Toxicology Reports 3 (2016) 46–54

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice

Sandra L. Rebholz[a], Thomas Jones[a], Robert L. Herrick[b], Changchun Xie[b], Antonia M. Calafat[c], Susan M. Pinney[b], Laura A. Woollett[a,*]

[a] Department of Pathology and Laboratory Medicine, University of Cincinnati, College of Medicine, Cincinnati, OH, United States
[b] Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH, United States
[c] Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

ARTICLE INFO

Article history:
Received 21 September 2015
Received in revised form
10 November 2015
Accepted 10 November 2015
Available online 18 November 2015

Keywords:
Perfluorooctanoic acid
C8
PFC
PFAS
Cholesterol
Dietary fat

ABSTRACT

Perfluorooctanoic acid (PFOA) is a man-made surfactant with a number of industrial applications. It has a long half-life environmentally and biologically. Past studies suggest a direct relationship between plasma cholesterol and PFOA serum concentrations in humans and an inverse one in rodents fed standard rodent chow, making it difficult to examine mechanisms responsible for the potential PFOA-induced hypercholesterolemia and altered sterol metabolism. To examine dietary modification of PFOA-induced effects, C57BL/6 and BALB/c mice were fed PFOA in a fat- and cholesterol-containing diet. When fed these high fat diets, PFOA ingestion resulted in marked hypercholesterolemia in male and female C57BL/6 mice and less robust hypercholesterolemia in male BALB/c mice. The PFOA-induced hypercholesterolemia appeared to be the result of increased liver masses and altered expression of genes associated with hepatic sterol output, specifically bile acid production. mRNA levels of genes associated with sterol input were reduced only in C57BL/6 females, the mice with the greatest increase in plasma cholesterol levels. Strain-specific PFOA-induced changes in cholesterol concentrations in mammary tissues and ovaries paralleled changes in plasma cholesterol levels. mRNA levels of sterol-related genes were reduced in ovaries of C57BL/6 but not in BALB/c mice and not in mammary tissues. Our data suggest that PFOA ingestion leads to hypercholesterolemia in mice fed fat and cholesterol and effects are dependent upon the genetic background and gender of the mice with C57BL/6 female mice being most responsive to PFOA.

1. Introduction

Perfluorooctanoic acid (PFOA) is a saturated eight carbon chain organic acid, perfluoroalkyl substance (PFAS) with all hydrogen replaced by fluorine. PFOA is primarily used to produce other PFASs for stain- and stick-resistant coatings (Teflon), water-resistant coatings (Gor-Tex), and food contact paper [29,4,42]. Because of their widespread use over the past several decades and relatively long half-lives [45] some of these PFASs, including PFOA, are prevalent throughout the world from populated, industrialized locations to remote locations [3,11]. Consequently, PFOA exposure is widespread throughout the US general population [7,39]. Though essentially everyone has measurable levels of PFOA due to their detection in almost every ecosystem worldwide, serum levels are somewhat greater in areas near facilities that use PFOA [13,51] and considerably higher in perfluoroalkyl production workers [9,43,44,46,47,56,57,66]. Though PFOA production in the US has decreased markedly in the past several years, along with serum concentrations in people in the US (NHANES), and should cease in 2015 (EPA Stewardship Plan), PFOA remains a human health concern due to continuing production and use worldwide, relatively long half-lives in humans [45], persistence in the environment, and degradation of other PFAAs into PFOA [4].

Controversy exists over whether PFOA is hypo- or hypercholesteremic. Human gene expression data from different studies suggest a hypercholesteremic hypothesis. PFOA upregulated cholesterol biosynthesis genes in human hepatocytes [5,50]. In a study of population co-exposed to PFOA and perfluorooctanoate sulfonate, expression of genes involved in reverse cholesterol transport expression, a cause of hypercholesterolemia, were reduced with PFOA exposure [17]. In addition, PFOA exposure has been significantly associated with an increased risk of clinical hypercholesterolemia [60,18] and increased plasma cholesterol concentrations in national

* Corresponding author at: Department of Pathology and Laboratory Medicine, 2180 E. Galbraith Rd, Reading Campus, University of Cincinnati Medical Center, Cincinnati, OH 45237-0507, United States. Fax: +1 513 558 1312.
E-mail address: laura.woollett@uc.edu (L.A. Woollett).

http://dx.doi.org/10.1016/j.toxrep.2015.11.004

population studies [39,14,20,75], in communities near perfluoroalkyl production facilities [60,18,16,19] and in perfluoroalkyl workers [9,26,56,57]. While most studies have been cross-sectional, significant associations between PFOA and cholesterol have been found in exposed communities [16,71] and occupational cohorts as well [9,56,57] though increases attributable to PFOA are small compared to other hypercholesteremic factors [44]. In many studies, effect estimates, and resultant cholesterol increases, are strongest at serum PFOA concentrations below 10 ng/mL (corresponding to 95% of US population exposure) although they remain significant for all concentrations, with the inflection point ≈25–40 ng/mL [60,18]. Regardless, though direction, magnitude and significance of the PFOA-cholesterol association vary across studies, especially for HDL and LDL, human experimental and epidemiological data suggest PFOA is indeed hypercholesterolemic.

A change in plasma cholesterol levels is often an indication of a change in hepatic sterol balance [59] and can lead to altered sterol metabolism in a variety of extra-hepatic tissues, including steroidogenic tissues. Hepatic sterol balance plays a key role in maintaining sterol balance in the whole body [59]. A positive sterol balance would occur with increased dietary cholesterol and will often lead to a reduction in LDL receptor (LDLR) expression levels which will in turn lead to fewer LDL particles being taken up by the liver and an increase in plasma LDL-cholesterol levels [12]. As most LDL is taken up by the liver [72], a reduction in uptake would lead to an increase in plasma levels. A change in sterol balance can also occur if sterol synthesis or bile acid production rates are altered. As the peripheral tissues take up a majority of LDL-cholesterol via receptor-independent processes or via receptors that are independent of tissue sterol balance, an increase in circulating lipoprotein-cholesterol would lead to an increase in uptake by peripheral tissues. An increase in tissue cholesterol could directly affect tissues, such as ovaries that synthesize estradiol and indirectly affect tissues that rely on the steroid products produced by the other tissues, including mammary tissues that rely on estradiol.

In studies with rodents, PFOA-treated animals often become hypocholesterolemic, opposite of that which occurs in humans [2,22,28,35–37,52,73]. In most of these previous studies, rodents consumed a standard chow diet containing 4% fat and very little cholesterol. It has been proposed that the effect occurs because PFOA will activate peroxisome proliferator-activated receptor α (PPARα) more readily in rodents than in humans, and PPARs can be hypolipidemic [24,2,38,54,68]. However, other studies suggest that some PFOA metabolic effects are PPARα-independent [55,27,54]. How animals respond to PFOA when sterol balance is altered with dietary factors and cholesterol, as occurs in humans, is unknown, though it appears that dietary fat can modify the effect of PFOA on other parameters [61].

Thus, the purpose of the current study was to determine the impact of dietary PFOA when fed in combination with a diet containing fat and cholesterol, more similar to that consumed by humans, using strains of mice that respond differently to PFOA with respect to sexual maturity [74] and to dietary factors [30,48], and have been shown previously to become hypocholeserolemic when exposed to PFOA [52,73]. The use of mice that become hypercholesteremic in response to PFOA and a high-fat diet would be very useful in assessing human PFOA response and addressing the contradictions between human and rodent data.

2. Materials and methods

2.1. Animals and diets

Male and female C57BL/6 and BALB/c mice were purchased from The Jackson Laboratories (Bar Harbor, ME) at weaning, randomly separated into 8 groups, and fed pelleted chow upon arrival (Harlan, Madison, WI); 8 groups consisted of 2 different sexes, two different strains of mice, and two different PFOA treatments. Mice were housed 3 per cage in a temperature and humidity controlled room and subjected to 12 h of light and 12 h of darkness. Four to five days after arrival, mice were weighed and distributed between cages to ensure similar initial body weights per treatment, and began consuming one of two diets (Research Diets, Inc.); we chose to study younger mice just prior to sexual maturity as PFOA has been described as an endocrine disruptor and could impact metabolism in endocrine tissues as they mature [1,74]. Both diets contained 0.25% cholesterol and 32% fat (kcal) with soybean oil as the primary oil. One diet was the control diet and contained no added PFOA. The other diet contained 3.5 mg PFOA (Sigma–Aldrich)/kg diet. We chose this amount of PFOA to add because we wanted mice to consume 0.5 mg PFOA/kg body weight and adult mice consume ≈3.5 g high fat diet [33]. Thus, a 22 g mouse would consume ≈12.3 μg PFOA per whole body per day or ≈0.56 mg PFOA per kg body weight per day. PFOA was added to the diet and not gavaged daily so that mice would be exposed to PFOA throughout the day and mimic human exposure to PFOA. Weekly individual body weights and food consumption of each cage were obtained to ensure adequate growth and to determine the dose of PFOA consumed each day per kg body weight, respectively; each group consisted of 2 cages of 3 mice each (6 male and 6 female mice of each strain per treatment). Animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

After 5 weeks, body fat mass was measured by MRI (Echo). The following week the mice were fasted 6 h, anesthetized, exsanguinated and tissues collected rapidly and stored as needed. Diethyl ether was used as an anesthesia (prior to the AVMA report in 2013) to ensure accurate PFOA measurements in plasma not confounded by interfering anesthetics. Additional care was taken so blood and plasma were collected and stored using syringes and tubes made of polypropylene. Plasma from each animal per group was pooled, stored frozen, and shipped to the Centers for Disease Control and Prevention (CDC) where it was analyzed for PFOA using online solid phase extraction coupled with reversed-phase high-performance liquid chromatography isotope-dilution tandem mass spectrometry as described [27]; samples were pooled in this initial study to ensure adequate volumes for all plasma measurements proposed. To ensure that the measured PFOA concentrations fell within the calibration range, necessary dilution of the plasma was performed. Blanks and quality control materials were analyzed along with the study samples to ensure the accuracy and reliability of the data [27].

2.2. Tissue and plasma steroid levels

Plasma cholesterol levels in each sample were measured enzymatically in duplicate (Thermo Electron). Plasma from each animal per group was pooled and separated into lipoproteins by fast-protein liquid chromatography (FPLC) using two Sephadex columns in tandem [31]. Pieces of liver (males and females) and mammary tissue and one ovary (females) were collected after exsanguination, saponfied in alcoholic KOH, sterol extracted, and mass of sterol measured by gas liquid chromatography using stigmastanol as an internal standard. Estradiol levels were measured in duplicate in plasma of some of the females (2–5 mice per group) by EIA (Cayman Chemical).

2.3. Real time PCR

Livers, ovaries, and pieces of mammary tissues were collected rapidly after exsanguination, snap frozen in liquid nitrogen, and stored at −80 °C until use. Tissue RNA was isolated using TRIzol® and stored in FORMAzol® at −80 °C. RNA was treated with RNase-