The effects of perfluorinated chemicals on adipocyte differentiation *in vitro*

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ABSTRACT

The 3T3-L1 preadipocyte culture system has been used to examine numerous compounds that influence adipocyte differentiation or function. The perfluoroalkyl acids (PFAAs), used as surfactants in a variety of industrial applications, are of concern as environmental contaminants that are detected worldwide in human serum and animal tissues. This study was designed to evaluate the potential for PFAAs to affect adipocyte differentiation and lipid accumulation using mouse 3T3-L1 cells. Cells were treated with perfluorooctanoic acid (PFOA) (5–100 µM), perfluorononanoic acid (PFNA) (5–100 µM), perfluorooctane sulfonate (PFOS) (50–300 µM), perfluorohexane sulfonate (PFHxS) (40–250 µM), the peroxisome proliferator activated receptor (PPAR) PPARα agonist Wyeth-14,643 (WY-14,643), and the PPARγ agonist rosiglitazone. The PPARγ agonist was included as a positive control as this pathway is critical to adipocyte differentiation. The PPARα agonist was included as the PFAA compounds are known activators of this pathway. Cells were assessed morphometrically and biochemically for number, size, and lipid content. RNA was extracted for qPCR analysis of 13 genes selected for their importance in adipocyte differentiation and lipid metabolism. There was a significant concentration-related increase in cell number and decreased cell size after exposure to PFOA, PFHxS, PFOS, and PFNA. All four PFAA treatments produced a concentration-related decrease in the calculated average area occupied by lipid per cell. However, total triglyceride levels per well increased with a concentration-related trend for all compounds, likely due to the increased cell number. Expression of mRNA for the selected genes was affected by all exposures and the specific impacts depended on the particular compound and concentration. Acox1 and Gapdh were upregulated by all six compounds. The strongest overall effect was a nearly 10-fold induction of Scd1 by PFHxS. The sulfonated PFAAs produced numerous, strong changes in gene expression similar to the effects after treatment with the PPARγ agonist rosiglitazone. By comparison, the effects on gene expression were muted for the carboxylated PFAAs and for the PPARα agonist WY-14,643. In summary, all perfluorinated compounds increased cell number, decreased cell size, increased total triglyceride, and altered expression of genes associated with adipocyte differentiation and lipid metabolism.

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1. Introduction

Perfluoroalkyl acids (PFAAs) are straight chain organofluorine chemicals that are used in commercial applications including paint additives, fire-fighting foams, surfactants, and water and stain repellants. Many PFAAs persist in the environment and numerous studies have demonstrated the environmental accumulation and toxicity of PFAAS (Lau et al., 2007; Lindstrom et al., 2011). The majority of work on PFAAs has been done on perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS), but data from the National Health and Nutrition Examination Survey (NHANES) indicate that in addition to PFOA and PFOS, perfluorononanoic acid (PFNA) and perfluorohexane sulfate (PFHxS) are routinely found in human serum (Kato et al., 2011).

Peroxisome proliferator activated receptors (PPARs) are a class of nuclear receptors with three subtypes, PPARα, PPARβ, and PPARγ, each with distinct expression and physiological roles (Escher and Wahli, 2000). The PPARγ pathway is a major regulator of adipocyte differentiation and lipid metabolism (Casals-Casas and Desvergne, 2011). The PPARα pathway plays a role in maintaining lipid homeostasis directly regulating genes involved in fatty acid uptake and

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