.e., liver, skeletal muscle, and adipose tissues) and eventually leads to the classic triad of hyperinsulinemia, hyperglycemia and hypertriglyceridemia, which are the characteristic features of type 2 diabetes [3].

Obesity is a multifactorial disease in which, besides genetic predisposition, excessive food intake combined with physical inactivity has generally been implicated. However, it was recently suggested that food pollutants could contribute to the etiology of obesity and type 2 diabetes [4]. This hypothesis is based on several findings including the speed with which the incidence of obesity and its related metabolic disorders increases that excludes the only involvement of genetic factors and strengthens the environmental origin hypothesis. Furthermore, the production and usage of synthetic chemicals that have paralleled the incidence of diabetes[5] support the hypothesis that the exposure to chemicals may have damaged physiological mechanisms regulating glucose and lipid metabolism[6]. This assumption is also based on epidemiological studies, which associated the prevalence of type 2 diabetes with elevated body burdens of chemicals [4 7], and experimental studies in rodents,

which established a causal relationship between exposure to chemicals and the development of metabolic disorders including obesity and insulin resistance [8-10].

Chemical pollutants end up in the plates

Based on their biodegradability, chemicals may be either highly persistent in the environment or non-persistent. Persistent Organic Pollutants (POPs) are chemicals created intentionally by industrial activities such as the polychlorobiphenyls (PCBs) made for their flame-retardant properties. Other POPs may be unwanted industrial by-products such as the dioxins which result from incomplete combustion during industrial processes. Production of POPs is limited (eg, dioxins) or was banned (eg, PCBs) in the 1970s because of accumulating evidences of their deleterious effects on wildlife[11]. However, owing to their persistence, all environmental compartments remain contaminated. These highly lipophilic compounds accumulate higher up the food chain in a process called bioaccumulation, being present in virtually all categories of foods and especially in fatty foods [12]. Other chemicals are short-lived compounds, but, because of their massive industrial production, particularly in the plastic industry, they are omnipresent in the environment. For example, bisphenol A (BPA) used in the manufacture of polycarbonate is constitutive of many food containers. Similarly, resins containing BPA are used in the lining of cans. Phthalates are another class of chemicals that is widely used in plastics to make them soft and flexible. BPA and phthalates can leach from food and beverage containers and packaging to cause contamination by migration, especially when heated[13 14]. Thus, diet is the main route of exposure to pollutants whether persistent or not. In addition, pollutants can transfer from the mother to the fetus through the placenta during pregnancy and through breastfeeding[15].

Chemical pollutants, endocrine and metabolic disruption

Many chemicals are considered as Endocrine Disrupting Compounds (EDCs). Although the definition of an EDC is still a matter of debate, the WHO definition states that an endocrine disrupter is an exogenous substance or a mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations [16]. There are multiple mechanisms through which EDCs may interfere with the endocrine system. In addition to acting as direct agonist or antagonist compounds through binding to nuclear receptors, EDCs may affect the synthesis, transport, metabolism and excretion of hormones, thus altering the plasma concentrations of natural hormones. Originally, EDCs have been charged to contribute, among others, to declining sperm counts in the offspring of exposed pregnant mothers and increased incidence of male children born with genital malformations [17]. It was next suggested that some EDCs may as well exhibit metabolic disrupting activities through interaction with nuclear receptors that were hormone receptors, xenosensors or liposensors such as the peroxisome proliferator receptors (PPARs)[18]. A subset was identified as obesogens because they promote adipocyte differentiation and fat storage[19]. These nuclear receptors are expressed in the metabolically active tissues, both at central and peripheral levels, and they play crucial roles in integrating the complexities of homeostasis and development, including insulin secretion and sensitivity to this hormone which is instrumental in the regulation of glucose and lipid metabolisms and in adipogenesis[18]. For example, estrogens play a pivotal role in regulating energy homeostasis[20]. Besides, inappropriate activation of androgen receptor (AR), thyroid hormone receptor (TR) and glucocorticoid receptor (GR) also leads to impaired metabolic homeostasis [18 21 22 23]. Furthermore, the xenosensors activated by numerous steroids and EDCs are involved in metabolic disease states such as steatosis, obesity and insulin resistance in addition to their primarily role in xenobiotic detoxification [24-26]. As such, the final consequences of an

exposure to chemicals will depend on the complexities of the interactions between the diverse members of the nuclear receptor family to convey normal metabolic regulations[18]. Also, chemicals might target different nuclear receptors depending on the metabolic tissues (as described above) and their integrative effects would rely on cross-talk with the other nuclear receptors, as shown with the obesogen tributyltin (TBT). TBT is a powerful biocide used as an "antifouling" agent in paints for ships' hulls, and now banned from use, which acts as an adipogenic agonist through interference both with estrogen signalling and the PPARγ pathway [19]. On top of this, POPs like dioxins might also display inflammatory activities favouring the development of resistance to insulin in adipose tissues [25]. It is also important to keep in mind that these events depend on sex, the developmental age, and the food matrix (particularly the lipid content).

Chemical pollutants and the limits of the current threshold model used in risk assessment

Human health risk assessments focused on single chemicals by setting up Tolerable Daily Intake (TDI) reference doses that are defined by international agencies, such as the U.S. Environmental Protection Agency (EPA) or the European Food Safety Agency (EFSA). TDI reference doses derived from the no-observed-adverse-effect levels (NOAELs) or the lowest-observed-adverse-effect levels (LOAELs) in animal studies. The supposedly most sensitive animal is being used and the most sensitive endpoint is being considered for the establishment of either the NOAEL or the LOAEL. Then this value is to be divided by an uncertainty factor ranging from 100 to 1000 to take into account interspecies as well as interindividual variation[27]. Thus, the TDI is an estimate of the amount of a given chemical in food or drinking water that can be ingested daily over a lifetime without a significant health risk. While these

reference doses are protective from a toxicological point of view, they may not sufficiently protect the population from an endocrine and metabolic point of view, and it was recently proposed that the principles of endocrinology should be considered in the making of the regulatory decisions on EDCs[28]. Specifically, since EDCs mimic/antagonize hormones, chemicals defined as EDCs should behave as hormones in many features, that is, they should act at low doses, exhibit non-linear, and often non-monotonic dose-response curves and their effects should be life-stage dependent in the context of multi-hormonal exposure. Consistently, adverse metabolic effects have been documented using doses near and even below the TDIs, especially for BPA[29 30]. Moreover, the population is exposed to a cocktail of pollutants[13], which resulting effect cannot be predicted on the basis of the effects of each individual pollutant, eluding possible agonist, antagonist or synergistic endocrine/metabolic interactions between chemicals in a context of multi-exposure. For dioxins and related POP congeners whose toxic effects are presumed to result from the binding to the xenosensor aryl hydrocarbon receptor (AhR), it is assumed that the combined effects of the congeners produce additive effects[31]. A concentration addition model was also proposed for mixtures of anti-androgenic compounds[32]. These concepts are largely debated nowadays because the biological effects of one particular EDC are different depending on the presence of the other EDCs. Moreover, the effects would be different depending on the doses of the other EDCs and on related endogenous hormone levels making uncountable the number of situations[33-36]. Thus, while the dose is a critical factor in toxicology, the vulnerability of the target as well as the possible complex non-monotonous relationship between dose and effect observed for some pollutants have to be taken into account with EDCs. An additional degree of complexity was further provided when interactions were evidenced between obesity and serum concentrations of pollutants on the prevalence of type 2 diabetes[37]. Interactions were also observed in animal experiments and it was found that the metabolic deleterious impact of

BPA was aggravated in animals fed a high-fat diet[38]. Thus, exposure to pollutants and diet imbalance may be two sides of the same coin as suggested by Barouki *et al* [39] to pinpoint that adaptation to either stress is sharing many of the same characteristics as chemicals originally identified for their endocrine disrupting properties are also capable of metabolic disturbances[18].

Furthermore, recent reports indicated that EDCs could regulate patterns of gene expression through epigenetic modifications such as DNA methylation and histone modifications. The process does not directly modify the DNA sequence but it alters the methylated or acetylated environment of the DNA sequence, facilitating/repressing transcription factors' access to the promoters of the targeted genes and altering gene expression[40 41]. Thus, pollutants through epigenetic mechanisms could interfere with the metabolic programming trajectories set up during the maternal life, a highly vulnerable period of development, as described in case of nutritional stress with long lasting health effects affecting the entire life of the individuals. This is the DOHaD hypothesis for Developmental Origins of Health and Disease[42]. Long-lasting effects may also pass the generations, and individuals may suffer from exposure of their grandparents. For example, in utero exposure of the F1 generation to TBT induces hepatic steatosis and adipose tissue expansion in the non-exposed subsequent generations[8]. Transgenerational obesogenic effects were also described in the F3 generation originated from F1 generation rats *in utero* exposed to either mixtures of plasticizers (BPA, phthalates), or the insecticide dichlorodiphenyltrichloroethane (DDT)[43].

Development of a proof-of-concept study

In this context of uncertainties related to the impact of pollutants on human health, notably during fetal and perinatal periods considered of very high vulnerability, it is essential

to study the cocktail effect of low-dosed chemicals. While it is hardly possible to estimate the risk for all scenarios, relevant mixtures of well-characterized pollutants may be studied by taking into account parameters such as geographical locations, sex and diet habits. Having this in mind, we recently developed an experimental model,[44] which is original because of the life-long exposure to pollutants, encompassing maternal life (preconception, gestation and lactation). In addition, mice were fed a high-fat high sucrose diet (HFSD) to mimic Western diet and reflect occidental diet habits. Thus, these mice are at high risk of insulin resistance and other metabolic disorders[45]. The mixture comprised pollutants largely occurring in foods, especially fatty food, either persistent (PCB153; 2, 3, 7, 8-tetrachlorodibenzo-p-dioxine, TCDD) or not persistent (BPA; Di-2-EthylHexylPhtalate, DEHP) and activating a broad range of signalling pathways. They are classified as EDCs and are of high concern for human health because of their established links with metabolic diseases in epidemiological and experimental studies[18 37]. Specifically, TCDD mainly acts through binding the AhR and exerts estrogenic-like effects depending on the hormonal context [46]; PCB153 is a non-dioxin-like PCB with estrogenic-like activities and is considered as a surrogate for the total PCB exposure [47]; BPA is the archetypal example of an estrogenomimetic EDC interacting with several nuclear receptors to promote insulin secretion, obesity and hepatic insulin resistance[48]; DEHP is a well-known activator of the PPARs[18]. Pollutants in the mixture were low-dosed, in the range of the TDI defined by international agencies for humans, and thus at least 500 fold lower than the NOAEL for each chemical supposedly defined on the most sensitive species and on the most sensitive end-point. As a preliminary study in the setting of the experimental model, we used a mixture with doses in the range of the NOAEL for each pollutant, and a general toxicity related to litter size and pup survival was observed[44]. No such toxicity was described using the mixture in the TDI range. However, we observed significant sex-dependent metabolic modifications in the absence of weight modification, underscoring the

importance of studying both males and females. In males, although pollutants did not alter glucose and insulin metabolic tests or plasma cholesterol levels, we observed changes in cholesterol metabolism with a decrease in hepatic total cholesterol together with enhanced expression of genes encoding the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoAR) which is the rate limiting-enzyme in cholesterol synthesis, and proteins involved in the bile salt pathway[44]. In females, there was deterioration of glucose tolerance, and we hypothesized that this effect was related to the twofold induction of estrogen sulfotransferase (EST/SULT1E1), impairing estrogen signalling in the liver. Indeed, through conjugation of a sulfonate group, EST/SULT1E1 inactivates estrogens preventing their binding to the high-affinity estrogen receptor, thus modulating estrogen bioavailability[49]. We interpreted these data as an indication that the cocktail of pollutants could have opposed the well-described protective effects of estrogens from metabolic disorders such as insulin resistance, in females. More experiments will aid in defining whether these events represented an adverse or a compensatory adaptive response to the mixture of pollutants.

Conclusion

Collectively, our experimental model is a proof-of-concept that low doses of pollutants, in the range of doses considered ineffective for humans, are in fact not harmless when exposure is chronic and when pollutants are administered in combination in a context of obesity caused by a calorie-rich diet. Of course, there are limitations in these experiments. We did not assess individually each pollutant that made up the mixture; nor did we yet get data with other concentrations of the mixture. Indeed, the two higher doses tested which were in the range of the NOAEL doses for each pollutant and 10-times higher, resulted in general toxicity with a reduced litter size and lowered pup survival[44]. Another limitation is that these

data were yielded in a rodent model. However, unless it is considered that the adverse effects seen in rodents are not expected to occur in humans (and the epidemiologic studies performed so far do not fit this hypothesis), the principle of precaution should guide the making of decisions, especially when considering women of childbearing age and young infants particularly exposed because of mouthing activities. Substitute replacement, while promising, should be carried out with caution because interference with the endocrine system was reported for several structural analogues of BPA[50]. In the meantime, it is estimated that removing BPA from food sources would allow sparing $ 2 billion annually in the US[51] because of prevention of childhood obesity and of its complications over the life course. Effective solutions rely on a breakthrough of ideas for better life as well as economic benefits. They require huge support from governments and economic players to contribute to the improvement of public health.

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