Glucose and Lipid Homeostasis in Adult Rat Is Impaired by Early-Life Exposure to Perfluorooctane Sulfonate

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ABSTRACT: Perfluorooctane sulfonate (PFOS), which belongs to the degradation product of many perfluorinated compounds, is on the list of persistent organic pollutants (POPs) and is currently detected in both wildlife and humans. The consequence of gestational and lactational exposure to PFOS on prediabetes effect in offspring was investigated in rats in the present study. Maternal rats were treated with vehicle, 0.5 mg/kg/day or 1.5 mg/kg/day PFOS respectively from gestation day 0 to postnatal day 21. The glucose and lipid metabolism effects were investigated on the offspring in adulthood. The gestational and lactational exposure to PFOS led to low body weight from birth to weaning, and evoked signs of a prediabetic state, with elevated fasting serum insulin and leptin level, impaired glucose tolerance, though the fasting serum glucose and glycosylated serum protein level were normal. Abnormal lipid homeostasis was also observed by the phenomenon of hepatic steatosis and increased gonadal fat pad weight. However, the circulating serum level of fasting triglyceride and cholesterol level were no different from controls. Our results suggested that developmental exposure to PFOS may contribute to glucose and lipid metabolic disorder in adulthood. © 2011 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2011.

Keywords: perfluorooctane sulfate; developmental exposure; diabetes; energy metabolism; low birth weight

INTRODUCTION

Type 2 diabetes mellitus, characterized by energy metabolic disorder, has become one of the most serious threats worldwide due to the substantial morbidity and mortality, as well as the great burden on financial cost. The hereditary factors, sedentary lifestyle and socioeconomic status have been well established as important contributors. However, there is increasing evidences suggesting that early life events have a significant impact on the origins of diabetes,
which gained far less interest and attention in comparison to those traditional risk factors. As one example, it is extensively observed in epidemiologic studies that inadequate maternal nutrition or relative low weight at birth and during infancy is associated with increased incidence of type 2 diabetes, adiposity, and metabolic syndrome in adulthood (Lithell et al., 1996; Rich-Edwards et al., 1999). Recent attention has turned to the maternal transfer of persistent organic pollutants (POPs) (Lee et al., 2007a; Wang et al., 2008; Sharp 2009) due to their bioaccumulations in human and animal tissues and potential impacts on human health. Studies published in recent years indicated that serum POPs levels are strongly associated with the prevalence of type 2 diabetes, as well as insulin resistance and adverse lipid profiles (Lee et al., 2006; Lee et al., 2007b). Several POPs can even increase diabetes risk at low doses similar to current human exposure levels (Lee et al., 2010).

Although most studies exploring the relationship between POPs and metabolic disorders focus on the exposure in adulthood, the relationships may be more important when exposure occurs during development. It is generally accepted that individuals are more sensitive to the effects of chemical exposure during vital developmental periods. If the adverse effects induced by adulthood exposure were documented, the outcome would be more serious when the exposure occurs during development. For instance, it has been confirmed in some animal studies that neonatal low-dose pesticide exposure disrupts energy homeostasis in a persistent manner (Lassiter and Brimijoin, 2008; Lassiter et al., 2008) or culminates in a metabolic pattern characteristic of prediabetes (Slotkin et al., 2005).

Perfluorooctane sulfonate (PFOS), which belongs to the degradation product of many perfluorinated compounds, is on the list of POPs. PFOS is currently used in a variety of commercial applications and has been detected in both wildlife and human beings (Calafat et al., 2007; Lau et al., 2007). The potential risk for PFOS exposure is emphasized by the fact that the developing fetus and infants are at high risk for PFOS exposure, as evidenced by the detection of PFOS in both umbilical cord blood and breast milk (Inoue et al., 2004; Karrman et al., 2007). PFOS is associated with adverse health effects, including hepatotoxicity, immunotoxicity, developmental toxicity and etc. (Lau et al., 2007). The association of PFOS exposure with energy metabolic disorder has also been emphasized due to its biological role as activator of peroxisome proliferator-activated receptors (PPARs) (Shipley et al., 2004; Bjork et al., 2008), which plays a key role in lipid metabolism. Previous study has reported PFOS could disturb thyroid hormone homeostasis (Lau et al., 2003; Luebker et al., 2005b), and apparent increase in liver glycogen stores was also observed in the rat pups with gestational and lactational PFOS exposure (Luebker et al., 2005a), all of which are correlated with energy metabolism. Recent epidemiological study suggests a significant positive association between homeostasis model assessment of insulin resistance and PFOS concentration in adult (Lin et al., 2009), and similar directions of association were also observed in another study (Nelson et al., 2010), even though the trend did not come close to statistical significance. The relationship between environmental PFOS exposure and abnormal plasma lipid level were also observed consistently in adult humans (Chateau-Degat et al., 2010), while animal studies demonstrated PFOS administration was negatively correlated with serum cholesterol level (Seacat et al., 2003; Seacat et al., 2002). The relationship between the PFOS exposure and the effects on energy metabolism may be more important if the exposure occurs during critical developmental period. We therefore suspect developmental PFOS exposure could lead to long-term adverse consequences with related to energy metabolism.

In this study, we tested the hypothesis that gestational and lactational PFOS exposure leads to deleterious long-term effects on glucose and lipid metabolism in adulthood. The effects on glucose and lipid metabolism were evaluated by measurement of body weight, serum parameters, glucose tolerance test, the change of adipose tissue, and fat accumulation in liver.

**METHODS**

**Animals and Treatment**

Male and Female SPF Wistar rats (weighing 200 ± 20 g) were purchased from Hubei center for disease control and prevention and were maintained at ABSL-3 Laboratory of Animal Experiment Center of Wuhan University. All rats were housed in polypropylene plastic cages at 20–24°C on a 12-h light/12-h dark cycle and were fed standard rodent chow containing 52% carbohydrates, 22.1% protein, and 5.28% fat. All animals had access to food and water ad libitum. All experiments were carried out humanely and with regard for alleviation of suffering, and the experimental procedures were reviewed and approved by an institutional committee for animal care and use.

All the animals were allowed to acclimatize for one week before experiments started. Two females and one male were placed together in one cage for breeding. Mating was confirmed by the appearance of a vaginal plug on the following morning. Eighteen pregnant females were assigned at random to three groups and were housed separately. Each pregnant rats were given daily oral doses of vehicle control, PFOS at 0.5 mg/kg/day and 1.5 mg/kg/day respectively from gestation day 0 (GD 0) to postnatal day (PND) 21. Dams were allowed for spontaneous delivery, pups were sexed and randomly selected and reassigned to ensure there are equal pups per litter (n = 10 pups per litter, 5 female, 5 male). The weights of pups per litter were
measured on PND 0, 5, 10, 15, and 21. On PND 21, all pups were weaned. All the rats subjected to the subsequent experiments are selected randomly. Pups were maintained until 19 week after weaning; they were then euthanized by carbon dioxide asphyxiation and all sacrificed. The blood was collected from the angular vein in 10 and 15 weeks after weaning, the serum was separated by centrifugation and then stored at −80°C until use.

**Chemicals and Dosing**

PFOS (purity >98%) was purchased from Fluka Chemical (Buchs, Switzerland). Tween 20 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Briefly, groups of 10 pregnant wistar rats (maternal rats) were given daily oral doses of vehicle control (0.5% Tween 20 in water) or K⁺PFOS (solubilized in 0.5% Tween 20 in water due to limited water solubility of K⁺PFOS) at 0.5, and 1.5 mg/kg/day from GD 0 to PND 20.

**Determination of PFOS Concentrations in Serum and Liver**

Serum and liver samples for PFOS analysis were obtained from pups on PND 0 and PND 21. All the samples were snap frozen at approximately −80°C until processing for analysis. PFOS in the serum and liver samples were extracted using a previously published method (Hansen et al., 2001). Briefly, serum samples or liver homogenate aliquots were mixed with 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate and 2 mL of 0.25 M sodium carbonate buffer in 15 mL polypropylene tubes for extraction. After being thoroughly mixed, 5 mL of methyl tert-butyl ether (MTBE) was added to the solution, and the mixture was shaken for 20 min. After being separated by centrifugation, an exact volume of MTBE (4 mL) was removed from the solution, and the process was repeated twice. The solvent was allowed to evaporate under nitrogen, then reconstituted in 1 mL of methanol and passed through a 0.2 μm nylon filter into an autosampler vial. Analysis was then performed using LC/MS (Agilent 1100 LC-MSD-trap-XCT, Agilent 1100 Series LC/MS Ion-Trap, Agilent, Palo Alto, CA) with a sample volume of 5 mL. Separation was achieved on an Inertsil ODS-3 column (2.1 mm × 150 mm, 5 μm), being carried out using a mobile phase of 1.0 mM ammonium acetate/methanol (v/v) at a flow rate of 0.2 mL/min. The gradient profile was as follows: linear increase from 40 to 75% methanol solution for 5–12 min, then hold at 75% for 3 min. PFOS was quantitatively analyzed by single mass mode using characteristic ions at m/z 498.9 (Martin et al., 2004; Tseng et al., 2006).

**Serum Analysis**

To measure serum hormones, the blood of offspring was collected from the angular vein after fasting for 16 h. Blood was left at 37°C for 1 h and then at 4°C overnight. The serum was separated by centrifugation at 4000 rpm for 20 min at 4°C. All the serum was stored at −80°C till analysis. Fasting serum triglyceride and total cholesterol were measured by Hitachi Modular 7600 Clinical Analyzer (Hitachi Ltd, Tokyo, Japan). Insulin and leptin were measured by radioimmunoassay (Millipore, St. Charles, USA). Adiponectin was measured by enzyme linked immunoabsorbent assay (Millipore, St. Charles, USA). Glycosylated serum protein (GSP) was measured using a NBT reduction assay kit (Nanjing Jianchen Bioengineering, Nanjing, China).

**Liver Triglyceride Extraction and Analysis**

Liver triglyceride extraction was performed as previously reported (Hara and Radin, 1978). Briefly, Frozen liver samples (200–600 mg) were cut into small pieces in chloroform and methanol (2:1 ratio) and incubated overnight at 4°C. The mixture was filtrated in order to remove tissue debris, then 0.9% NaCl was added, and all samples were centrifuged at 1500 r.p.m. for 10 min. Lipids in the organic phase were transferred to a new tube, air-dried and redissolved with X-Triton, methanol and tert-butyl alcohol (1:1:3 ratio). Triglyceride was determined using a commercial enzymatic assay kit (Nanjing Jianchen Bioengineering, Nanjing, China).

**Fasting Blood Glucose Level and Glucose Tolerance Test**

For fasting blood glucose level, animals were fasted overnight for 16 h, and glucose levels were determined from whole venous blood using an automatic glucose monitor (Ascensia BRIOTM Blood Glucose Monitoring System, Bayer HealthCare LLC). For glucose tolerance tests, animals were fasted overnight for 16 h, and then given with 2 g/kg body weight of glucose by gavage. Glucose levels were determined in blood collected from the angular vein immediately before and 15, 30, 60, and 120 min after the gavage. For quantitative comparison, the areas under OGTT curve (AUC) were calculated.

**RNA Extraction, Reverse Transcription, and Real-Time PCR**

Liver samples were collected at the time of sacrifice (22-week-old rats). Total RNA of liver tissue was isolated using SV Total RNA Isolation System (Promega, Madison, WI) according to the manufacturer’s protocol. Reverse transcription for cDNA synthesis was performed using Transcript™ RT/RI Enzyme Mix (TransGen Biotech, Beijing, China). Real-time PCR was carried using Power SYBR Green PCR Master Mix reagents (TransGen Biotech, Beijing, China) in an ABI PRISM 7900 sequence detection system (Applied Biosystems, Framingham, MA) according
to the manufacturer’s protocol. The primer sequences of SREBP-1c (Accession no. XM_213329.5) gene were: forward, 5’- CAGGCAGGCCCCTGGACAG -3’; reverse, 5’- GTGCGTTCTCACACGCGT -3’ (product sizes 149 bp).

The condition for real-time PCR was: 95 °C for 30s followed by 40 cycles at 95 °C for 5 s, and 60 °C for 30s. Differences in gene expression between groups were calculated using cycle time (Ct) values, which were normalized against β-actin and expressed as relative values (mean ± S.E.M) compared to the control.

**Pancreas Immunofluorescence and Assessment of Pancreatic β-Cell Area**

Pancreas were removed at the time of sacrifice (22-week-old rats) and fixed overnight in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Following rehydration and permeabilization (1% Triton X100), sections were incubated with anti-insulin (Millipore, Billerica, USA) and antigliucagon (Abcam, Cambridge, UK) antibodies overnight at 4°C. Detection was performed with Alexa Fluor® 488 (green) and Alexa Fluor® 555 (Johansson et al.) conjugated secondary antibodies (The Cell Signaling Technology, Danvers, USA). The methods for quantification of β-cell area used in this study have been described previously (Alonso-Magdalena et al., 2010).

**Adipose Tissue H&E and Liver Oil Red O Staining**

The sections of rat liver and epididymal adipose tissue were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Generally, five independent rats per group were used in order to examine the morphological changes, H&E staining for the adipose tissue and Oil red O staining for the liver were carried out separately. Adipocyte cross-sectional areas were measured in 100 cells per rat using Image J software. Integrated optical density for each Oil red section was measured by Image-Pro Plus software 7.0.

**Data Analysis**

Statistical analysis was performed using SPSS13.0. The interactions with gender were first determined using multivariate analysis of variance (ANOVA). If no gender interaction was observed, male and female data were combined for analysis, then statistical significance was determined using a one-way analysis of variance (ANOVA) (P ≤ 0.05). When significant differences were detected, Dunnett’s Comparison was used to compare treatment group and controls. Normality test had been done for the serum parameters using one-sample Kolmogorov-Smirnov test. Data are presented as mean ± S.E.M.

**TABLE I. Birth weight at birth and during lactation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.5 mg/kg</th>
<th>1.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (PND 0)</td>
<td>6.7 ± 0.4</td>
<td>5.9 ± 0.4</td>
<td>5.7 ± 0.1*</td>
</tr>
<tr>
<td>Body weight (PND 5)</td>
<td>11.3 ± 0.2</td>
<td>10.7 ± 0.1*</td>
<td>9.9 ± 0.2*</td>
</tr>
<tr>
<td>Body weight (PND 10)</td>
<td>20.5 ± 0.3</td>
<td>19.1 ± 0.3*</td>
<td>18.9 ± 0.4*</td>
</tr>
<tr>
<td>Body weight (PND 15)</td>
<td>31.0 ± 1.0</td>
<td>27.9 ± 0.3*</td>
<td>27.7 ± 0.6*</td>
</tr>
<tr>
<td>Body weight (PND 21)</td>
<td>41.8 ± 0.9</td>
<td>39.2 ± 0.3*</td>
<td>38.5 ± 0.8*</td>
</tr>
</tbody>
</table>

The data are collected as an average per litter. Each data represents mean ± S.E.M (n = 6 litter per group (60 pups per group, as there are 10 pups in each litter)), *P < 0.05 compared with control group.

**RESULTS**

There were no statistical interactions related to gender in the current study, so genders were combined for the analysis of all of the glucose and lipid homeostasis data.

**Low Body Weight During Development with Matched PFOS Contents**

All neonates, both in 0.5 and 1.5 mg/kg groups, were born alive and appeared active. The majority successfully survived through lactation period and the survival rates are comparable among groups (survival rate at weaning were 98.7%, 98.8%, and 98.8% for control, 0.5 and 1.5 mg/kg groups). Gestational PFOS exposure significantly reduced the birth weight of offspring in 1.5 mg/kg group. The 0.5 mg/kg group showed a similar but nonsignificant pattern, the detailed birth weight data are displayed in Table I. The postnatal growth of pups exposed to PFOS appeared stunted. Body weight of pups during lactation in PFOS treated group significantly lagged behind those of the controls. The difference among PFOS-treated and control groups diminished from nine week after weaning, which demonstrate a catch-up effect in adulthood, the detailed body weight data are displayed in Table II. The PFOS concentration (Data are normally distributed as determined by normality test, P = 0.092) in the serum and liver were presented in Table III. The mean PFOS content of serum and liver in control group was lower than the limit of detection on either PND 0 or 21. With the increasing dosage of PFOS exposure, PFOS content was significantly raised in serum and liver tissues with a significant dose-dependent manner. What’s more, there was a time-related increase in PFOS concentration both in serum and liver as the continuance of PFOS exposure in the lactational period.

**Impairment of Glucose Tolerance**

To assess the impact on glucose tolerance, rats were subjected to an oral glucose tolerance test (OGTT). At 10 weeks after weaning, area-under-curve (AUC) value
revealed a slightly but significantly impaired glucose tolerance at 1.5 mg/kg group (Fig. 1A). At 15 weeks after weaning, the enlarged AUC was observed mainly at 0.5 mg/kg group. 1.5 mg/kg group also showed a tendency toward glucose intolerance with the AUC values averaged 737 (mmol/L/min) compared with 685 (mmol/L/min) in controls, though the difference was not statistically significant (Fig. 1B). The fasting glucose concentration was measured at 10 and 15 weeks after weaning respectively, but there was no differences among groups. The fasting serum glucose intolerance with the AUC values averaged 737 (mmol/L/min) compared with 685 (mmol/L/min) in controls, though the difference was not statistically significant (Fig. 1B). The fasting glucose concentration was measured at 10 and 15 weeks after weaning respectively, but there was no differences among groups. The fasting serum glucose levels during the previous one to two weeks, the results also measured in order to reflect the fluctuation of blood glucose levels. The data suggest that gestational and lactational exposure to PFOS may lead to glucose intolerance later in life.

**Disturbed Serum Fasting Hormone Level**

Normality test was done for all the serum parameters before they were analyzed by ANOVA test. All the data are normally distributed (P value for serum insulin, leptin and adiponectin are 0.396, 0.083, and 0.508 respectively). At 18 weeks after weaning, the fasting serum insulin level was significantly increased in 1.5 mg/kg group (Fig. 2A) as measured by radioimmunoassay, while the 0.5 mg/kg group was comparable to the control. The insulin resistance index also increased in 1.5 mg/kg group at 18 week after weaning as measured by IR = insulin × glucose/22.5 (Fig. 2B). The 1.5 mg/kg group showed peripheral insulin resistance though these evidences are indirect. In parallel to the results of serum insulin, the fasting serum leptin level also increased in 1.5 mg/kg group (Fig. 2C). Adiponectin, a major adipokine secreted specially by adipocyte for the purpose of increasing insulin sensitivity, is usually suppressed in insulin resistance individuals. In this study the fasting serum adiponectin level was decreased in both 0.5 and 1.5 mg/kg group as measured by ELISA (Fig. 2D). We also examined the β-cell area in pancreas, but there was no difference in β-cell area among groups (data not shown).

**Hepatic Steatosis and Increased Gonadal Fat Weight**

To determine if there exist disturbance on hepatic fat accumulation, liver samples at 22 week of age (~19 week after weaning) from three groups were examined for oil red O

**TABLE III. Mean PFOS concentrations in serum and liver of rat pups on PND 0 and PND 21**

<table>
<thead>
<tr>
<th>Age</th>
<th>Treatment</th>
<th>PFOS in serum (µg/ml)</th>
<th>PFOS in liver (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ND*</td>
</tr>
<tr>
<td>PND 0</td>
<td>Control</td>
<td></td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg body wt/day</td>
<td>3.98 ± 0.80*</td>
<td>10.49 ± 0.80*</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/kg body wt/day</td>
<td>36.25 ± 4.26*</td>
<td>114.93 ± 6.14*</td>
</tr>
<tr>
<td>PND 21</td>
<td>Control</td>
<td></td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg body wt/day</td>
<td>11.00 ± 1.35*</td>
<td>42.22 ± 2.55*</td>
</tr>
<tr>
<td></td>
<td>1.5 mg/kg body wt/day</td>
<td>71.35 ± 3.27*</td>
<td>139.68 ± 4.38*</td>
</tr>
</tbody>
</table>

Values are presented as the mean ± SEM of six rats per group, the serum samples of PND 0 are pooled by litters.
*Represents the value lower than the limit of detection.
*P < 0.05.
staining. 1.5 mg/kg group showed obvious abnormal fat accumulation [Fig. 3(A,B)], while control and 0.5 mg/kg groups did not find the histological change. In accordance with hepatic steatosis, the lipogenic gene SREBP-1c mRNA increased nearly twofold in 1.5 mg/kg group livers compared with control [Fig. 3(C)], the triglyceride in the liver tissue was also significantly elevated in 1.5 mg/kg group livers compared with control [Fig. 3(E)]. Overnight fasting serum triglyceride and serum cholesterol levels were also tested, and no significant differences were observed (data not shown). These results suggest that exposure to PFOS during gestation and lactation may lead to abnormal hepatic fat accumulation, though the fasting serum triglyceride and cholesterol levels were normal.

Rats both in the 0.5 and 1.5 mg/kg groups also displayed a significant increase of epigonadal fat pad weight when sacrificed at 19 week after weaning [Fig. 3(D)]. However, this augment in adipose tissue mass did not reflect in the mean adipocyte size as determined by histological and computational analysis. The adipocyte size was not increased compared with the control (data not shown).

DISCUSSION

Since PFOS is a pervasive environment pollutant widely used in industrial and commercial products, exposure during fetus or infant’s developmental periods can be expected and indeed has been documented (Butenhoff et al., 2006; Apelberg et al., 2007). PFOS has been shown, in rodent studies, to induce developmental effects including reduced birth weight, decreased gestational length and developmental delay etc. (Grasty et al., 2003; Lau et al., 2003; Thibodeaux et al., 2003; Luebker et al., 2005b) However, most of these developmental effects were observed during developmental periods neglecting the potential effects in adulthood. In this study, we sought to profile the potential prediabetic effects in adulthood induced by developmental exposure to PFOS. We first demonstrated that the lipid and glucose homeostasis in adulthood can be affected by gestational and lactational exposure to PFOS.

The PFOS exposure rats in this study exhibited reduced glucose tolerance, and higher levels of serum insulin, implying insulin resistance, and impaired glucose homeostasis. This enhanced insulin level might be a compensation for an aggravated condition of insulin resistance, to maintain satisfactory blood glucose level. In addition, fasting serum leptin level was also elevated, a key character of leptin resistance, in 1.5 mg/kg PFOS treated group. A major function of leptin is regulating glucose homeostasis and insulin sensitivity in peripheral tissues (Tuduri et al., 2009). The elevated level of serum leptin may affect energy metabolism, aligning with the result of glucose intolerance. Adiponectin, secreted by adipocyte involving in insulin action sensitivity, is usually decreased in type 2 diabetes and insulin resistance ones (Hotta et al., 2000). Accordingly, the fasting serum adiponectin level is also down-regulated. On the basis of these results, we concluded that exposure to PFOS during development may develop impaired glucose homeostasis in adult rat, which is a hallmark of prediabetic state.

A disorder of lipid metabolism was observed in 1.5 mg/kg group, characterized by fatty accumulation in liver (hepatic steatosis), elevation of SREBP-1c mRNA level and increasing of gonadal fat pad weight. The transcription factor SREBP-1c controls the entire pathway of lipogenesis (Shimano et al., 1999; Shimano, 2001), and activated SREBP-1c contribute to insulin resistance in liver (Ide et al., 2004). Thus, the hepatic steatosis may be caused by the metabolic reprogramming induced by developmental
PFOS exposure. It was suggested that individuals with fatty accumulation in liver has a high risk of developing type 2 diabetes, which is great possible because of the presence of associated glucose intolerance or insulin resistance (Cankurtaran et al., 2007; Adams et al., 2009). Thus, the lipid metabolic disorder in liver is highly associated with the above-mentioned results of impaired glucose homeostasis. The increased gonadal fat pad weight also made us to suspect if this was due to lipogenesis effect induced by hyperglycaemia, while no significant difference among groups was observed. So we speculated the increased gonadal fat pad weight was due to the increased number of adipocyte through proliferation but not the enhanced lipogenesis effect induced by insulin. Anyway, the increased gonadal fat pad weight contributes to the conclusion of the disturbed of lipid homeostasis. The change of serum lipid level was not observed in this study, though early reported health effects in animal studies indicated that administered high PFOS in adulthood induced hypolipidemia (Seacat et al., 2002, 2003). A basic awareness should be noticed that the rats in present study experienced PFOS exposure during gestational and lactational periods, which is totally distinguished from the exposure in adulthood. So the underlying mechanism and the health outcome might be different.

Low weight at birth and during lactation was observed at the present PFOS exposure level. The mean serum PFOS concentration on PND 21 for the rats’ pups of 0.5 and 1.5 mg/kg group reached 11 μg/mL and 71 μg/mL respectively, which is sufficient to ensure that all neonates survive the developmental periods meanwhile. This result was comparable with early rat experiments with similar maternal PFOS level (Chang et al., 2009). Exposure to PFOS during gestation has been widely reported to be associated with decrements in birth weight in rats (Grasty et al., 2003; Lau et al., 2003; Thibodeaux et al., 2003; Luebker et al., 2005a,b), but the results observed in the human epidemiological studies display an inconsistent pattern, for some epidemiological studies indicated that gestational and lactational PFOS exposure was negatively correlated with body weight at birth (Apelberg et al., 2007; Stein et al., 2009; Washino et al., 2009) and even during infancy (Andersen et al., 2010), while some indicated the correlation does not exist (Fei et al., 2007; Monroy et al., 2008). The epidemiologic observations that low birth weight or relative thinness at birth and during infancy is associated with increased incidence of coronary heart disease, stroke, type 2 diabetes mellitus, adiposity, the metabolic syndrome and osteoporosis in adult life have been extensively replicated (Hales
et al., 1991; Robinson et al., 1992; Phipps et al., 1993; McCance et al., 1994; Lithell et al., 1996; Rich-Edwards et al., 1999; Gluckman et al., 2008). Since the low birth weight is a common developmental effect of PFOS and the well established role of low birth weight as a risk factor for type 2 diabetes in adulthood, it is plausible that low weight during development may be a link between developmental PFOS exposure and the impairment of energy homeostasis observed in this study.

The serum PFOS concentrations observed in this study are about two to three orders of magnitude higher in comparison with the concentrations observed in the general population as reported by numerous epidemiological studies (Apelberg et al., 2007; Betts, 2007; Fei et al., 2007; Stein et al., 2009), so the adverse health effects associated with current PFOS exposure dosage seems unlikely to occur in the general population with a relatively lower exposure level. However, we should also notice that human beings might be more sensitive than the rodents due to their much more lengthy serum elimination half-lives up to several years for PFOS (Olsen et al., 2007), and we should also be aware of another important message that there are considerable individual differences in PFOS exposure levels (Olsen et al., 2009). In addition, although PFOS have been phased out by some manufacturers in the U.S. and in some European countries, it is still produced elsewhere, and other chemical compounds with similar structure can also break down to PFOS (Organisation for Economic Co-operation and Development 2002). So the potential detrimental health effect associated with developmental PFOS exposure may need to be further investigated in the future.

The evidences of developmental toxicity of PFOS emerged in adult life are limited. To our knowledge, this is the first study to access the energy metabolic effects on adulthood of developmental exposure to PFOS. Our data are consistent with our hypothesis that developmental exposure to PFOS disrupted the energy homeostasis in adult life. Since low birth weight is a risk factor for many chronic diseases in adulthood, we should pay more attention to the effects in adulthood of developmental exposure to PFOS.
and a longer time observation than the present study is also preserved. Furthermore, it is necessary to explore the specific developmental windows during gestation and lactation. It was previously reported that the non observed adverse effects level (NOAEL) for developmental toxicity of PFOS was established of 0.4 mg/kg in rats (Luebker et al., 2005a).

However, the long-term metabolic effects in adulthood were detected closely near to the NOAEL originally established. We suggested that it is necessary to re-examine the NOAEL for developmental toxicity of PFOS and the long-term effects in adulthood should be pay more attention.

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REFERENCES


Lassiter TL, Ryde IT, Mackillop EA, Brown KK, Levin ED, Seidler FJ, Slotkin TA. 2008. Exposure of neonatal rats to...


