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Perfluorooctanoic acid exposure disturbs glucose metabolism in mouse liver

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ABSTRACT

Environmental pollutants such as perfluorooctanoic acid (PFOA) can influence human metabolism processes and are associated with certain metabolic diseases. To investigate the effect of PFOA on liver glucose homeostasis, adult male Balb/c mice were orally administered 1.25 mg/kg of PFOA for 28 d consecutively. Compared with the control mice, the body weights of the PFOA-treated mice were unchanged following exposure. However, PFOA exposure increased fasting blood glucose levels and decreased glycogen and glucose content in the liver of treated mice, but did not influence blood insulin significantly. The increased blood glucagon might contribute to the hyperglycemia observed in the PFOA-treated group compared with the control group. In addition, pyruvate tolerance tests supported enhanced glucose production ability in PFOA-exposed mice. Consistent with the increase in blood glucose and decrease in hepatic glucose and glycogen, PFOA exposure decreased the protein level of glycogen synthase in the mouse liver, but increased the level of glucokinase. Furthermore, liver pyruvate, as well as mRNA levels of enzymes involved in the Krebs cycle, such as citrate synthase, isocitrate dehydrogenase, and alpha-ketoglutarate dehydrogenase, increased in the PFOA-treated group. PFOA exposure did not affect muscle glucose or glycogen levels. Indirect calorimetry showed higher VO_2 consumption and respiratory quotient values in the PFOA-treated group compared with the control group, implying that PFOA treatment might promote energy consumption in mice, with a reliance on carbohydrates as a primary source of energy. Thus, our findings indicate that subacute exposure to PFOA might enhance glycogenolysis and gluconeogenesis and promote carbohydrate consumption.

1. Introduction

Over the last few decades, due to the rise in hypertension, diabetes, and other cardiovascular diseases, metabolic disorders have become of increasing concern (Meo, 2016; Yi et al., 2017). Genetic, dietary, and behavioral factors, such as a lack of physical activity and overeating, all contribute to metabolic syndromes (Lyche et al., 2011). In addition to conventional risk factors, epidemiological studies have also suggested that exposure to environmental chemicals can lead to metabolic dysfunctions, including insulin resistance and type 2 diabetes (Kim et al., 2014; Pestana et al., 2014; Yanagisawa et al., 2014).

Perfluoroalkyl substances (PFASs) are a class of synthetic surfactants widely applied in industrial and commercial fields. Since PFASs have strong C−F bonds, they exhibit high environmental persistence, bioaccumulation, and long-distance transport (Cui et al., 2010). Perfluorooctanoic acid (PFOA), a well-known PFAS, has been routinely detected in environmental samples, wildlife, and humans (Lau, 2012). A series of laboratory studies demonstrated that PFASs can cause a variety of adverse health outcomes, including lipid metabolism disturbance, liver enlargement, developmental toxicity, neurotoxicity, endocrine disruption, and reproductive toxicity (Butenhoff et al., 2004; Kennedy et al., 2004; Lau et al., 2007; White et al., 2011). However, the effect of PFAS exposure on carbohydrate metabolism and related diseases remains controversial. Epidemiologic studies on the association between PFASs and diabetes are inconsistent. High serum PFOA content among occupational workers has been reported to confer an elevated risk of diabetes mortality (Leonard et al., 2008; Steenland and Woskie, 2012). In contrast, no association between PFOA concentration and blood glucose levels and type 2 diabetes have been found in the general population (Frisbee et al., 2009; MacNeil et al., 2009; Steenland et al., 2009a,b; Melzer et al., 2010; Karnes et al., 2014). Although no direct epidemiological correlation has been reported, PFASs may be associated with the risk factors of type 2 diabetes, such as glucose homeostasis and metabolic disorders (Lin et al., 2009).

In laboratory animal studies, body weight loss and dyslipidemia have been observed after PFAS exposure, and are suggested to be due to the activation of the PPARα pathway in the liver (Lau et al., 2007). The activation of PPARα reduces glucose utilization and increases fatty acid

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uptake and oxidation in the hepatocytes, leading to the accumulation of lipid intermediate metabolites (Reddy and Hashimoto, 2001; Finck et al., 2002). Previous research has indicated that levels of fatty acid metabolites appear to be elevated in the liver, whereas glucose levels appear to be suppressed in both serum and liver, with a significant downregulation of many hepatic genes involved in glucose metabolism, following PFOA exposure (Tan et al., 2013). Conversely, our earlier study found higher blood glucose in PFOA-treated mice (Yan et al., 2015). However, these impairments of glucose homeostasis have been observed under relatively high doses of PFOA, and have been accompanied by significant decreases in body weight. The effect of relatively low doses of PFASs on glucose metabolism remains unclear. In the present study, we sought to determine the influences of PFOA on glucose homeostasis, as well as on hepatic mRNA and proteins levels of key enzymes involved in glucose metabolism, in mice after low dose PFOA exposure (1.25 mg/kg/d consecutively for 28 d).

2. Materials and methods

2.1. Animal treatment

Male Balb/c mice (aged 6–8 weeks) were purchased from Weitong Lihua Laboratory Animal Center (Beijing, China). Mice were housed in cages at 20–26°C with 50–70% relative humidity and a 12:12 h light:-dark cycle. After adaptation for one week, 40 mice were randomly divided into two groups (control group administrated with Milli-Q water and PFOA group treated with 1.25 mg/kg/d of PFOA consecutively for 28 d (96% purity, Sigma-Aldrich, St. Louis, MO, USA). Body weights of mice were recorded every week. At the end of exposure, blood from the ophthalmic vein was collected and animals were sacrificed via cervical dislocation. Livers were removed, weighed, and stored at − 80 °C until further use. The blood was collected, centrifuged, and stored at − 80 °C until further analysis. All animal care and treatment protocols were approved by the Committee on the Ethics of Animal Experiments from the Institute of Zoology, Chinese Academy of Sciences.

2.2. Determination of PFOA content in serum and liver

The levels of PFOA in serum and liver were analyzed using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) (Pan et al., 2017).

2.3. Levels of serum glucagon and insulin

Serum glucagon and insulin concentrations were measured using a glucagon ELISA kit and mouse ultrasensitive insulin ELISA kit, respectively (ALPCO Diagnostics, NH, USA).

2.4. Glucose, pyruvate, and insulin tolerance tests

Another 40 mice with the same groups and under the same treatment as above were used for glucose (GTT), pyruvate (PTT) and insulin tolerance tests (ITT) (Yan et al., 2015). We performed the GTT (n = 5 animals per group) on day 25, and the PTT (n = 10 animals per group) and ITT (n = 5 animals per group) on day 27. The animals were sacrificed on day 29. The GTT, PTT, and ITT were performed via intraperitoneal injection with glucose (2 g/kg, 16 h fasting), pyruvate (1 g/kg, 16 h fasting), and insulin (0.75 IU/kg, 4 h fasting), respectively (0.75 IU/kg, 4 h fasting). Values for the area under the curve (AUC; arbitrary units) in GTT, PTT, and ITT were also calculated. Blood glucose baseline levels were determined with blood taken from the tail vain using an Accu-Chek Performa Glucometer (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's recommendations, and blood glucose levels were measured again at 15, 30, 60, 90, and 120 min after challenge.

2.5. Tissue glycogen, glucose, pyruvate, lactate, cAMP measurement and enzyme activity assay

Another 24 mice were randomly divided into two groups and were under the same exposure as mentioned above. At the end of the exposure experiment, the animals were starved for 4 h, with half in each group then challenged with insulin. The animals were used for tissue glycogen, lactate, glucose, pyruvate and cAMP level detection, as well as enzyme activity, mRNA level, and Western blot analysis. All parameters were detected 10 min after the insulin challenge. The contents of glycogen, lactate, glucose, and pyruvate in the liver and thigh muscle after PFOA exposure were analyzed via commercial kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). cAMP content were measured via commercial ELISA kit from Bio-Swamp (Wuhan, China). The enzyme activities of hexokinases (HKs), 6-phosphofructokinase (PFK), and pyruvate kinases (PK) were analyzed in the liver and muscle using commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. qPCR and Western blot analysis

The mRNA levels of glucose transporter-2 (GLUT2), GLUT4, citrate synthase (CS), isocitrate dehydrogenase (ICDH), and alpha-ketoglutarate dehydrogenase (α-KGDHC) in the liver and muscle were detected with qPCR. The protein levels of glycogen synthase (GS) and its phosphorylated protein P-GS (S641), protein kinase B (PKB, or Akt) and its phosphorylated protein P-PKB (or P-Akt) (S473), GS kinase-3 beta (GSK3β) and its phosphorylated protein P-GSK3β (S9), cAMP regulatory element-binding protein (CREB) and its phosphorylated protein P-CREB (S133), as well as glucose-6-phosphatase (G6Pase) and glucokinase (GK) were detected in the liver using Western blot analysis. The qPCR and Western blot analyses were performed as per our previous study (Yan et al., 2015). Detailed information on the antibodies and qPCR primers are given in the Supplementary materials in Tables S1 and S2, respectively.

2.7. Oxygen consumption analysis

Another 18 mice under the same PFOA exposure as above were used for oxygen consumption analysis. After four weeks of PFOA exposure, indirect calorimetry was performed (Phenomaster/Labmaster, TSE systems, Bad Homberg, USA) on nine mice from each group (Castaneda et al., 2011). During the experiment, mice were individually housed in standard units, with ad libitum access to standard food and water. After a 24-h acclimation period, calorimetry measurements in every unit were performed and the assay continued for 24 h. The measured indexes included O_2 consumption (VO_2, mL/h/kg), CO_2 elimination (VCO_2, mL/h/kg), respiratory quotient (RQ), and food intake (g/d).

2.8. Statistical analysis

Statistical analyses between two groups were performed by two-tailed unpaired t-tests, whereas analyses among more than two groups were performed by one-way ANOVA and Tukey's post hoc tests. All statistical analyses were performed using SPSS 17.0 Software (SPSS, Inc., Chicago, IL, USA). Data were represented as means with standard errors (mean ± S.E.). A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Effect on PFOA on liver weight and internal content

The body weights of mice under PFOA treatment (1.25 mg/kg/d) did not change compared with that of the control group (Fig. 1a),

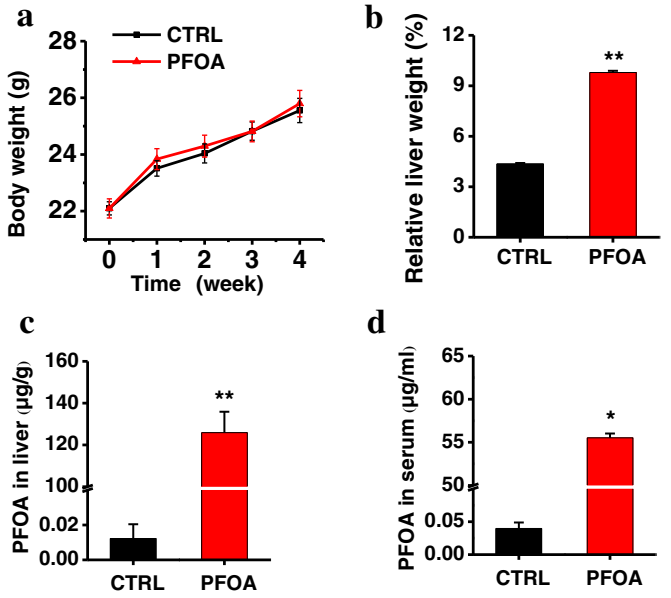

Fig. 1. Body weight (a) and relative liver weight (liver weight/body weight) (b) in mice after PFOA treatment. Results are means ± SE (n = 20). Levels of PFOA in liver (c) and serum (d) (mean ± SE, n = 3). Mice were treated with 1.25 mg/kg/d PFOA for 28 d. $^{*}p < 0.05$, $^{**}p < 0.01$ indicate significant differences between the control and exposure groups.

whereas relative liver weights increased significantly (Fig. 1b). To determine the level of internal exposure, the content of PFOA in the serum and liver of mice was analyzed with HPLC-MS/MS. Results showed that PFOA levels were higher in the liver (125.9 ± 10.0 μg/g) than in serum (55.5 ± 0.50 μg/mL) (Fig. 1c, d) following treatment.

3.2. Increase in initial blood glucose and glucagon levels and increase in glucose tolerance in mice after PFOA exposure

Exposure to PFOA led to a high level of fasting glycemia, with a 53.1% increase in the PFOA-treated group compared with the control group (Fig. 2a). However, no significant change in blood insulin was observed between the two groups (Fig. 2b). Although PFOA-exposed mice were under a state of high fasting blood glucose, the GTT results showed that the peak blood glucose levels were not higher in the PFOA-treated mice than in the control group after glucose injection (Fig. 2c). After pyruvate stimulation, the PFOA-treated group showed higher blood glucose levels from the start to the end of the assay (120 min after injection) (Fig. 2d). The ITT results showed that blood glucose in the PFOA-treated mice decreased to the same level as that observed in the control group 30 min after the insulin challenge, although PFOA-treated mice showed higher initial glucose levels. However, the glucose levels tended to increase again in the PFOA treatment group than control after 30 min after the insulin challenge, and at 120 min, PFOA-treated mice showed higher glucose levels than control significantly (Fig. 2e). Higher AUC of GTT and PTT was observed in PFOA treatment group than that in control, but no difference was observed in AUC of ITT between the two groups, indicating that mice under PFOA treatment still responded sensitively to insulin challenge (Supplementary material, Fig. S1). Although blood insulin was not significantly changed after PFOA treatment, blood glucagon levels increased markedly in the treated group compared with that in the control group (Fig. 2f).

3.3. PFOA exposure affected liver carbohydrate content in mice

Our results showed a significant reduction in liver glycogen content in the PFOA-treated group compared with the control group, which was opposite to the effect of insulin, which showed an increase (albeit non-significant) in the hepatic glycogen level 10 min after insulin stimulation (Fig. 3a). There was a significant decrease in liver glucose content in PFOA-exposed mice, though no liver glucose level change was observed 10 min after insulin stimulation (Fig. 3b). The level of pyruvate in the PFOA-treated group was significantly higher than that in the control group, and insulin stimulation exerted no obvious effect on pyruvate (Fig. 3c). Liver lactate content exhibited a decreasing trend in PFOA-exposed mice compared with that observed in the control. Similar to the effect of PFOA, insulin stimulation decreased the lactate levels compared with that in the control group (Fig. 3d). Levels of glycogen, glucose, pyruvate, and lactate were also measured in muscle. There was no significant difference between the PFOA-treated and control groups after 4 h starvation, though a decreasing trend was observed in muscle glycogen. Insulin stimulation did not change glycogen, glucose, pyruvate, and lactate levels either in muscle (Supplementary material, Fig. S2).

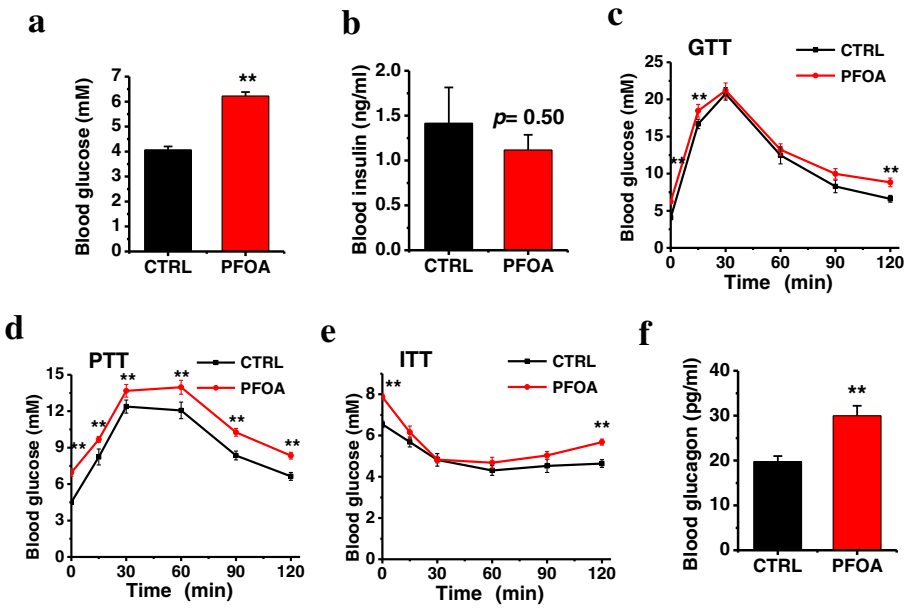

Fig. 2. Fasting blood glucose (a), insulin (b), and blood glucose concentration changes after the glucose tolerance test (GTT) (c), pyruvate tolerance test (PTT) (d), insulin tolerance test (ITT) (e), and the blood glucagon level (f). Results are means ± SE (n = 10). $^{*}p < 0.05$ and $^{**}p < 0.01$ indicate significant differences between the control and exposure groups.

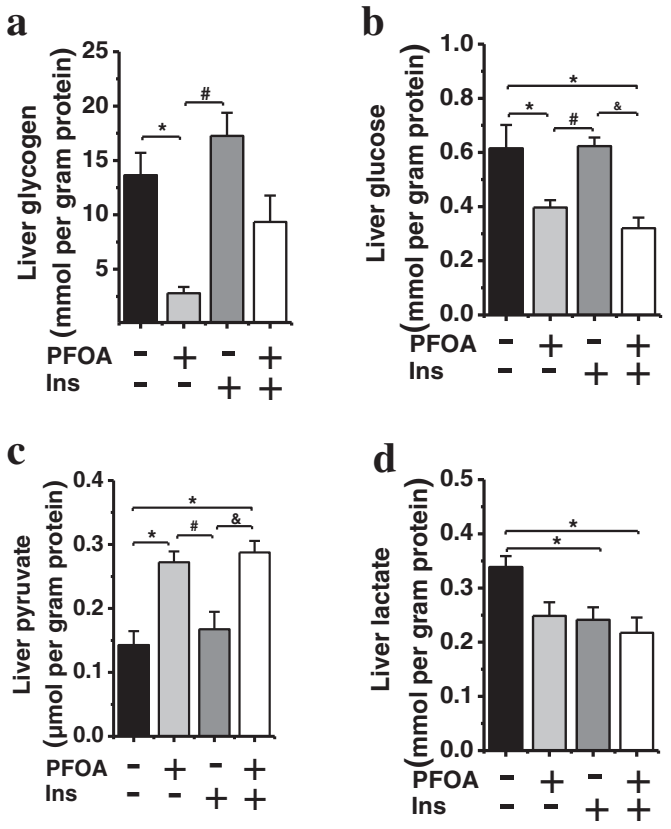

Fig. 3. Levels of glycogen (a), glucose (b), pyruvate (c), and lactate (d) in the mouse liver after 4 h starvation ($n = 6$). Animals were treated with 0 and 1.25 mg/kg/d of PFOA for 28 d in the insulin challenge groups, with parameters determined 10 min after the challenge. Results are means ± SE. $p < 0.05$ indicates significant differences and is shown with shown with * (compared with control), # (compared with PFOA group) and & (compared with insulin group).

3.4. PFOA exposure decreased GS with increased expression of GK and G6Pase in the mouse liver

Western blot analysis showed that the expressions of total GS and P-GS (S641) significantly decreased in the livers of 1.25 mg/kg/d PFOA-treated mice (Fig. 4a, b). Ten minutes after insulin stimulation, no significant increases in P-GS (S641) or total GS levels were observed. There were significant increases in the levels of P-PKB (S473) but no significant changes in total PKB between the PFOA-treated and control groups (Fig. 4c, d). Insulin stimulation showed similar effects as PFOA exposure, and tended to increase the levels of P-PKB (S473). In addition, PFOA exposure significantly increased the phosphorylated levels of downstream protein GSK3β (P-GSK3β (S9)) (Fig. 4c, e). Similar to PFOA, insulin stimulation also tended to increase P-GSK3β levels. Furthermore, the expression levels of G6Pase and GK were significantly increased in the liver after PFOA exposure, but were not significantly changed after insulin stimulation (Fig. 4f, g). The level of cAMP increased in the liver after PFOA exposure; and though the CREB level decreased, the ratio of P-CREB/CREB was enhanced after PFOA treatment (Supplementary material, Fig. S3).

3.5. PFOA exposure induced an acceleration in glucose transport and catabolism

Significant increase in the mRNA levels of GLUT2 was observed in the liver of mice, after PFOA treatment (Fig. 5a). However, the mRNA level of GLUT4 was unchanged in the muscle after treatment. The mRNA levels of CS, ICDH, and α-KGDHC increased significantly in the livers of PFOA-treated mice, but showed no significant changes in the muscles (Fig. 5b). The enzyme activities of PFK and PK in the liver were decreased in the PFOA-treated group compared with the control group, whereas the activities of hepatic HKs were unchanged. The activities of these three enzymes in muscle were all elevated in the PFOA-treated group compared with the control group (Fig. 5c).

3.6. PFOA exposure increased oxygen consumption and respiratory quotient

To explore oxygen consumption at the physiological level, VO_2, VCO_2, and RQ were measured using indirect calorimetry. Results showed that PFOA-treated mice exhibited higher VO_2 compared with that of the control group (Fig. 6a, b). The increase in oxygen consumption mainly occurred in the 12 h dark cycle period, with no difference observed during the 12 h light cycle period between the PFOA-treated and control groups (Fig. S4a). Similar to oxygen consumption, the RQ values for PFOA-treated mice over the 24-h measurement period were higher than those of the control group (Fig. 6c). In addition, the RQ values for the PFOA-exposure group significantly increased during the dark cycle period, whereas there was no difference during the light cycle period (Fig. S4b). Food intake was also measured and no significant differences were observed between the two groups during the 24-h measurement period ($p = 0.63$) (Fig. 6d).

4. Discussion

The most common toxic effects of PFOA in rodents are dyslipidemia and hepatomegaly (Lau et al., 2007). However, the effect of PFOA on glucose metabolism is controversial, with both hypoglycemia and hyperglycemia reported (Tan et al., 2013; Yan et al., 2015). The disturbance of glucose metabolism in these studies is often accompanied by body weight loss. Previously, we performed exposure study with various doses of PFOA, included 0.08, 0.31, 1.25, 5 and 20 mg/kg BW/d for 28 d consecutively, and many general parameters including body weights, organ weights and serum biochemical parameters were all evaluated (Yan et al., 2014). Under the 5 mg/kg dose, the body weight of the exposed animals decreased significantly, with nearly 13% decrease compared with control group. The more than 10% weight decrement means a systematic toxic effect caused by 5 mg/kg dose PFOA treatment, and given the fact that blood glucose is very sensitive, the glucose metabolism alteration under 5 mg/kg dose may be not special. Based on the above consideration, we carried more detailed study on glucose metabolism with 1.25 mg/kg exposure group. During the four weeks of 1.25 mg/kg PFOA exposure, no differences in body weight were observed between the treatment and control groups. The hepatomegaly observed in our study was consistent with other PFOA exposure research (Lau et al., 2007). In the current study, internal PFOA content was analyzed at the end of exposure, with 55.5 ± 0.50 μg/mL and 125.9 ± 10.0 μg/g PFOA recorded in the serum and liver, respectively. Although the serum PFOA content (55.5 μg/mL) in mice in our study was 61-fold higher than the geometric mean (0.899 μg/mL) reported in occupational workers (Olsen et al., 2003), when the highest concentration of PFOA (114.1 μg/mL) in occupational workers is considered (Ehresman et al., 2007), the value of the highest serum PFOA content in occupational workers is only about 2-fold of that in mice serum in our study. Under 1.25 mg/kg PFOA treatment, fasting blood glucose in mice increased 53.1% compared with that of the control. To test if PFOA-treated mice were still sensitive to insulin stimulation after PFOA exposure, we performed ITTs. Blood glucose decreased rapidly in the PFOA-treated mice, reaching the same level as that of the control group 30 min after insulin infusion. Although they exhibited higher initial and end glucose levels during ITT, the rapid decrease in blood glucose after insulin challenge did not support insulin resistance in PFOA-treated mice. Results indicated that after PFOA exposure, hyperglycemic mice still exhibited a sensitive insulin response, although the hyperglycemia did not stimulate an increase in blood insulin. We

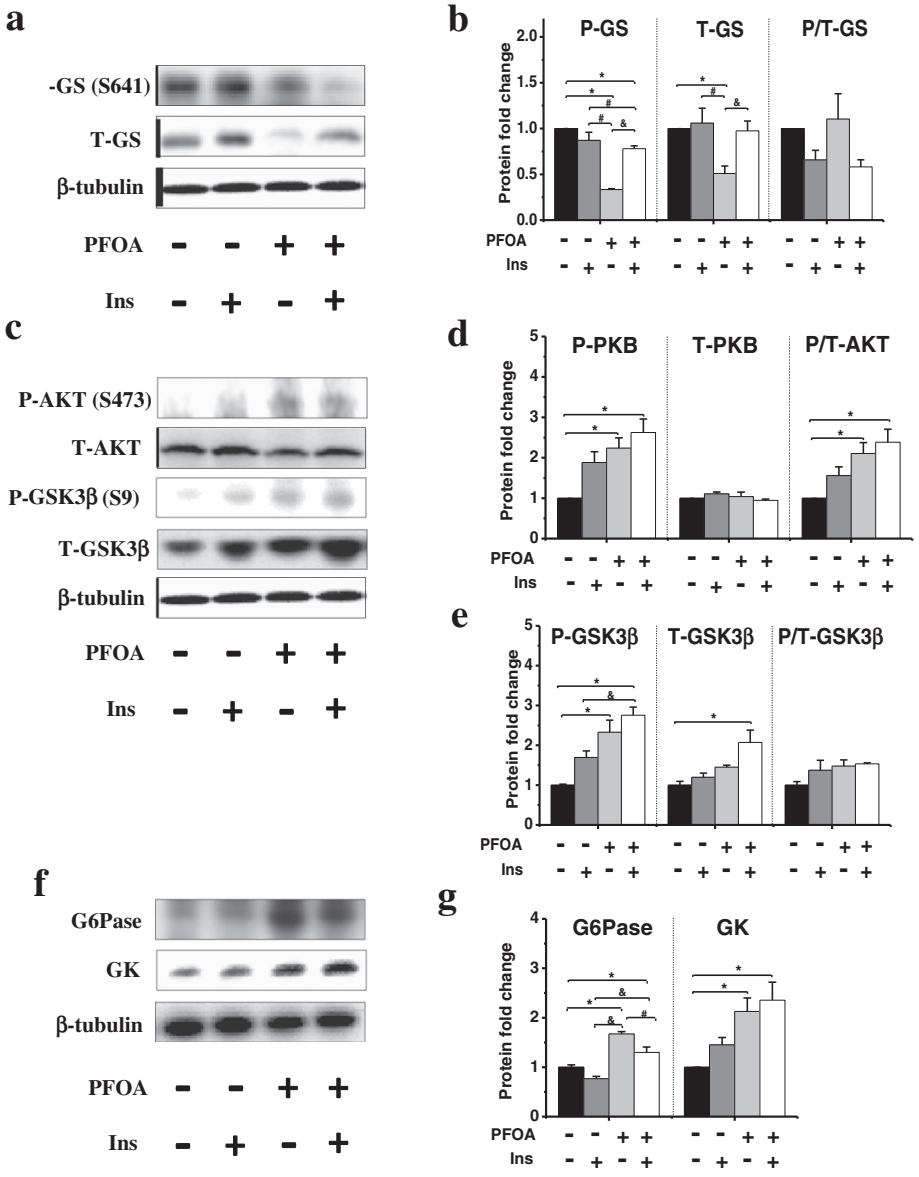

Fig. 4. Western blot results in the mouse liver after 4 h starvation. Total glycogen synthase (T-GS), phosphorylated protein P-GS (S641) (a) and their fold change (b); Total protein kinase B, phosphorylated protein P-PKB (S473), total glycogen synthase kinase-3 beta (T-GSK3β), phosphorylated protein P-GSK3β (S9) (c), and their fold change (d and e); glucose-6-phosphatase (G6Pase), glucokinase (GK) (f), and their fold change (g). Data are means ± SE (n = 6). $P < 0.05$ indicates significant differences and is shown with * (compared with control), # (compared with PFOA group) and & (compared with insulin group).

also carried out GTTs and PTTs. After D-glucose injection, the peak level of blood glucose was similar in the PFOA-treated and control groups, and decreased from the peak to the level before the injection. These results imply that glucose stimulation induced blood insulin release from pancreatic β cells. For the PTTs, the higher blood glucose levels from the start to the end of the assay in the PFOA-treated mice compared with the control mice reflected a vigorous hepatic gluconeogenesis ability. Both the GTT and PTT results did not support disturbance in the function of β cells in pancreatic islets by PFOA. The elevated blood glucose levels and the above challenge tests demonstrated that PFOA

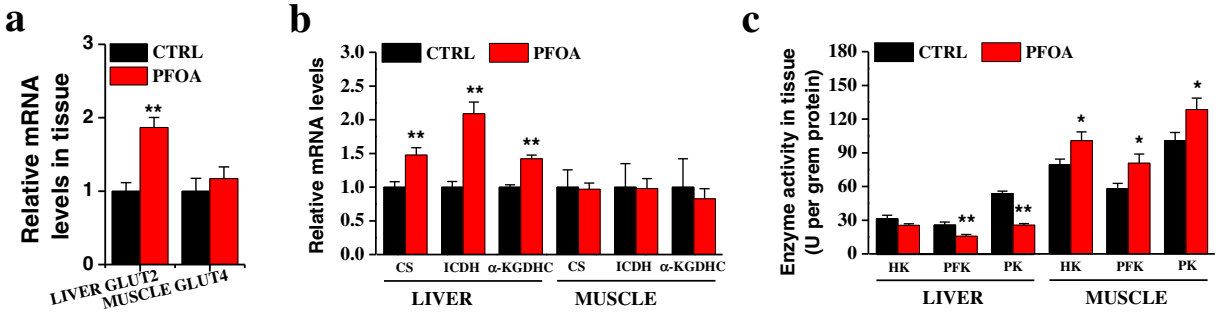

Fig. 5. Expression levels of glucose transporter-2 (GLUT2) and GLUT4 (a), citrate synthase (CS), isocitrate dehydrogenase (ICDH), and alpha-ketoglutarate dehydrogenase (α-KGDHC) (b); enzyme activities of hexokinase (HK), 6-phosphofructokinase (PFK), and pyruvate kinase (PK) (c) in liver and muscle after PFOA treatment. Data are means ± SE (n = 6). $^{*}p < 0.05$; $^{**}p < 0.01$ indicate significant differences between the control and exposure groups.

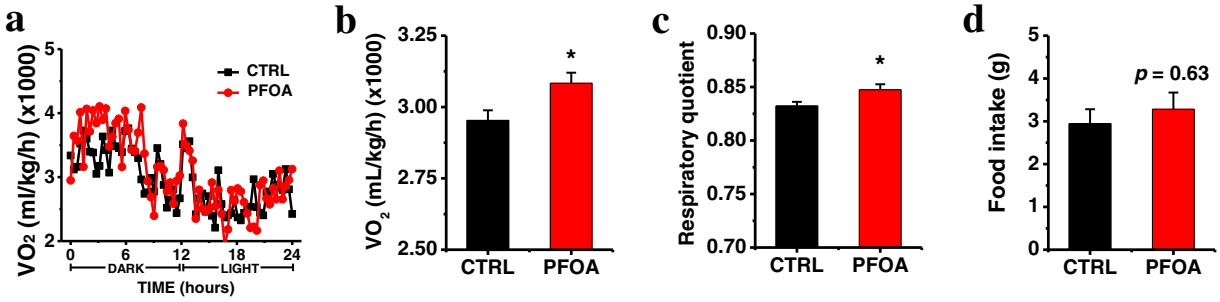

Fig. 6. Energy expenditure (a), total oxygen consumption (b), total respiratory quotient (c), and food intake (d). Results are means ± SE ($n = 9$). $^{*}p < 0.05$; $^{**}p < 0.01$ indicate significant differences between the control and exposure groups.

treatment resulted in increased hepatic glucose products in mice. Previous research has shown that stress can induce glucagon increase, which, in turn, orchestrates hyperglycemia (Harp et al., 2016). An increase in blood glucagon levels was observed in the PFOA-treated mice in the current study. Elevated blood glucagon, which counteracts the action of insulin on glucose synthesis (Pilkis et al., 1988), might be a contributor to hyperglycemia in PFOA-treated mice, and the high glucose tolerance in PFOA-exposed mice might be an adaptive or stress-induced response.

The liver plays a key role in glucose metabolism. It maintains blood glucose levels within a narrow range by balancing glucose uptake and storage via glycogenesis and glucose release via glycogenolysis and gluconeogenesis (Nordlie et al., 1999; Adeva-Andany et al., 2016). Four key enzymes (GK, G6Pase, GS, and glycogen phosphorylase) are involved in the conversion regulation of glycogen to glucose in the liver. GS controls the rate of hepatic glycogen synthesis from glucose-6-phosphate (G6P) and is opposed by glycogen phosphorylase. The activity of GS is controlled by phosphorylation and de-phosphorylation. Its de-phosphorylation can be induced by the activation of PKB (P-PKB (S473)) and inhibitory phosphorylation of GSK-3β by insulin (Radziuk and Pye, 2001; Hemmings and Restuccia, 2015). For free glucose production, glucose-6-phosphate is hydrolyzed to glucose by G6Pase in both gluconeogenic and glycogenolytic processes (Wang et al., 2015). In contrast, GK promotes glucose phosphorylation to G6P, which is the first step in glycolysis and enhances glycogen deposition in hepatocytes (Meijer, 2002; Ferrer et al., 2003; Francini et al., 2015). The cAMP/protein kinase A/CREB pathway also plays an important role in gluconeogenesis (Staehr et al., 2004). cAMP mediates phosphorylation of CREB, which powerfully controlled the transcription of several key unidirectional enzymes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase and G6Pase (Koo et al., 2005).

The effect of PFOA on hepatic glucose metabolism is shown in Fig. 7. In the treated mice, PFOA exposure resulted in an increase in fasting blood glucose levels, decrease in glycogen and glucose content in the liver, decrease in hepatic T-GS and P-GS protein levels, and increase in G6Pase protein levels. In addition, the transcriptional level of transmembrane carrier protein GLUT2 also increased in the liver, which facilitates glucose flux across the plasma membrane of the hepatocytes (Thorens, 1996). These results suggest that PFOA reduced glycogen in the liver by accelerating glycogenolysis and reducing glycogenesis. Muscle is another major tissue for glycogen deposition. In the present study, muscle glycogen levels tended to decrease after PFOA exposure, whereas exposure did not affect the levels of muscle glucose. Consistently, the mRNA level of GLUT4, which is the main transmembrane carrier protein of glucose in muscle (Huang and Czech, 2007), was unchanged.

Glucose production (and excretion into blood) by the liver is strongly inhibited, whereas glycogen production is markedly promoted, by high concentrations of blood insulin. Insulin elicits a diverse array of biological responses by binding to its specific receptor, which then commences various protein phosphorylation cascades (Saltiel and Kahn, 2001). The activation of PKB and successive inhibition of GSK3β by phosphorylation are important in the transmission of the insulin signal (Cheatham et al., 1994). In the current study, phosphorylated P-PKB (S473) and GSK3β (P-GSK3β (S9)) showed an increasing tendency in the mouse liver in both the insulin stimulation group and PFOA treatment group, implying that the ability to stimulate GS enzyme activation was increased. However, given the decrease in glycogen and glucose levels, as well as the decrease in total GS in the PFOA-treated liver, PFOA appears to exert a reverse effect to insulin stimulation.

Glucagon stimulate hepatic glucose primarily through via cAMP/PKA/CREB pathway (Chen et al., 2005), the increased glucagon, cAMP,

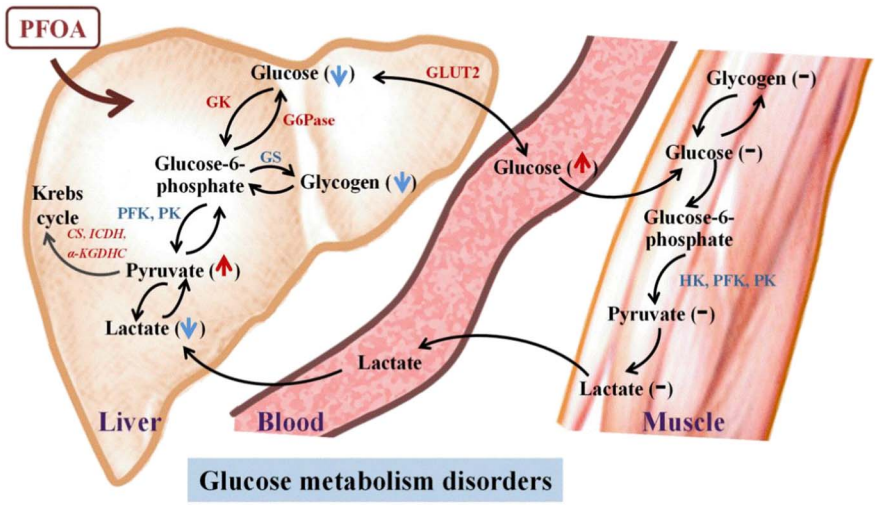

Fig. 7. Schematic describing the effect of PFOA on hepatic glucose metabolism. GS, glycogen synthase; G6Pase, glucose-6-phosphatase; GK, glucokinase; PFK, 6-phosphofructokinase; PK, pyruvate kinase; GLUT2, glucose transporter-2; CS, citrate synthase; ICDH, isocitrate dehydrogenase; α-KGDHC, alpha-ketoglutarate dehydrogenase. Glucose metabolism substrates are labelled with (↑), (↓), and (−), in which red (↑) means increase, blue (↓) means decrease, and (−) means no change detectable after PFOA treatment compared with the control. Enzymes involved in glucose metabolism are labelled red for an increase and blue for a decrease after PFOA treatment compared with the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

P-CREB/CREB ratio, and G6Pase in our study supported a vigorous hepatic gluconeogenesis in PFOA-treated mice. HK, PFK, and PK, are the first, third, and last enzymes along the glycolysis pathway, and thus can limit the overall glycolytic rate (Xie et al., 2016). Pyruvate serves as a substrate of the Krebs cycle, with CS, ICDH, and α-KGDHC also involved. Lactate participates in the Krebs cycle by converting to pyruvate and by the de novo synthesis of glucose via the gluconeogenesis pathway (Adeva-Andany et al., 2016). Here, PFOA exposure increased both pyruvate and lactate levels in the liver. Furthermore, CS, ICDH, and α-KGDHC were also increased in the livers of PFOA-treated mice compared with those of the control group, indicating that pyruvate oxidation by the Krebs cycle was enhanced in the liver after PFOA exposure. We further explored the effect of PFOA on the enzyme activities of PFK and PK in the glycolytic pathway. The decrease in enzyme activity showed that the ability to convert G6P to pyruvate decreased in livers of the PFOA-treated group compared with the control group. After PFOA treatment, no changes in pyruvate or lactate level in the muscle, or in mRNA levels of CS, ICDH and α-KGDHC, were observed. Given the relatively stable levels of glucose, glycogen, pyruvate, and lactate in the muscle after PFOA treatment, we inferred a weak effect on muscle glucose metabolism by PFOA. The weak effect might be due to less accumulative characteristics in muscle than in enriched protein tissues such as liver and serum (Luebker et al., 2002; Jones et al., 2003; Sheng et al., 2016). However, PFOA increased the enzyme activities of HK, PFK and PK in the muscle. Given that these enzymes catalyze irreversible reactions from G6P to pyruvate in the glycolysis pathway (Xie et al., 2016), this result implies that the conversion ability of G6P to pyruvate in muscle was enhanced after PFOA treatment. Insulin stimulation did not change pyruvate levels in either the liver or muscle, but a decrease trend in lactate was observed in the liver after PFOA treatment.

Given the effects of dyslipidemia, which is a common phenomenon after PFOA exposure, and hyperglycemia in our study, we examined whether the use of energy source changed by PFOA treatment. The RQ value varies with carbohydrate and lipid oxidation, reflecting the use of lipids or carbohydrates as a primary energy source in organisms. It usually ranges from 1.0, which represents the value expected for pure carbohydrate oxidation, to approximately 0.7, which represents the value expected for pure fat oxidation (Westbrook et al., 2009). In the present study, RQ values for PFOA-treated mice were moderately higher than those of the control group during the 24-h measurement period. These data imply that PFOA exposure might increase mouse oxygen consumption, and consumption might be focused on carbohydrates as the primary source of energy. But given the fact that the difference in RQ values between control and PFOA-treated mice is marginal, any interpretation on its physiological significance should be careful. Earlier studies have shown that higher fasting RQ values, which indicate decreased fat oxidation, are linked to weight gain, metabolic inflexibility, and insulin resistance (Zurlo et al., 1990; Ukropcova et al., 2007). Compared with the control group here, however, weight did not increase in the mice exposed to PFOA, and the exposed animals still responded to the insulin challenge sensitively. Increases in movement and feeding can lead to high VO_2 in mice (Westbrook et al., 2009). In the present study, no average daily feed intake change was observed during the 24-h indirect calorimetry experiment, although movement measurement in PFOA-treated mice was not conducted. Whether PFOA treatment increases movement activity, and thus contributes to higher oxygen consumption in mice, needs further exploration.

In conclusion, PFOA exposure induced high fasting blood glucose and decreased the contents of glycogen and glucose in the liver, accompanied by a decrease in glycogen synthase and increase in G6Pase. These results suggest that PFOA might increase the rate of glycogenolysis in the liver and glucose release from the liver into the blood, and enhance the downstream glycolytic pathway and Krebs cycle. In addition, PFOA treatment might promote energy consumption in mice, with a reliance on carbohydrates as a primary source of energy.

Conflict of interest

We declare that we have no conflict of interest.

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Appendix A. Supporting data

Supplementary Tables S1 and S2 provide detailed information on the antibodies and primers used for Western blot and qPCR. AUC of glucose tolerance test (GTT), pyruvate tolerance test (PTT), insulin tolerance test (ITT) are shown in Fig. S1. Levels of glycogen, glucose, pyruvate, and lactate in muscle after 4 h starvation are shown in Fig. S2. Levels of cAMP, total cAMP regulatory element-binding protein (T-CREB), phosphorylated protein P-CREB (S133) and their fold change in the mouse liver after 4 h starvation are shown in Fig. S3. Oxygen consumption and the respiratory quotient in light and dark conditions are shown in Fig. S4. Supplementary data associated with this article can be found in the online version, at doi: https://doi.org/10.1016/j.taap.2017.09.019.

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