INTRODUCTION

Trans-fatty acids (TFA) are unsaturated fatty acids with at least one unsaturated, nonconjugated double bond in the trans (rather than the typical cis) configuration [1]. Food products that contain TFAs will have a crispier taste and a longer storage life. Therefore, TFAs are regarded as substitute goods for saturated fatty acids and are widely used in processed foods, such as margarine, cocoa butter replacer, most commercial baked goods and snack foods. However, recent data regarding Trans fatty acids (TFAs) have implicated this lipid as being particularly deleterious to human health [2]. Chardigny et al. [3] commented in their research that epidemiological studies have shown a positive association between cardiovascular disease (CVD) risk and TFA intake from industrial sources. Some research has also suggested that trans fatty acids may increase lipoprotein (a) and C-reactive protein concentrations, insulin resistance, visceral adiposity, metabolic syndrome, inflammatory factors, and diabetes, although these data are by no means consistent and when observed tend to occur at relatively high trans fatty acid intakes [4,6].

Malondialdehyde (MDA) and superoxide dismutase (SOD) are the most commonly used indicators to evaluate human oxidative stress, and their changes in level can reflect the dynamic balance process of free radical production and elimination in the body [7,8]. MDA is the final product of lipid peroxidation, and its content can be effectively reflected the tissue lipid peroxidation. SOD and Glutathione peroxidase (GSH-Px) are antioxidants two enzymes in the system. SOD and catalase (CAT) are important enzyme proteins for scavenging free radicals of reactive oxygen species, and have a strong defense against the oxidative process and phagocytosis after cell injury. SOD plays an important role in the process of removing reactive oxygen species [9]. Besides, GSH-Px can catalyze the decomposition of hydrogen peroxide in the body and it’s very important to stop the lipid peroxidation caused by free radicals [10]. Therefore, measurement of MDA, SOD, and GSH-Px can reflect the oxidative stress level in tissues.

Adenosinetriphosphatase (ATPase), including Na+ K+-ATPase, Mg2+-ATPase, and Ca2+-ATPase, plays a role in the regulation of ion concentration on the cell. ATPase which belongs to the biomembrane system maintains the ion homeostasis, synaptic transmission and chemical gradient [11]. Therefore, the change of ATPase levels can reflect biological functions.

With the improvement of people’s living standards, the traditional dietary structure has been constantly changing, and, the intake of TFAs is on the rise in various countries. However, studies on the effects of TFAs on antioxidant system and ATPase levels in organism have not appeared. Therefore, in our study, we hypothesized that long-term intake of TFAs may cause damage to the liver and kidney. This experiment aimed to explore the effects of TFAs on the content of MDA, activities of antioxidant enzymes (SOD and GSH-Px) and ATPase in liver and kidney of rats.

MATERIALS AND METHODS

Materials and animals

Forty-eight male Wistar rats (180-220 g) were selected from the Laboratory Animal Center of Shandong University. The rats were housed in a controlled room with a temperature of (23 ± 2) ºC, humidity of (60 ± 10)%, and a 12-hour light and dark cycle. During the experiment, the rats were fed in cages with basic feed, and could drink and eat freely. The protocol was approved by the Institutional Animal Ethics Committee, and all animal studies complied with the Chinese Animal Care and Use Guideline in China (approval number: 20130001).

TFAs (mass fraction: 40%) were purchased from Yuan Cheng Group (Wuhan, China). The test kits of total protein (TP), SOD, GSH-Px, MDA, and ATPase were all purchased from Nanjing Jiancheng Bioengineering Institute, China. All other reagents were of analytical-reagent grade.

Experimental design

After a week of acclimatization, forty-eight rats were randomly divided into four groups (control group, low dose TFA group, medium dose TFA group and high dose TFA group) according to their weight. Rats in control group received 0.2 ml/kg corn oil once a day. For TFA exposure groups, TFA were dissolved in corn oil when the rats were fed. Rats in low, medium and high dose groups received 50, 100 and 150 mg/kg TFA respectively once a day. The exposure time lasted 12 weeks, all solutions above were administrated via oral gavage.

Rats in each group fasted 24 h after the last administration, then the rat was decapitated, the liver and kidneys were quickly removed, cleaned of adhering matters and washed with normal saline. Organs were weighed and recorded quickly. All tissue samples were stored at -20°C until required analysis.

MDA, SOD, and GSH-Px analyses

The activity of SOD, GSH-Px and the content of MDA in liver and kidney tissues were determined according to the instructions of the relevant kit [12].
ATPase analyses
The activity of ATPase was measured by the level of inorganic phosphorus.

Statistical analysis
Data are expressed as means ± SD. The statistical results were tested by using one-way analysis of variance (ANOVA), and multiple comparisons between groups were made by using Student-Newman-Keuls (SNK). P values <0.05 were considered to be statistically significant.

RESULTS

Physical assessment
During the experiment, it was observed that the rats in the control group were in good health. In addition to eating and drinking normally, all rats had normal activity and shiny fur. While with the increase of the duration of TFA exposure, the rats in the experimental group showed signs of malaise, anorexia, slow movement and the fur was sparser and duller. The average weight changes of the rats are shown in Table 1. The weight gain of rats in M-TFA exposure group and H-TFA exposure group was significantly lower than that in control group from the third week (p<0.05).

Effects of TFA on antioxidant enzymes and MDA in liver and kidney of rats
The effects of TFAs on the activities of antioxidant enzymes and the content of MDA in rat liver are shown in Figure 1. Compared to the control rats, the activity of SOD in medium and high dose TFA groups and the activity of GSH-Px in all TFA groups were significantly decreased (p<0.05), while the level of MDA in all TFA groups showed a significant increase (p<0.05). As shown in Figure 2, exposure of the rats to TFAs significantly impaired the activity of SOD and GSH-Px in their kidney. The activity of SOD in the medium and high dose TFA groups, and GSH-Px in all TFA groups were significantly decreased (p<0.05), compared with the control group. And MDA level was also higher in the medium and high dose TFA groups (p<0.05).

Effects of TFA on ATPase in rat liver and kidney
The effects of TFAs on the ATPase activities in rat liver are shown in Figure 3. As compared to those activities in the control rats, the activities of Na+ K+-ATPase and Ca²⁺-ATPase were significantly reduced in all TFA groups (p<0.05). Besides, administration of TFAs caused a significant reduction of Mg²⁺-ATPase in all TFA groups (p<0.05).

Figure 1)
Effects of TFAs on the activities of SOD (A) and GSH-Px (B) and the content of MDA (C) in rat liver. For all Figures, each point or column represents the mean ± SD.

Figure 2)
Effects of TFAs on the activities of SOD (A) and GSH-Px (B) and the content of MDA (C) in rat kidney. For all Figures, each point or column represents the mean ± SD.

**TABLE 1**
Average weight changes of rats at different stages of exposure

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th week</th>
<th>3rd week</th>
<th>6th week</th>
<th>9th week</th>
<th>12th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>196.5±12.4</td>
<td>287.7±25.0</td>
<td>328.0±31.0</td>
<td>374.2±32.2</td>
<td>433.1±31.3</td>
</tr>
<tr>
<td>L-TFA</td>
<td>186.3±7.3</td>
<td>271.0±27.5</td>
<td>313.8±31.1</td>
<td>352.0±42.8</td>
<td>425.6±64.8</td>
</tr>
<tr>
<td>M-TFA</td>
<td>196.1±14.5</td>
<td>258.8±32.7</td>
<td>303.2±39.3</td>
<td>339.1±47.9</td>
<td>391.8±56.5</td>
</tr>
<tr>
<td>H-TFA</td>
<td>187.3±8.0</td>
<td>263.6±22.5</td>
<td>285.2±32.9</td>
<td>327.4±30.1</td>
<td>375.5±39.9</td>
</tr>
</tbody>
</table>

Note: Values represent mean ± SD. * p<0.05, significantly different from control values.
ATPase, and Ca\(^{2+}\)-ATPase in rat kidney. Compared to those activities in the control rats, the activities of Na\(^{+}\) K\(^{-}\)-ATPase and Ca\(^{2+}\)-ATPase were significantly reduced in all TFA groups (p<0.05). What’s more, exposure to TFAs caused a significant reduction of Mg\(^{2+}\)-ATPase in medium and high dose TFA groups (p<0.05).

**DISCUSSION**

The results of this study have shown that the intake of TFAs which is considered a general health risk factor can induce damage to the liver and kidney. Machado et al. [13] found that excessive intake of TFA would increase the probability of developing nonalcoholic fatty hepatitis and reduce the fat content in body fat. Recommendations to reduce TFA intake have warned some official or international organizations (e.g. Food and Drug Administration, European Food Safety Authority) to take new preventive measures.

The change of animal weight during the experiment can reflect the general growth and development of animals in the stage of poisoning. We found that intake of TFAs could result in a decline in body weight gain in rats. Results showed that oral intake of TFAs might influence food intake and absorption in rats, leading to growth retardation.

Zarrouk et al. [14] commented in their research that studies have shown a positive association between TFAs, oxidative stress, LPO, and the risk of cognitive disorders. Oxidative stress arises when the generation of reactive oxygen species (ROS) exceeds the scavenging activity of the antioxidant system. Oxidative stress can damage lipids, nucleic acids, and proteins [15]. SOD can catalyze the conversion of reactive superoxide anions into hydrogen peroxide, which is an essential reactive oxygen species (ROS). CAT can contribute to the reduction of glutathione, then reduce the H\(_2\)O\(_2\) to O\(_2\), and GSH-Px has similar ability to CAT in eliminating hydrogen peroxide in cells [16], so the activity of these two enzymes can effectively reflect the resistance of the organism Oxidation state [17]. Besides, MDA is the end product of lipid peroxidation (LPO), and can be used to evaluate the severity of LPO. In normal conditions, combination of antioxidants, such as SOD, in cells could erase active oxygen radicals so their activities could reflect the ability of antioxidant in body. Additionally, antioxidation is a parameter of significance to evaluate health and immunity of body that ability of body to eliminate surplus oxygen radical in normal conditions [18].

The results of our study showed that administration of TFAs decreased the activities of GSH-Px and SOD, and increased the level of MDA in rat liver and kidney. It is indicated that TFA intake caused an increase of the LPO level, broke the balance of the generation and elimination of ROS and resulted in oxidative damage to rat liver and kidney. The results are in agreement with the research of ingesting a large amount of TFA can directly attack the components of hepatic cell membranes and the membrane fluidity, causing oxidative stress, activating cell signaling pathways and leading to the occurrence and development of insulin resistance [19].

There are many causes of the reduction of ATPase activity, such as intracellular calcium overload, cholesterol level, oxidative damage, and the damage of free radicals to ATPase which may be one of the main ways to reduce ATPase activity [20]. Studies have shown that elevated cholesterol levels and oxidative damage can affect the activity of Na\(^{+}\) K\(^{-}\)-ATPase [20,21]. It has also been found that the fatty acids in axial direction can influence the activity of Na\(^{+}\) K\(^{-}\)-ATPase by changing the membrane fluidity [22]. The results of this study showed that the activity of Na\(^{+}\) K\(^{-}\)-ATPase, Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase in liver and kidney tissues of rats in the Trans fatty acid group was lower than that of the control group. This indicates that the intake of trans-fatty acids can reduce the activity of ATPase in liver and kidney of rats, thus affecting the ion transport and signal transmission in tissues. The decrease of Na\(^{+}\) K\(^{-}\)-ATPase activity will lead to the disorder of excitatory amino acid uptake, which will lead to a variety of pathological lesions [21]. The Ca\(^{2+}\)Mg\(^{2+}\)-ATPase can hydrolyze ATP and pump intracellular Ca\(^{2+}\) out of the cell to maintain low intracellular Ca\(^{2+}\) concentration, which is one of the important mechanisms for cell homeostasis. The decreased activity of this enzyme can lead to the increase of Ca\(^{2+}\) in cells, and the Ca\(^{2+}\) overload in cells is an important way of cell damage. Teixeira et al. [23] commented in their research that long-term consumption of hydrogenated oil in rats decreased the activity of Na\(^{+}\) K\(^{-}\)-ATPase in the hippocampus and cortex of rats, and decreased the learning and memory ability of rats. However, whether the effect of trans-fatty acids on ATPase activity indicates the metabolic changes needs further study.

In conclusion, the intake of TFAs can cause oxidative damage and a disorder of the antioxidant system in rat liver and kidney. TFAs can reduce the activity of Na\(^{+}\) K\(^{-}\)-ATPase and Ca\(^{2+}\) Mg\(^{2+}\)-ATPase in liver and kidney. Thus, long-term and large-amount intake of TFAs may cause an impairment of rat liver and kidney but further researches are required to give a detailed explanation of the mechanisms responsible for their effects.

At present, the intake of TFAs is on the rise in many countries. Therefore, in order to protect the health of residents, countries should develop corresponding preventive measures to monitor trans-fatty acids in combination with the actual situation, and, in daily life, people should pay attention to healthy diet and reduce the intake of Trans fatty acids as much as possible.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**