Published in final edited form as:

Toxicol Lett. 2011 September 10; 205(3): 265–272. doi:10.1016/j.toxlet.2011.06.015.

Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPAR α

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Abstract

Perfluorooctanoate, a peroxisome proliferator-activated receptor alpha (PPAR α) agonist, has the potential to lower testosterone levels as a result of testicular toxicity. To elucidate the mechanism and impact of PPAR α on this reproductive toxicity, ammonium perfluorooctanoate (APFO) at doses of 0, 1.0 (low) mg/kg/day, or 5.0 (high) mg/kg/day was orally given daily to 129/sv wild-type (*mPPAR α*), *Ppara*-null and PPAR α -humanized (*hPPAR α*) mice for 6 weeks. Both low- and high-dose APFO significantly reduced plasma testosterone concentrations in *mPPAR α* and *hPPAR α* mice, respectively. These decreases may, in part, be associated with decreased expression of mitochondrial cytochrome P450 side-chain cleavage enzyme, steroidogenic acute regulatory protein or peripheral benzodiazepine receptor as well as microsomal cytochrome P450_{17 α} involved in the steroidogenesis. Additionally, both doses increased abnormalities in sperm morphology and vacuolated cells in the seminiferous tubules of both mouse lines. In contrast, APFO caused only a marginal effect either on the testosterone synthesis system or sperm and testis morphology in *Ppara*-null mice. These results suggest that APFO may disrupt testosterone biosynthesis by lowering the delivery of cholesterol into the mitochondria and decreasing the conversion of cholesterol to pregnenolone and androstane in the testis of *mPPAR α* and *hPPAR α* mice, which may, in part, be related to APFO-induced mitochondrial damage.

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Conflict of interest statement
None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxlet.2011.06.015.

Keywords

Perfluorooctanoic acid; Peroxisome proliferator-activated receptor; alpha; Reproductive toxicity; Testosterone

1. Introduction

Perfluorooctanoate (PFOA) has been used primarily as a surface-active agent in the production of various fluoropolymers, including tetrafluoroethylene (Kennedy et al., 2004). This chemical is also an end product of many fluorochemical compounds in the natural environment (Giesy and Kannan, 2002). Because of its persistent nature, PFOA is found in the atmosphere and surface water (Nakayama et al., 2005).

PFOA is rapidly absorbed in humans and persists with a half-life thought to be in the range of 2.3–3.8 years (Olsen et al., 2007; Lau et al., 2007). Its easy uptake and bioaccumulation may increase the potential for toxicity. Accordingly, PFOA is detected in sera of the general population in many countries such as the United States (median, 4.4–41.5 ng/ml), Columbia (5.6–6.2 ng/ml), Brazil (<20ng/ml), Italy (<3 ng/ml), Poland (18.4–23.2 ng/ml), Belgium (2.4–4.3 ng/ml), Germany (mean in this case, 5.2–9.4 ng/ml), India (<3–3.5 ng/ml), Malaysia (<3 ng/ml), Korea (28.5–30.9 ng/ml), and Japan (<6.8–12.3 ng/ml) (Kannan et al., 2004; Wilhelm et al., 2009).

Epidemiologically, PFOA is reported to have developmental toxicity potential. Apelberg et al. (2007) observed small negative associations between PFOA concentrations of cord serum and birth weight and size. In this regard, PFOA also has a similar effect in rodents: gestational exposure to PFOA reduced neonatal survival and pup weight gain which were observed to be dependent on peroxisome proliferator-activated receptor α (PPAR α) (Abbott et al., 2007). In addition, PFOA was reported to directly decrease testosterone levels in testis of rats (Biegel et al., 1995) as well as testicular toxicity such as Leydig cell adenomas (Jensen and Leffers, 2008). While the mechanism of this reproductive toxicity is still not fully elucidated, Zhao et al. (2010) found that PFOA inhibited 3 β - and 17 β -hydroxysteroid dehydrogenase (3 β HSD and 17 β HSD, respectively) activities in rat Leydig cells.

PFOA is a PPAR α and PPAR γ agonist (Ikeda et al., 1985; Vanden Heuvel et al., 2006) that exerts morphological and biochemical effects characteristic of receptors (Nakagawa et al., 2011). These receptors have attracted enormous attention due to their key role in regulating adipogenesis, lipid metabolism, insulin sensitivity, inflammation and blood pressure (Guan, 2004). Most notably, PPAR α regulates the metabolism of cholesterol (Marrapodi and Chiang, 2000), the precursor of sex hormone synthesis. Therefore, any agonist of PPAR α could possibly impact sex hormone synthesis. PFOA activated mouse PPAR α more strongly than human PPAR α (Nakamura et al., 2009; Nakagawa et al., 2011), and the expression of human PPAR α is thought to be 1/10 that of experimental animals (Palmer et al., 1998). In investigating the human PPAR α function, PPAR α -humanized (PPAR $\alpha^{\text{Tet-OF}}$) mice have been established through insertion of human PPAR α in the liver of *Ppara*-null background mice (Cheung et al., 2004). This animal model expresses human PPAR α only in the liver, not in testis, suggesting that it is easy to understand how human as well as mouse PPAR α

expressed in mouse liver is related to PFOA-induced testis toxicity using the PPAR α ^{Tet-OF} (*hPPAR α*) together with wild-type (*mPPAR α*) and *Ppara*-null mice (Lee et al., 1995).

Thus, in the present study, the involvement of mouse and human PPAR α in PFOA-induced reproductive toxicity such as testosterone levels and its molecular mechanism, sperm characteristics or testis morphology were investigated using wild-type (*mPPAR α*), *Ppara*-null and humanized *PPAR α* (*hPPAR α*) mice.

2. Materials and methods

2.1. Chemicals

Ammonium pentadecafluorooctanoate (APFO) [CF₃(CF₂)₆COONH₄, molecular weight 431.10] was purchased from Tokyo Kasei (Tokyo, Japan), and the purity was over 98.0%.

2.2. Animals

The animal study was conducted according to the Guidelines for Animal Experiments of the Nagoya University Animal Center. Male wild-type (*mPPAR α* , *n* = 27), *Ppara*-null (*n* = 26, Lee et al., 1995) and PPAR α -humanized (*hPPAR α*) (*n* = 28, Cheung et al., 2004) mice on a 129/Sv background were used in this study. All mice were housed in cages in a clean room under controlled temperature (23–25°C), relative humidity (57–60%), and 12h light/12h dark cycle, and had free access to food and tap water as described elsewhere (Nakamura et al., 2009).

2.3. Treatments

Each genotyped group at eight-weeks-old was randomly divided into 3 groups (*n* = 8–10), and APFO was orally administered at doses of 0, 1.0 mg/kg d or 5.0 mg/kg d for 6 weeks (42 consecutive days). The reason why these doses were selected was described elsewhere (Nakagawa et al., 2011). Mice of the control group ingested a vehicle (distilled water) the same as for APFO treatment. Sixteen hours after the last administration, all mice were weighed and sacrificed by decapitation. Blood was collected into heparinized tubes. The following organs were removed and weighed as quickly as possible: epididymis, testes, seminal vesicle and prostate. Each right testis was fixed in modified-Davidson's formalin (Latendresse et al., 2002), and the remaining organs and plasma were kept at –80°C until use.

2.4. Epididymal sperm count

The sperm suspension from epididymis was diluted with saline containing 0.5% formalin. The number of sperm was counted using a Neubauer-type hemocytometer (Erma, Tokyo, Japan). Epididymal sperm counts were expressed as the number of sperm per gram of cauda epididymis.

2.5. Epididymal sperm motility analysis

The left cauda epididymis was weighed, placed in a dish containing 2 ml Hank's solution at 37 °C, and then cut into small pieces. The sperm were gently filtered through gauze under a

microscope on a warming plate (Tokai Hit Co., Fujinomiya, Japan) while maintaining the temperature at 37 °C, and filtrates (spermatozoa) were used to manually count motile sperm.

2.6. Epididymal sperm abnormalities

A drop of sperm suspension was uniformly smeared on three glass slides per sample. After being air-dried, the smeared slides were fixed in methanol and then stained according to Bryan's method (Bryan, 1970). Based on the method of Wyrobek and Bruce (1975), abnormalities in sperm morphology were examined under the microscope.

2.7. Hormone assay

The plasma testosterone was directly extracted from 10 µl plasma with 2 ml hexane-ethyl ether mixture (3:2). Concentrations of testosterone in the plasma were measured by a testosterone EIA kit (Cayman, Ann Arbor, MI).

2.8. Real-time quantitative PCR analysis

mRNA levels of several important enzymes, carrier proteins, or receptors involved in the testicular biosynthetic pathway of testosterone were measured by qPCR according to the method of Zhang et al. (2007). The primers used are listed in Supplemental Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for data analysis. All genes of mRNA expression levels were normalized to GAPDH expression levels.

2.9. Western blotting

The testis from mice was homogenized with a 3-fold volume of 10 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose. The protein concentration of each sample was measured with a Protein Assay Kit (Bio-Rad, Tokyo, Japan). After electrophoresis in 10% or 12.5% SDS-PAGE, proteins were transferred onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA), and important enzymes and carrier protein shown to decrease in mRNA expression were detected using their antibodies according to the method of Ramdhan et al. (2009). Each band was quantified using densitometry with the Lane & Spot Analyzer version 5.0 (ATTO, Tokyo, Japan), and the mean strength of the control group was assigned a value of 1.0. As an internal standard, GAPDH protein was also stained.

2.10. Histopathological evaluation of testes

Right testes fixed in modified-Davidson's formalin (Latendresse et al., 2002) were placed in 10% formalin solution. Tissue samples were embedded in paraffin and cut at 5 µm thickness, stained with hematoxylin & eosin, and then examined with a light microscope.

2.11. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using Tukey-Kramer HSD test to compare the effects of APFO among each treated group of each genotyped mouse, and among the control group of three genotyped mice. *P* values less than 0.05 were considered statistically significant. Because variables of testosterone levels

and sperm abnormalities were not normally distributed, log transformation was performed before the statistical analyses.

3. Results

3.1. Body and organ weights

During the oral exposure to APFO for 6 weeks, no overt sign of poisoning was found in all mice. No significant difference was noticed in the body weight among the three mouse groups which were not influenced by APFO treatments (Table 1). In the control group, there were no differences in testis and epididymis weights among the three groups, whereas the weight of the seminal vesicle + prostate gland was significantly higher in the *mPPAR α* mice than in *Ppara-null* and *hPPAR α* mice. No dose influenced testis weight, while high-dose APFO decreased both weights of epididymis and seminal vesicle + prostate gland only in the *mPPAR α* mice.

3.2. Sperm count, abnormalities and motility, and plasma testosterone

APFO treatment did not influence sperm count (Fig. 1A) nor motility (data not shown) in all mice. In contrast, APFO treatment increased sperm abnormalities in *mPPAR α* (1.4- and 1.5-fold at low and high dose, respectively) and dose-dependently in *hPPAR α* (1.3- and 2.6-fold) mice (Fig. 1B). In contrast, APFO treatments dose-dependently decreased plasma testosterone in the *mPPAR α* (by 57% and 37% at low and high dose, respectively), and in *hPPAR α* mice decreased it by 31% and 29%, respectively (Fig. 1C). These treatments tended to decrease plasma testosterone in *Ppara-null* mice, but the small changes were not statistically significant.

3.3. Mechanism of decrease in plasma testosterone

Using real-time quantitative PCR, the mRNA levels for several genes associated with testicular cholesterol synthesis, transport, and testosterone biosynthesis similar to the previous studies were examined (Ramdhan et al., 2009; Zhang et al., 2007).

3.3.1. Cholesterol biosynthesis—In Leydig cells of testes, de novo cholesterol is an essential substrate for testosterone production. Both the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase involved in testicular cholesterol biosynthesis were measured. These enzymes were not changed after treatments of APFO in three mouse groups, though the HMG-CoA reductase levels of the control group were significantly higher in *hPPAR α* mice than *mPPAR α* and *Ppara-null* ones (Fig. 2A and B).

3.3.2. Cholesterol transport—Peripheral benzodiazepine receptor (PBR) and steroidogenic acute regulatory protein (StAR) play key regulatory roles in cholesterol transport from the outer to the inner mitochondrial membrane. High-dose APFO inhibited the expression of StAR mRNA in the testis of *mPPAR α* mice, and the low and high dose in *hPPAR α* mice (Fig. 2C). PBR mRNA levels in testis of the control group were higher in *hPPAR α* mice than *mPPAR α* and *Ppara-null* mice. The high dose decreased the levels only in *hPPAR α* mice (Fig. 2D).

3.3.3. Testosterone biosynthesis—Cytochrome P450 side-chain cleavage enzyme (P450_{scc}) is responsible for conversion of cholesterol to pregnenolone. The expression of this enzyme was significantly lower in *hPPARα* mice than in *mPPARα* and *Ppara*-null animals (Fig. 2E). High-dose APFO treatment significantly suppressed P450_{scc}-mRNA expression in the testes from *mPPARα* mice.

Pregnenolone is converted into testosterone via several enzymes such as cytochrome P450 17α-hydroxylase/C17–20 lyase (P450_{17α}), 3β-HSD and 17β-HSD. The expression of P450 17α-mRNA was clearly lower in control *Ppara*-null and *hPPARα* mice than in *mPPARα* mice (Fig. 2F). High-dose APFO decreased this enzyme level in *mPPARα* and *hPPARα* mice. Low-and high-dose APFO treatment significantly decreased the expression of 3β-HSD in *hPPARα* mice, although they showed no difference in their expression in untreated mice (Fig. 2G). Expression levels of 17β-HSD mRNA were lower in the low-dose groups of *Ppara*-null mice (Fig. 2H). APFO treatments did not have any effect on aromatase mRNA levels in all mouse lines (data not shown).

3.4. Mitochondrial elements

Messenger RNAs encoding uncoupling protein 2 (UCP2) (Kizaki et al., 2002; Negre-Salvayre et al., 1997) and superoxide dismutase 1 (SOD1) which are involved in inhibiting oxidative stress (Salvemini et al., 1999), and carnitine palmitoyltransferase 1 (CPT1), which mediates the transport of long-chain fatty acids across the membrane, were also measured. A decrease in the expression of these genes is indicative of mitochondrial damage (McGarry and Brown, 1997). No dose influenced UCP2 mRNA levels in all mouse groups (data not shown), whereas low-and/or high-dose APFO decreased CPT1 and SOD1 mRNA levels in *mPPARα* and *hPPARα* mice (Fig. 2I and J).

3.5. Steroidogenic protein expression in testes

In order to confirm the effects of APFO on steroidogenic gene products, protein expression levels of StAR, P450_{scc}, and P450_{17α} were determined by Western blot analysis. By comparison with the respective control, low-and/or high-dose APFO significantly decreased StAR and P450_{17α} protein in *mPPARα* and *hPPARα* mice, whereas P450_{scc} protein was significantly decreased only in the APFO high-dose group of *mPPARα* mice (Fig. 3A and B).

3.6. Histopathological evaluation of testes

In testes of the control group, there were no obvious abnormal findings in control groups of *mPPARα* mice (Fig. 4), while a few seminiferous tubules with vacuoles were observed in control *Ppara*-null and *hPPARα* mice. The APFO dose at 5.0 mg/kg appeared to increase abnormal seminiferous tubules with vacuoles or lack of germ cells in *mPPARα* and *hPPARα* mice. The necrotic cells were also observed in testes of *mPPARα* mice after 5.0 mg/kg APFO exposure. However, no obvious effects of APFO treatment were morphologically observed in those of *Ppara*-null mice.

4. Discussion

The present study demonstrated that 6-week administration of APFO to male mice had adverse effects on their reproductive system. The APFO exposure significantly reduced the reproductive organ (epididymis and seminal vesicle + prostate gland) weights of *mPPARα* mice and the plasma testosterone levels of both *mPPARα* and *hPPARα* mice, and it increased sperm abnormalities in epididymis and testicular morphological abnormalities such as vacuoles in seminiferous tubules in both strains. These changes in reproductive organ weights and the sperm abnormalities in the APFO-treated mice may be partially related to the reduction in testosterone, because these phenotypic changes are known to depend heavily on androgen (George and Wilson, 1994; McLachlan et al., 2002). Interestingly, these changes were not observed in *Ppara*-null mice, suggesting the involvement of either mouse or human hepatic PPARα in such APFO-induced reproductive toxicity.

The mechanism of APFO-induced decreased plasma testosterone in *mPPARα* and *hPPARα* mice is of great interest and may be due to altered expression of steroidogenic enzymes in the testis. Although testosterone is mainly synthesized from cholesterol in Leydig cells, APFO did not influence HMG-CoA synthase and reductase, suggesting this chemical might not influence cholesterol de novo synthesis in the Leydig cells, though APFO decreased plasma total cholesterol levels in *mPPARα* and *Ppara*-null mice (Nakagawa et al., 2011). Cholesterol produced in the cells is transported into mitochondria via StAR in concert with PBR (Hauet et al., 2005), and converted to pregnenolone by catalytic action of P450_{scc} (Jefcoate et al., 1992; Hanukoglu, 1992). Decrease in the expressions of these three genes in *mPPARα* and/or *hPPARα* mice may be related to that of testosterone, because they are thought to be rate limiting in testosterone production (Papadopoulos, 1993; West et al., 2001; Caron et al., 1997; Payne, 1990).

Because PBR, StAR, and P450_{scc} are in mitochondria, the decreases in *mPPARα* and *hPPARα* mice could damage mitochondria in Leydig cells. Indeed, altered expression of CPT1 and SOD1, which are also expressed in mitochondria, regulate lipid metabolism (McGarry and Brown, 1997) and remove oxidative stress (Salvemini et al., 1999), respectively, suggesting mitochondrial damage. These results suggest that increase oxidative stress may be produced with APFO treatment, and that the defense mechanisms are lower in the testis of *mPPARα* and *hPPARα* mice. This hypothesis is supported by the finding that perfluorododecanoic acid, structurally similar to PFOA, inhibited 3'-5'-cyclic adenosine monophosphate-stimulated steroidogenesis in mouse Leydig tumor cells by reducing the expression of StAR, where reactive oxidative stress (ROS) and H₂O₂ produced played an important role (Shi et al., 2010). However, it is doubtful whether oxidative stress could be produced in the Leydig cells, because the cells of *Ppara*-null and *hPPARα* mice are similar in that they lack PPARα protein in the Leydig cells. Nevertheless, the toxic effects of APFO were observed only in the latter mice. In the cis-permethrin study, Zhang et al. (2007) reported damage of the mitochondrial membrane in Leydig cells as revealed by electron microscopy, and decreased testicular expression of PBR, StAR and P450_{scc} mRNA. Therefore, it is likely that the mitochondrial membrane of Leydig cells was also damaged after APFO exposure.

Pregnenolone is converted to androstendione in smooth endoplasmic reticulum (sER) via two pathways: one is via dehy-droepiandrosterone by P450_{17 α} converted to androstendione by the catalytic activity of 3 β -HSD (Payne and Youngblood, 1995). The second pathway is via progesterone catalyzed by 3 β -HSD, followed by conversion to androstendione by P450_{17 α} . In the sER, androstendione is converted to testosterone by 17 β -HSD (Payne and Youngblood, 1995). Low- and high-dose APFO significantly decreased the expression of P450_{17 α} protein in *mPPAR α* and *hPPAR α* mice, though only the high dose decreased both mRNAs, and that of 3 β -HSD mRNA in the testes of *hPPAR α* mice, which may also be involved in repression of plasma testosterone. Low-dose APFO decreased the plasma 17 β -HSD mRNA level in *Ppara*-null mice, but since this influence could not be observed dose-dependently, it may be ignored.

The testosterone in Leydig cells is converted to estrogen by aromatase (Carreau, 2002; Genissel et al., 2001) and thus aromatase activity is an important factor in regulating testosterone levels. Liu et al. (1996) reported that exposure to over 2 mg/kg APFO for two weeks elevated liver aromatase activity in rats which was related with plasma estradiol levels, but did not influence the activity in testes. Unfortunately, we did not measure liver aromatase mRNA as well as plasma estradiol levels. In our experiment, APFO may increase liver aromatase mRNA.

In the current study, an increase in abnormal sperms and the incidence of abnormal seminiferous tubules with vacuoles or lack of germ cells were observed in APFO-exposed *mPPAR α* and *hPPAR α* mice. However, these findings were not observed in *Ppara*-null mice. *PPAR α* is expressed in interstitial Leydig cells or seminiferous tubule cells of testis in *mPPAR α* mice, but not in the testis in *hPPAR α* , similar to *Ppara*-null mice (Cheung et al., 2004). Nevertheless, APFO caused reproductive impairment of *hPPAR α* mice similar to *mPPAR α* , suggesting that some toxic molecule(s) such as ROS in relation to hepatic *PPAR α* may be produced in the liver and circulated in the body, because a common point between *mPPAR α* and *hPPAR α* mice was that both had *PPAR α* in the liver, and the activation produced ROS by induction of the receptor-regulated ROS-generating genes (Nakajima et al., 2010). In the liver, however, APFO may not cause mitochondrial damage in *mPPAR α* mice because of the induction of UCP2; and this chemical indeed induced mitochondrial damage in *hPPAR α* mice because of the weak induction of UCP2 compared to that of *mPPAR α* mice (Nakagawa et al., 2011), while in the testis, this gene could not be induced in both strain, which may result in adverse effects in this tissue. Of course, further studies are warranted to assess whether some reactive species to attack mitochondria of the Leydig cells were produced in the liver, and the decreased testosterone in *mPPAR α* and *hPPAR α* mice may also be involved in the morphological changes caused by APFO.

Finally, we did not investigate differences of APFO kinetics among the three genotyped mice used in this experiment. As shown in the previous report (Nakagawa et al., 2011), lipid levels such as triglyceride and total cholesterol in the liver and plasma were different among these mouse strains. How these differences are involved in those of testis morphology would also warrant future study.

5. Conclusion

APFO might adversely affect mitochondria of Leydig cells, which may decrease testosterone levels in plasma, followed by reproductive organ damage and sperm abnormalities. These effects were prominent in *mPPAR α* mice and *hPPAR α* mice, but only marginal in *Ppara*-null mice. Interestingly, these findings were observed after relatively high-dose (5.0 mg/kg) APFO treatment but minimal with a low dose (1.0 mg/kg).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations:

3β-HSD	3 β -hydroxysteroid dehydrogenase
17β-HSD	17 β -hydroxysteroid dehydrogenase
CPT1	carnitine palmitoyltransferase I
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HMG-CoAR	3-hydroxy-3-methylglutaryl coenzyme reductase
HMG-CoAS	cytosol 3-hydroxy-3-methylglutaryl coenzyme synthase
P450_{17α}	cytochrome P450 17 α -hydroxylase/C17–20 lyase
P450_{scc}	cytochrome P450 side-chain cleavage enzyme
PBR	peripheral benzodiazepine receptor
SOD1	superoxide dismutase 1
PPARα	peroxisome proliferator-activated receptor alpha
StAR	steroidogenic acute regulatory protein
APFO	ammonium perfluorooctanoate
PFOA	perfluorooctanoate

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Fig. 1. Effects of APFO exposures on plasma hormone levels, sperm number and quality. (A) Plasma testosterone concentrations; (B) sperm abnormalities; (C) sperm numbers. Values are expressed as means $\pm$ SD for 8–10 mice per group. *Significantly different from respective control group ($P < 0.05$).

Fig. 2. Real-time quantitative PCR analysis of testicular mRNA encoding enzymes involved in testosterone biosynthesis and mitochondrial function from control and APFO-exposed mice. Values are expressed as means $\pm$ SD for 8–10 mice per group. *Significantly different from *mPPARα* control group ($P < 0.05$); #Significantly different from *Ppara-α-null* control group ($P < 0.05$); Significantly different from respective control group ($P < 0.05$).

Fig. 3. Western blot analysis of StAR, P450_{scc}, and P450 17α protein expression levels in the testes of control mice and 1.0 mg/kg, 5.0 mg/kg dose APFO-exposed mice (8–10 per group). (A) Western blot analyses of StAR, P450_{scc}, and P450_{17α}. (B) Each band obtained by immunoblot analysis was quantified by densitometric analysis. Histogram shows the relative densitometric ratio (mean ± SD) of StAR, P450_{scc}, and P450_{17α} to GAPDH. †Significantly different from respective control group ($P < 0.05$).

Fig. 4. Light microscopic findings of testis. Bar in the photos indicates 100 μ m. Testis from *mPPARα* and *hPPARα* mice exposed to 5.0 mg/kg d PFOA showed vacuoles. Arrows show seminiferous tubules with vacuoles.

Table 1:

Effects of PFOA exposure on body and reproductive organ weights (g) in mice.

	<i>mPPARα</i>	<i>Ppara</i> -null	<i>hPPARα</i>
Body weight(g)			
0.0 mg/kg	24.8 ± 1.0[8]	24.2 ± 2.4[8]	23.5 ± 1.3[9]
1.0 mg/kg	24.7 ± 2.2[9]	23.6 ± 2.8[9]	22.3 ± 1.7[9]
5.0 mg/kg	23.5 ± 1.7[10]	25.6 ± 2.5[9]	23.5 ± 2.1[10]
Testis weight, g(g/body weight × 100)			
0.0 mg/kg	0.18 ± 0.02 (0.74 ± 0.08)	0.19 ± 0.03 (0.77 ± 0.09)	0.23 ± 0.03 (0.97 ± 0.14)
1.0 mg/kg	0.17 ± 0.03 (0.69 ± 0.10)	0.18 ± 0.01 (0.78 ± 0.08)	0.20 ± 0.03 (0.91 ± 0.12)
5.0 mg/kg	0.17 ± 0.02 (0.74 ± 0.06)	0.19 ± 0.04 (0.73 ± 0.17)	0.22 ± 0.03 (0.92 ± 0.08)
Epididymis weight, g (g/body weight × 100)			
0.0 mg/kg	0.060 ± 0.007 (0.241 ± 0.026)	0.049 ± 0.005 (0.203 ± 0.018)	0.059 ± 0.008 (0.250 ± 0.043)
1.0 mg/kg	0.056 ± 0.007 (0.224 ± 0.017)	0.049 ± 0.006 (0.209 ± 0.021)	0.051 ± 0.003 (0.231 ± 0.024)
5.0 mg/kg	0.048 ± 0.006* (0.204 ± 0.025)	0.049 ± 0.007 (0.191 ± 0.029)	0.051 ± 0.011 (0.216 ± 0.042)
Seminal vesicle + prostate gland weight, g (g/body weight × 100)			
0.0 mg/kg	0.26 ± 0.03 (1.05 ± 0.13)	0.21 ± 0.03 [#] (0.86 ± 0.12) [#]	0.19 ± 0.04 [#] (0.79 ± 0.16) [#]
1.0 mg/kg	0.24 ± 0.04 (0.96 ± 0.10)	0.21 ± 0.07 (0.88 ± 0.33)	0.20 ± 0.03 (0.91 ± 0.10)
5.0 mg/kg	0.18 ± 0.02* (0.75 ± 0.08) [*]	0.20 ± 0.03 (0.77 ± 0.12)	0.18 ± 0.02 (0.78 ± 0.06)

Body weights were also used elsewhere (Nakagawa et al., 2011). Angle brackets represent numbers of mice used. Data represent mean ±SD.

^{*} Significantly different from respective control group ($P < 0.05$).[#] Significantly different from the control group of wild-type mice ($P < 0.01$).