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Adverse effects of triclosan on kidney in mice: Implication of lipid metabolism disorders

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ABSTRACT

Triclosan (TCS) is a ubiquitous antimicrobial used in daily consumer products. Previous reports have shown that TCS could induce hepatotoxicity, endocrine disruption, disturbance on immune function and impaired thyroid function. Kidney is critical in the elimination of toxins, while the effects of TCS on kidney have not yet been well-characterized. The aim of the present study was to investigate the effects of TCS exposure on kidney function and the possible underlying mechanisms in mice. Male C57BL/6 mice were orally exposed to TCS with the doses of 10 and 100 mg/(kg•day) for 13 weeks. TCS was dissolved in dimethyl sulfoxide (DMSO) and diluted by corn oil for exposure. Corn oil containing DMSO was used as vehicle control. Serum and kidney tissues were collected for study. Biomarkers associated with kidney function, oxidative stress, inflammation and fibrosis were assessed. Our results showed that TCS could cause renal injury as was revealed by increased levels of renal function markers including serum creatinine, urea nitrogen and uric acid, as well as increased oxidative stress, pro-inflammatory cytokines and fibrotic markers in a dose dependent manner, which were more significantly in 100 mg/(kg•day) group. Mass spectrometry-based analysis of metabolites related with lipid metabolism demonstrated the occurrence of lipid accumulation and defective fatty acid oxidation in 100 mg/(kg•day) TCS-exposed mouse kidney. These processes might lead to lipotoxicity and energy depletion, thus resulting in kidney fibrosis and functional decline. Taken together, the present study demonstrated that TCS could induce lipid accumulation and fatty acid metabolism disturbance in mouse kidney, which might contribute to renal function impairment. The present study further widens our insights into the adverse effects of TCS.

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Introduction

Triclosan (TCS) is a synthetic chemical which has gained enormous popularity in healthcare and in commerce owing to its antibacterial properties since 1970s (Yueh and Tukey, 2016). With the massive use of TCS, its increasing presence in water resources as well as in farming soils, its potential for bioaccumulation in tissues and toxicity in organisms arouse concerns to human and environmental health (Fang et al., 2010). TCS have been found in various human body fluids ranging from nmol/L to μmol/L concentrations in different regions of the world, indicating the potential of lifetime exposure to TCS for the general population (Weatherly and Gosse, 2017). Compelling data from animal studies have shown that TCS perturbs microsomal detoxification, causes hepatotoxicity, leads to central nervous system suppression, endocrine disruption and disturbance of immune function (Anderson et al., 2016; Clayton et al., 2011; Hao et al., 2019; Paul et al., 2010; Tabari et al., 2019; Wang et al., 2017).

The mammalian liver, kidney and intestinal tract play important roles in metabolism and elimination of xenobiotics. Pharmacokinetic studies indicated that after being absorbed, TCS is rapidly metabolized in the liver to glucuronide and sulfate conjugates that are removed in feces and urine (Fang et al., 2016). Unchanged TCS have also been identified in urine and feces (Moss et al., 2000). Several studies suggested that TCS exposure could enhance liver cell proliferation, fibrogenesis and oxidative stress in mice, acting as a promoter of liver tumor (Tang et al., 2018; Wang et al., 2017; Yueh et al., 2014). Another study demonstrated that diet containing 10 and 80 μg/mL TCS for 3 weeks increases colonic inflammation and colon tumorigenesis in mice (Yang et al., 2018). Kidney plays a vital role in the elimination of metabolic wastes in the form of urine. Because of the high rate of perfusion, active transport capacity, and concentrating functions, kidneys are often exposed to higher concentrations of chemicals than other organs (Kluwe and Hook, 1980). Besides, overwhelming evidence has recently accumulated in support of the prooxidative action of TCS (Teplova et al., 2017; Yueh et al., 2014), while oxidative stress can also contribute to renal function decline via inflammation, glomerular filtration barrier damage and fibrosis (Ratliff et al., 2016). Therefore, it could be speculated that kidney might also be vulnerable to the direct or indirect action of TCS. At present, several studies have partially revealed the effects of TCS on mammalian kidney. In one study, rats that exposed to TCS at 200 mg/(kg•day) developed alterations in the histological structure and functions of renal cortex (Hassan et al., 2014). Occurrence of fibrogenesis in kidney was also observed in mice treated with a chow diet containing 0.08% TCS for 8 months (Yueh et al., 2014). However, the systematic and complex data concerning the actions and mechanisms of TCS on kidney are still largely missing.

Abnormal metabolic processes have been considered to be one of the critical characteristics and mechanisms of renal response to environmental pollutants (Xu et al., 2018a, 2018b). Since renal tubular cells have high basal levels of energy consumption, thus preferring fatty acid oxidation (FAO) as the major energy source (Simon and Hertig, 2015). Therefore, any effect on energy metabolism is important to physiological function of kidney. Especially, disturbances of lipid metabolism and fatty acid metabolism have been implicated in the process of kidney fibrosis (Kang et al., 2015). To date, however, metabolomics analysis in TCS-induced kidney injury has not been performed especially on the consequences from lipid metabolism disorder and potential energy metabolism disruption. In the present study, we used a mouse model involving TCS exposure to investigate the biological effects induced by TCS on kidney. The potential mechanism was evaluated from the perspectives of lipid homeostasis and energy metabolism.

1. Materials and methods

1.1. *Animal experiment*

Animal experiment was approved by the Animal Ethics Committee of Hong Kong (Hong Kong, China). Male C57BL/6 mice (4 weeks old, $n = 30$) were purchased from the Laboratory Animal Services Centre of The Chinese University of Hong Kong (Hong Kong, China). The mice were maintained under a 12-hr dark/light cycle at the temperature of (25 ± 2)°C and humidity of 45%-65% with free access to food and water. After acclimatization for two weeks, the mice were randomly divided into three groups (10 mice per group) for exposure with vehicle control (corn oil containing 0.5% DMSO), TCS at doses of l0 mg/kg body weight per day (mg/(kg•day)) and 100 mg/(kg•day). Stock solution of TCS was prepared with DMSO. Different doses of TCS were mixed with corn oil and given to the mice by gavage every day for 13 weeks. Body weight was recorded every week. At the end of 13 weeks, all the mice were sacrificed, and samples including serum and kidney were collected and stored in −80°C until analysis.

1.2. *Histological analysis*

Kidney tissues were sectioned at 8 μm and stained with hematoxylin and eosin (H&E) staining to assess kidney injury following TCS exposure. Histological analysis was conducted with the Leica DM 2500 microscope (Leica, Wetzlar, Germany).

1.3. *Serum biochemistry assessment*

The serum levels of creatinine, blood urea nitrogen (BUN) and uric acid were measured using commercial ELISA kits (Shanghai Enzyme-linked Biotechnology, China) according to the manufacturer's protocol. Briefly, serum samples were diluted 5-fold with sample diluent solution before use. Then, 50 μL of diluted specimen and undiluted standards were pipetted into appropriate wells and 100 μL of horseradish peroxidase labeled enzyme conjugate reagent was then added to the reaction mixture. Subsequent steps were performed as described in the kit, and the result was read at 450 nm with a microtiter well reader (PerkinElmer Inc., MA, USA).

1.4. *Chemical analysis in kidney and serum by mass spectrometry*

Kidney tissues (30 mg) were homogenized with ice-cold water containing 80% methanol (1:20, *m/V*). The samples were further lysed by three cycles of freezing and thawing. The homogenates were then centrifuged at 16,000 ×*g* for 15 min (4°C), and the supernatants were collected and evaporated to dryness. The dried samples were re-suspended in 200 µL of 50% methanol containing internal standard 4-Chlorophenylalanine (IS, 4-Cl-Phe, 1 ng/µL) and centrifuged at 15,000 ×*g* for 10 min (4°C). The supernatant was withdrawn for analysis.

Determination of TCS in mouse serum was conducted as follows. Briefly, 100 µL of methanol containing 4-Cl-Phe (1 ng/µL) was added to 25 µL of serum sample for protein precipitation. Then, samples were centrifuged at 15,000 ×*g* for 10 min (4°C), and the supernatant was subjected to analysis.

Samples were characterized using an Ultimate 3000 UHPLC-TSQ Quantiva Triple Quadrupole Mass Spectrometer (Thermo Fisher Scientific Inc. USA) for quantification of targeted chemicals under selective reaction monitoring mode. The ion transitions of the target analytes were listed in **Appendix A Table S1**. The instrument conditions were set as previously reported (Cao et al., 2020).

1.5. *Profiling of triglyceride and acylcarnitine in kidney tissues*

Kidney tissues (30 mg) were homogenized in 600 µL of ice-cold methanol and 150 µL of water, and then 450 µL of chloroform was added. The samples were further lysed by three cycles of freezing and thawing. Then, 150 µL of water was added to promote phase separation, followed by centrifugation at 16,000 ×*g* for 15 min (4°C). The bottom layers were collected and dried in a Max-Up (NB-504CIR) IR vacuum concentrator (N-Biotek, GyeongGi-Do, Korea) at 4°C. The dried samples were reconstituted in 200 µL of ACN/isopropanol/H_2O (65:30:5, *V/V/V*) containing 1,2-dinonadecanoyl-sn-3-phophocholine (PC 19:0/19:0) for instrumental analysis.

Lipidomic profiling was achieved on an Orbitrap Fusion Tribird MS system (Thermo Fisher Scientific Inc., USA) equipped with a heated electrospray ionization (HESI) source under positive ionization mode. Samples were separated through a BEH C18 column (2.1 mm × 100 mm, 1.7 µm, Waters Corporation, UK). The instrument conditions were set as previously reported (Zhao et al., 2018). The collected data were imported into the LipidSearch software (Thermo Fisher Scientific Inc., USA) for the identification of triglyceride (TG) and acylcarnitine (AcCa). The exported peak area was used for analysis.

1.6. *Assessment of inflammation, lipid peroxidation and lipid accumulation*

Measurement of inflammatory factors including interleukin 1 beta (IL-1β), IL-6 and tumor necrosis factor alpha (TNFα) in kidney samples were performed with commercial ELISA kits (Shanghai Enzyme-linked Biotechnology, China) according to the manufacturer's protocol. Evaluation of lipid peroxidation was performed by measuring malondialdehyde (MDA) with the commercial TBARS Assay kit (Cayman Chemical, USA) according to the manufacturer's protocol. The total TG levels in kidney samples were determined with a Triglyceride Colorimetric Assay kit (Cayman Chemical, USA) according to the manufacturer's protocol.

1.7. *Quantitative gene expression analysis*

Total RNA was extracted from 20 mg of frozen kidney samples with the RNAsio Plus reagent (TaKaRa, Japan). cDNA was synthesized using the PrimeScript RT reagent kit (perfect real-time) (TaKaRa, Japan) according to the manufacturer's instructions. Real-time polymerase chain reaction (RT-qPCR) analysis were performed with TB Green Premix Ex Taq (TaKaRa, Japan) on a PikoReal^{TM} Real-Time PCR System (Thermo Scientific, USA). All the primers were obtained from the Beijing Genomics Institute (Beijing, China) and the primer sequences were listed in **Appendix A Table S2**. Gene expression levels were calculated using the comparative *Ct* method. *Gapdh* was used as the reference gene.

1.8. *Statistical analysis*

All values were presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using the SPSS 19.0 software (IBM, USA). Statistically significant differences were determined by one-way ANOVA followed by Tukey's multiple comparison test. *P* values less than 0.05 were considered to be of significance.

2. Results

2.1. *Effects of TCS on renal index in mice*

TCS was administered intragastrically to mice at three dosages [vehicle control, 10 and 100 mg/(kg•day)] for 13 weeks. As shown in Fig. 1a and **Appendix A Table S3**, no significant difference in body weight, kidney weight and kidney organ coefficient were observed in TCS-exposed groups compared with the vehicle control group. Serum level of unconjugated TCS was (110.0 ± 11.4) nmol/L for mice received 10 mg/(kg•day) TCS, and (646.3 ± 43.6) nmol/L for 100 mg/(kg•day) TCS-exposed mice (Fig. 1b). Concentrations of serum markers for kidney function (BUN, creatinine and uric acid) of the 100 mg/(kg•day) group were significantly higher than those of the controls. Of these, creatinine increased by 19.4% in 10 mg/(kg•day) group and 24.1% in 100 mg/(kg•day) group, BUN increased by 26.8% in 100 mg/(kg•day) group but has no significant change in 10 mg/(kg•day) group, and uric acid increased by 9.8% in 100 mg/(kg•day) group but has no significant change in 10 mg/(kg•day) group (Fig. 1c). RT-qPCR analysis revealed significant increase in mRNA levels of *Kim-1* (kidney injury molecule) in 100 mg/(kg•day) TCS exposure group with a fold change of 6.65 (Fig. 1d). Histological examination indicated that kidney from 100 mg/(kg•day) group showed alterations in morphology, including expansion of Bowman's space, damage and necrosis in glomerulus (Fig. 1e).

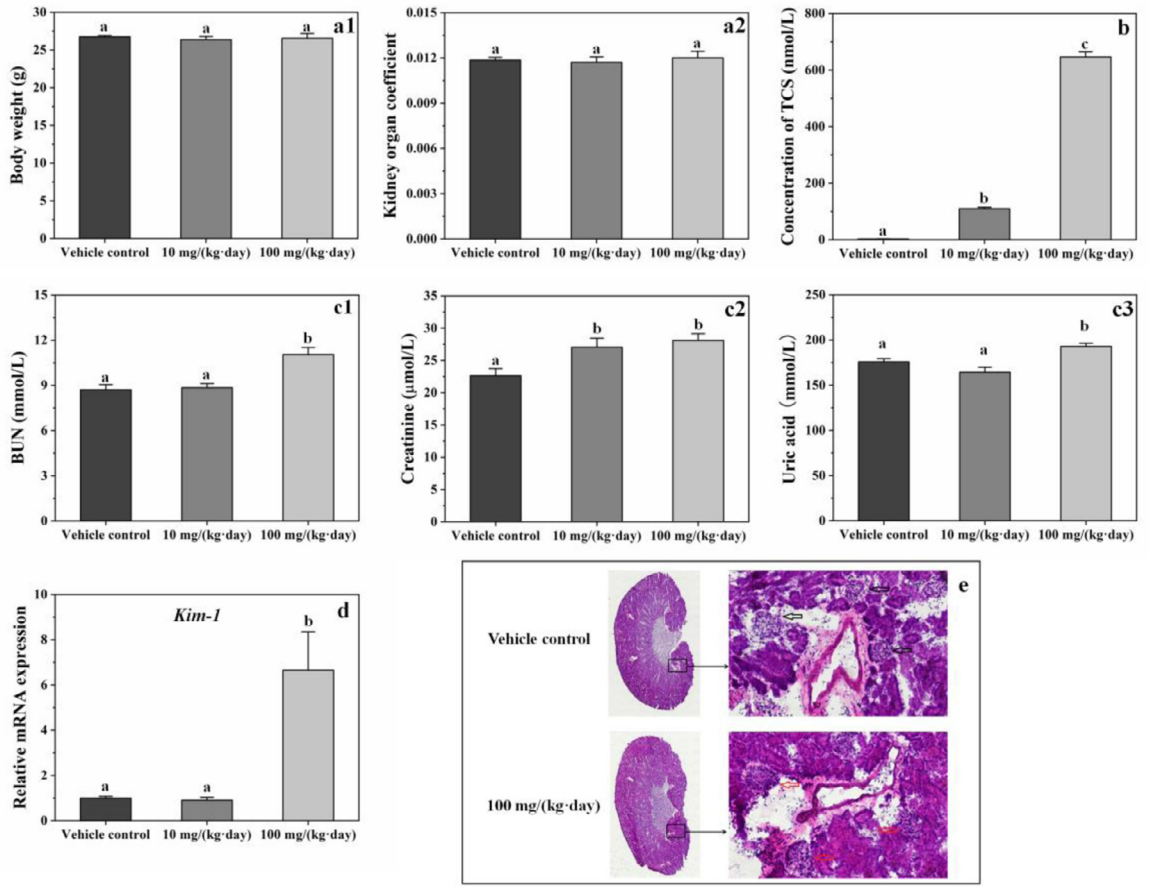

Fig. 1 – TCS treatment induced renal injury in mice. (a) Comparison of (a1) body weight and (a2) kidney organ coefficient between vehicle control and TCS-treated mice. (b) Concentrations of unconjugated TCS in mouse serum. (c) Quantification of serum renal function markers: (c1) BUN; (c2) Creatinine; (c3) Uric acid. (d) Alterations in expression level of kidney injury molecule (Kim-1) gene. Values shown throughout the figures are the mean ± SEM ($n = 10$ per group). (e) Histological analysis with H&E staining of kidney from vehicle control and 100 mg/(kg·day) TCS-treated mouse. Black arrows delineate normal glomerulus. Red arrow indicates damage and necrosis in glomerulus. Different letters indicate significant difference at $P < 0.05$, as determined by one-way ANOVA with Tukey's post-hoc test.

2.2. *TCS exposure caused pro-inflammatory, pro-fibrotic response and oxidative stress in kidney*

To follow up on kidney injury observed in TCS-exposed mice, we investigated markers of inflammation, oxidative stress and fibrosis which were known to be involved in kidney injury. The levels of pro-inflammatory cytokines were mostly elevated in kidneys of TCS treatment group. Particularly, IL-6 and TNFα were significantly upregulated in kidneys of 100 mg/(kg•day) TCS-treatment group as measured by ELISA assays (Fig. 2a). In agreement with these results, *Tgfβ1*, a well-established marker of fibrosis and inflammation, was also significantly upregulated (by 56.4%) in kidneys of 100 mg/(kg•day) group (Fig. 2b). Other markers of extracellular matrix deposition including *Col1a1*, *Timp1* and *α-Sma* also showed significant elevations of 63.5%, 252.9%, and 22.7% in the 100 mg/(kg•day) TCS-treated animals compared with the vehicle controls, respectively (Fig. 2b). Seen from Fig. 2c, expression of the oxidative stress responsive genes *Ho-1* and the mitochondrial superoxide dismutase *Sod2* were significantly altered in 100 mg/(kg•day) group. Moreover, mRNA expression levels of the NADPH oxidase subunits *Gp91phox* and *p47phox* were upregulated in kidneys of 100 mg/(kg•day) TCS-treated mice. Lipid peroxidation as assessed by MDA level also showed 14.2% and 49.9% increase in the treated groups (Fig. 2d), which were consistent with the oxidative stress related genes.

2.3. *TCS exposure induced triglyceride and cholesterol accumulation in mouse kidney*

It has been proposed that excess accumulation of TG and cholesterol induces cellular lipotoxicity, potentially contributing to the development of renal fibrosis (Moorhead et al., 1982; Weinberg, 2006). Herein, we assessed the contents of TG and cholesterol in kidney. By LC-MS profiling of the lipids, a total of 100 individual TG species was identified. The result showed that TCS exposure markedly increased the total abundance of TG (relative to IS) in mouse kidneys (Fig. 3a and b). The commercial enzymatic assay further confirmed the upregulation of TG in TCS-treated kidneys, showing 24.9% and 56.3% in-

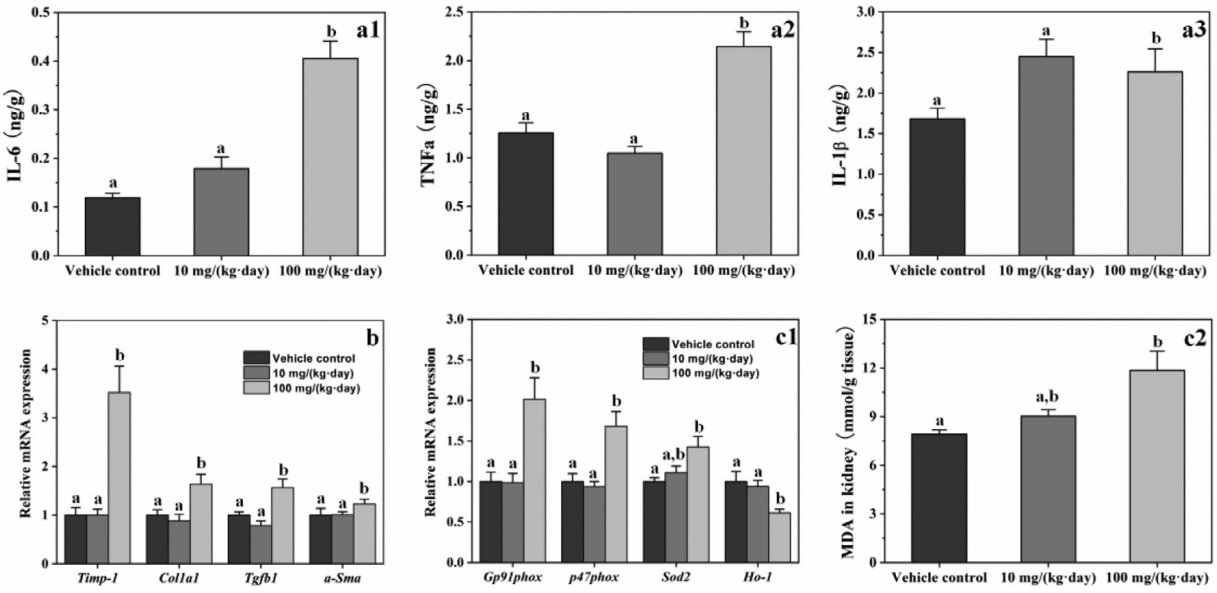

Fig. 2 – TCS exposure mediated pro-inflammatory, pro-fibrotic and oxidative response in kidney. (a) The levels of pro-inflammatory cytokines (a1) IL-6, (a2) TNFα, and (a3) IL-1β in vehicle control and TCS exposure groups. (b) Relative expression of genes associated with fibrosis, including Timp-1, Col1a1, Tgfβ1 and α-Sma. (c) Assessment of oxidative stress by (c1) RT-qPCR analysis of Gp91phox, p47phox, Sod2, Ho-1 genes and (c2) quantification of MDA levels in kidney. Values shown are the mean ± SEM (n = 10 per group). Different letters indicate significant difference at $P < 0.05$, as determined by one-way ANOVA with Tukey's post-hoc test.

crease in a dose-dependent manner (Fig. 3c). Quantification of free cholesterol level in kidney tissues by LC-MS showed a 54.0% elevation of free cholesterol in 100 mg/(kg•day) TCS-exposed mouse kidney (Fig. 3d).

2.4. *TCS exposure altered fatty acid metabolism in mouse kidney*

We performed quantification of non-esterified fatty acids (NEFAs) as well as AcCa by LC-MS. The result revealed that levels of saturated NEFAs including capric acid (C10:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) increased in TCS-exposed mouse kidneys in a dose-dependent manner, while levels of several unsaturated NEFAs like palmitoleic acid (C16:1), oleic acid (C18:1), linolenic acid (C18:3) and arachidonic acid (C20:4) decreased (Fig. 4a). Levels of long-chain AcCa also significantly increased in TCS-exposed mouse kidneys (Fig. 4b and **Appendix A Table S4**). Quantitative gene expression analysis showed that *Cd36*, which encodes a protein for fatty acid uptake, was upregulated by 25.5% in 100 mg/(kg•day) TCS-exposed mouse kidney. Besides, lower expression of *Cpt2, Pgc-1α* and *Acox1* were observed in TCS-exposed mouse kidneys (Fig. 4c).

3. Discussion

In 2016, the U.S. Food and Drug Administration banned the use of TCS in over-the-counter handwashing products. However, the legality of using TCS in cosmetics and mouthwashes still maintained. Thus, human exposure to TCS could be a persistent problem. Urinary excretion has been found to be a major route of TCS elimination by human (Sandborgh-Englund et al., 2006). Several previous researches have revealed to some extent that TCS exposure could induce nephrotoxicity (Hassan et al., 2014; Yueh et al., 2014). However, the obtained information was limited, especially in respect to metabolic process, such as fatty acid and lipid metabolism, which are responsible for maintaining normal physiological function of kidney. In this study, we investigated the effects of TCS exposure in mouse kidney by LC-MS based metabolomics and biological assays, aiming to elucidate the effects and the underlying mechanisms of TCS exposure on mouse kidney from a new perspective.

Although, the serum concentration of TCS in our study seemed to be comparable to or higher than that previously reported in human blood samples of different population (Allmyr et al., 2006, 2008, 2009), this study might be representative of the higher end of the exposure range in human beings in some scenarios, for example occupational exposure. In addition, it is challenging to use yet-to-be-defined relevant levels of TCS in animal studies and to translate findings to human health, our study is more intended to conduct a mechanism-based study in mice that defines the pathological condition in the kidney elicited by TCS exposure. The biochemical analysis results revealed upregulated levels of serum BUN, creatinine and uric acid in 100 mg/(kg•day) TCS treated groups in comparison with the vehicle control group. These results were similar to previous report that significant increase in the levels of BUN, creatinine and uric acid were observed in rats treated with TCS at 200 mg/(kg•day) for six weeks (Hassan et al., 2014). Previous data reported by Yueh et al. (2014) showed that 8.3%

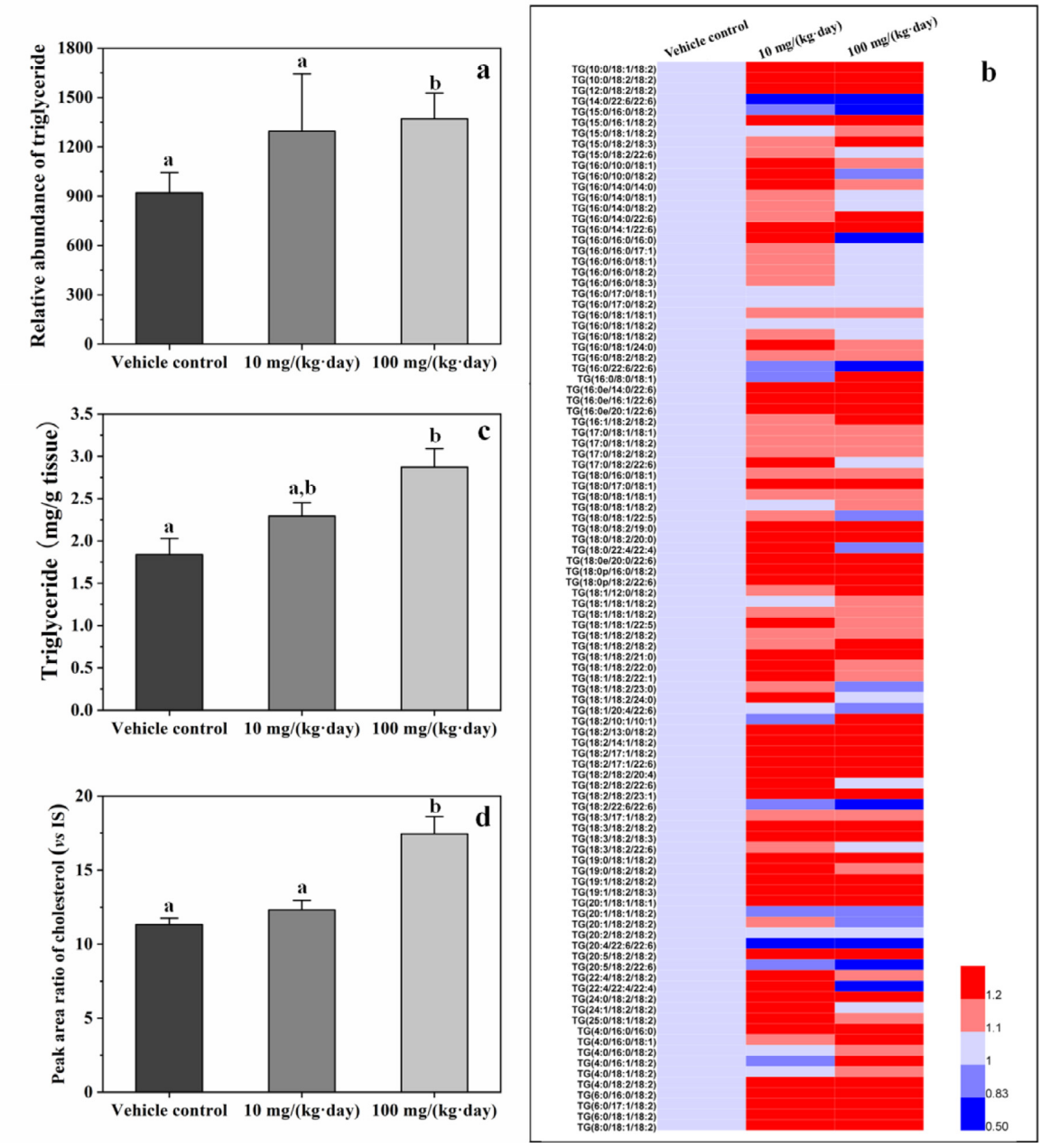

Fig. 3 – Lipid accumulation in kidney of TCS-treated mice. (a) Total MS peak area ratio of TG (vs IS) in kidneys of vehicle control and TCS-treated mice. (b) Heatmap analysis of identified individual TG species. (c) Quantification of TG contents in kidney tissues by commercial kit. (d) Quantification of cholesterol by LC-MS. Values shown are the mean ± SEM ($n = 10$ per group). Different letters indicate significant difference at $P < 0.05$, as determined by one-way ANOVA with Tukey's post-hoc test.

of mice treated with a chow diet containing 0.08% TCS for 8 months developed renal fibrosis, suggesting the pro-fibrotic response induced by TCS in kidneys. Herein, we further verified that the expression levels of the genes related to renal fibrosis were mostly elevated in 100 mg/(kg•day) TCS-treated mice, which are concomitant with signs of pro-inflammation and oxidative stress. These findings were in consistent with the result that was found in liver (Yueh et al., 2014). Renal fibrosis is considered to be the hallmark of virtually all progressive renal disease (Boor et al., 2010). Oxidative stress and activation of pro-inflammatory pathways are believed to be involved in the progression of renal injury (Duni et al., 2019). In particular, we found significantly reduced expression of *Ho-1*, an endogenous cytoprotective enzyme in TCS-treated mice. Previous reports have shown that the *Ho-1* deficient mice displayed reduced stress defense and a pro-inflammatory tendency (Poss and Tonegawa, 1997). We speculated that TCS exposure might transcriptionally deactivated the *Ho-1* gene and

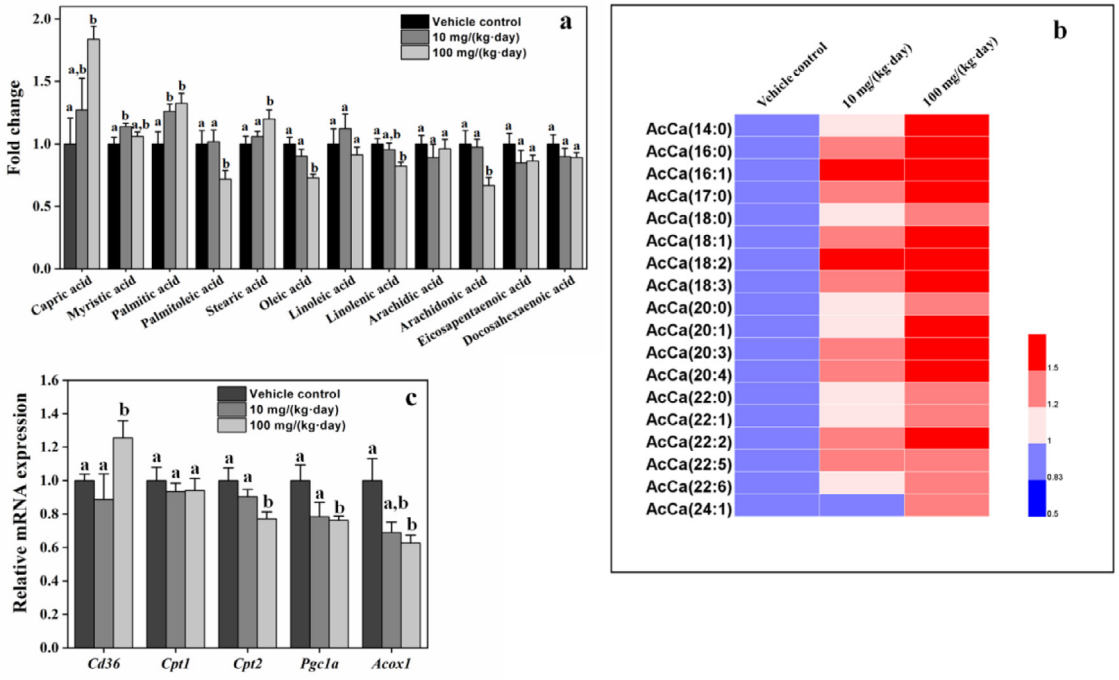

Fig. 4 – TCS exposure induced disturbance of fatty acid metabolism. (a) Comparison of NEFAs in vehicle control and TCS-treated mice by LC-MS. (b) Heatmap analysis of identified AcCa species. (c) Relative mRNA levels of genes related to fatty acid uptake and fatty acid oxidation. Values shown are the mean ± SEM (n = 10 per group). Different letters indicate significant difference at $P < 0.05$, as determined by one-way ANOVA with Tukey's post-hoc test.

thus limited the capacity to upregulate *Ho-1* in response to oxidative stress, thus contributing to the sustain of oxidative stress.

Dysregulations in lipid metabolism has been proposed to be linked with dysfunction in various tissues (Ertunc and Hotamisligil, 2016). In our previous study, we found that TCS exposure-mediated lipid metabolism disorders might contribute to the hepatotoxicity in mice (Huang et al., 2019). Metabolic syndromes like lipid metabolism and fatty acid metabolism disorders might also contribute to the pathogenesis and progression of kidney disease, of which oxidative stress and insulin resistance may mediate the lipid-induced renal damage (Bobulescu, 2010). In present study, significant increases in renal TG and cholesterol content were observed in TCS-treated mouse kidneys. Renal tubule epithelial cell lipid accumulation has attracted substantial attention in recent years, especially in the context of diabetic and acute kidney disease (Izquierdo-Lahuerta et al., 2016; Kang et al., 2015). Clinical observations indicated a potential association between lipid levels and chronic kidney disease development. Ectopic lipid accumulation in nonadipose tissues including kidney has been proposed to induce cellular lipotoxicity, potentially contributing to the development of renal fibrosis (Izquierdo-Lahuerta et al., 2016; Wahl et al., 2016). Besides, emerging data also strongly suggested a role of impaired cholesterol homeostasis within glomerular cells as an important mediator of chronic kidney disease (Merscher et al., 2014). What's more, the elevation of TG and cholesterol might also play a part in the increased expression of pro-fibrotic growth hormones, pro-inflammatory cytokines, and oxidative stress (Tovar-Palacio et al., 2012). Significant increased levels of saturated NEFAs including C10:0, C14:0, C16:0 and C18:0 were also observed in kidney of TCS-exposed mice. The increased cellular internalization of dominant saturated FAs has shown to contribute to kidney deterioration through apoptosis, mitochondrial superoxide generation and endoplasmic reticulum stress (Lee et al., 2017; Sieber et al., 2010). Saturated FAs can stimulate the release of inflammatory extracellular vesicles in cultured proximal tubular epithelial cells, leading to lipotoxicity (Cobbs et al., 2019). In this context, our observations implied that oral TCS exposure might lead to renal lipotoxicity.

Compelling experimental evidence showed that FAO disorders profoundly influence the fate of tubular epithelial cells by promoting the transformation of epithelial cells to mesenchymal cells, inflammation, and eventually fibrosis (Simon and Hertig, 2015). Kidney is one of the most energy consuming tissues in the body, with approximately two-third of its oxidative substrate are fatty acids (Nieth and Schollmeyer, 1966). Elevated expression level of *Cd36* might improve the availability of fatty acids in kidney of TCS-exposed mice. In normal tissues, NEFAs are esterified to fatty acyl-CoAs, which are then transported into the mitochondria for β-oxidation and converted into fatty acetyl-CoA to feed the TCA cycle. The carnitine palmitoyltransferase-1 (CPT1), CPT2 and the carnitine system were critical in this process (Knottnerus et al., 2018). As shown in Fig. 5, we found that TCS exposure led to significant increase of almost all long-chain AcCa species in the kidney. Lower mRNA expression of enzymes involving in mitochondrial FAO (*Pgc-1α*, *Cpt2*) suggested the inhibition of mitochon-

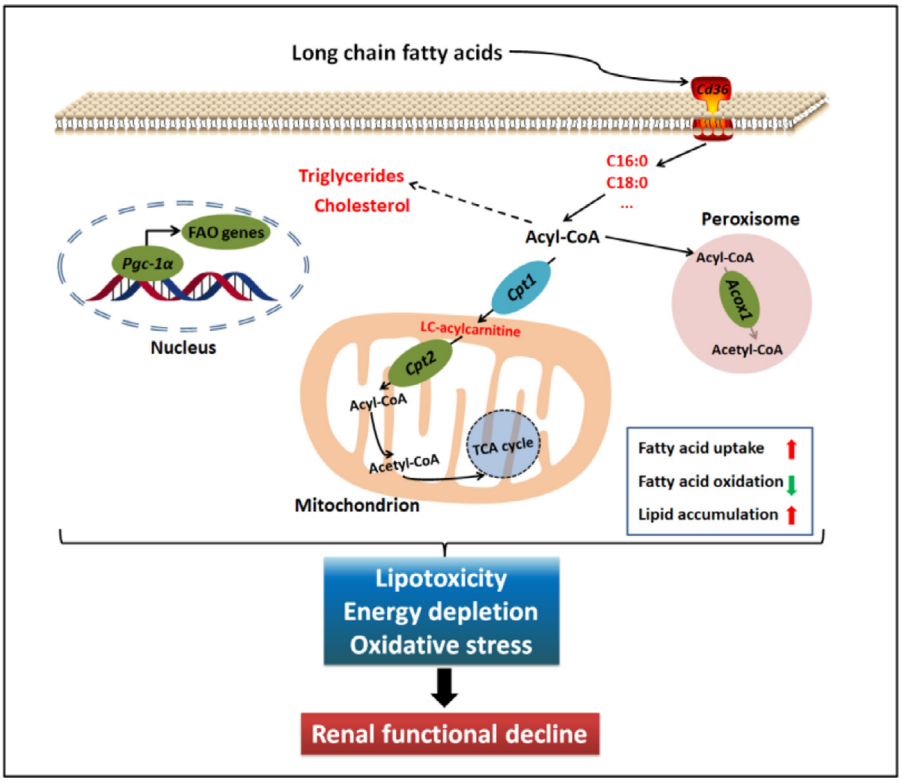

Fig. 5 – Proposed mechanism underlying lipid metabolism disorder in TCS-exposure induced kidney injury. Upon TCS exposure, downregulated Pgc-1α resulted in decreased transcription of genes related to fatty acid oxidation, which might lead to energy depletion. The increased fatty acid uptake and downregulated fatty acid utilization lead to excess saturated NEFAs and lipid accumulation, which would possibly induce lipotoxicity in kidney. Impaired mitochondrial and peroxisomal FAO depletes energy production and elevates oxidative stress, and that would cause the tubular necrosis and kidney dysfunction. The downregulated genes were marked with green oval and blue means no significant changes. The upregulated metabolites were marked in red and black means not assessed in this work.

drial FAO, which is a possible mechanism for the accumulation of AcCa. The downregulation of *Acox1*, which encodes the enzyme that catalyze the first and rate-limiting enzyme of the peroxisomal FAO of very long-chain FAs (Vluggens et al., 2010), further demonstrated the inhibition of FAO. Defective FAO would cause energy depletion, resulting in increased apoptosis and dedifferentiation, which in turn contributes to fibrosis and chronic renal disease progression (Kang et al., 2015). As one of the most potent pro-fibrotic cytokines, TGFβ1 is also thought to induce metabolic reprogramming in renal cells, especially by reducing FAO and increasing lipid accumulation (Kang et al., 2015). Besides, previous studies also suggested that lower FAO appears to contribute to the development of tubulointerstitial fibrosis, while genetic or pharmacological improvement of FAO protects animals from kidney fibrosis (Kang et al., 2015). A grade-dependent metabolomics study of human kidney cancer tissues revealed that the FAO pathway was inhibited in kidney tumors in proportion with grade (Afshinnia et al., 2018). Another study about the lipid profiles across different stages of human chronic kidney disease also showed impaired fatty acid β-oxidation in advanced chronic kidney disease (Wettersten et al., 2015). Taken these findings into consideration, we believed that impairment of FAO induced by TCS exposure might correlate with the upregulated expression of *Tgf*β1 and contribute directly or indirectly to the kidney fibrosis.

It has been known that the peroxisome proliferator-activated receptor gamma (PPARγ) is involved in fatty acid storage and metabolism, and takes part in pathological conditions such as inflammation, oxidative stress and so on. The ability of TCS to bind to PPARγ has been proved by *Fang et al.* in an in vitro model (Fang et al., 2015). Besides, a previous publication concerning the involvement of PPARγ in the mechanism of action of TCS showed that TCS exposure decreases the expression of *Ppar*γ in skeletal muscle and adipose tissue of pregnant mice (Hua et al., 2017). These findings demonstrated the possible role of PPARγ in TCS-induced effects. Our previous study on liver revealed similar effect that a 50% decrease of *Ppar*γ gene expression was observed in mouse liver exposed to 100 mg/(kg•day) of TCS. Therefore, it might be speculated that PPARγ receptor might be involved in TCS-induced renal injury, which needs to be verified by further studies.

4. Conclusions

Collectively, this study revealed the effects of TCS exposure in mouse kidney. We found that TCS administration induced

function alterations, oxidative stress, pro-inflammatory and fibrotic response in mouse kidney. The underlying mechanism was assessed in respective to lipid and fatty acid metabolism. Our results suggested that the lipid accumulation related lipotoxicity and the decreased FAO might play a causative role in TCS-induced nephrotoxicity. Our findings provide new insights into the effects and molecular mechanisms of TCS exposure.

Acknowledgments

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jes.2021.11.032.

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