Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 66 (2003) 749–756
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The relationship between liver peroxisome proliferation and adipose tissue atrophy induced by peroxisome proliferator exposure and withdrawal in mice

Yi Xie*, Qian Yang, B. Dean Nelson, Joseph W. DePierre

Unit for Biochemical Toxicology, Wallenberg Laboratory, Department of Biochemistry and Biophysics, Stockholm University, S-106 91 Stockholm, Sweden

Received 10 October 2002; accepted 19 May 2003

Abstract

We have previously demonstrated that severe adipose tissue atrophy occurs upon dietary treatment of mice with potent peroxisome proliferators (PPs). This atrophy occurs subsequent to peroxisome proliferation in the liver and may represent a novel addition to the pleiotropic effects exerted by PPs. In the present study we have characterized the recovery of mice from such atrophy following cessation of exposure. Following termination of treatment with perfluorooctanoic acid (PFOA) for 7 days, the adipose tissue atrophy was rapidly reversed, beginning on 2–5 days of recovery and being complete within 10 days. In contrast, hepatic peroxisome proliferation recovered much more slowly, indicating that these processes are not strictly coordinated. Analysis of lipoprotein lipase and hormone-sensitive lipase activities in adipose tissue revealed that the decrease and increase in these activities, respectively, caused by PFOA were both reversed within 10 days of recovery. Overall, these data provide further support for our previous conclusion that the adipose tissue atrophy induced by PFOA is caused, at least in part, by changes in the activities of lipoprotein lipase and hormone-sensitive lipase. The serum level of cholesterol, which increased after termination of PFOA treatment, returned to normal with a time-course similar to the recovery of adipose tissue weight, although hepatic peroxisome proliferation was still present. The possible relationship between the reduction in serum cholesterol and/or in its availability to peripheral tissues and the associated atrophy of adipose tissues caused by PPs is discussed.

Keywords: Adipose tissue; Peroxisome proliferator; Perfluorooctanoic acid; Lipoprotein lipase; Hormone-sensitive lipase; Cholesterol

1. Introduction

Peroxisome proliferators (PPs) constitute a large family of widespread foreign compounds, including many industrial chemicals and important clinical drugs. Discovery of the peroxisome proliferator-activated receptor-alpha (PPARα), a member of the superfamily of nuclear hormone receptors, and studies on PPARα-null mice have revealed that PPs exert many of their effects by activating this receptor (for a review, see Ref. [1]).

Alteration in adipose tissues due to exposure to xenobiotics is of interest with regards to diseases, such as obesity and type II diabetes. Recently, it has been observed in our laboratory [2] and others [3–6] that severe adipose tissue atrophy occurs in mice upon treatment with certain potent peroxisome proliferators (PPs). This phenomenon exhibits dose- and time-courses similar to those of hepatic peroxisome proliferation [2].

In addition to causing peroxisome proliferation, hepatomegaly and upregulation of hepatic peroxisomal fatty acid β-oxidation in rodents, many PPs exert a clinically useful hypolipidemic effect in both humans and rodents [7,8]. It has been demonstrated that PPs exert their hypolipidemic effect *via* both PPARα-dependent and -independent mechanisms. Downregulation of the apo C-III protein and upregulation of hepatic lipoprotein lipase (LPL), leading to increased lipid clearance by the liver, are PPARα-dependent [9–12]. On the other hand, inhibition

* Corresponding author. Tel.: +46-8-164189; fax: +46-8-153024.
E-mail address: yi@dbb.su.se (Y. Xie).

Abbreviations: PFOA, perfluorooctanoic acid; PP, peroxisome proliferator; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CPT, carnitine pamitoyltransferase; COT, carnitine octanoyltransferase.

0006-2952/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-2952(03)00386-1

of phosphatidylethanolamine methyltransferase by fibrate and perfluorooctanoic acid (PFOA), resulting in decreased very low density lipoprotein (VLDL) secretion, is PPARα-independent [12,13]. This later phenomenon may explain the fact that PPs exert a hypolipidemic effect on human beings, even though these substances apparently do not cause peroxisome proliferation in human liver.

In a previous investigation we found that peroxisome proliferation induced in mouse liver by PFOA persists for at least 20 days after cessation of exposure [14]. To further elucidate the relationship between peroxisome proliferation and adipose tissue atrophy, the recovery of adipose tissue weight in mice treated for 7 days with PFOA was further characterized. Interestingly, in contrast to hepatic peroxisome proliferation, adipose tissue atrophy was totally reversed within the 10-day recovery period, and the activities for the enzymes in adipose tissue which govern fatty acid uptake into and release from this tissue, i.e. lipoprotein lipase and hormone-sensitive lipase (HSL), respectively, normalized. In addition, the circulating levels of plasma lipids (triglyceride and cholesterol), a possible link between the liver and adipose tissue, were also monitored during the treatment and recovery period. These results suggest a close relationship between adipose tissue weight loss and regain and the circulating level of cholesterol.

2. Materials and methods

2.1. Chemicals

PFOA was purchased from Aldrich Chemical Company (Steinheim, Germany). All other materials and chemicals were of analytical grade and obtained from common commercial sources.

2.2. Animals and treatment

Male C57BL/6 mice (B&K Universal AB, Sweden) weighing 24–28 g (about 8–10 weeks old) were housed in groups of four. These animals were maintained on a 12-hr light:12-hr dark cycle at 25° and given free access to laboratory chow (Rat and Mouse Standard Diet, B&K Universal AB, Sweden) and water. These studies were approved by the Northern Stockholm Ethical Committee for Animal Experimentation.

The PFOA-containing diet was prepared as described previously [14]. Briefly, PFOA was dissolved in 20 mL acetone and mixed with 500 g powdered food to give a final concentration of 0.02% PFOA (w/w). The chow was dried in a ventilated hood until no smell of acetone was detectable (>24 hr). Since mice scatter powdered food, both the normal and PFOA-containing chows were prepared in the form of pellets to allow determination of food consumption. The daily food and water consumption were determined by weighing, as was total body weight. For monitoring recovery, the animals were fed a diet containing 0.02% PFOA (w/w) for 7 days, after which they received normal chow for 2–10 days. For observation of the time-course of alteration in serum lipids, the mice in one group were administered this PFOA-containing diet for 2, 5 or 7 days.

At the end of this feeding period, the mice were bled under ether anaesthesia and then sacrificed by cervical dislocation. The liver and epididymal and retroperitoneal adipose tissues were dissected out and weighed. The blood from each mouse was allowed to clot at room temperature, after which the serum was collected by centrifugation and stored at −80° until used for analysis. Each liver was homogenized individually in ice-cold 50 mM potassium phosphate buffer (pH 7.5) with a Polytron (Model PT3000, Kinematica AG) to obtain a 20% (w/v) homogenate. The adipose tissue samples to be employed for assaying LPL were de-fatted according to Saha *et al.* [15] prior to being stored at −80°.

2.3. Assays of enzyme activities and lipolytic activity

All assays were performed employing established procedures. Carnitine pamitoyltransferase (CPT) and carnitine octanoyltransferase (COT) [16] were assayed employing liver homogenates. Lipoprotein lipase activity in de-fatted adipose tissue was determined as described by Del Prado *et al.* [17]. Briefly, dibutyri-fluorescine was used as a substrate for LPL and the fluorescine liberated by enzymatic hydrolysis of the substrate was determined. The LPL activity was expressed as nanomoles of fluorescent products produced/per hour per epididymal tissue.

HSL activity was obtained from the difference in the amount of glycerol released by the lipolytic activity of epididymal tissue with and without epinephrine stimulation *in vitro*, according to the procedure of Herrera *et al.* [18], with calculation as described by Brandes *et al.* [19].

2.4. Serum fractionation

Serum was fractionated by discontinuous density gradient ultracentrifugation as described earlier [20], with slight modification. In brief, sera from four control or PFOA-treated (7 days) mice were pooled and 1 mL of sample was layered onto a density gradient. Centrifugation was performed according to the reported procedure, with the modification that 1 mL sucrose solution with a density of 1.21 g/mL (200 mg sucrose dissolved in 1 mL premixed NaCl- and NaBr-containing solution with a density of 1.184 g/mL [20]) was added to the bottom of the tube in order to separate out a fraction exhibiting a density higher than that of the high density lipoprotein (HDL) fraction.

2.5. Assays of the triglyceride and cholesterol contents of serum and subfractions thereof

The triglyceride content was determined by monitoring the oxidation of NADH at 340 nm in the coupled

enzymatic system described by Nemeth *et al*. [21]. This measurement was performed in duplicate on microplates and the triglyceride content expressed as mg/dL serum.

Cholesterol was determined by monitoring H_2O_2 production in a coupled reaction involving cholesterol esterase and cholesterol oxidase, as described by Heider and Boyett [22]. This measurement was also performed in duplicate on microplates and the cholesterol content also expressed as mg/dL serum.

Protein was quantitated according to Lowry *et al*. [23], utilizing bovine serum albumin as the standard.

2.6. Analysis of plasma lipoproteins

For determination of plasma apolipoprotein levels, whole serum and the subfractions thereof were electrophoresed on a 4–20% (linear gradient) polyacrylamide gel. These gels were subsequently stained with Coomassie Brilliant Blue R.

2.7. Statistical analysis

Each experimental group contained four animals and the data are presented as means ± SD. Significant differences between groups were identified by one-way ANOVA followed by planned multiple comparison according to the least significant difference method (Statistica software, Stat Soft). Student's *t*-test was used for statistical comparisons (for further details, see the figure and table legends).

3. Results

3.1. General observations during administration of PFOA to mice

Upon treatment of mice with 0.02% (w/w) PFOA for 7 days, none of the animals demonstrated any apparent signs of toxicity, such as sores, poor grooming, lethargy or other behavioral changes. Although total food intake did not decrease during this 7-day treatment, total water intake and body weight were significantly reduced (22%). In contrast, a significant increase in water intake (40%) (6.88 ± 0.87 g/mouse per day vs. 9.65 ± 2.09 g/mouse per day, $P < 0.01$) was observed during the first 2 days of recovery, after which water intake returned to normal levels. Food intake was not altered during recovery, although body weight recovered rapidly (Fig. 1).

3.2. Effect of PFOA administration and subsequent withdrawal on liver and adipose tissue weights

Like total body weight, the weights of the epididymal and retroperitoneal adipose tissue pads increased rapidly after cessation of PFOA treatment (Fig. 1). This recovery was statistically significant after 2–5 days and complete after 10 days. Indeed, retroperitoneal adipose tissue pad weight was even greater than that of the control after 10 days of recovery (Fig. 1). In contrast, the increased liver weight caused by PFOA had not returned to normal 10 days after cessation of treatment, suggesting that recovery of the hepatic and adipose tissues are not tightly coupled.

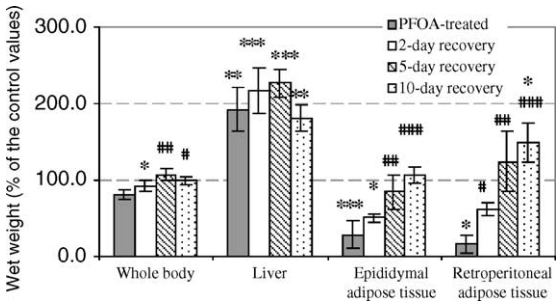

Fig. 1. Effects of PFOA administration and subsequent withdrawal on the body, liver and adipose tissue weights of mice. The results shown are the means ± SD (the bars) of the values for four animals. Values for untreated controls are set to 100%. Differences were analyzed statistically employing the one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the control (untreated) value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to the PFOA-treated (7 days) value. For further details, see Section 2.

3.3. Regulation of liver mitochondrial and peroxisomal fatty acid β-oxidation upon administration and subsequent withdrawal of PFOA

In light of the persistence of the hepatomegaly, mitochondrial and peroxisomal fatty acid β-oxidation were monitored in whole liver homogenates by assaying CPT and COT activities, commonly employed as indicators of peroxisome proliferation. CPT and COT facilitate the transport of long and medium chain acyl-CoA into mitochondria and peroxisomes, respectively. Both carnitine acyltransferases facilitate the generation of energy by β-oxidation of fatty acids in the organelles in which they are present (for a review, see Ref. [24]). As we have found previously, PFOA treatment induced both of these activities (Fig. 2). Subsequent withdrawal of PFOA resulted in significant reductions in both activities, this decrease being much more pronounced after 10 days of recovery in the case of COT (from a maximum of 9- to 5-fold above the control value) than CPT (from a maximum of 4- to 3-fold above the control value). The fact that neither of these activities had returned to the control level after 10 days of recovery is in agreement with the persistence of the peroxisome proliferation caused by PFOA we reported earlier [14].

3.4. Restoration of lipolytic (HSL) and lipoprotein lipase (LPL) activities in epididymal tissue during recovery from PFOA administration

We have demonstrated previously that adipose tissue weight loss resulting from PFOA treatment is a consequence of fat depletion, rather than a decrease in adipocyte

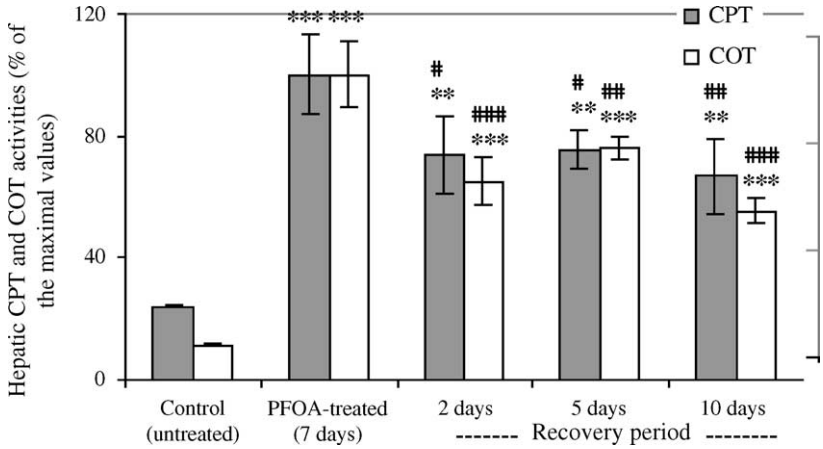

Fig. 2. Effects of PFOA administration and subsequent recovery for up to 10 days on mouse hepatic CPT (filled columns) and COT (open columns) activities. The results shown are the means ± SD (the bars) of the values for four animals. Differences were analyzed statistically employing the one-way ANOVA. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the control (untreated) value. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared to the PFOA-treated (7 days) value. For further details, see Section 2.

number [2]. This explains why, under these circumstances, total LPL activity during treatment and after withdrawal is a better measure than the corresponding activity per gram tissue, since changes in the latter reflect primarily changes in the fat content (and thus weight) of adipose tissue rather than cellular variation in protein expression. Furthermore, the activity of HSL in the phosphorylated and unphosphorylated states differs [25]. Thus, this lipolytic activity was determined as the difference between the rates of glycerol released per gram tissue *in vitro* in the presence and absence of epinephrine, which activates HSL maximally. Downregulation of LPL (essential for fatty acid uptake) and upregulation of HSL (rate-limiting for fatty acid release) in adipocytes was found to, at least in part, explain this adipose tissue atrophy.

In the present study, the HSL activity of epididymal adipose tissue (reflected by glycerol released *in vitro*) was determined following dietary administration of 0.02% (w/w) PFOA and subsequent withdrawal. After 7 days of treatment, this activity was significantly elevated (about 30%, not shown [2]). However, activities fell during 10 days of recovery to a level significantly lower than that observed in control animals (Table 1), supporting the idea that HSL plays a role in PP-induced loss and subsequent regain of adipose tissue.

Furthermore, following withdrawal of PFOA, the total LPL activity of epididymal adipose tissue increased rapidly on the second day of recovery and was significantly elevated after 5–10 days compared to the PFOA-treated group (Fig. 3). This is exactly the same pattern observed for the recovery of adipose tissue weight (see above). Thus, the rapid recovery of adipose tissue weight following cessation of treatment with PFOA might be a consequence of the rapid return of both downregulated LPL and upregulated HSL activities to control levels.

Table 1
In vitro HSL activity of the epididymal adipose tissue of untreated mice and of mice treated for 7 days with PFOA and then allowed to recover for 10 days

HSL activity (μmol glycerol released/g wet epididymal adipose tissue—3 hr)		
Control mice (untreated)	PFOA-treated mice	Mice treated with PFOA and allowed to recover for 10 days
4.07 ± 1.08	30% higher than the control value[a]	1.63 ± 1.06[*]

The values shown are the differences between the hormone-stimulated and unstimulated release of glycerol. All values are means ± SD for four animals. Differences were analyzed statistically employing the paired Student's *t*-test.

[a] See Ref. [9].

[*] $P < 0.05$ compared to the control (untreated) value. For further details, see Section 2.

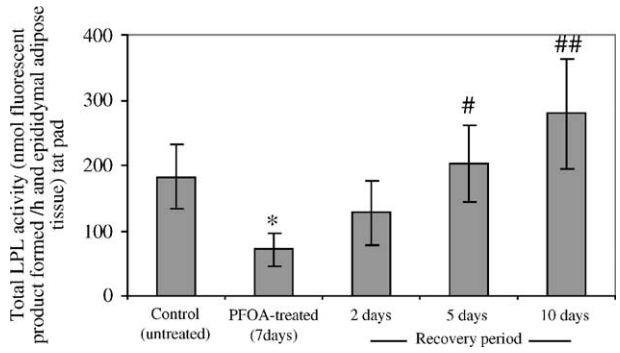

Fig. 3. Effects of PFOA administration and subsequent recovery for up to 10 days on the total LPL activity of epididymal adipose tissue. The results shown are the means ± SD (the bars) of the values for four animals. Differences were analyzed statistically employing the one-way ANOVA. $^{*}P < 0.05$ compared to the control (untreated) value. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to the PFOA-treated (7 days) value. For further details, see Section 2.

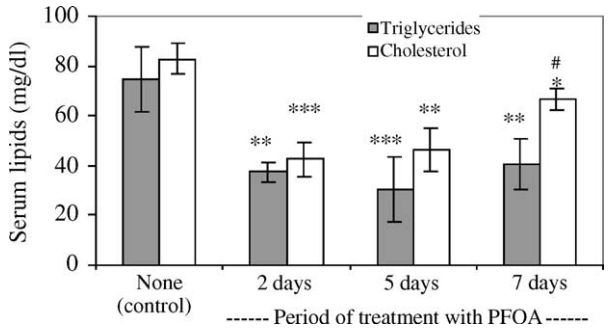

Fig. 4. The time-course of the effect of PFOA administration on serum levels of triglyceride (filled column) and cholesterol (open column) in mice. The results shown are the means ± SD (the bars) of the values for four animals. Differences were analyzed statistically employing the one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the control (untreated) value. $^{\#}P < 0.05$ compared to the PFOA-treated (2 days) value. For further details, see Section 2.

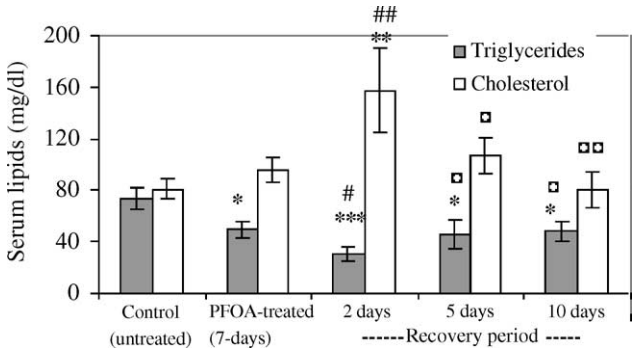

Fig. 5. Recovery of serum levels of triglyceride (filled column) and cholesterol (open column) after withdrawal of PFOA. The results shown are the means ± SD (the bars) of the values for four animals. Differences were analyzed statistically employing the one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to the control (untreated) value. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to the PFOA-treated (7 days) value. $\square P < 0.05$ and $\square\square P < 0.01$ compared to the value for the 2-day recovery group. For further details, see Section 2.

3.5. Time-course of the effect of PFOA administration on serum levels of triglycerides and cholesterol

In order to establish a possible relationship between the hepatomegaly and adipose tissue atrophy caused by PFOA treatment, serum lipids, a possible link between these two tissues, were quantitated. The serum triglyceride content was significantly reduced during the entire 7-day period of treatment with PFOA (Fig. 4), in agreement with the effects of other PPs as well. Although serum cholesterol was also significantly decreased by the second day of treatment, this value increased again to 80% of the control by the seventh day of treatment. This rebound suggests that PFOA might cause changes in addition to the normal hypolipidemic effect of PPs.

3.6. Serum levels of cholesterol and triglycerides during recovery from PFOA treatment

Following termination of PFOA treatment, serum cholesterol continued to increase (2-fold) during the first 2 days of recovery and thereafter began to fall, returning to normal levels within 10 days (Fig. 5). The rate in this decrease was similar to the rate observed for adipose tissue weight gain, indicating a possible relationship between these two parameters.

In contrast to these changes in serum cholesterol following withdrawal of PFOA from the diet, the PFOA-induced decrease in triglycerides was relatively preserved during the entire 10-day recovery period, with the lowest level actually being obtained after 2 days of recovery (Fig. 5). This relative preservation of PFOA-induced changes was also observed with hepatic CPT and COT activities and the liver weight.

3.7. Effects of PFOA on the levels of lipids and apolipoprotein in individual subfractions of serum

Changes in different lipoprotein particles occurring during PFOA treatment and subsequent recovery were also explored to further clarify the alteration in details. After PFOA treatment for 7 days, the decrease in total serum cholesterol content was relatively evenly distributed in all serum subfractions (Fig. 6A). Separation of the serum proteins present in the different subfractions by SDS–PAGE (Fig. 6B) revealed that, although the total amount of apo-B100 protein was significantly decreased, the total amount of apo-B48 was not altered. However, apo-B48 was significantly enriched in the bottom subfraction of the plasma, suggesting the redistribution of apo-B48 to a denser particle. This finding is consistent with the observation by Okochi *et al.* [12] that PFOA added *in vitro* can cause apo-B48 lipoprotein to dissociate from lipids, resulting in the lipid-poor apo-B48-containing particles in this bottom fraction. This is in agreement with the significant decrease in the cholesterol content of this bottom subfraction in response to PFOA treatment as well.

In addition, exposure to PFOA slightly reduced the total level of A–I apolipoprotein in whole serum and in the HDL subfraction, while dramatically decreasing the level of this protein in the low density lipoprotein (LDL) subfraction (Fig. 6B).

4. Discussion

In the present investigation, we observed that, although decreasing somewhat, both the liver weight and hepatic fatty acid β-oxidation of mice treated with PFOA remain elevated for at least 10 days after cessation of treatment. These findings are consistent with our previous observation that the 13-fold increase in hepatic peroxisomal lauoryl-CoA oxidase activity observed in mice exposed to PFOA decreases significantly within 10 days of recovery, but is not restored to normal until after 20 days [14]. Furthermore, similar to the persistent hepatic peroxisome proliferation, the decreased plasma triglyceride level evoked by

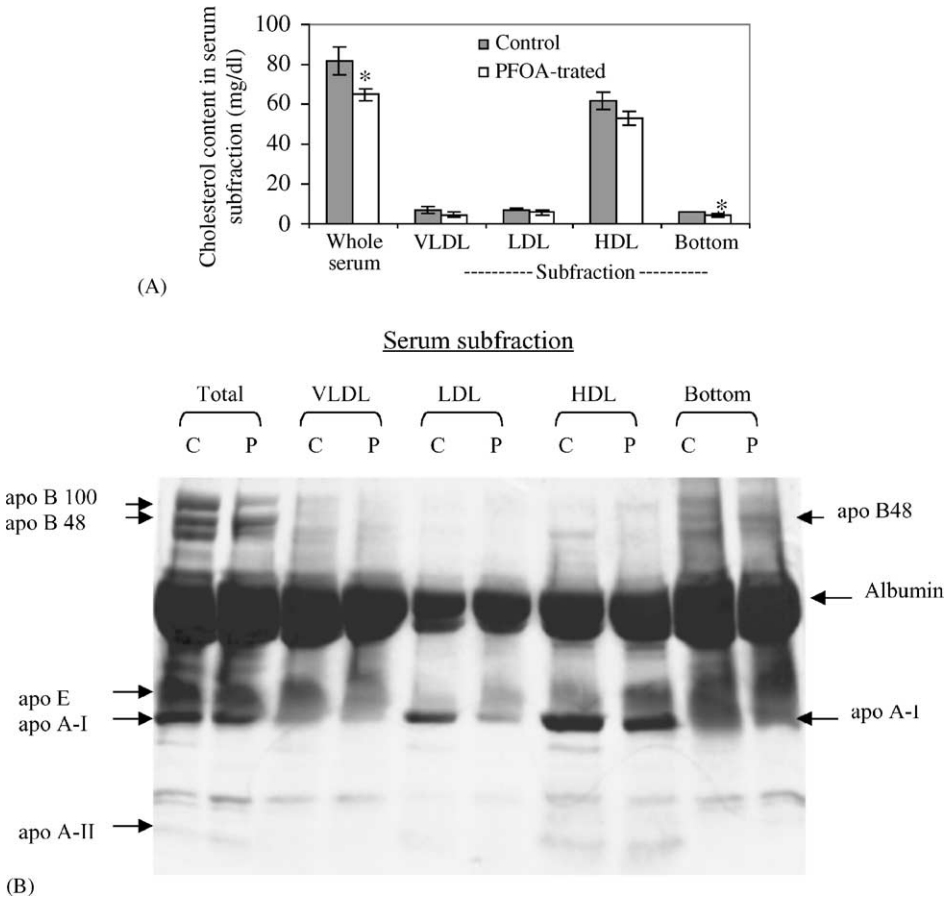

Fig. 6. (A) The cholesterol contents in subfractions of serum from control (untreated) (filled columns) and PFOA-treated mice (open columns). (B) Protein compositions of the lipoproteins present in total and different subfractions of serum from control (C) and PFOA-treated mice (P). After 7 days of treatment, sera from four mice in each group were pooled and separated by ultracentrifugation and the individual subfractions thus obtained subsequently analyzed. The profile shown is representative of independent analyses performed on three different groups of mice. Differences were analyzed statistically employing the paired Student's *t*-test. $^{*}P < 0.05$ compared to the control (untreated) value. For further details, see Section 2.

PFOA treatment remains low throughout the 10-day recovery period, suggesting that the liver weight, peroxisome proliferation and serum triglyceride level are coordinated, at least to some extent, during the recovery period.

We observed previously that the time-course of adipose tissue atrophy upon PP treatment closely resembles that of hepatic peroxisome proliferation [2]. However, in contrast to the persistent hepatomegaly and hepatic peroxisome proliferation caused by PFOA in mice, adipose tissue atrophy is rapidly and completely reversed within 10 days after termination of treatment, i.e. hepatic peroxisome proliferation and adipose tissue weight are not strictly coordinated during this period. In addition, the previously observed alterations in LPL and HSL activities during PFOA-induced adipose tissue atrophy [2] are also rapidly reversed during recovery. These findings further indicate that these activities play an essential role in adipose tissue atrophy and restoration.

Since hepatic capacity for fatty acid β-oxidation is retained during recovery, uptake and/or release of triglycerides from adipocytes *via* altered LPL and HSL activities may contribute to the persistent low level of serum triglyceride.

Interestingly, the elevation of serum cholesterol 2 days after cessation of PFOA treatment is normalized following 10 days of recovery, suggesting a close relationship between serum cholesterol level and adipose tissue weight. That both LPL and HSL activities and serum cholesterol levels exhibit the same time-course as adipose tissue recovery raises question of possible relationships between these phenomena.

To explain our observations, we have previously suggested a mechanism (for details, see Ref. [2]) based on the involvement of insulin in regulation of both LPL and HSL activities. It has been reported that both of these activities can be influenced by the insulin sensitivity of adipose tissue, which synergistically influences PPARγ-dependent regulation of LPL expression [26] and noradrenaline-stimulated phosphorylation of HSL [25]. Furthermore, it has been demonstrated that the insulin receptors are located in a special plasma membrane domain, the so-called caveolae [27], and that alteration of the lipid composition and, especially, depletion of cholesterol in the caveolae can influence insulin signaling [28]. Thus, a decrease in the cholesterol content of adipocyte membrane

caveolae will probably attenuate insulin signaling [28], thereby leading to the changes in LPL and HSL activities observed here.

As is also the case for other PPs, PFOA enhances hepatic clearance of the lipid particle remnant *via* PPARα-dependent processes [9–11,29], as well as reducing triglyceride and cholesterol secretion from the liver in the form of lipoprotein particles, VLDL [12,13]. Consequently, net hepatic cholesterol secretion is reduced, resulting in a decrease in the serum cholesterol content during the first 5 days of treatment. In addition, in primary cultures of rat hepatocytes PFOA can dissociate apo-B48 from lipids during the assembly of VLDL particles [12]. This PPARα-independent effect can further impair LDL formation and, subsequently, LDL particle uptake *via* the apo-B-dependent LDL receptor. The occurrence of such a phenomenon is supported by the observation that upon serum subfractionation, apo-B48-containing particles are shifted to the fraction of highest density, indicating impairment of LDL formation and/or uptake *in vivo*.

The observed cholesterol ''rebound'' phenomenon in Fig. 4 might be due to the accumulation of impaired LDL cholesterol in serum. However, all of these effects reduce the supply of serum cholesterol to adipose tissues, although by different mechanisms. Thus, both PPARα-dependent and -independent effects of PFOA might decrease the cholesterol availability to adipocytes. An intricate synergism may be suggested between PPARα-dependent (increased hepatic remnant clearance, cholesterol excretion from the body and reverse cholesterol transport from peripheral tissues to the liver) and PPARα-independent (apo-B48 dissociation, reduced VLDL secretion and its effects on LDL uptake) mechanisms. Preliminary experiments on PPARα-null mice (unpublished data) are also supportive of the occurrence of both PPARα-dependent and -independent pathways in the phenomena studied here.

Furthermore, the reported enhancement of ABCA1-dependent reverse cholesterol transport upon treatment with PPs (for a review, see Ref. [30]) may well increase serum HDL cholesterol levels and also favour removal of cholesterol from the plasma membrane, especially, from caveolae [31,32]. Further experiments are obviously required to test this hypothesis, for example, direct determination of the level of cholesterol and insulin signaling in the adipocyte plasma membrane.

Together, these effects may lower the cholesterol contents of certain peripheral tissues, and especially of the caveolae [30], thus, affecting insulin signaling in the plasma membrane of adipocytes.

In summary, the recovery experiments described here reveal that persistent hepatic peroxisome proliferation and regain of adipose tissue occur after cessation of PFOA treatment, i.e. these phenomena are not strictly coordinated. The changes in the weight of adipose tissue involve alterations in LPL and HSL activities, as well as, possibly, changes in the availability of circulating cholesterol.

Will these effects of PPs influence adipose tissues in humans as well? Since PPs exert hypolipidemic effects both in humans and rodents [33], the associated risk for health problems, such as insulin resistance in adipocytes, may also occur in human beings. Fortunately, instead of apo-B48, human liver secrets apo-B100, which is not dissociated from lipids by PFOA treatment, so that there should be no direct effect of PFOA on cholesterol uptake by human adipose tissues. However, the hypolipidemic effect occurs in humans as well. Is this phenomenon a friend or foe? The answer to this question is strongly dependent on the particular situation. Potent PPs may function as effective anti-atherosclerotic and anti-diabetes drugs for hyperlipidemic patients, while adversely disturbing lipid metabolic balance in healthy individuals.

Acknowledgments

The Knut and Alice Wallenberg Foundation (Stockholm) and the Environmental Fund of the Swedish Association of Graduate Engineers (Stockholm) supported this study financially. We greatly appreciate Professor Bo Angelin's reading our manuscript and valuable suggestions for improvement. We are also highly grateful to Jarl Olsson for his help with the animals.

References

[1] Corton JC, Lapinskas PJ, Gonzalez FJ. Central role of PPARalpha in the mechanism of action of hepatocarcinogenic peroxisome proliferators. Mutat Res 2000;448:139–51.
[2] Xie Y, Yang Q, Nelson BD, DePierre JW. Characterization of the adipose tissue atrophy induced by peroxisome proliferators in mice. Lipids 2002;37:139–46.
[3] Madsen L, Guerre-Millo M, Flindt EN, Berge K, Tronstad KJ, Bergene E, Sebokova E, Rustan AC, Jensen J, Mandrup S, Kristiansen K, Klimes I, Staels B, Berge RK. Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance. J Lipid Res 2002;43:742–50.
[4] Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulain P, Derudas B, Herbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 2000; 275:16638–42.
[5] Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S, Ezaki O. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 2000;49:1534–42.
[6] Mancini FP, Lanni A, Sabatino L, Moreno M, Giannino A, Contaldo F, Colantuoni V, Goglia F. Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats. FEBS Lett 2001; 491:154–8.
[7] DePierre JW, Sohlenius AK, Cai Y, Eriksson AM, Andersson K, Sundberg C. Peroxisome proliferation in response to xenobiotics. Biochem Soc Trans 1995;23:425–9.
[8] Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 1998;98:2088–93.
[9] Malmendier CL, Lontie JF, Delcroix C, Dubois DY, Magot T, De Roy L. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic

patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. Atherosclerosis 1989;77: 139–49.

[10] Staels B, Vu-Dac N, Kosykh VA, Saladin R, Fruchart JC, Dallongeville J, Auwerx J. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. J Clin Invest 1995;95:705–12.

[11] Auwerx J, Schoonjans K, Fruchart JC, Staels B. Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. Atherosclerosis 1996;124(Suppl):S29–37.

[12] Okochi E, Nishimaki-Mogami T, Suzuki K, Takahashi A. Perfluorooctanoic acid, a peroxisome-proliferating hypolipidemic agent, dissociates apolipoprotein B48 from lipoprotein particles and decreases secretion of very low density lipoproteins by cultured rat hepatocytes. Biochim Biophys Acta 1999;1437:393–401.

[13] Nishimaki-Mogami T, Suzuki K, Takahashi A. The role of phosphatidylethanolamine methylation in the secretion of very low density lipoproteins by cultured rat hepatocytes: rapid inhibition of phosphatidylethanolamine methylation by bezafibrate increases the density of apolipoprotein B48-containing lipoproteins. Biochim Biophys Acta 1996;1304:21–31.

[14] Sohlenius AK, Lundgren B, DePierre JW. Perfluorooctanoic acid has persistent effects on peroxisome proliferation and related parameters in mouse liver. J Biochem Toxicol 1992;7:205–12.

[15] Saha SK, Moriya M, Ohinata H, Kuroshima A. Lipid interference with fluorometric assay of DNA in adipose tissues under various conditions. Jpn J Physiol 1994;44:421–31.

[16] Bieber LL, Abraham T, Helmrath T. A rapid spectrophotometric assay for carnitine palmitoyltransferase. Anal Biochem 1972;50:509–18.

[17] Del Prado M, Hernandez-Montes H, Villalpando S. Characterization of a fluorometric method for lipoprotein lipase. Arch Med Res 1994; 25:331–5.

[18] Herrera E, Lasuncion MA, Castro M, Gomez-Coronado D, Martin A, Quack G. Studies with etofibrate in the rat. Part I. Effects on glycerol, free fatty acid and triacylglycerol metabolism. Biochim Biophys Acta 1988;963:42–52.

[19] Brandes R, Arad R, Bar-Tana J. Adipose conversion of cultured rat primary preadipocytes by hypolipidemic drugs. Biochim Biophys Acta 1986;877:314–21.

[20] Converse CA, Skinner ER. Lipoprotein analysis: a practical approach. New York: Oxford University Press; 1992.

[21] Nemeth PM, Hitchins OE, Solanki L, Cole TG. Fluorometric procedures for measuring triglyceride concentrations in small amounts of tissue and plasma. J Lipid Res 1986;27:447–52.

[22] Heider JG, Boyett RL. The picomole determination of free and total cholesterol in cells in culture. J Lipid Res 1978;19:514–8.

[23] Lowry OH, Rosebrough NJ, Farr AL, Randall AJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.

[24] Berge RK, Aarsland A. Correlation between the cellular level of long-chain acyl-CoA, peroxisomal beta-oxidation, and palmitoyl-CoA hydrolase activity in rat liver. Are the two enzyme systems regulated by a substrate-induced mechanism? Biochim Biophys Acta 1985;837: 141–51.

[25] Stralfors P, Bjorgell P, Belfrage P. Hormonal regulation of hormone-sensitive lipase in intact adipocytes: identification of phosphorylated sites and effects on the phosphorylation by lipolytic hormones and insulin. Proc Natl Acad Sci USA 1984;81:3317–21.

[26] Zhang B, Berger J, Zhou G, Elbrecht A, Biswas S, White-Carrington S, Szalkowski D, Moller DE. Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. J Biol Chem 1996;271:31771–4.

[27] Gustavsson J, Parpal S, Karlsson M, Ramsing C, Thorn H, Borg M, Lindroth M, Peterson KH, Magnusson KE, Stralfors P. Localization of the insulin receptor in caveolae of adipocyte plasma membrane. FASEB J 1999;13:1961–71.

[28] Parpal S, Karlsson M, Thorn H, Stralfors P. Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J Biol Chem 2001;276:9670–8.

[29] Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta 1996;1302: 93–109.

[30] Fielding CJ. Caveolae and signaling. Curr Opin Lipidol 2001;12: 281–7.

[31] Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995;36:211–28.

[32] Yokoyama S. Apolipoprotein-mediated cellular cholesterol efflux. Biochim Biophys Acta 1998;1392:1–15.

[33] Hennuyer N, Poulain P, Madsen L, Berge RK, Houdebine LM, Branellec D, Fruchart JC, Fievet C, Duverger N, Staels B. Beneficial effects of fibrates on apolipoprotein A–I metabolism occur independently of any peroxisome proliferative response. Circulation 1999;99: 2445–51.