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Review

Childhood obesity and environmental pollutants: a dual relationship

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Summary. The rise in obesity rates is an alarming global health concern. Despite obesity is mainly due to an unbalanced energy intake and expenditure, several recent studies suggest that it could be a consequence of exposure during the critical developmental windows to environmental chemicals disrupting endocrine function. This suggests that a shift is occurring in the human body pathways used to integrate changing nutritional and environmental variables and to maintain metabolic balance and body weight. This review highlights the role of pesticides, in particular endocrine disrupter ones, on obesity pathogenesis in childhood and summarizes the current under-standing of the major environmental influences on pediatric obesity. (www.actabiomedica.it)

Key words: children, endrocrine disrupters, environment, obesity, pesticides, pollution

Introduction

As reported by literature data, we are in the midst of a worldwide pandemic of obesity. The actual trend of obesity development should be interrupt, otherwise in the next 50 years we will become an obese species (1). Currently, 34% of American adults are obese and 68% are overweight, as defined by the World Health Organization (WHO) categories based on Body Mass Index (BMI). WHO has also estimated worldwide that over-weighted outnumbered the malnourished: for the first time in human kind history, people on the planet are mainly overweight than underfed (2). The epidemic diffusion of obesity has not spared children and during the last ten years it has emerged that diseases related to obesity, such as the metabolic syndrome (MetS), diabetes mellitus type 2 (T2DM), and nonalcoholic fatty liver disease (NAFLD) are not just pertinence of the adulthood but also of the childhood. The thermodynamic or "calories in-calories out" pathway is mainly used to explain the increased rate of obesity, so the Scientific Community agrees that people become obese because the energy intake exceeds the expenditure (3) However, questions such as *"why are we all in a positive energy balance?"* and *"why is getting obese different from a subject to another, given the same amount of calories' excess?"* rise. It is important to bear in mind that the biochemical nature of the calories introduced with the diet plays a very important role in their stored pathways, as well as in the regulation of appetite and satiety (4). Environmental and genetic factors play a crucial role too, either when considered individually or due to the "gene-by-environmental" interaction. Some exogenous compounds can disrupt normal hormone signaling system, so called endocrine disrupting compounds (EDCs). They are ubiquitous in the environment and their main role on fetal life and childhood seem to be related with the increasing rates of premature birth, low birth weight, disorders of sex development and obesity. The influence of environmental compounds on epigenetics offers a complementary mechanism for hereditary complex and multifactorial process such as obesity, that is independent of gene sequence variation. EDCs could also influence

the weight amount with other direct effects, especially during childhood. The purpose of this review is to focus on the relationship between childhood obesity and environment pollutants.

Pesticides and endocrine-distrupting compounds

Pesticides are a group of heterogeneous chemicals that have an important public health benefit by increasing food production and decreasing food-borne and vector-borne diseases. On the other hand, they may represent a health risk according to the type of agent and exposure.

Several classes of compounds are used to destroy organisms competing for food supply. More than 20.000 pesticide products are used as insecticides, herbicides, rodenticides, fungicides, nematocides, wood preservatives, plant growth regulators, and fumigants. Products are ubiquitous in the environment and can be found in water, food, homes, schools, gardens, workplaces, and lawns.

Although the toxic actions of pesticides are targeted at specific pest species, they may have potential adverse effects on humans health that are still not completely characterized, especially in infants and young children (5).

Most of the pesticides, especially the organic compounds primarily composed by carbon, exhibit two main mechanisms of action: the first one is direct and non-genomic, acting as oxidative stress and leading to apoptosis; the second one, indirect and genomic, acts on permanent modification of gene transcription (6). Once released, they break down very slowly, persisting in air, water, soil and living organisms, influencing the food chain.

Exposure to these persistent chemicals has been associated with health effects including cancer (7-9), reproductive defects (10), and obesity (11). McGlynn et al. in 2006 evaluated the persistent organochlorinated pesticides levels in lipid serum of subjects with liver cancer, according to previous results on rodents, showing that dichlorrodiphenyldichloroethylene (DDT) and dichlorrodiphenyldichloroethylene (DDE) exposure can cause tumors. They measured serum concentrations of DDT and DDE by gas chromatography-mass spectrometry in participants of the Nutritional Intervention Trials in Linxian (China). The case group included 168 individuals who developed liver cancer during the trials, and the control group included 385 individuals frequency-matched on age and sex who were alive and well at the end of the study. In multivariable-adjusted models, the risk of developing liver cancer increased with increased serum DDT concentration; in contrast there was no statistically significant association between liver cancer and serum DDE concentration. A calculation of crude liver cancer risk found that there would be 26 liver cancers per 100,000 persons per year in the lowest quintile of DDT exposure versus 46 liver cancers per 100,000 persons per year in the highest quintile of DDT exposure (12).

Regarding reproductive defects, the review of Martenies et al. summarized most recent evidences related to pesticide exposures and commonly used semen quality parameters (13). Seventeen studies were included in the review, 15 of which reported significant associations between exposure to pesticides and semen quality indicators. Specific pesticides targeted for study included DDT, hexachlorocyclohexane (HCH), and abamectin. Overall, a majority of the studies reported significant inverse associations between pesticide exposure and sperm parameters as decrease in sperm concentration and decreased motility. An association between pesticide exposure and sperm morphology was less clear, with only two studies reporting an association (13). Wei et al. had recently reviewed studies suggesting a possible link between exposure to dichlorophenol pesticides and obesity in adults. Study participants aged 20-85 years were selected from the U.S. National Health and Nutrition Examination Survey, and were categorized as obese and non-obese based on BMI. Creatinine-corrected urinary concentrations of dichlorophenols were determined to assess level of exposure to environmental pesticides. Significantly higher geometric means of urinary concentrations of dichlorophenols were seen in obese compared to non-obese adults. A dose-dependent increase in the prevalence of obesity was also observed after adjustment for confounding variables (14). These are some examples of the possible link between pesticides' exposure and health problems: most of the mechanisms are not clear thus a number of studies are ongoing trying to elucidate them.

Table 1. Chemical classification of main pesticides and mechanism of action

Chemical structure	Mechanism of action
Organophosphates and Carbamates	Inhibit acetyl cholinesterase activity at nerve endings, determining an excess of neurotransmitter acetylcholine and a depolarizing blockade of neural transmission. Carbamates effects are more reversible because their half-life is lower than the half-life of organophosphates.
Organochlorines	Interfere with nerve cell membrane cationic transport, and effect neural irritability and excitation of the central nervous system.
Pyrethrins	Cause allergic reactions. Naturally emitted by plants and rapidly metabolized by mammals; commonly used in anti-lice shampoo and as topic treatment for scabies. Repellants, such as diethyltoluamide, are also included and are used to reduce the risk of vector-borne diseases (Lyme disease, Rocky Mountain spotted fever) and insect stings. Ingestion and less common dermal exposure can cause toxic encephalopathy and seizures.
Chlorophenoxy compounds	Herbicides primarily irritate skin and respiratory tract in acute exposure and act through different mechanisms. Until recently arsenical pesticides were used for wood preservation like chromium arsenate. They can cause central nervous system depression at certain doses.

Compounds that could interfere at several control points in the hormone signaling pathways, changing the physiological hormonal and homeostatic system, are the so called EDCs. EDCs include not only pesticides, but also persistent organic pollutants (dioxins and polychlorinated biphenyls), several industrial chemicals (phthalates and brominated flame retardants), heavy metals (arsenic and cadmium) (6), and phytoestrogens (isoflavones, coumestans and lingams); the last ones are present in vegetable food commodities such as soy.

When present in the body, they can interfere via different mechanisms such as agonists/antagonists of receptors, interference with the hypothalamus-pituitary axis and inhibition of hormone biosynthesis (15). To date, most studies have focused on the effects that EDCs pose on endocrine and reproductive processes regulated by hormonal signaling mediated by members of the family of nuclear receptors (NRs), in particular the estrogen receptors (ERs) and the androgen receptor. In addition, the thyroid hormone receptors, and more recently retinoid X receptor (RXR), and peroxisome proliferator-activated receptors (PPARs) are shown to be targets for EDCs action. EDCs can affect these systems in several different ways, e.g. by directly interfering with receptor signaling or by activating other signaling pathways, in particular that of the aryl hydrocarbon receptor (AhR), involved in the metabolism of many xenobiotic substances (6).

Main biological mechanisms predisposing obesity seem to be related to the activation of PPARs on adipocytes and the thyroid and steroid hormones alterations (Table 2).

EDCs spread, as obesity, is increased rapidly during the last decades. This induced the Scientific Community to speculate on the presence of a link between these two factors. Some EDCs are now definitely considered as “obesogenic substances” that are molecules regulating inappropriately lipid metabolism and adipogenesis to promote obesity (16).

Childhood obesity and EDCs exposure: a strong link influenced by growth, environment and epigenetics

A constant interaction exists between environment and gene expression. It is known that environment can strongly influence epigenetic mechanisms leading to reprogramming of inheritance and promoting disease development. Recently, epidemiological studies identified a significant negative correlation between DNA methylation and plasma pesticides concentration, demonstrating that environment influences DNA composition (6).

Greenlandic Inuit have some of the highest reported persistent organic pollutants (POPs) levels worldwide. Rusicki et al. evaluated the relationship between plasma POPs concentrations and global DNA

Table 2. Classes of EDCs involved in obesity pathogenesis and hypothesized mechanism of action

Chemical structure	Use and Mechanism of action
Persistent Organochlorine Compounds	Main components: DDE – metabolite of DDT – hexachlorobenzene (HCB) and polychlorinated biphenyls (PBCs). The last were banned in 1970s, but we can still find them in environment because of their bioaccumulation and long half-life. They were contented, as lubricant, in electrical appliances. DDT was also banned in 1970s, and was an insecticides as HCB. They show an anti-androgenic activity and are thyroid hormone disruptors and pregnant X receptor/ constitutive androstane receptor (PXR/CAR) inducers.
Phthalates	Used as plasticizers and stabilizers in the manufacture of consumer products such as children toys and food packaging. Their probable endocrine-disrupting mechanisms are related to an anti-androgenic activity and PPARs agonist function.
Bisphenol A	Used as a monomer in polymerization reaction to produce polycarbonate plastics used in baby bottles, medical tubing, food packaging. Its main disrupting mechanisms are to inhibit adiponectin and to stimulate the release of inflammatory adipokines, such as interleukine-6 (IL-6) and tumour necrosis factor alpha (TNF-α) from human adipose tissue. In addition, it may acts as an estrogenic receptor agonist.
Brominates flame retardants	Poly-brominates diphenylethers (PBDEs) and hexabromocyclododecane (HBCD) are used as flame retardant in several products. They show anti-androgenic activity and are thyroid hormone disruptors.
Perfluorinated alkyl acids	Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are members of the fluorine-containing chemicals family and they make materials strain resistant. The probable mechanism of action by which they cause obesity is their behaviour as PPAR-α agonist.

methylation (percent 5-methylcytosine) in DNA extracted from blood samples from 70 Greenlandic Inuit. Blood samples were collected under the Arctic Monitoring and Assessment Program and previously analyzed for a battery of POPs. To estimate global DNA methylation was used pyrosequencing via Alu and LINE-1 assays of bisulfite-treated DNA. They found that global methylation levels were inversely associated with blood plasma levels for several POPs (17).

Epigenetics are commonly defined as changes in gene expression that occur without modifications in DNA sequence and can be transmitted through mitosis and/or meiosis. They consist of three main mechanisms, such as DNA methylation, histone modification and micro-RNA production, which could lead to a gene overexpression or silencing (11). Even if genetic inheritance is the same in every cell, epigenetics modify the process that regulates different cellular phenotypes expression.

The DNA changes can be environmentally induced and inherited by multiple generations independently of subsequent individual exposure (18).

Two of the main potential windows for epigenetic dysregulation are fetal life and puberty during which the programming of main adult outcome happens, due to the rapid increase in cellular (and DNA) turnover and cell growth.

Obesity can be considered a multifactorial condition, so it cannot be explained only by DNA sequence. Epigenetic dysregulation due to EDCs and Obesogens offers a complementary mechanism for gene expression and inheritance that is independent of DNA sequence variation.

In-utero exposure and breastfeeding

Changes in central endocrine regulatory systems occur during pregnancy by early life exposure to EDCs because chemicals cross the placenta and reach the fetus proportionally to the maternal exposure (24). A non-monotonic dose-response relationship (typical in toxicology) between EDCs exposure levels *in-utero* and obesity development in children was demonstrated. Studies investigating the effects of *in-utero*

EDCs exposure suggested these substances may cause permanent physiological changes starting with influence on birth weight and, subsequently, predisposing to later weight gain (19). Unfortunately there are few data about the correlation between birth weight and EDCs exposure during pregnancy. Govart et al. in 2012 published a review examining the hypothesis that the combination of PCBs and DDE adversely affects birth weight according to projects as EU (European Union) ENRIECO (ENvironmental Health RIsks in European Birth Cohorts) and EU OBELIX (OBesogenic Endocrine disrupting chemicals: LInking prenatal eXposure to the development of obesity later in life) (20,21).

They collected maternal and cord blood and breast milk samples of 7,990 women enrolled in 15 study populations from 12 European birth cohorts from 1990 through 2008. Using identical variable definitions, they performed for each cohort linear regression of birth weight on estimates of cord serum concentration of PBC-153 and p,p´-DDE adjusted for gestational age and a priori selected covariates.

Results showed that birth weight decline of 150 g per 1 μg/L increase in PCB-153 cord serum concentration after adjustment for potential confounders in 12 of 15 study populations (22).

The influence of maternal occupational exposure to various chemicals on fetal growth was also investigated in 4,680 pregnant women participating in the Generation R Study: a population-based prospective cohort study from early pregnancy onwards in the Netherlands. Mothers evaluated had a paid employment during pregnancy and had a spontaneously conceived singleton live born pregnancy (n ¼ 4,680). A job exposure matrix was used, linking job titles to expert judgment on exposure to chemicals in the workplace. Fetal growth characteristics were repeatedly measured by ultrasound and were used in combination with measurements at birth. Placental weight was obtained from medical records and hospital registries. Linear regression models for repeated measurements were used to study the associations between maternal occupational exposure to chemicals and intrauterine growth.

It was observed that maternal occupational exposure to polycyclic aromatic hydrocarbons, phthalates, alkylphenolic compounds and pesticides adversely influenced several domains of fetal growth (weight, head circumference and length) and placental weight (23).

Both maternal overfeeding and poor nutrition before and during pregnancy, mainly related to the geographical location, can influence offspring phenotype in utero and after birth (24). An example can be represented by the DNA hypomethylation that results from a decrease in dietary sources of methyl group donors in conjunction with decreased availability of B vitamins (25). Adverse or undernourished environment during *in-utero* life, characterized by altering fetal nutrition, maternal diseases (as diabetes or obesity), exposure to drugs and toxics (as smoke and EDCs), can cause permanent metabolic programming aimed at conserving nutrients and predisposing to obesity in later life.

It is known that Low Birth Weight (LBW) new born present IR due to reduction of nourishments and insulin secretion during pregnancy. After birth IR persists, predisposing to health complications as T2DM, Methabolic Syndrome (MS) and Obesity (26).

This introduces the recent evidence of the "intra-uterine programming of adiposity" also named as "Barker hypothesis" (27). Barker's hypothesis suggests that under-nutrition and other toxic insults in utero and during infancy can permanently change the body's structure, physiology and metabolism. The lasting or lifelong effects of under-nutrition will depend on the developmental period at which it occurs. In early gestation it will reduce body's size permanently, whereas in late gestation it will have deep effects on body form without necessarily reducing body size. These effects include altered gene expression, reduced cell numbers, imbalance between cell types, altered organ structure, pattern of hormonal release and hormonal responses (28).

To examine the effects of prenatal exposure to POPs on rapid growth in the first 6 months of life and overweight at 14 months of age, Valdi et al. recently evaluated a large Spanish birth cohort study. DDE, HCB and PCBs were measured in maternal serum collected in the first trimester of pregnancy during 2003-2008. Rapid growth was defined as a z-score weight gain >0.67 SD between 6 months of age and birth. Overweight at 14 months was defined as a BMI z-score ≥85th percentile. Generalized linear models

examined the association between POPs and rapid growth (N = 1285) and overweight (N = 1198).

The analysis population included 24% rapid growers and 30% overweight infants. DDE and HCB were positively associated with rapid growth and with overweight. There was some indication that infant sex and exclusive breastfeeding duration may modify the effects of DDE, and that maternal pre-pregnancy BMI status may influence the effects of HCB. PCBs were not related to postnatal growth (29).

According to what explained above, EDCs exposure during pregnancy plays a role on obesity predisposition influencing birth weight, reduction of lean mass, different adiposity deposition with high percentage of abdominal fat, increasing of the waist circumference(30). More studies are needed to explain mechanism on the basis of this relationship.

Exposure to contaminants continues during breastfeeding. The decision to breastfeed and the continuation of breastfeeding are inherently markers of multivalent cultural lifestyle, and environmental characteristics, all of which can vary by location, cultural context, and individual mother-infant dyads.

In humans, numerous published studies indicate that breastfeeding protects against the development of later obesity (31-33). Although the magnitude of this protective effect differs by time of follow up, study size, and study location, the consensus from reviews and meta-analyses is that breastfeeding provides a weak to moderate protection against later overweight (34). While current data suggest that the immunological, physiological, nutritional and psychological benefits of breastfeeding far outweigh any risk from contaminants, it is clear that breast milk is commonly contaminated with high levels of pesticides. Serum concentrations of these compounds have been found to be significantly higher ($p<0.0001$) in breastfed than in bottle fed infants (35). A 1995 Victorian (Australian) survey found that organic compounds were detectable in nearly all breast milk samples and that infant's measured intake of some of them from breast milk greatly exceeded the adult acceptable daily intake (ADI) level (36). Over the past few decades, however, levels of organic compounds, PCBs, and dioxins have declined in breast milk in countries where these chemicals have been banned or otherwise regulated (37).

During breastfeeding infants may ingest EDCs and their metabolites present in the breast milk, because its fat content allows for the accumulation of substances with high lipid solubility. For example, a mother's breast milk level of DDE which is lipophilic, may be 6 to 7 times higher than that found in her blood (38).

EDCs in breast milk are not limited to those used locally near the mother's residence or workplace but are influenced by the different kind of food place of origin. For instance, the breast milk of mothers in Finland contained chlordane in a 1980s study, although chlordane was not used in Finland. The maternal exposures were attributed to the consumption of chlordane-contaminated fish from the Baltic Sea (39). In another investigation, similar breast milk DDE/DDT ratios were found in Saudi mothers who lived in regions with different usage patterns of DDT: Riyadh, where DDT use was banned; and Al- Ehssa, where DDT was used regularly to control leishmaniasis. The study estimated that 97.2% from the Riyadh region and 99.2% of the infants who resided in the Al-Ehssa region had DDE levels 20 g/kg of body weight per day, suggesting that DDT intake was similar in both regions (40).

Multiple pesticides may be present at the same time in breast milk according to food consumption. Bedi et al. recently published an interesting overview on how high the pesticide food contamination is in Punjab state (India) due to increasing demands of food for growing population and the consequent indiscriminate use of pesticides. The estimated daily intake value of DDT was higher than the FAO/WHO permissible tolerable daily intake one for few infants. Analysis of 53 human breast milk samples for pesticide residues revealed the presence of several different compounds derived from HCH, DDE, DDT and endrin and the occurrence of β-endosulfan, endosulfan sulphate, cypermethrin and chlorpyrifos for the first time in human breast milk in Punjab. With increase in parity, HCH and DDT residue burden in donor's milk decreased (41). The quantity of pesticide passed to the infant via breast milk is influenced by many variables such as maternal age and parity, maternal body burden of the chemical, and breastfeeding patterns. In 1999 Kostiniak et al. evaluated the breast milk composition

of lactating female of the New York State, focusing on specific alimentary consumption, reproductive and lactation history. They found that organic compounds concentration increased with the increasing concentration of milk lipid, increased as a function of maternal age and varied inversely with parity. In addition, the total number of months of lifetime lactation varied inversely with the total organic compounds concentration in breast milk (42).

After birth: characteristics of children and contaminant exposure (dose, absorption, metabolism)

Children are more exposed than adults to environmental contaminants for several reasons. First of all children have greater energy and water/Kg requirements than adults: when compared to adults, infants drink more water, eat more food and breath more air in relation to their body weight, being exposed to higher pesticides doses (5). Furthermore, gut surface is higher in infants than in adults and milk diet can promote absorption of substances like metals (lead, cadmium and mercury) explaining the major susceptibility of children to these toxicants (43). The ratio of surface area to body mass of children is 2.7-fold greater compared to adults with higher skin permeability . Moreover, children have more skin cuts, abrasions, and rashes than adults (44). These characteristics, combined with infants crawling behavior, determine a greater potential for dermal exposure to contaminants on floors, carpets, lawns and soil (45). Due to their behavior and outdoor activities, children are more exposed than adults during first childhood to contaminants present in air, water and soil. Generally, they are closer to the ground, often mouthing objects and fingers, with higher risk of toxicants ingestion and inhalation. Furthermore, during the first year of life, the fat body percentage increases from 13.4% to 22.4% and, as mentioned above, the EDCs exposure during this period could be high. This combination could lead to lifelong EDCs storage, caused by the high EDCs lipophilic nature and their long half-life. The larger the EDCs storage is, the higher the endocrine effects are, both local (in adipose tissue) and systemic (like steroids and thyroid hormones effects) increasing the potential obesity complications (44,45).

Furthermore, EDCs are metabolized and distributed differently from adults. Renal function is still immature showing lower ability to excrete metabolites in urine. Liver has greater relative weight, showing higher clearance ability and more potential bioactivity of exposed toxicants (43). On the opposite side, immaturity of liver enzymatic systems leads to slower metabolism, less activation and less ability to detoxify the body in children (5). In addition, the ability of serum proteins to bind substrates is limited.

Puberty

Recently, it was shown that being overweight is associated with earlier puberty in girls. The Frisch hypothesis suggested that menarche occurs at a critical weight (originally defined as 48 kg) and it seems recently to be supplemented by the endocrine role of adipose tissue. Proposed physiologic mediators of the link between obesity and pubertal timing include leptin, adipocytokines and gut peptides (18).

Focusing on leptin, it seems to be the signal to the hypothalamus GnRH pulse generator indicating that there are sufficient energy stores in the adipose tissue for fertility to commence, which is necessary but not sufficient for the initiation of puberty. Adipose tissue is also related to increasing IR, which lowers sex hormone binding globulin (SHBG) levels and leads to increased bioavailability of sex steroids. In addition, adipose tissue has aromatase action, which increases androgen conversion to estrogens. The higher estrogen levels could promote earlier onset of breast development and menarche in girls (46).

Several studies have identified environmental exposures and EDCs as likely contributors to the international secular trend in earlier pubertal development. Specifically, genome wide methylation studies have suggested that epigenetic mechanisms are intrinsically involved in the neuroendocrine control of female puberty (18). The 'perfect storm' may be that EDCs directly worsen obesity as well as directly impact endocrine function. The resultant obesity and change in the hormonal milieu feedback may act additively or synergistically to disrupt normal pubertal development (46).

EDCs' direct influence on endocrine system

EDCs present a non-development influence on obesity, independent from epigenetic mechanisms previously described. The major pathway by which Obesogens contribute to the etiology of obesity is by directly promoting adipogenesis. Main mechanisms of this promotion are: increase either adipocyte number or size with activation of nuclear receptors (eg. RXR and PPAR gamma), promotion of adipose cell lines at the expense of other cell lines, regulation of signaling pathways that commit adipose cell lines over other cell lines, enhanced differentiation of pre-adipose to adipose tissue through activation of PPAR gamma, promotion of increased storage of fat, and potential epigenetic mechanisms that promote factor transcription activation of adipogenic genes.

There may be a 'critical window' of susceptibility for each EDCs, that could interact with other EDCs, and impact the total body burden for an individual, depending on timing and dose of each exposure. Exposure to these chemicals may change adipocyte metabolism thus potentiating the combination of obesity and environmental exposures (46).

Recently Kim et al. analyzed the effects of POPs on human adipose cells and rodent adipose tissue, using human multipotent adipose-derived stem cells. They carried out large-scale gene expression analysis to identify the major pathways modified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated PCB congener 126 (PCB-126), and PCB-153 and to evaluate their toxic effects. The effects of TCDD on gene expression and adipose tissue histology were also assessed in mice.

The most significantly regulated genes in both precursor cells and adipocytes were those involved in the inflammatory/immune response, cancer, and metabolism pathways. Interestingly, the fold induction and the number of modulated genes were higher in precursors than in adipocytes, suggesting that the former could be more sensitive to the effect of pollutants. When cells were treated with combination of pollutants, the effects of the AhR ligands TCDD and PCB-126 were dominant compared with those of the non-dioxin-like PCB-153. The effects of AhR ligands were reduced by the AhR antagonist α-naphthoflavone. The regulation of inflammatory pathway was observed in wild-type adipose tissue but not in AhR-knockout mice.

Both in vitro and in vivo studies showed that adipose cells were targets of AhR ligands and suggest that inflammation is one of the main regulated pathways. These observations suggest a possible contribution of pollutants to low-grade AT inflammation that accompanies the pathogenesis of metabolic diseases (47).

It is well known that adipose tissue has its own endocrine activity. Two of the main hormones produced are leptin and adiponectin, implied in energy balance regulation. Leptin is synthesized proportionally to the body mass and regulates appetite and energy intake increasing energy expenditure and reducing food intake (48). Adiponectin has two main functions: to reduce glucose output by enhancing liver insulin sensitivity, and to increase fatty acid oxidation and glucose metabolism in muscles. It is the hormone of weight loss, presenting catabolic functions and showing a high serum level during weight decreasing. Low adiponectin levels are related to IR, pro-inflammatory tissue activity, obesity, MetS, T2DM so factors that reduce adiponectin production could predispose to these pathologies (49).

Recently, a strong correlation between IR and serum concentrations of persistent organic pollutants (POPs), especially for organic compounds, was found. In particular, the association of obesity and diabetes seems to become stronger with the POPs levels' increasing in blood circulation. The most possible hypothesis is that EDCs can alter the normal metabolism of glucose and lipids by antagonism of PPARs, promoting insulin resistance and modulating gene activity, including those genes that alter insulin action (50,51).

Recently, 90 diabetes-free subjects were analyzed during 20 years follow-up. They were a stratified random sample, enriched with overweight and obese persons. POPs measured in 1987-88 (year 2) sera included 8 organochlorine (OC) pesticides, 22 polychlorinated biphenyls (PCBs), and 1 polybrominated biphenyl (PBB). Body mass index (BMI), triglycerides, HDL-cholesterol, LDL-cholesterol, and homeostasis model assessment value for insulin resistance (HOMA–IR) were study outcomes at 2005-06. Among OC pesticides, p,p'-DDE most consistently

predicted higher BMI, triglycerides, and HOMA-IR and lower HDL-cholesterol at year 20 after adjusting for baseline values. Persistent PCBs with Ṣ7 chlorides predicted higher BMI, triglycerides, and HOMA-IR and lower HDL-cholesterol at year 20 with similar dose-response curves (52).

Several EDCs bind PPARs involved on adipocyte differentiation and maturation and lipid metabolism. PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor super-family and act as fatty acid sensors to control many metabolic programs that are essential for systematic energy homeostasis, including adipocyte differentiation, inflammation and energy homeostasis, lipoprotein metabolism, and fatty acids oxidation (53). All PPAR isoforms (α, β and γ) have a highly conserved structure composed of five different domains. The activation of target gene transcription depends on the binding of the ligand to the receptor that induces a conformational change in the ligand-binding domain (LBD) of the receptor and facilitates recruitment of co-activator molecules. In this context, several studies have identified a series of endogenous and synthetic ligands for PPARs such as numerous EDCs (16,54).

In addition, EDCs can be accumulated in adipose tissue and this can lead to interactions and modifications of the endocrine activity of adipose tissue and on the homeostatic systems of weight control (48). On the contrary, the more adipose tissue is represented, the more EDCs can be accumulated, triggering a vicious circle (5).

The exposure to Bisphenol A (BPA) could have an "obesogenic" function affecting the glucose transport in adipocytes and, at high doses, inhibiting the synthesis of adiponectin (48). It could also bind the estrogen receptors (ER) expressed in pancreatic isles and adipocytes. Authors (55) reported that, binding these receptors, BPA could stimulate IR by a hyper production and secretion of insulin. Other recent studies have demonstrated that BPA stimulates the releasing of two pro-inflammatory cytokines: TNF α and IL-6 both associated to the pro-inflammatory status existing in metabolic pathologies and increasing risks of cardiovascular disease, obesity and diabetes(56). Furthermore, high levels of TNF α, can also down-regulate the glucose transporter, increasing IR, and stimulate lipolysis, increasing plasmatic fatty acids by themselves. In summary, BPA, by increasing TNF α and IL-6 and by decreasing adiponectin, seems to predispose to the development of obesity and in particular to MetS.

Phthalates also show an obesogenic action, due to their anti-androgenic activity and their ability to reduce testosterone levels, leading to increase obesity, IR and T2DM prevalent in male subjects (57). A large study on NHANES data (1999-2002) investigated the association between urinary phthalate metabolite concentrations and BMI/waist circumference (WC) finding an important gender difference especially in WC. This exploratory, cross-sectional analysis revealed a number of interesting associations with different phthalate metabolites and obesity outcomes, including notable differences by gender and age subgroups**.** Using multiple regression they analyzed associations between six phthalate metabolites measured in BMI WC in 4369 participants aged 6-80. The most consistent associations were in males aged 20-59; there were no important associations among children, but several inverse associations among 60-80 year olds (58). A positive relationships between urinary concentrations of monoethyl phthalate and body size measures in overweight children was detected in an interesting prospective analysis published in 2012. Urinary concentrations of nine phthalate metabolites and anthropometrical data, including BMI and WC were measured among 387 Hispanic and Black, New York City children who were between six and eight years at cohort enrollment (2004-2007). Relationships between baseline metabolite concentrations and body size characteristics obtained one year later were examined using multivariate-adjusted geometric means for each body size characteristic by continuous and categories of phthalate metabolite concentrations. Dose response relationships were seen with monoethyl phthalate and the sum of low molecular-weight phthalates and body mass index and waist circumference among overweight children (14). These results are probably associated with the anti-androgens activity of several phthalates and the consequent concentration of endogenous levels of hormones. Higher androgen levels are associated with smaller WC in males, and with higher BMI, polycystic ovarian syndrome and MetS in females (53,54,59).

Conclusion

The progressive increase in obesity rates worldwide suggests that a fundamental shift is occurring in how the human body integrates changing nutritional and environmental variables to maintain metabolic balance and body weight**.** EDCs spread, as obesity, has been increased rapidly during the last decades. The presence of a link between these two factors seems certain and some EDCs are definitely considered as "obesogenic substances": molecules that inappropriately regulate lipid metabolism and adipogenesis to promote obesity**.** Accumulating evidence indicates that prenatal, early life and pubertal exposure to EDCs has the potential to affect fetal and later growth and child development. In addition, several physical characteristics and behaviors of growing-up children could predispose to higher EDCs contact and storage in adipose tissue, interacting with its endocrine activity and homeostatic systems of weight control. The more adipose tissue is represented, the more EDCs can be accumulated, triggering a vicious circle and increasing also the IR risk.

In conclusion, obesity is considerable now more than ever, a multi-factorial disease, based on the interruption of an healthy balance between food intake and physical activity and influenced by genetic predisposition and environmental factors.

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