Endocrinology, 2021, Vol. 162, No. 12, 1–13
https://doi.org/10.1210/endocr/bqab194
Mini-Review

Mini-Review

PFAS and Potential Adverse Effects on Bone and Adipose Tissue Through Interactions With PPARγ

Andrea B. Kirk,[1] Stephani Michelsen-Correa,[2] Cliff Rosen,[3] Clyde F. Martin,[4] and Bruce Blumberg[5]

[1]US Environmental Protection Agency, Washington, District of Columbia 20460, USA; [2]EPA Office of Chemical Safety and Pollution Prevention, Biopesticides and Pollution Prevention Division, Washington, District of Columbia 20460, USA; [3]Tufts University School of Medicine, Boston, Massachusetts 02111, USA; [4]Texas Tech University, Lubbock, Texas 79409, USA; and [5]University of California, Irvine, Irvine, California 92697, USA

ORCiD numbers: 0000-0002-1182-3104 (A. B. Kirk); 0000-0002-1053-217X (S. Michelsen-Correa); 0000-0003-3436-8199 (C. Rosen); 0000-0002-5449-0653 (C. F. Martin); 0000-0002-8016-8414 (B. Blumberg).

Abbreviations: AO, adverse outcome; AOP, adverse outcome pathway; ATSDR, Agency for Toxic Substances and Disease Registry; BMD, bone mineral density; BMI, body mass index; FA, fatty acid; MIE, molecular initiating event; MSC, mesenchymal stem cell; PFAS, perfluoroalkyl and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUnDA, perfluoroundecanoic acid; PPARα, peroxisome proliferator–activated receptor α; PPARγ, peroxisome proliferator–activated receptor γ; RXR, retinoid X receptor; TBT, tributyltin; TZDs, thiazolidinediones; US EPA, US Environmental Protection Agency

Received: 28 June 2021; Editorial Decision: 1 September 2021; First Published Online: 4 September 2021; Corrected and Typeset: 13 October 2021.

Abstract

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a widely dispersed, broad class of synthetic chemicals with diverse biological effects, including effects on adipose and bone differentiation. PFAS most commonly occur as mixtures and only rarely, if ever, as single environmental contaminants. This poses significant regulatory questions and a pronounced need for chemical risk assessments, analytical methods, and technological solutions to reduce the risk to public and environmental health. The effects of PFAS on biological systems may be complex. Each may have several molecular targets initiating multiple biochemical events leading to a number of different adverse outcomes. An exposure to mixtures or coexposures of PFAS complicates the picture further. This review illustrates how PFAS target peroxisome proliferator–activated receptors. Additionally, we describe how such activation leads to changes in cell differentiation and bone development that contributes to metabolic disorder and bone weakness. This discussion sheds light on the importance of seemingly modest outcomes observed in test animals and highlights why the most sensitive end points identified in some chemical risk assessments are significant from a public health perspective.

Key Words: peroxisome proliferator activated receptor, PFOA, PFAS, bone, adipose, PPAR

ISSN Online 1945-7170

Perfluorinated compounds (PFAS) constitute a large class of thousands of synthetic chemicals whose long and extensive use in consumer goods and industry has led to their ubiquitous presence in the environment. PFAS have been used in firefighting foam, cookware coatings, water-repellant fabrics and stain-prevention additives for carpeting, clothing, and vehicle interiors and cosmetics. The broad dispersal of PFAS, their potential to bioaccumulate, and their toxicity are a growing public health and regulatory concern. Biological effects of PFAS exposure are diverse, with reports of hepatic (1, 2), renal (3), developmental (4), reproductive (5, 6), and immune system effects (7). In this context the terms "*developmental*" and "*developmental study*" refer to studies of the effects of early (in utero or perinatal) chemical exposures on the biological development of an organism. Changes in serum lipoproteins (8), body mass, adiposity, and bone structure and quality have also been linked to PFAS exposure (4, 9). State and federal regulatory agencies have found it challenging to determine which PFAS to assess and at what level does exposure pose a minimal risk. The rapid pace of research also complicates risk assessments for such a large group of co-occurring chemicals (10) as new research may rapidly outdate even recent decisions.

State and federal regulatory efforts focus on PFAS as water contaminants, but other exposure pathways exist, including dietary (11) and inhalation routes (12). The precise number of contaminated water systems is uncertain because testing for PFAS is not required. Although, the number of US water systems where PFAS have been detected has reached into the thousands (13). The exact percentage of systems is not currently known. When tests are performed, they are often limited to a small standard set of PFAS for which analytical methods are available (14, 15). PFAS have been detected in surface and/or groundwater across the United States and on every continent (eg, North America, Europe (16), Africa (17), Asia (18), Australia (19), South America (20), as well as in the Artic (21).

When developing drinking water standards, state and federal agencies have sometimes treated PFAS or small sets of PFAS as a single "class" with one-for-one additive toxicities. For example, the US Environmental Protection Agency (US EPA) has developed health advisories of 70 parts per trillion for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) and 70 parts per trillion total health advisories when the 2 are present in mixtures (22). The advantage of using additive toxicities or otherwise treating all PFAS as a single class is the relative simplicity, making it the most expedient and feasible method for regulatory purposes (23). However, not all PFAS share a common mechanism of action or ultimate tissue distributions (24). Therefore, the additive toxicities approach may not be appropriate for all cases from a scientific viewpoint although they may be so from a policy view. Additionally, the broad classification of "PFAS" includes a diversity of structures as can be visualized through the US EPA CompTox Dashboard (25). Some basic structural types are shared by groups of PFAS (Fig. 1).

Some PFAS chemicals have more in common with non-PFAS contaminants such as tributyltins or phthalates than with other PFAS. An alternative classification approach might divide PFAS into subclasses according to common interactions with a single molecular target, structural commonalities, or shared adverse outcome (AO), and apply either toxic equivalency factors or generalized concentration addition modeling for setting standards for mixed PFAS (26, 27). An additional consideration is the preference of different PFAS for different tissues. For example, evidence so far points to bone as a preferential sink for PFOA, lungs for perfluorobutanoic acid, and liver and brain for perfluorohexanoic acid (24). Identifying common mechanisms and tissue sinks would allow more granular, target-specific classification of various PFAS that would aid in risk assessments for mixtures of PFAS. This paper will discuss issues surrounding nuclear-receptor–PFAS interactions and provide a more detailed exploration of the potential human health significance of interactions between PFOA and peroxisome proliferator-activated γ receptor (PPARγ).

Using Adverse Outcomes Networks to Chart Routes Through Complex Mechanisms

Adverse outcome pathways (AOPs) chart the progression from chemical interaction with a molecular target through a series of key events to an eventual AO (28, 29). The interaction that begins the sequence is termed the molecular initiating event (MIE). Some xenobiotics may induce a single MIE, as might happen with competitive inhibition. This would be seen in the case of perchlorate competing with iodide for uptake into a thyroid epithelial cell. Key events that followed would be reduction of iodide availability, reduced synthesis of thyroid hormone, reduced receptor interaction, and reduced gene transcription of genes regulating neurodevelopment to the final AO of reduced IQ (30). With PFAS the numbers of molecular initiating events may be considerably larger than the one seen with iodide uptake inhibition, both because of the thousands of different branched and linear PFAS chemicals known to exist and because a particular PFAS may induce more than one MIE, forming a network rather than a straight path. A PFAS that might be a minor contributor to one end point might be a major contributor to another. If we could identify MIEs common to specific groupings of PFAS, it might be possible to predict their toxicology-related attributes,

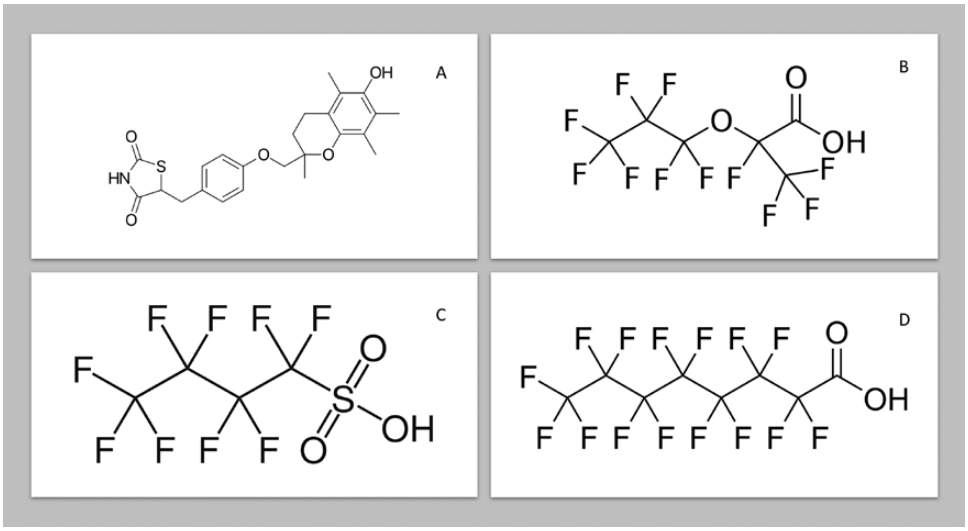

Figure 1. Examples of structural differences among perfluoroalkyl and polyfluoroalkyl substances. (A) Troglitazone; (B) GenX; (C) PFOS; and (D) PFOA.

focus and prioritize research efforts, and improve decisions on, for example, remediation of soils or water systems contaminated with mixtures of PFAS.

Perfluoroalkyl and Polyfluoroalkyl Substances and Nuclear Receptors

Individual PFAS can trigger several MIEs through interactions with more than one major nuclear transcription factor (31). For example, PFOA has been shown to interact with peroxisome proliferator–activated receptor alpha (PPARα), estrogen receptor α, constitutive activated receptor (32), the human pregnane X receptor (33), and PPARγ (34). Interaction with any of these transcription factors may trigger specific and sometimes intersecting biochemical pathways and affect multiple biological systems. This is a common feature of endocrine-disrupting chemicals (35).

An individual AOP that traces the progression from an MIE to an outcome that is harmful in a singular aspect to an individual organism or population might not capture other important end points. Assessing the toxicity of a chemical ideally requires an analysis of the totality of responses, particularly where there are multiple adverse impacts. Analyzing potential pathways and outcomes may also help focus research directions and risk characterization for this and other complex groups of chemicals.

PFAS interactions with PPARγ have been selected as an example for more in-depth discussion for the following reasons:

1. A number of PFAS interact with PPARγ;
2. Two key developmental studies (4, 9) show abnormalities in bone development, a PPARγ -influenced outcome, in PFOA dosing experiments;
3. The outcomes measured (anomalies in bone development) in these 2 studies were selected as points of departure for a PFOA oral exposure of intermediate duration minimal risk level derived by the Agency for Toxic Substances and Disease Registry (ATSDR) (36) as well as a health advisory by the US EPA (22).
4. PPARγ activation increases adipogenesis, in part because it drives the determination of mesenchymal stem cells (MSCs) toward adipogenic and away from osteogenic fates. This may be factor in the current obesity epidemic and should be considered for public health reasons.

Brief Introduction to Peroxisome Proliferator–Activated Receptors

PPARs are members of the nuclear receptor superfamily. They are ligand-modulated transcription factors whose actions have far-reaching effects on many developmental and homeostatic functions (37). To bind to DNA, PPARs must first dimerize with a retinoid X receptor (RXR) to form a heterodimer. PPARs possess 2 DNA-binding zinc fingers and receive ligands through a ligand-binding domain, which leads to activation or repression of gene expression depending on the amount and nature of cofactors present, which can be either coactivators or corepressors (38). PPARs can receive many different endogenous and synthetic agonists. Endogenous agonists include intermediates of fatty acid (FA) metabolism and oxidation and include unsaturated, branched oxidized FAs as well as nitro FAs (39). Exogenous examples include many drugs, including some used for the control of diabetes and hypertension (40). PPAR activity may be induced by direct ligand-receptor interaction or through interaction with PPAR-related cofactors (41). Some agonists, including certain PFAS, can target more than one PPAR

(Table 1), or even other nuclear hormone receptors, which may result in diverse and widespread biological effects on the receiving organism. The PPARs themselves, PPARα, PPARβ/δ, and PPARγ have differing tissue distributions and control different sets of genes.

PPARα plays important roles in FA oxidation, and its expression is concentrated in tissues with heavy metabolic demand and large numbers of mitochondria such as the heart, liver, kidney, and brown adipose tissue (42). PPARα also plays an important role in regulation of drug efflux transporters at the blood-brain barrier. Because these transporters remove lipophilic xenobiotics from the brain, including drugs for treatment of brain cancer, epilepsy, and depression (among others), exposure to environmental PPARα agonists may potentially reduce the efficacy of some pharmaceuticals (43).

PPARβ (or *PPARδ*) is widely distributed, with concentrations highest in intestinal epithelium, liver, and skin (44), and plays roles in energy metabolism in skeletal muscle, and may play a role in T-cell development (45). PPARβ has been implicated in the development of nonalcoholic fatty liver disease (46) and some cancers (47).

PPARγ is highly expressed in bone marrow (48), where it is essential for differentiation of MSCs to adipocytes and away from the osteocyte pathway. Inappropriate expression or regulation of PPARγ in the bone marrow can result in the dysregulation of skeletal and adipose development and homeostasis. The PPARγ ligand-binding pocket is unusually large for a nuclear receptor and can accommodate a variety of structures (49) including a variety of xenobiotics. Because we will be focusing on bone and adipose tissue, the role of PPARγ in other tissue types will be discussed later (50, 51).

PPARγ has 2 known human isoforms, PPARγ1 and PPARγ2. PPARγ2 is not normally expressed at appreciable levels in MSCs, which will be the focus of our discussion, but its expression is induced by activation of PPARγ1 (52). Induction of PPARγ2 expression is a critical step in the commitment of MSCs to the adipocyte rather than osteoblast lineage within bone marrow. Recent in vitro studies using human MSCs indicate that PFAS, particularly PFOA, exposure at environmentally relevant concentrations (10 nM to 10 μM) may alter osteogenic and adipogenic processes (53). PPARγ is a key regulator of adipogenesis (54) and plays a crucial role in the development of white adipose tissue; in the absence of PPARγ, white adipose tissue does not form (55).

PPARγ plays a role in several aspects of bone development, including prenatal and perinatal ossification. PPARγ deficiency has been shown to result in increased osteoblastogenesis and increased bone mass (56). Cartilage-specific deletion of PPARγ in mice led to abnormally developed growth plates, reduced ossification, and reduced trabecular thickness (57). Alterations in PPARγ activity can be detrimental to adults as well. Increased PPARγ activity impaired skeletal homeostasis, increased bone resorption, and drove aberrant bone modeling in mice (58). Differences in strength or duration of PPARγ activation by PFAS may be driven by variability in receptor affinity but might also be dependent on other target interactions. PFAS-PPARγ interaction may lead to reduced osteoblast numbers and increased osteoclast activation. Altered ratios of these cells may lead to reduced ability to ossify cartilage or maintain bone mass, leading to an AO of possible altered developmental trajectory in the latter, or osteoporosis and increased risk of fracture in the former.

PPARγ agonists cause bone loss through at least 2 mechanisms: 1) via their suppressive effects on osteoblast differentiation (59) in favor of adipocyte formation (60, 61); and 2) by increased bone resorption (58). In respect to the former, MSC fate is determined by the balance between runt-related transcription factor 2 (Runx2) whose expression results in commitment to osteogenesis (62), and PPARγ2 activation, which results in commitment to adipogenesis (63, 64). Allocation of MSC to the adipogenic compartment may ultimately reduce the number of osteoblasts available for bone formation, while at the same time increasing numbers of adipose cells. Once an MSC has committed to the adipogenic lineage, it is no longer responsive to bone differentiation signals. Larger-scale allocation of MSCs to adipogenesis may be a mechanistic explanation for the increased adiposity and the appearance of bone abnormalities observed in test animals dosed with

Table 1. Perfluoroalkyl and polyfluoroalkyl substances that have shown in vitro receptor interactions or change in receptor proteins

PFAS	Known receptor(s)	Citation
PFOA	PPARα	(34)
	PPARγ	
	PPARβ/δ	
PFOA	HNF4α	(100)
PFOA	CAR	(101)
PFOA	Vitamin D receptor	(102)
PFOS	PPARα	(34)
	PPARγ	
PFOS	HNF4α	(100)
PMOH (GenX)	PPARγ	(103)
PFBA	PPARα	(32)
PMPP	PPARγ	(103)

Abbreviations: PFAS, perfluoroalkyl and polyfluoroalkyl substances; PMOH, perfluoro-2-methyl-3-oxahexanoic acid; PMPP, 3H-perfluoro-3-[(3-methoxypropoxy) propanoic acid].

PFOA. This was first shown with tributyl tin (65). PPARγ activation by certain ligands has been shown to skew the ratios of adipocytes and osteogenic cells and cause skeletal fragility as well as weight gain. This is exemplified by thiazolidinediones (TZDs), a drug class that selectively activates PPARγ (66, 67).

While activation of PPARγ is well established in promoting MSC differentiation toward adipogenesis, other factors are likely to be involved. For example, it has recently been demonstrated that activation of RXR, but not PPARγ, can commit mouse MSCs to the adipogenic lineage (63). These authors did not explicitly test whether RXR activators also decreased osteogenesis, but this can be reasonably be inferred.

Although skeletal tissue is a shared target for at least some PPARγ-activating agents, obesity and metabolic syndrome may be far more important end points than skeletal effects from a public health perspective. The most sensitive end point identified both by the US EPA (22) and ATSDR (36) for PFOA was immunotoxicity, which is also PPARγ influenced (68, 69). This may be a consequence of reduction of osteoblast cytokine support of B lymphopoesis (70, 71) or through other cytokine support of B lymphopoesis (70, 71) or through other mechanisms (72). PPARγ agonists are showing promise as attenuators of the immune response, through suppression of inflammatory pathways (73, 74), which may be helpful for treatment of autoimmune diseases, although cardiotoxicity has been a major concern. Because PPARγ suppresses some aspects of the immune response (69), it should not be surprising if exogenous agents that activate PPARγ such as PFOA show similar effects. However, at the time of ATSDR (36) and US EPA's (22) document completion, there were insufficient data with which to derive a human equivalency dose for immune effects, and bone effects were modeled instead.

Lessons from Peroxisome Proliferator–Activated Receptor–Activating Drugs

If a xenobiotic or drug has been identified as a PPARγ agonist, we can look to reported adverse effects for insights into what AOs we might expect to see with exposures to PPARγ agonistic PFAS. Examples of exogenous PPARγ agonists are tributyltins (TBT), including dibutyl tin, tetrabutyltin, and triphenyl tin (75, 76), phthalates (77) and TZD drugs used for control of diabetes (75, 78). Li et al (79) showed that PPARγ activation by TBT is sufficient for adipocyte differentiation. TBT can still activate reporter genes through the RXR half of the heterodimer, but only TBT activation of PPARγ can differentiate preadipocytes into adipocytes Some TZDs have shown evidence of cardiotoxicity and been withdrawn from the market. Interestingly, PFOA has also shown evidence of cardiotoxicity, with dosed mice showing histopathological abnormalities and mitochondrial dysfunction (80). Some epidemiological studies have shown increased risk of cardiovascular disease in a PFOA-exposed population (81); however, others have shown associations with hypercholesterolemia, but not with coronary artery disease (82).

Peroxisome Proliferator–Activated Receptor γ Agonists

PPARγ agonists have been used pharmacologically to manage diabetes (83) and been proposed for use in mitigating oxidative damage in degenerative neurological diseases such as Alzheimer, Parkinson, and Huntington disease (84). TZD drugs (rosiglitazone, pioglitazone, troglitazone) are selective PPARγ agonists (85) that were developed for the treatment of type 2 diabetes and introduced to the market in the 1990s. They share a common core structure but differ in side chains (Fig. 2). The common core aspect is thought to be the section that binds to PPARγ independent of chain length (86), while the side chains may influence binding affinity, and thus potency and/or duration of response.

TZDs produce side effects similar to adverse effects seen with some PFAS exposures. Among these are increased risk of fracture in TZD-treated patients (87, 88), as well as obesity, hepatic steatosis (89, 90), and changes in blood lipids (91). These side effects fall within a pattern of possible expectations because while activation of PPARγ will increase insulin sensitivity, it should also activate other PPARγ-involved mechanisms, including those that regulate adipogenesis, bone development, growth, and homeostasis.

Adverse effects on bone in the form of increased risk of fracture in women have been observed in patients taking thiazolinedione PPARγ agonists such as rosiglitazone for treatment of diabetes. Pioglitazone, a less powerful PPARγ agonist, is still in use, although it also decreases bone mineral density (BMD) and, like other PPARγ agonists, increases bone marrow adiposity (92). While the clinical significance of increased bone marrow adiposity in itself is uncertain, increased marrow adiposity is associated with decreased BMD and greater fracture risk (93, 94) and is an expected consequence of PPARγ activation. Indeed, osteoporosis has been referred to as "obesity of bone" (95).

Dosing C57BL/6 adult male mice with rosiglitazone, a strong PPARγ agonist, resulted in loss of cortical bone mass, along with decreased osteoblastic activity, and reduced calcium, ash, and phosphate content (96). A study designed to evaluate the effects of rosiglitazone on male young, young adult, and elderly mice showed the strongest effects on BMD in adult and aged animals.

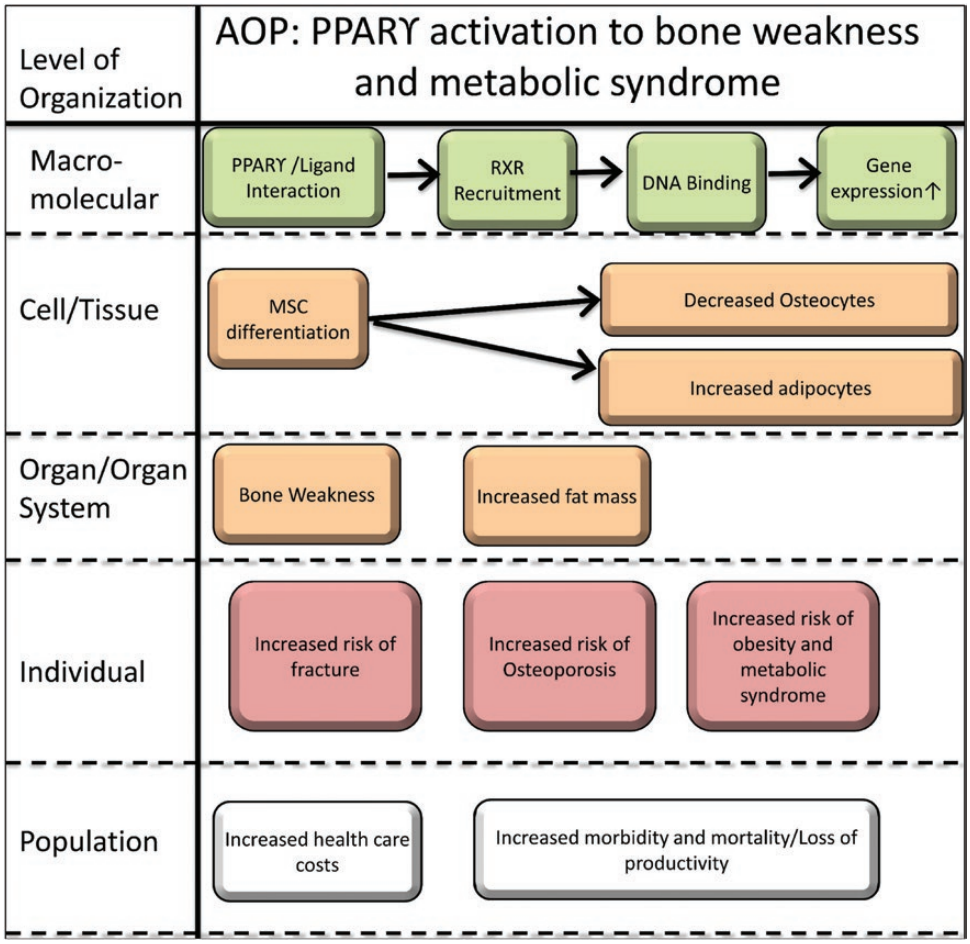

Figure 2. Simplified example of a perfluoroalkyl and polyfluoroalkyl substance–related adverse outcome pathway.

Young animals (male) experienced early closure of the epiphyses (97). This differs from skeletal effects seen in PFOA-dosed C57BL/6 female mice that were dosed through gestation and lactation. The PFOA-dosed mice showed decreased tibial BMD, but not significantly different from controls. There are a number of possible reasons for this even though the strain was the same as used by Broulík and colleagues (96). First, the bone density of PFOA-dosed mice was evaluated for more than 1 year after dosing ceased. Second, PFOA-dosed mice were 10% heavier at adulthood than were control mice. The increased body weight may have been a significant factor in determining bone density, and the mice in the study by Koskela et al (4), the key study used by ATSDR (36) in determining a minimal risk level for PFOA, were female. There is evidence that PPARγ activity differs between males and females, possibly because of interactions with estrogen, at least where immune function is concerned (98). Finally, rosiglitazone is a nanomolar affinity ligand for PPARγ (99) whereas PFOA activates PPARγ only at tens of micromolar, a concentration at which it may hit other cellular pathways.

Dual Peroxisome Proliferator–Activated Receptor α/γ Agonists

A number of PFAS appear to be dual PPARα/γ agonists (see Table 1), and their effects may parallel those of the Glitazar drug family, which bind both PPAR isoforms. Both PPARα and PPARγ play important roles in bone modeling and homeostasis and may present opposing effects (104). In contrast to activation of PPARγ (which reduces bone formation), PPARα activation has been demonstrated to increase differentiation of osteoblast precursors including the MC3T3-E1 cell line and in primary osteoblasts (105), potentially leading to increased bone precursor differentiation. There is also strong concern that dual PPARγ/α activators may be cardiotoxic (106) or pose other risks including carcinogenesis and kidney damage (107).

Other Peroxisome Proliferator–Activated Receptor γ–Activating Xenobiotics

Other environmental contaminants or agents, such as tributyltins (75, 79), phthalates, and other chemicals (77),

activate PPARγ and induce related adverse effects, some of which may persist in subsequent generations (108).

Assessing Perfluoroalkyl and Polyfluoroalkyl Substances by Peroxisome Proliferator–Activated Receptor γ Affinity

The affinity of PFAS for PPARγ may be one of several possible approaches for assessment of relative risks to development and long-term health posed by perfluorinated substances. Such approaches have been applied to drug discovery, particularly to the identification of potential PPAR ligands (109) and other efforts to identify PPARγ agonists and antagonists (110, 111). It is important to note that PPARγ activation is only one of several PFAS mechanisms of action, and that PPARγ affinity may not represent a clear picture of overall toxicity. Riu and colleagues (110) characterized PPARγ modes of xenobiotic binding by using nuclear receptor-based affinity columns to evaluate the binding affinities of halogenated bisphenols to PPARγ and to the estrogen receptor. More highly halogenated bisphenols showed greater affinity for PPARγ. They further found that "bulkier" bisphenol A analogues had greater PPARγ activation and that the halogenated bisphenol A promoted adipocyte differentiation of 3T3L1 preadipocytes (112).

Other Peroxisome Proliferator–Activated Receptor γ–Influenced Effects

It is important to note that bone is one of many PPARγ-related end points. Other pathways may increase risk of coronary artery disease, metabolic syndrome, kidney disease (113), or impaired learning and memory and immune function (114), age-related macular degeneration (115), and cancer, including breast cancer (116). The effects of enhanced cancer risk is thought to be due to the influence of PPARγ on angiogenesis (117, 118), as has also been proposed for macular degeneration. PPARγ is highly expressed in skeletal muscle (119), where it regulates lipid metabolism and insulin sensitivity (98). PPARγ also plays roles in kidney homeostasis (113) and innate immune responsiveness (69). In addition to its roles in normal skeletal development, and adipogenesis, PPARγ is also important for thermoregulation and cold adaptation (120) and mammary gland development (121). PPARγ may also play an important role in the development of nonalcoholic fatty liver disease (122).

Additional end points for PPARγ disruption may include delayed mammary gland development (121, 123) and shorter durations of breastfeeding (124). PFOA exposure has also been shown to suppress STAT5B (101), which would not be unexpected because STAT5B is suppressed by activation of PPARγ (125) as well as PPARα (126). STAT5B is important in mammary gland development and mammary tumorigenesis (127). Suppression of STAT5B following PFAS exposure represents an additional possible key event along an AOP as STAT5B is important in mammary gland development, growth, immune function (128) and may be important in learning, memory, and behavior (129). Suppression of STAT5 and other molecular events may need to be explored separately and the outcomes integrated into a larger, overall understanding of PFAS toxicity. Last, PPARγ has been detected throughout the male reproductive system (51), which might explain some of the reproductive effects of PFAS exposure. The effects of PFOA and PFOS on male reproduction have been recently reviewed (130). We hope to address the potential significance of exogenous PPARγ agonists on tissues other than bone and fat in a future publication.

Associations Between Perfluoroalkyl and Polyfluoroalkyl Substances Exposures and Bone Effects in Human Populations

A number of recent epidemiology studies support associations between PFAS exposures and bone alterations both in children and adults. A study of young men (aged 18-21 year) with long-term residence in areas of Veneto, Italy, where PFAS had contaminated drinking water for decades (131) were found to have reduced bone quality as measured by quantitative ultrasound. Quantitative ultrasound indices can evaluate BMD, microarchitecture, and mechanical parameters and are used to predict risk of osteoporosis and bone fracture (132). Decrements in BMD were also associated with serum PFOA, PFOS, and perfluorodecanoate during middle childhood (133) among children in the Boston area. In representative US cohorts, PFAS exposures are also associated with reduced BMD and increased risk of osteoporosis in women (134). Among overweight and obese individuals, higher PFOA, PFOS, and perfluorononanoic acid (PFNA) plasma levels were associated with lower BMD and increased rates of BMD decline during weight loss (11). A recent study (135) provides evidence of relative potency for PFAS disruption of bone homeostasis with perfluoroundecanoic acid (PFUnDA) > PFOA > PFNA > PFOS > perfluorohexane sulfonate (PFHxS).

Associations Between Perfluoroalkyl and Polyfluoroalkyl Substance Exposures and Adipose Tissue–Related Effects in Human Populations

A number of studies of human exposures have observed associations between some PFAS and increased risk of obesity or elevated body mass index (BMI). Maternal

PFOA and PFOS serum concentrations have been associated with increased obesity/overweight among 5-year-old Norwegian children (136). Associations between higher childhood BMI and PFOA exposure, but not with other PFAS, have been noted, with stronger effects in boys (137) in the Cincinnati, Ohio, area. PFOA, but not PFOS, has been associated with increased BMI and waist circumference among children aged 12 to 18 years participating in the US National Health and Nutritional Survey between 1999 and 2021 (138). Prenatal exposures to PFOA and PFHxS have been associated with increased central adiposity and risk of obesity/overweight among 12-year-old children whose mothers lived in an area with PFAS-contaminated drinking water (139). Children whose mothers' serum was tested for PFAS during the first trimester showed a higher incidence of obesity when they were evaluated during early childhood (140). A study of young Danish women exposed to PFOA during gestation found an increased risk of elevated BMI at age 20 (141). Children's serum concentrations were not associated with increased adiposity or body weight, indicating that prenatal exposures may be more significant (142). A clinical study examining the effects of energy restricted diets on weight changes revealed that participants (particularly women) with the highest plasma levels of PFAS had lower resting metabolic rates and regained weight more quickly after caloric restriction than did those with the lowest plasma PFAS levels (142). In utero exposures of rodents have also provided evidence supporting a critical window of exposure for increased body weight as well as a possible role for PFOA in increased adiposity later in life (143). Disruption of metabolic cytokines, including adiponectin, have been observed in PFOA-exposed women (144). For a recent review of chemical induction of obesity see Amato et al (145).

Concerns for Vulnerable Populations

Traditionally, developing fetuses, infants, and pregnant women are considered to be the most vulnerable populations when considering allowable exposures for human populations. PFAS exposures among these groups would certainly merit additional consideration, especially in light of the important developmental roles played by PPARs. For example, PPARγ has been shown to be important for placental and heart development (146) and helps regulate angiogenesis (147). If we are looking exclusively at bone outcomes, vulnerable populations might be those with commonly recognized risk factors for osteoporosis: females, older individuals, immobility, poor nutrition, etc, but also individuals with chronic kidney disease, whose BMD declines as the disease progresses (148, 149). Those with diabetes (150), taking glucocorticoids (151) or those with nutritional challenges, including gastric bypass surgery patients (152), and others predisposed to osteoporosis are vulnerable to PPARγ disruption.

A number of polymorphisms in PPARγ have been identified, and some of these have been explored for links with increased risk for diabetes or metabolic syndrome (153-156). Of particular interest from our present perspective are the reduced BMD (157) and increased risk of vertebral fracture in individuals heterozygous for 3 different polymorphisms (rs12497191, rs4135263, and rs1151999). Individuals with these polymorphisms had an increased risk of vertebral fractures (odds ratio = 1.48-1.76, $P = .005$-$.04$) compared to those homozygous for the most common allele (158). The most extensively studied PPARγ polymorphism, rs1801282, in which a proline has been replaced by an alanine at codon 12, exon B, is associated with a greater risk of diabetes. The rs1801282 polymorphism is most common in White individuals (155), with about 77% being homozygous for the most common alleles, 12%j to 20% heterozygous, and around 1% homozygous in a German population (158). This particular polymorphism is far less prevalent among people of Asian and African ancestry. Associations of other PPARγ polymorphisms with obesity and osteoporosis, as well as their functional significance, are uncertain to date.

Conclusions

PFAS exert toxic effects through interaction with nuclear receptors including the PPARs and may play a role in increased adipogenesis and decreased bone quality. There are other PPAR-related pathways, and many other means through which a particular PFAS may induce bioactivity in a manner that is detrimental to an organism. PFAS are found in thousands of forms and configurations, usually in mixtures, making traditional, single-chemical risk evaluations difficult. Chemicals targeting PPARs, and there is evidence of at least several that do, may lead to changes in cell differentiation and bone development that contribute to metabolic disorder and bone weakness. It is hoped that this discussion sheds light on the importance of some seemingly modest outcomes observed in test animals and describes why the most sensitive end points identified in some chemical risk assessments are significant from a public health perspective.

Acknowledgments

The views expressed in this manuscript are solely those of the authors and do not represent the policies of the US EPA. Mention of trade names of commercial products should not be interpreted as an endorsement by the US EPA.

Additional Information

Correspondence: Andrea Kirk, PhD, US EPA Headquarters, William Jefferson Clinton Bldg, 1200 Pennsylvania Ave NW, Mail Code 5201P, Washington, DC 20460, USA. Email: kirk.andrea@epa.gov.

Disclosure Statement: The authors have nothing to disclose.

Data Availability: Data sharing is not applicable to this article because no data sets were generated or analyzed.

References

1. Tang J, Jia X, Gao N, et al. Role of the Nrf2-ARE pathway in perfluorooctanoic acid (PFOA)-induced hepatotoxicity in Rana nigromaculata. *Environ Pollut*. 2018;**238**:1035-1043.
2. Bassler J, Ducatman A, Elliott M, et al. Environmental perfluoroalkyl acid exposures are associated with liver disease characterized by apoptosis and altered serum adipocytokines. *Environ Pollut*. 2019;**247**:1055-1063.
3. Wang J, Zeng XW, Bloom MS, et al. Renal function and isomers of perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS): Isomers of C8 Health Project in China. *Chemosphere*. 2019;**218**:1042-1049.
4. Koskela A, Finnilä MA, Korkalainen M, et al. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. *Toxicol Appl Pharmacol*. 2016;**301**:14-21.
5. Song P, Li D, Wang X, Zhong X. Effects of perfluorooctanoic acid exposure during pregnancy on the reproduction and development of male offspring mice. *Andrologia*. 2018;**50**(8):e13059.
6. Zhang H, Lu Y, Luo B, Yan S, Guo X, Dai J. Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery. *J Proteome Res*. 2014;**13**(7):3370-3385.
7. Frawley RP, Smith M, Cesta MF, et al. Immunotoxic and hepatotoxic effects of perfluoro-n-decanoic acid (PFDA) on female Harlan Sprague-Dawley rats and B6C3F1/N mice when administered by oral gavage for 28 days. *J Immunotoxicol*. 2018;**15**(1):41-52.
8. Graber JM, Alexander C, Laumbach RJ, et al. Per and polyfluoroalkyl substances (PFAS) blood levels after contamination of a community water supply and comparison with 2013-2014 NHANES. *J Expo Sci Environ Epidemiol*. 2019;**29**(2):172-182.
9. Lau C, Thibodeaux JR, Hanson RG, et al. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol Sci*. 2006;**90**(2):510-518.
10. Xiao F. Emerging poly- and perfluoroalkyl substances in the aquatic environment: a review of current literature. *Water Res*. 2017;**124**:482-495.
11. Hu XC, Tokranov AK, Liddie J, et al. Tap water contributions to plasma concentrations of poly- and perfluoroalkyl substances (PFAS) in a nationwide prospective cohort of U.S. women. *Environ Health Perspect*. 2019;**127**(6):67006.
12. Liu X, Guo Z, Folk EE IV, Roache NF. Determination of fluorotelomer alcohols in selected consumer products and preliminary investigation of their fate in the indoor environment. *Chemosphere*. 2015;**129**:81-86.
13. Social Science Environmental Health Research Institute 2021. PFAS contamination site tracker. Accessed June 4, 2021. https://www.northeastern.edu/environmentalhealth/mapping-the-expanding-pfas-crisis/
14. Guelfo JL, Adamson DT. Evaluation of a national data set for insights into sources, composition, and concentrations of per- and polyfluoroalkyl substances (PFASs) in U.S. drinking water. *Environ Pollut*. 2018;**236**:505-513.
15. Shoemaker J, Tettenhorst D. *Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)*. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment; 2018.
16. Shafique U, Schulze S, Slawik C, Böhme A, Paschke A, Schüürmann G. Perfluoroalkyl acids in aqueous samples from Germany and Kenya. *Environ Sci Pollut Res Int*. 2017;**24**(12):11031-11043.
17. Chirikona F, Filipovic M, Ooko S, Orata F. Perfluoroalkyl acids in selected wastewater treatment plants and their discharge load within the Lake Victoria basin in Kenya. *Environ Monit Assess*. 2015;**187**(5):238.
18. Duong HT, Kadokami K, Shirasaka H, et al. Occurrence of perfluoroalkyl acids in environmental waters in Vietnam. *Chemosphere*. 2015;**122**:115-124.
19. Hepburn E, Madden C, Szabo D, Coggan TL, Clarke B, Currell M. Contamination of groundwater with per- and polyfluoroalkyl substances (PFAS) from legacy landfills in an urban re-development precinct. *Environ Pollut*. 2019;**248**:101-113.
20. Nascimento RA, Nunoo DBO, Bizkarguenaga E, et al. Sulfluramid use in Brazilian agriculture: a source of per- and polyfluoroalkyl substances (PFASs) to the environment. *Environ Pollut*. 2018;**242**(Pt B):1436-1443.
21. Butt CM, Berger U, Bossi R, Tomy GT. Levels and trends of poly- and perfluorinated compounds in the Arctic environment. *Sci Total Environ*. 2010;**408**(15):2936-2965.
22. US Environmental Protection Agency. Lifetime health advisories and health effects support documents for perfluorooctanoic acid and perfluorooctane sulfanate. *Fed Regist*. 2016;**81**(101):33251.
23. Bălan SA, Mathrani VC, Guo DF, Algazi AM. Regulating PFAS as a chemical class under the California Safer Consumer Products Program. *Environ Health Perspect*. 2021;**129**(2):25001.
24. Pérez F, Nadal M, Navarro-Ortega A, et al. Accumulation of perfluoroalkyl substances in human tissues. *Environ Int*. 2013;**59**:354-362.
25. Williams AJ, Grulke CM, Edwards J, et al. The CompTox chemistry dashboard: a community data resource for environmental chemistry. *J Cheminform*. 2017;**9**(1):61.
26. Howard GJ, Webster TF. Generalized concentration addition: a method for examining mixtures containing partial agonists. *J Theor Biol*. 2009;**259**(3):469-477.
27. Watt J, Webster TF, Schlezinger JJ. Generalized concentration addition modeling predicts mixture effects of environmental PPARγ agonists. *Toxicol Sci*. 2016;**153**(1):18-27.
28. Ankley GT, Bennett RS, Erickson RJ, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem*. 2010;**29**(3):730-741.

29. Carusi A, Davies MR, De Grandis G, et al. Harvesting the promise of AOPs: an assessment and recommendations. *Sci Total Environ*. 2018;**628-629**:1542-1556.
30. Kirk AB. Environmental perchlorate: why it matters. *Anal Chim Acta*. 2006;**567**(1):4-12.
31. Vanden Heuvel JP, Thompson JT, Frame SR, Gillies PJ. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. *Toxicol Sci*. 2006;**92**(2):476-489.
32. Bjork JA, Butenhoff JL, Wallace KB. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. *Toxicology*. 2011;**288**(1-3):8-17.
33. Zhang YM, Dong XY, Fan LJ, et al. Poly- and perfluorinated compounds activate human pregnane X receptor. *Toxicology*. 2017;**380**:23-29.
34. Takacs ML, Abbott BD. Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/delta, gamma) by perfluorooctanoic acid and perfluorooctane sulfonate. *Toxicol Sci*. 2007;**95**(1):108-117.
35. Gore AC, Chappell VA, Fenton SE, et al. EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocr Rev*. 2015;**36**(6):E1-E150.
36. Agency for Toxic Substances and Disease Registry. 2021. Toxicological profile for perfluoroalkyls. Accessed August 30, 2021. https://www.atsdr.cdc.gov/ToxProfiles/tp200.pdf
37. Abbott BD. Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. *Reprod Toxicol*. 2009;**27**(3-4):246-257.
38. Poulsen Ll, Siersbæk M, Mandrup S. PPARs: fatty acid sensors controlling metabolism. *Semin Cell Dev Biol*. 2012;**23**(6):631-639.
39. Schopfer FJ, Cole MP, Groeger AL, et al. Covalent peroxisome proliferator-activated receptor gamma adduction by nitro-fatty acids: selective ligand activity and anti-diabetic signaling actions. *J Biol Chem*. 2010;**285**(16):12321-12333.
40. Ammazzalorso A, Maccallini C, Amoia P, Amoroso R. Multitarget PPARγ agonists as innovative modulators of the metabolic syndrome. *Eur J Med Chem*. 2019;**173**:261-273.
41. Wang W, Wong CW. Statins enhance peroxisome proliferator-activated receptor gamma coactivator-1alpha activity to regulate energy metabolism. *J Mol Med (Berl)*. 2010;**88**(3):309-317.
42. Braissant O, Foufelle F, Scotto C, Dauça M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology*. 1996;**137**(1):354-366.
43. More VR, Campos CR, Evans RA, et al. PPAR-α, a lipid-sensing transcription factor, regulates blood-brain barrier efflux transporter expression. *J Cereb Blood Flow Metab*. 2017;**37**(4):1199-1212.
44. Girroir EE, Hollingshead HE, He P, Zhu B, Perdew GH, Peters JM. Quantitative expression patterns of peroxisome proliferator-activated receptor-beta/delta (PPARbeta/delta) protein in mice. *Biochem Biophys Res Commun*. 2008;**371**(3):456-461.
45. Mothe-Satney I, Murdaca J, Sibille B, et al. A role for peroxisome proliferator-activated receptor beta in T cell development. *Sci Rep*. 2016;**6**:34317.
46. Chen J, Montagner A, Tan NS, Wahli W. Insights into the role of PPARβ/δ in NAFLD. *Int J Mol Sci*. 2018;**19**(7):1893.
47. Liu Y, Deguchi Y, Tian R, et al. Pleiotropic effects of PPARD accelerate colorectal tumorigenesis, progression, and invasion. *Cancer Res*. 2019;**79**(5):954-969.
48. Li Y, Jin D, Xie W, et al. PPAR-γ and Wnt regulate the differentiation of MSCs into adipocytes and osteoblasts respectively. *Curr Stem Cell Res Ther*. 2018;**13**(3):185-192.
49. Bourguet W, Germain P, Gronemeyer H. Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. *Trends Pharmacol Sci*. 2000;**21**(10):381-388.
50. Liu LL, Xian H, Cao JC, et al. Peroxisome proliferator-activated receptor gamma signaling in human sperm physiology. *Asian J Androl*. 2015;**17**(6):942-947.
51. Santoro M, De Amicis F, Aquila S, Bonofiglio D. Peroxisome proliferator-activated receptor gamma expression along the male genital system and its role in male fertility. *Hum Reprod*. 2020;**35**(9):2072-2085.
52. Luo H, Zhou Y, Hu X, et al. Activation of PPARγ2 by PPARγ1 through a functional PPRE in transdifferentiation of myoblasts to adipocytes induced by EPA. *Cell Cycle*. 2015;**14**(12):1830-1841.
53. Liu S, Yang R, Yin N, Wang YL, Faiola F. Environmental and human relevant PFOS and PFOA doses alter human mesenchymal stem cell self-renewal, adipogenesis and osteogenesis. *Ecotoxicol Environ Saf*. 2019;**169**:564-572.
54. Rosen ED, Hsu CH, Wang X, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes Dev*. 2002;**16**(1):22-26.
55. Tontonoz P, Spiegelman BM. Fat and beyond: the diverse biology of PPARgamma. *Annu Rev Biochem*. 2008;**77**:289-312.
56. Akune T, Ohba S, Kamekura S, et al. PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. *J Clin Invest*. 2004;**113**(6):846-855.
57. Monemdjou R, Vasheghani F, Fahmi H, et al. Association of cartilage-specific deletion of peroxisome proliferator-activated receptor γ with abnormal endochondral ossification and impaired cartilage growth and development in a murine model. *Arthritis Rheum*. 2012;**64**(5):1551-1561.
58. Wan Y, Chong LW, Evans RM. PPAR-gamma regulates osteoclastogenesis in mice. *Nat Med*. 2007;**13**(12):1496-1503.
59. Ali AA, Weinstein RS, Stewart SA, Parfitt AM, Manolagas SC, Jilka RL. Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation. *Endocrinology*. 2005;**146**(3):1226-1235.
60. Gimble JM, Robinson CE, Wu X, et al. Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol*. 1996;**50**(5):1087-1094.
61. Li CH, Ren XM, Guo LH. Adipogenic activity of oligomeric hexafluoropropylene oxide (perfluorooctanoic acid alternative) through peroxisome proliferator-activated receptor γ pathway. *Environ Sci Technol*. 2019;**53**(6):3287-3295.
62. Komori T, Yagi H, Nomura S, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell*. 1997;**89**(5):755-764.
63. Shoucri BM, Martinez ES, Abreo TJ, et al. Retinoid X receptor activation alters the chromatin landscape to commit

mesenchymal stem cells to the adipose lineage. *Endocrinology*. 2017;**158**(10):3109-3125.
64. Lecka-Czernik B, Moerman EJ, Grant DF, Lehmann JM, Manolagas SC, Jilka RL. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology*. 2002;**143**(6):2376-2384.
65. Kirchner S, Kieu T, Chow C, Casey S, Blumberg B. Prenatal exposure to the environmental obesogen tributyltin predisposes multipotent stem cells to become adipocytes. *Mol Endocrinol*. 2010;**24**(3):526-539.
66. Bruedigam C, Eijken M, Koedam M, et al. A new concept underlying stem cell lineage skewing that explains the detrimental effects of thiazolidinediones on bone. *Stem Cells*. 2010;**28**(5):916-927.
67. Ahmadian M, Suh JM, Hah N, et al. PPARγ signaling and metabolism: the good, the bad and the future. *Nat Med*. 2013;**19**(5):557-566.
68. Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol*. 2002;**2**(10):748-759.
69. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature*. 1998;**391**(6662):79-82.
70. Zhu J, Garrett R, Jung Y, et al. Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells. *Blood*. 2007;**109**(9):3706-3712.
71. Kfoury Y, Scadden DT. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell*. 2015;**16**(3):239-253.
72. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L, Padula SJ. The nuclear receptor PPAR gamma and immunoregulation: PPAR gamma mediates inhibition of helper T cell responses. *J Immunol*. 2000;**164**(3):1364-1371.
73. Shigenobu T, Ohtsuka T, Shimoda M. The prevention of tracheal graft occlusion using pioglitazone: a mouse tracheal transplant model study. *Transpl Immunol*. 2019;**53**:21-27.
74. Nor Effa SZ, Yaacob NS, Mohd Nor N. Crosstalk between PPARγ ligands and inflammatory-related pathways in natural T-regulatory cells from type 1 diabetes mouse model. *Biomolecules*. 2018;**8**(4):135.
75. Kanayama T, Kobayashi N, Mamiya S, Nakanishi T, Nishikawa J. Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor gamma/retinoid X receptor pathway. *Mol Pharmacol*. 2005;**67**(3):766-774.
76. Grün F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. *Endocrinology*. 2006;**147**(6 Suppl):S50-S55.
77. Watt J, Schlezinger JJ. Structurally-diverse, PPARγ-activating environmental toxicants induce adipogenesis and suppress osteogenesis in bone marrow mesenchymal stromal cells. *Toxicology*. 2015;**331**:66-77.
78. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci U S A*. 1997;**94**(9):4312-4317.
79. Li X, Ycaza J, Blumberg B. The environmental obesogen tributyltin chloride acts via peroxisome proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1 preadipocytes. *J Steroid Biochem Mol Biol*. 2011;**127**(1-2):9-15.
80. Salimi A, Nikoosiar Jahromi M, Pourahmad J. Maternal exposure causes mitochondrial dysfunction in brain, liver, and heart of mouse fetus: an explanation for perfluorooctanoic acid induced abortion and developmental toxicity. *Environ Toxicol*. 2019;**34**(7):878-885.
81. Shankar A, Xiao J, Ducatman A. Perfluorooctanoic acid and cardiovascular disease in US adults. *Arch Intern Med*. 2012;**172**(18):1397-1403.
82. Winquist A, Steenland K. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. *Environ Health Perspect*. 2014;**122**(12):1299-1305.
83. Willson TM, Lambert MH, Kliewer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu Rev Biochem*. 2001;**70**:341-367.
84. Corona JC, Duchen MR. PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. *Free Radic Biol Med*. 2016;**100**:153-163.
85. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem*. 1995;**270**(22):12953-12956.
86. Khazaee M, Christie E, Cheng W, Michalsen M, Field J, Ng C. Perfluoroalkyl acid binding with peroxisome proliferator-activated receptors α, γ, and δ, and fatty acid binding proteins by equilibrium dialysis with a comparison of methods. *Toxics*. 2021;**9**(3):45.
87. Colhoun HM, Livingstone SJ, Looker HC, et al; Scottish Diabetes Research Network Epidemiology Group. Hospitalised hip fracture risk with rosiglitazone and pioglitazone use compared with other glucose-lowering drugs. *Diabetologia*. 2012;**55**(11):2929-2937.
88. Billington EO, Grey A, Bolland MJ. The effect of thiazolidinediones on bone mineral density and bone turnover: systematic review and meta-analysis. *Diabetologia*. 2015;**58**(10):2238-2246.
89. Watkins SM, Reifsnyder PR, Pan HJ, German JB, Leiter EH. Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. *J Lipid Res*. 2002;**43**(11):1809-1817.
90. Füchtenbusch M, Standl E, Schatz H. Clinical efficacy of new thiazolidinediones and glinides in the treatment of type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2000;**108**(3):151-163.
91. Deeg MA, Tan MH. Pioglitazone versus rosiglitazone: effects on lipids, lipoproteins, and apolipoproteins in head-to-head randomized clinical studies. *PPAR Res*. 2008;**2008**:520465.
92. Pop LM, Lingvay I, Yuan Q, Li X, Adams-Huet B, Maalouf NM. Impact of pioglitazone on bone mineral density and bone marrow fat content. *Osteoporos Int*. 2017;**28**(11):3261-3269.
93. Paccou J, Penel G, Chauveau C, Cortet B, Hardouin P. Marrow adiposity and bone: review of clinical implications. *Bone*. 2019;**118**:8-15.
94. Muruganandan S, Govindarajan R, Sinal CJ. Bone marrow adipose tissue and skeletal health. *Curr Osteoporos Rep*. 2018;**16**(4):434-442.
95. Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol*. 2006;**2**(1):35-43.
96. Broulík PD, Sefc L, Haluzík M. Effect of PPAR-γ agonist rosiglitazone on bone mineral density and serum adipokines in C57BL/6 male mice. *Folia Biol (Praha)*. 2011;**57**(4):133-138.

97. Lazarenko OP, Rzonca SO, Hogue WR, Swain FL, Suva LJ, Lecka-Czernik B. Rosiglitazone induces decreases in bone mass and strength that are reminiscent of aged bone. *Endocrinology*. 2007;**148**(6):2669-2680.
98. Park HJ, Park HS, Lee JU, Bothwell AL, Choi JM. Sex-based selectivity of PPARγ regulation in Th1, Th2, and Th17 differentiation. *Int J Mol Sci*. 2016;**17**(8):1347.
99. Thompson PW, Bayliffe AI, Warren AP, Lamb JR. Interleukin-10 is upregulated by nanomolar rosiglitazone treatment of mature dendritic cells and human CD4+ T cells. *Cytokine*. 2007;**39**(3):184-191.
100. Beggs KM, McGreal SR, McCarthy A, et al. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. *Toxicol Appl Pharmacol*. 2016;304:18-29.
101. Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. *Toxicology*. 2017;**387**:95-107.
102. Di Nisio A, Rocca MS, De Toni L, et al. Endocrine disruption of vitamin D activity by perfluoro-octanoic acid (PFOA). *Sci Rep*. 2020;10(1):16789.
103. Behr AC, Plinsch C, Braeuning A, Buhrke T. Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). *Toxicol In Vitro*. 2020;62:104700.
104. Stunes AK, Westbroek I, Gustafsson BI, et al. The peroxisome proliferator-activated receptor (PPAR) alpha agonist fenofibrate maintains bone mass, while the PPAR gamma agonist pioglitazone exaggerates bone loss, in ovariectomized rats. *BMC Endocr Disord*. 2011;**11**:11.
105. Gong K, Qu B, Wang C, et al. Peroxisome proliferator-activated receptor α facilitates osteogenic differentiation in MC3T3-E1 cells via the sirtuin 1-dependent signaling pathway. *Mol Cells*. 2017;**40**(6):393-400.
106. Kalliora C, Kyriazis ID, Oka SI, et al. Dual peroxisome-proliferator-activated-receptor-α/γ activation inhibits SIRT1-PGC1α axis and causes cardiac dysfunction. *JCI Insight*. 2019;**5**(17):e129556.
107. Rubenstrunk A, Hanf R, Hum DW, Fruchart JC, Staels B. Safety issues and prospects for future generations of PPAR modulators. *Biochim Biophys Acta*. 2007;**1771**(8):1065-1081.
108. Chamorro-García R, Sahu M, Abbey RJ, Laude J, Pham N, Blumberg B. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. *Environ Health Perspect*. 2013;**121**(3):359-366.
109. Temporini C, Brusotti G, Pochetti G, Massolini G, Calleri E. Affinity-based separation methods for the study of biological interactions: the case of peroxisome proliferator-activated receptors in drug discovery. *Methods*. 2018;**146**:12-25.
110. Riu A, le Maire A, Grimaldi M, et al. Characterization of novel ligands of ERα, Erβ, and PPARγ: the case of halogenated bisphenol A and their conjugated metabolites. *Toxicol Sci*. 2011;**122**(2):372-382.
111. Yu C, Chen L, Luo H, et al. Binding analyses between human PPARgamma-LBD and ligands. *Eur J Biochem*. 2004;**271**(2):386-397.
112. Riu A, Grimaldi M, le Maire A, et al. Peroxisome proliferator-activated receptor γ is a target for halogenated analogs of bisphenol A. *Environ Health Perspect*. 2011;**119**(9):1227-1232.
113. Corrales P, Izquierdo-Lahuerta A, Medina-Gómez G. Maintenance of kidney metabolic homeostasis by PPAR gamma. *Int J Mol Sci*. 2018;**19**(7):2063.
114. Nobs SP, Kopf M. PPAR-γ in innate and adaptive lung immunity. *J Leukoc Biol*. 2018;**104**(4):737-741.
115. Yao PL, Peavey J, Malek G. Leveraging nuclear receptors as targets for pathological ocular vascular diseases. *Int J Mol Sci*. 2020;**21**(8):2889.
116. Saez E, Rosenfeld J, Livolsi A, et al. PPAR gamma signaling exacerbates mammary gland tumor development. *Genes Dev*. 2004;**18**(5):528-540.
117. Fauconnet S, Lascombe I, Chabannes E, et al. Differential regulation of vascular endothelial growth factor expression by peroxisome proliferator-activated receptors in bladder cancer cells. *J Biol Chem*. 2002;**277**(26):23534-23543.
118. Forootan FS, Forootan SS, Gou X, et al. Fatty acid activated PPARγ promotes tumorigenicity of prostate cancer cells by up regulating VEGF via PPAR responsive elements of the promoter. *Oncotarget*. 2016;**7**(8):9322-9339.
119. Loviscach M, Rehman N, Carter L, et al. Distribution of peroxisome proliferator-activated receptors (PPARs) in human skeletal muscle and adipose tissue: relation to insulin action. *Diabetologia*. 2000;**43**(3):304-311.
120. Van Nguyen TT, Vu VV, Pham PV. Transcriptional factors of thermogenic adipocyte development and generation of brown and beige adipocytes from stem cells. *Stem Cell Rev Rep*. 2020;**16**(5):876-892.
121. White SS, Calafat AM, Kuklenyik Z, et al. Gestational PFOA exposure of mice is associated with altered mammary gland development in dams and female offspring. *Toxicol Sci*. 2007;**96**(1):133-144.
122. Skat-Rørdam J, Højland Ipsen D, Lykkesfeldt J, Tveden-Nyborg P. A role of peroxisome proliferator-activated receptor γ in non-alcoholic fatty liver disease. *Basic Clin Pharmacol Toxicol*. 2019;**124**(5):528-537.
123. Tucker DK, Macon MB, Strynar MJ, Dagnino S, Andersen E, Fenton SE. The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. *Reprod Toxicol*. 2015;**54**:26-36.
124. Romano ME, Xu Y, Calafat AM, et al. Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. *Environ Res*. 2016;**149**:239-246.
125. Shipley JM, Waxman DJ. Simultaneous, bidirectional inhibitory crosstalk between PPAR and STAT5b. *Toxicol Appl Pharmacol*. 2004;**199**(3):275-284.
126. Shipley JM, Waxman DJ. Down-regulation of STAT5b transcriptional activity by ligand-activated peroxisome proliferator-activated receptor (PPAR) alpha and PPARgamma. *Mol Pharmacol*. 2003;**64**(2):355-364.
127. Barash I. Stat5 in the mammary gland: controlling normal development and cancer. *J Cell Physiol*. 2006;**209**(2):305-313.
128. Hwa V. STAT5B deficiency: impacts on human growth and immunity. *Growth Horm IGF Res*. 2016;**28**:16-20.
129. Furigo IC, Ramos-Lobo AM, Frazão R, Donato J Jr. Brain STAT5 signaling and behavioral control. *Mol Cell Endocrinol*. 2016;**438**:70-76.
130. Tarapore P, Ouyang B. Perfluoroalkyl chemicals and male reproductive health: do PFOA and PFOS increase risk

for male infertility?. *Int J Environ Res Public Health*. 2021;**18**(7):3794.
131. World Health Organization. *Keeping Our Water Clean: the Case of Water Contamination in the Veneto Region, Italy*. World Health Organization; 2017.
132. Chin KY, Ima-Nirwana S. Calcaneal quantitative ultrasound as a determinant of bone health status: what properties of bone does it reflect? *Int J Med Sci*. 2013;**10**(12):1778-1783.
133. Cluett R, Seshasayee SM, Rokoff LB, et al. Per- and polyfluoroalkyl substance plasma concentrations and bone mineral density in midchildhood: a cross-sectional study (Project Viva, United States). *Environ Health Perspect*. 2019;**127**(8):87006.
134. Khalil N, Chen A, Lee M, et al. Association of perfluoroalkyl substances, bone mineral density, and osteoporosis in the U.S. Population in NHANES 2009-2010. *Environ Health Perspect*. 2016;**124**(1):81-87.
135. Banjabi AA, Li AJ, Kumosani TA, Yousef JM, Kannan K. Serum concentrations of perfluoroalkyl substances and their association with osteoporosis in a population in Jeddah, Saudi Arabia. *Environ Res*. 2020;**187**:109676.
136. Lauritzen HB, Larose TL, Øien T, et al. Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. *Environ Health*. 2018;**17**(1):9.
137. Braun JM, Eliot M, Papandonatos GD, et al. Gestational perfluoroalkyl substance exposure and body mass index trajectories over the first 12 years of life. *Int J Obes (Lond)*. 2021;**45**(1):25-35.
138. Geiger SD, Yao P, Vaughn MG, Qian Z. PFAS exposure and overweight/obesity among children in a nationally representative sample. *Chemosphere*. 2021;**268**:128852.
139. Liu Y, Li N, Papandonatos GD, et al. Exposure to per- and polyfluoroalkyl substances and adiposity at age 12 years: evaluating periods of susceptibility. *Environ Sci Technol*. 2020;**54**(24):16039-16049.
140. Bloom MS, Commodore S, Ferguson PL, et al. Association between gestational PFAS exposure and children's adiposity in a diverse population. *Environ Res*. 2021;**203**:111820.
141. Halldorsson TI, Rytter D, Haug LS, et al. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. *Environ Health Perspect*. 2012;**120**(5):668-673.
142. Liu G, Dhana K, Furtado JD, et al. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: a prospective study. *PLoS Med*. 2018;**15**(2):e1002502.
143. Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE. Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated serum leptin and insulin, and overweight in mid-life. *Mol Cell Endocrinol*. 2009;**304**(1-2):97-105.
144. Ding N, Karvonen-Gutierrez CA, Herman WH, Calafat AM, Mukherjee B, Park SK. Associations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS mixtures with in midlife women. *Int J Hyg Environ Health*. 2021;**235**:113777.
145. Amato AA, Wheeler HB, Blumberg B. Obesity and endocrine-disrupting chemicals. *Endocr Connect*. 2021;**10**(2):R87-R105.
146. Barak Y, Nelson MC, Ong ES, et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell*. 1999;**4**(4):585-595.
147. Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. *J Biol Chem*. 1999;**274**(13):9116-9121.
148. Bezerra de Carvalho KS, Vasco RFV, Custodio MR, Jorgetti V, Moysés RMA, Elias RM. Chronic kidney disease is associated with low BMD at the hip but not at the spine. *Osteoporos Int*. 2019;**30**(5):1015-1023.
149. Malmgren L, McGuigan F, Christensson A, Akesson KE. Reduced kidney function is associated with BMD, bone loss and markers of mineral homeostasis in older women: a 10-year longitudinal study. *Osteoporos Int*. 2017;**28**(12):3463-3473.
150. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int*. 2007;**18**(4):427-444.
151. Davidge Pitts CJ, Kearns AE. Update on medications with adverse skeletal effects. *Mayo Clin Proc*. 2011;**86**(4):338-343; quiz 343.
152. Blom-Høgestøl IK, Hewitt S, Chahal-Kummen M, et al. Bone metabolism, bone mineral density and low-energy fractures 10 years after Roux-en-Y gastric bypass. *Bone*. 2019;**127**:436-445.
153. Lv X, Zhang L, Sun J, et al. Interaction between peroxisome proliferator-activated receptor gamma polymorphism and obesity on type 2 diabetes in a Chinese Han population. *Diabetol Metab Syndr*. 2017;**9**:7.
154. Meirhaeghe A, Cottel D, Amouyel P, Dallongeville J. Association between peroxisome proliferator-activated receptor gamma haplotypes and the metabolic syndrome in French men and women. *Diabetes*. 2005;**54**(10):3043-3048.
155. Black MH, Wu J, Takayanagi M, et al. Variation in PPARG is associated with longitudinal change in insulin resistance in Mexican Americans at risk for type 2 diabetes. *J Clin Endocrinol Metab*. 2015;**100**(3):1187-1195.
156. Ogawa S, Urano T, Hosoi T, et al. Association of bone mineral density with a polymorphism of the peroxisome proliferator-activated receptor gamma gene: PPARgamma expression in osteoblasts. *Biochem Biophys Res Commun*. 1999;**260**(1):122-126.
157. Harsløf T, Tofteng CL, Husted LB, et al. Polymorphisms of the peroxisome proliferator-activated receptor γ (PPARγ) gene are associated with osteoporosis. *Osteoporos Int*. 2011;**22**(10):2655-2666.
158. Schäffler A, Barth N, Schmitz G, Zietz B, Palitzsch KD, Schölmerich J. Frequency and significance of Pro12Ala and Pro115Gln polymorphism in gene for peroxisome proliferation-activated receptor-gamma regarding metabolic parameters in a Caucasian cohort. *Endocrine*. 2001;**14**(3):369-373.