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Environmental Obesogens: Organotins and Endocrine Disruption via Nuclear Receptor Signaling

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Over the last two decades, the incidence of obesity and associated metabolic syndrome diseases has risen dramatically, becoming a global health crisis. Increased caloric intake and decreased physical activity are believed to represent the root causes of this dramatic rise. However, recent findings highlight the possible involvement of environmental obesogens, xenobiotic chemicals that can disrupt the normal developmental and homeostatic controls over adipogenesis and energy balance. Environmental estrogens, *i.e.* chemicals with estrogenic potential, have been reported to perturb adipogenic mechanisms using *in vitro* model systems, but other classes of endocrine-disrupting chemicals are now coming under scrutiny as well. Organotins represent one class of widespread persistent organic pollutants with potent endocrine-disrupting properties in both invertebrates and vertebrates. New data identify tributyltin chloride and triphenyltin chloride as nanomolar agonist ligands for retinoid X receptor (RXRα, RXRβ, and RXRγ) and peroxisome proliferator-activated receptor γ, nuclear receptors that play pivotal roles in lipid homeostasis and adipogenesis. The environmental obesogen hypothesis predicts that inappropriate receptor activation by organotins will lead directly to adipocyte differentiation and a predisposition to obesity and/or will sensitize exposed individuals to obesity and related metabolic disorders under the influence of the typical high-calorie, high-fat Western diet. The linking of organotin exposure to adipocyte differentiation and obesity opens an important new area of research into potential environmental influences on human health and disease. (*Endocrinology* 147: S50–S55, 2006)

GLOBAL OBESITY RATES have climbed steadily over the past decades such that 60 million people in the United States alone are currently defined as clinically obese (1). The dramatic rise in the incidence of childhood obesity is of particular concern because obesity is difficult to treat effectively once established. Obesity is associated with insulin resistance, dyslipidemia, and hypertension, all of which are prominent risk factors for the development of type 2 diabetes and cardiovascular disease (2).

The etiology of obesity in humans is complex. Current models ascribe high-density caloric and/or fatty diets coupled with decreased physical activity as the root causes (3). The super sizing of the Western diet and sedentary modern lifestyles make a compelling supporting argument. In contrast to the obvious contribution of diet and lifestyle, the role of genetic components in increasing obesity rates is less clear. Genetic variation contributes to an individual's propensity to develop obesity, but the pace of genetic changes at the population level is inadequate to explain the rapid increase in obesity rates in Western societies. Instead, interaction with the modern environment is postulated to expose inherent genetic differences. The thrifty-genotype hypothesis suggests that *in utero* fetal nutritional status determines the risk for obesity and associated metabolic syndrome diseases (4–9). In this view, early metabolic programming alters the range in adaptive responses to the environment, *e.g.* diet and exercise, favoring thrifty use of nutrients *in utero* and later in life, thereby leading to obesity and metabolic syndrome under conditions of nutritional excess (10). Plausible molecular mechanisms include imprinting of obesity-sensitive hormonal pathways or changes in cell type and number, *e.g.* adipocytes, established during development. Experimental evidence from animal models supports this hypothesis (11). Food use in both obese and diabetic animal models is more efficient under fasting or calorie-restricted conditions. This lends credence to the thrifty-genotype hypothesis that proposes an evolutionary advantage on genes that can promote both peripheral insulin resistance and favored fat storage during periods of famine (12, 13). Candidate genes whose misregulation is linked to obesity include leptin and proliferator-activated receptor γ (PPARγ), which regulate food intake, metabolic efficiency, and energy storage (14).

Environmental Obesogens

An emerging alternative view proposes that the environment plays another role in obesity and hypothesizes that metabolic programming of obesity risk may be linked to *in utero* or lifetime exposure to xenobiotic chemicals (15, 16). This hypothesis parallels models for the action of environmental estrogens that affect aspects of reproductive endocrinology and health (17). It is plausible and provocative to associate the recent increased incidence of obesity with a rapid increase in the use of industrial chemicals over the past 40 yr. This model predicts the existence of chemical obesogens, molecules that inappropriately regulate lipid metabolism and adipogenesis to promote obesity. Support for this model would require the identification of obesogens, their

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Abbreviations: C/EBP, CCAAT/enhancer binding protein; EDC, endocrine-disrupting chemicals; 11β-HSD, 11β-hydroxysteroid dehydrogenase; PPARγ, proliferator-activated receptor γ; RXR, retinoid X receptor; TBT, tributyltin; TZD, thiazolidinedione.

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molecular targets, and potential cellular mechanisms through which they might act.

Although until now data have been scant, some epidemiological and *in vitro* studies suggested a link between environmental chemical exposure and obesity. These serve as proof-of-principle for a chemical obesogen hypothesis. For instance, the risk of childhood obesity is associated with maternal smoking during pregnancy. Smoking before or during pregnancy, but not afterward, increased the odds ratio for obesity approximately 2-fold in school-age children. This suggests that early *in utero* developmental events, rather than familial lifestyle, are determinant (18, 19). Animal studies implicate prenatal nicotine exposure as a possible factor for this postnatal weight gain through modulation of cholinergic and catecholaminergic systems (20, 21). In addition, many known or suspected environmental endocrine-disrupting chemicals (EDCs) mimic natural lipophilic hormones that mediate their effects through members of the superfamily of nuclear receptor transcription factors. Environmental estrogenic chemicals, such as bisphenol A and nonylphenol, can promote adipocyte differentiation or proliferation of murine preadipocyte cell lines (such as 3T3-L1 cells). Treatment with bisphenol A in the presence of an induction cocktail MDI (containing the cAMP signaling activator isobutyl methylxanthine, dexamethasone, and insulin) augmented 3T3-L1 cell differentiation into adipocytes (22). Differentiation of 3T3-L1 followed by treatment with 4-nonylphenol or 4-*t*-octylphenol stimulated proliferation of differentiated adipocytes (23). However, it remains unclear whether these effects are mediated solely through an estrogen receptor signaling pathway. These observations are in conflict with the general conclusions from *in vivo* studies, which showed that estrogens are antiadipogenic in adults and that increased obesity is associated with inhibition rather than activation of estrogen signaling. Increased adiposity is observed under conditions of estrogen deficiency as seen in FSH receptor knockout (FORKO) (24) and aromatase knockout (ArKO) (25) mouse models or estrogen receptor α signaling knockout (αERKO) mice (26). Furthermore, other estrogenic compounds such as the phytoestrogen genistein inhibit adipocyte differentiation *in vitro* at levels encountered in the diet (27) and decrease adipose deposition in ovariectomized mice (28). One report finds that at high doses, genistein acts as a ligand for the key adipogenic transcription factor PPARγ to stimulate adipogenesis (29). Taken together, these results suggest that antagonists of estrogen signaling or PPARγ agonists are more likely obesogen candidates than are environmental estrogens.

Organotins as Endocrine Disruptors

Seen from this viewpoint, published data on the persistent environmental pollutant and potent endocrine disruptor tributyltin (TBT) hinted at a potential role as an obesogen. Organotins are tetravalent tin compounds with a variety of mono-, di-, tri-, or tetra-substituted organic functional groups. Increased substitution and alkyl chain length is generally associated with increased toxicity. Since the 1960s, organotins such as TBT have been employed as antifouling agents in paints for marine shipping and for a variety of other uses. Human exposure to non-point sources of organotins occurs through contaminated dietary sources (seafood and shellfish), as fungicides on food crops, and as antifungal agents in wood treatments, industrial water systems, and textiles (30). A variety of mono- and dialkyltins, which include significant contaminating trialkyl species, are also prevalently used as heat stabilizers in the manufacture of polyolefin (PVC) plastics, bringing them into closer contact with drinking water and food supplies. Measured exposure levels of organotins, such as dibutyltin and tributyltin, in wildlife and human tissue samples are in the range of 3–100 nM (31–33).

Historically, the ability of trialkyl organotins to cause imposex, the abnormal induction of male sex characteristics in female marine invertebrates represents one of the clearest examples of environmental endocrine disruption. Shortly after the wide-scale introduction of organotins into the marine environment, the first reports of imposex on female gastropods surfaced (34). Subsequent field and laboratory work identified TBT as the causative agent. Bioaccumulation of TBT was demonstrated to decrease the activity of P450 aromatase, the key step in conversion of androgens to estrogens, with a consequent increase in testosterone and decrease in estrogen levels (35). Recent reports demonstrated that TBT can also induce masculinization in fish species (36). In mammals, however, organotins have modest adverse effects on both the male and female reproductive tracts and do not affect sex ratios. Instead, hepatic, neural, and immunotoxicity are the predominant indicators of high-level organotin exposure (37, 38). *In vitro* experiments can demonstrate a direct inhibitory effect by organotins on mammalian aromatase albeit with IC_{50} values in the micromolar range or above, concentrations that are cytotoxic (39, 40). Cotreatment with reducing thiol compounds reversed the inhibitory effects, implicating active-site cysteine residues in the interaction (41). Hence, the mechanistic understanding of the endocrine-disrupting potential of organotins is based primarily on their actions on key steroid regulatory enzymes (*e.g.* aromatase activity) or general toxicity mediated via damage to mitochondrial functions and subsequent cellular stress responses (39, 40, 42–44). However, there is evidence that organotins alter transcriptional activity. For example, expression of the aromatase gene was down-regulated by TBT in human ovarian granulosa cells, similar to the effects of treatment with ligands for either PPARγ or retinoid X receptors (RXRs) (45–47). The potential to modulate sex steroid homeostasis through transcriptional regulation, particularly through a nuclear receptor-mediated signaling pathway, seemed an intriguing possibility as a contributing mechanism for organotin action in vertebrates.

Organotins Are RXR and PPARγ Agonists

New data from Nishikawa *et al.* (48, 49) and independently from our laboratory showed that organotins indeed function as agonist ligands for several nuclear receptors. Nishikawa's group employed an *in vitro* molecular interaction screen between nuclear receptors and coactivators (48), together with a yeast one-hybrid GAL4 DNA-binding domain-nuclear receptor ligand-binding domain (GAL4 DBD-NR LBD) system

(49), to test a high-priority list of known or suspected environmental endocrine disruptors for receptor-mediated activation. We employed a similar ligand screen with GAL4 DBD-NR LBD constructs in mammalian cell culture (50). Surprisingly, organotins such as TBT or triphenyltin act as potent nanomolar activators of both RXRs and PPARγ with equilibrium binding constants (K_d) from 12–20 nM (50). Ligand-binding studies and the pattern of ligand-dependent recruitment of coactivators followed those observed with other receptor-specific ligands, thereby establishing organotins as *bona fide* receptor ligands.

The ability of organotins to act as bifunctional ligands that regulate both RXR and PPARγ signaling is troubling. RXR plays a central role as the common heterodimeric partner to many other nuclear receptor partners in multiple hormonal signaling pathways. In permissive heterodimers, RXR-specific ligands (rexinoids) can contribute to regulation of gene expression. Therefore, inappropriate activation of RXR can be expected to lead to wide-ranging disturbances in the body's homeostatic hormonal controls. In particular, RXR-PPARγ has been shown to play a key role in adipocyte differentiation and energy storage and is therefore key to the control of whole-body metabolism (reviewed in Ref. 14). PPARγ activation increases the expression of genes that promote fatty acid storage and represses genes that induce lipolysis in adipocytes in white adipose tissue (51). Subsequently, PPARγ activation modulates gene expression leading to decreases in circulating glucose and triglycerides, depleting their levels in muscle and liver. Because of these properties, PPARγ ligands such as the thiazolidinediones (TZDs) are used to treat type 2 diabetes. TZDs reverse insulin resistance in the whole body by sensitizing the muscle and liver tissue to insulin (52). Unfortunately, an undesirable consequence of this increase in whole-body insulin sensitivity is an increase in fat mass through the promotion of triglyceride storage in adipocytes. Activation of thrifty genes such as PPARγ that divert metabolic energy stores toward fat under conditions of nutritional stress may have provided an early evolutionary advantage in a calorie-poor environment. In calorie-rich modern diets, such thrifty genes may instead lead to obesity and contribute to metabolic syndrome diseases (13, 53). RXR ligands also activate the RXR-PPARγ heterodimer and act as insulin-sensitizing agonists in rodents (54), underscoring the potential effects of both PPARγ and RXR agonists on diabetes and obesity.

Organotins Promote Adipogenesis *in Vitro* and *in Vivo*

The 3T3-L1 cell system is a well characterized model for adipogenesis with a program of adipocyte differentiation driven by several master transcriptional regulators (reviewed in Ref. 55) (Fig. 1, *bottom*). These include members of the CCAAT/enhancer binding protein family (C/EBPα/β/δ), PPARγ, and sterol regulatory element-binding protein 1. Expression of these genes changes dynamically and sequentially throughout the differentiation process. Early expressed targets for adipocyte differentiation include the growth

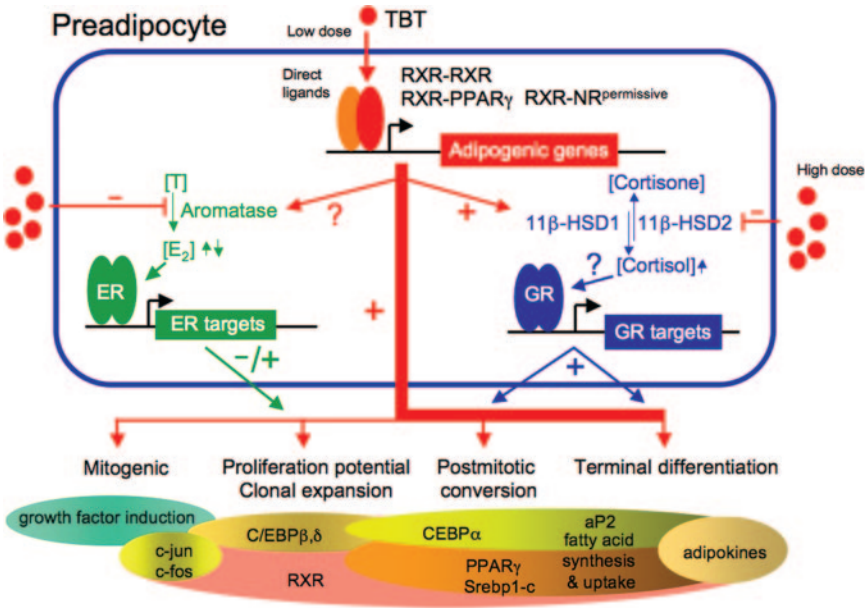

FIG. 1. Schematic depiction of the known and potential pathways through which TBT might act to modulate adipocyte differentiation and obesity. TBT at low nanomolar doses can activate RXR and PPARγ to directly modulate the activity of genes involved at multiple stages of adipocyte differentiation. These include early mitogenic genes such as c-*jun*, transcription factors responsible for clonal expansion and differentiation such as C/EBPβ and sterol regulatory element-binding protein 1 (Srebp-1c), as well as direct targets of PPARγ signaling such as adipocyte P2 (aP2), fatty acid synthase, and fatty acid transport protein. The *lower left* illustrates that TBT at high doses can inhibit aromatase enzyme activity directly, leading to decreased estradiol levels and down-regulation of estrogen receptor (ER) target genes. However, at low doses, aromatase transcription can be either up- or down-regulated by organotins in a tissue-specific manner depending on promoter usage. TBT at moderate to high doses inhibits the activity of 11β-HSD2, leading to decreased inactivation of cortisol, thereby increasing local glucocorticoid levels that could target late stages in adipocyte differentiation. Both 11β-HSD1 and -2 are themselves also potential targets of RXR-heterodimer-mediated transcriptional regulation. E_2, Estradiol; GR, glucocorticoid receptor; T, testosterone.

factor-responsive transcription factors c-myc, c-fos, and c-jun, followed by transient expression/switch in C/EBP factors from C/EBPβ/δ to C/EBPα. Subsequent induction of PPARγ promotes the expression of terminal adipocyte-specific genes such as *aP2*. Hence, effective adipocyte formation *in vitro* requires the adipogenic hormone cocktail MDI (isobutyl methylxanthine, dexamethasone, and insulin) to initiate the program and a PPARγ agonist for terminal differentiation.

The receptor activation data lead to the prediction that organotins would recapitulate the action of TZDs and rexinoids in the RXR-PPARγ signaling pathway. Indeed, TBT can complement the role of a PPARγ ligand in combination with the MDI induction cocktail to induce RXR-PPARγ target genes and efficiently drive adipocyte differentiation (48, 50, 56). Modest differentiation was also observed in the absence of MDI cocktail in a manner similar to other rexinoid ligands (50, 57). This result is significant because it implies that signaling through RXR and/or via its permissive heterodimeric partners is sufficient to drive preadipocytes through the complete differentiation process. TBT may be particularly potent in this respect because it is a dual ligand for both RXRs and PPARγ.

In vivo, acute exposure to TBT, TZDs, or rexinoids in adult mice resulted in coordinate regulation of lipogenic RXR-PPARγ target gene expression in adipose tissue and liver and modulated adipocyte differentiation factors such as C/EBPβ and sterol regulatory element-binding protein 1c (50). Furthermore, developmental exposure *in utero* led to a fatty liver (hepatic steatosis) phenotype and enhanced lipid staining of neonatal fat depots and resulted in a significant increase in the epididymal fat pad size of mice later in life (50). Whether this occurs through increased lipid storage, an increase in adipocyte number, or a combination of both is currently unresolved. However, organotin activation of RXR-PPARγ signaling represents a compelling mechanistic example of a class of environmental pollutants that have the ability to impact key adipogenic factors, fat depot size, and function. How significant these effects will be on long-term changes in lipid homeostasis, overall adipogenesis, and consequently altered risks for obesity and its associated metabolic disorders in humans remain to be determined.

Future Directions

This emerging paradigm for organotin action opens new avenues of investigation and the ability to reinterpret existing work. Several important questions will need to be addressed. Compared with known endogenous or synthetic ligands of RXR and PPARγ, organotins are structurally and chemically unique. Structure-activity relationships also suggest that the specificity of organotin binding is more relaxed than might be expected from highly discriminatory receptors such as RXR, although competition binding experiments indicate that the site overlaps at least partially with the classical receptor binding pocket (50). How exactly then do organotins bind with high affinity to their cognate receptors? Structural studies should yield important details about this interaction, may indicate novel ways to promote productive receptor-coactivator interactions, and could lead to the development of a new therapeutic class of insulin sensitizers.

The combinatorial role of RXRs as heterodimeric partner to many other nuclear receptors widens the possibility of organotins to perturb lipogenic signaling. Figure 1 summarizes the central role played by RXR-heterodimer signaling in adipogenesis and also highlights potential points of intersection with the sex steroid and glucocorticoid axes. Organotin activation of permissive RXR heterodimer combinations is observed for liver X receptor α and PPARδ among others. Stimulation of liver X receptor signaling is predicted to impact lipid homeostasis by regulating genes of cholesterol efflux, bile acid production, fatty acid synthesis, and lipid transporters (58). PPARδ, on the other hand, plays an opposing role to PPARγ by inducing fatty acid catabolism and regulating overall energy homeostasis (59). Hence, increased PPARδ activity leads to resistance to diet-induced obesity, whereas a decrease results in obesity, hyperlipidemia, and tissue steatosis in transgenic mouse models (59). The overall balance between PPARγ and PPARδ activation by organotins may therefore be critical. The physiological response of adipocytes, liver, muscle, and other relevant tissues will depend not just on the exposure level of specific organotins but also on the particular expression profiles of RXRs and their permissive heterodimeric partners. How these multiple pathways interconnect in response to organotins will undoubtedly be complex and the focus of future research.

Adding to this complexity is the perturbation of other nuclear hormone receptor signals, often in a tissue-specific manner. Disturbance in sex steroid levels, either through direct enzyme inhibition of aromatase or through transcriptional up- or down-regulation of aromatase promoters by organotins has been well documented (47) (60). The specific response of aromatase promoters to TBT in adipose tissues is currently unknown. Compounding these effects, testosterone biosynthesis is also targeted by organotins through inhibition of 17β-hydroxysteroid dehydrogenase (61). Additional enzymes involved in steroid metabolism may be misregulated by organotins as well. Hypercortisolism has been strongly associated with lipodystrophies such as central obesity and risk for the development of diabetes. Localized increases in active glucocorticoids and secretion of inflammatory cytokines from adipocytes and infiltrating macrophages are characteristics of obesity that negatively impact leptin and insulin signaling (62). Inappropriate peripheral regulation of 11β-hydroxysteroid dehydrogenase (11β-HSD) isoforms is believed central to these mechanisms. Type 1 (activating) and type 2 (inactivating) 11β-HSDs mediate the interconversion between cortisol (active) and cortisone (inactive). Both isoforms are sensitive at the transcriptional level to RXR heterodimer signaling (63–65), and recent work has demonstrated a direct inhibitory action by organotins on 11β-HSD2 (66). It is reasonable to hypothesize, therefore, that organotins may increase the set point for cortisol levels in tissues and produce broad ranging effects on glucocorticoid-sensitive pathways including obesity, hypertension, and diabetes.

Conclusions

The roles of environmental chemicals in the etiology of complex diseases such as obesity, type 2 diabetes, and cardiovascular disease are currently poorly understood. The link that has been forged between organotins and adipocyte differentiation opens an important new area of research into environmental influences on human health with respect to obesity and related metabolic disorders such as type 2 diabetes and cardiovascular disease. Mean measured levels of TBT in random human serum samples reach concentrations (~27 nM) (32) sufficient to activate high-affinity receptors such as RXR and PPARγ. This suggests that a significant fraction of the general population may be exposed to the obesogenic effects of these compounds, which is a potential cause for concern. Therefore, additional research directed at understanding the nature and action of chemical obesogens will illuminate the connection between health and the environment and may also reveal unappreciated new mechanisms regulating adipose tissue development, obesity, and diabetes. The existence of chemical obesogens in and of themselves suggests that the prevailing paradigm, which holds that diet and decreased physical activity alone are the causative triggers for the burgeoning epidemic of obesity, should be reassessed.

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