

## Effects of vitamin C supplementation on Chinese sausage-induced liver damage in rats

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### Abstract

Contamination of nitrosamine compounds in Chinese sausages and the relevant hepatotoxicity have been reported. This study was conducted to evaluate the effects of vitamin C (VC) supplementation on Chinese sausage contaminated with *N*-nitrosodimethylamine (NDMA)-induced liver damage and relevant metabolomic changes. Rats were orally administered with Chinese sausage contaminated with NDMA at levels overpassing its safety limit. In some experiments, VC was additionally administered *via* drinking water to the rats for 25 weeks. Serum samples were collected from the rats for the measurement of the metabolomic profiles by using gas chromatography–mass spectrometry. Compared with the group administered with Chinese sausages alone, in rats with additional VC treatment, all serum biochemical parameters indicating hepatic damage were significantly alleviated, which suggested an improved hepatic function by VC supplementation. Metabonomic analysis of the serum samples showed notable changes in metabolites in VC-supplemented group as compared to that with sausage alone, especially for those involved in the regulation of amino acid or energy or lipid metabolism and/or anti-oxidative function. This study indicates that VC supplement in parallel with long-term exposure to Chinese sausage may attenuate liver damage and promote the recovery of liver function.

*Keywords:* Chinese sausage; liver damage; metabolomics; *N*-nitrosodimethylamine; vitamin C

### Introduction

Long-term intake of animal-based pickled foods is a hazard to the human health. Many epidemiological studies indicate that consumption of processed meats is associated with increased risk of esophageal, gastrointestinal, nasopharyngeal and pancreatic tumours, as well as liver damage (IARC, 2018; Zhao *et al.*, 2020). Previously, we have observed that the intake of sausage contaminated with *N*-nitrosodimethylamine (NDMA) at excessive levels may induce liver damage (Rong *et al.*, 2018a). The hepatotoxicity of the Chinese sausages might be attributed to

some toxic substances, such as nitrosamine compounds, aldehydes and ketones in the animal-based pickled foods may also be causative (Lee, 2019). Excessive intake of these toxic substances usually increases the level of reactive oxygen species (ROS) in the body, which may cause oxidative damage of the cell membrane, DNA and tissues (including the liver) (Chain *et al.*, 2023). Chinese sausage is the commonest salted meat for Chinese residents to consume, however, the contamination of nitrosamines and their precursors (nitrate, nitrite, etc.) in Chinese sausage and their adverse health effects have become significant public concerns (Li *et al.*, 2021; Rong *et al.*, 2018b).

A number of studies suggest that the intake of fresh vegetables and fruits rich in antioxidants, especially vitamin C (VC), contributes to the prevention from various chronic non-communicable diseases (NCD), including tumours. Therefore, it is interesting whether diet intervention of VC is effective for prevention against sausage-induced liver damage. Recently, several evidence-based nutrition studies suggest that appropriate intake of VC may help alleviate cardiovascular diseases and some cancers (e.g. hepatocellular carcinoma, esophageal cancer and gastric cancer) (Berends *et al.*, 2019; Fu *et al.*, 2021; Miller *et al.*, 2022), and the involved mechanisms include reduced formation of nitrous acid and nitrosamines in the gastrointestinal tract (McCormick, 1954). It has been proposed that the ideal concentration of VC in the blood is 100  $\mu\text{M}$ , which is effective for suppressing nitrosylation (Frei *et al.*, 2012; Lbban *et al.*, 2023). Multiple studies further suggest that intake of 200 mg VC per day by an adult is safe (Fu *et al.*, 2021; Mosen, 2000), which may also maintain a plasma concentration of VC as 70  $\mu\text{M}$ , and its maximal interval level may be attained by oral administration (Myint *et al.*, 2008; Simon *et al.*, 2001). This level is also effective for reducing the risk of NCD, according to studies by the Second (Chinese) National Health and Nutrition Examination Survey (NHANES II).

Vitamin C, also named ascorbic acid, is an efficient and water-soluble antioxidant capable of eliminating free radicals and reactive oxygen species (ROS) (e.g. superoxide radicals, hydrogen peroxide, singlet oxygen and nitrite peroxide) (Larsson *et al.*, 2022). Therefore, VC may effectively protect cells from oxidative damage. Besides, it also enhances the activity of enzymes as scavengers of ROS, such as the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), so as to prevent lipid from peroxidation. It has been reported that appropriate administration of ascorbic acid can alleviate the toxicity of some substances, such as mercury, lead, arsenic, benzene, some drugs and biotoxins (Fu *et al.*, 2020; Preddy *et al.*, 2010). Therefore, we hypothesised that oral VC supplementation may reduce the liver damage induced by Chinese sausage.

The liver is a powerful and complicated metabolising organ, which is responsible for the metabolism of proteins, lipids, carbohydrates and vitamins, as well as the disposition of heavy metals and some drugs. Metabolomics is a high-throughput and sensitive method to uncover the alterations in the metabolism of organisms exposed to hazardous substances (Zhao *et al.*, 2019), which has also been applied in the field of nutrition and food safety (Sebedio, 2017). The use of metabolomics in nutrition research is called nutrimentalomics, focusing on changes in endogenous metabolites from nutrients and reflecting changes in biochemical processes in the body (Rangel-Huerta and Gil, 2016; Shibutami

and Takebayashi, 2021). Therefore, in this study, serum metabolomics was integrated into the investigation of the influence of long-term VC supplementation on the hepatotoxicity of the Chinese sausages contaminated with NDMA in rats.

## Materials and Methods

### Chemicals and reagents

The assay kits for the determination of the levels of albumin (ALB), VC, total cholesterol (TC), malondialdehyde (MDA, a lipid peroxidation product) and the activities of AST, ALT, T-SOD and GSH-PX were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

All chemicals and solvents were of analytical or chromatographic grade. L-ascorbic acid (99.0% purity) were obtained from Sigma-Aldrich Co. (Shanghai, China). Methanol, acetonitrile, pyridine, n-hexane, methoxylamine hydrochloride (97%) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from CNW Technologies GmbH (Düsseldorf, Germany). L-2-chlorophenylalanine was from Shanghai Hengchuang Bio-technology Co. Ltd. (Shanghai, China).

### Animals and treatment

A total of 135 6-week-old male Sprague-Dawley (SD) rats were housed in the specific pathogen-free quarters of the Laboratory Animal Centre of Southern Medical University (SCXK-2001-0015). Animals had free access to water and diet under standard conditions with a 12-hour light–dark cycle, humidity of 50–60% and temperature of  $20 \pm 4^\circ\text{C}$  (mean  $\pm$  SD). Protocols for the animal experiments were approved by the Ethics Committee of Animal Centre of Southern Medical University, in accordance with Chinese Guidelines for Experimental Animal Welfare, with all experimental procedures following the Guide for the Care and Use of Laboratory Animals.

After 2 weeks of acclimatization, the rats were randomly assigned to five groups, with 27 rats in each group. In the control group (CON), rats were fed with AIN-93G diet, while in the two sausage diet groups, S1 group and S2 group, sausage diet I and II, respectively, were used to feed the rats; in the two VC supplementation groups (S1 + VC and S2 + VC groups), rats were fed with sausage diet I and II supplemented individually with 0.25 mg/mL VC in drinking water. Each treatment was maintained for a period of 25 weeks. Animal diets were modulated based on the recommendation of American institute of

Nutrition (AIN-93) (see Table 1). Sausage diet I was a diet of 1 part Chinese sausage to 5.5 parts certified rat chow, with 7.37 µg/kg NDMA contained in the sausage sample, a level exceeding the tolerant limit (3 µg/kg established by the Ministry of Health of China). Sausage diet II was a diet of 1 part Chinese sausage to 2.7 parts certified rat chow, with the same concentration of NDMA in Chinese sausage as in sausage diet I. No significant energy and nutrient changes were found in all experimental group diets ( $P > 0.05$ ). All diets were commissioned by Guangdong Medical Experimental Animal Centre with vacuum packed, cobalt 60 irradiated and sterilised, and stored at 4°C in the dark. For the two VC supplemented groups, VC was supplied to rats in drinking water with a concentration of 0.25 mg/mL throughout the experiment, while sterile tap water was given in the other groups.

#### Sample collection and biochemical analysis

According to the data described in our previous report, (Rong *et al.*, 2018a), eight rats from each group ( $n = 8$ ) were randomly selected in the 7th, 17th and 25th week after treatment, and anaesthetised with chloral hydrate *via* intraperitoneal injection. The blood samples were collected from the aorta abdominalis before the rats were sacrificed. Serum was isolated from each blood sample through centrifugation at 3000 rpm for 15 min. One part of each serum (supernatant) sample was immediately used for biochemical analysis according to the instruction of biochemical kits, including those for AST, ALT, ALB, TC, total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and VC; the other part was transferred into Eppendorf tubes and stored at -80°C for gas chromatography–mass spectrometry (GC-MS) analysis.

**Table 1. Ingredients of rat diets (per 100g).**

Ingredients (g)	CON diet	Sausage diet I	Sausage diet II
Sausage*	—	18.0	36.0
Corn starch	39.75	39.75	39.75
Casein	20.0	16.2	12.4
Soybean oil	7.0	1.9	—
Sucrose	10.0	5.8	—
Maltodextrin	13.2	13.2	7.8
Mixed minerals	3.5	3.5	3.5
Mixed vitamins	1.0	1.0	1.0
L-cystine	0.3	0.3	0.3
Choline chloride	0.25	0.25	0.25
Fibre	5.0	5.0	5.0
Total	100	103	105

\*Sausage: The concentration of NDMA in the sausage sample was 7.37 µg/kg, which exceeds the tolerant limit of 3 µg/kg as established by the Ministry of Health of China.

#### Serum GC-MS analysis

##### Sample preparation

Serum samples were thawed at room temperature and then divided into aliquots of 50 µL. Each sample was mixed with 10 µL of 2-chloro-L-phenylalanine (in methanol) as an internal quantitative standard, vortexed for 10 seconds, then added with 150 µL of methanol/acetonitrile mixture (2:1, v:v) (precooled in ice) and vortexed for 1 min. The extract was homogenised for 5 min and centrifuged at 12,000 rpm at 4°C for 10 min; afterwards, the supernatant (150 µL) was transferred to a glass vial and dried in a freeze concentration dryer. Eighty µL of 15 mg/mL methoxylamine hydrochloride salt (dissolved in pyridine) was subsequently added, vortexed for 2 min and incubated at 37°C for 90 min. Finally, 80 µL of BSTFA (with 1% TMCS) was added, vortexed for 2 min, and then derivatised at 70°C for 60 min.

The quality control (QC) samples were prepared from a mixture of all sample extracts, treated and tested in the same way as for the samples. One QC sample was inserted and analyzed for every 10 samples.

##### GC-MS nontargeted metabolism analysis

Samples were analysed based on a gas chromatograph system (Agilent J&W Scientific, Folsom, CA, California, USA, model 7890B) coupled with a mass selective detector (Agilent, model 5977A), exactly in accordance to our recent descriptions (Rong *et al.*, 2018b).

##### Data analysis

The GC-MS raw data were processed by Chroma Time-Of-Flight (TOF) software (v 4.34, LECO, St Joseph, MI, USA), and LECO-Fiehn Rtx5 database was used for raw peaks extracting, data baselines filtering, deconvolution analysis and peaks identification. The metabolites from the GC-MS spectra were identified with a similarity more than 400. The resulting data were imported into SIMCA-P 13.0 (Umetrics, Umea, Sweden) where a non-supervised principal component analysis (PCA) was used to visualise general clustering change trends or outliers among the observations. To further distinguish the metabolic profiles between groups, the partial least squares-discriminant analysis (PLS-DA) models were utilised. The parameters of PLS-DA ( $R^2Y$ ,  $Q^2Y$ ) were used for the evaluation of the goodness of the fit and the predictive ability of models (Trygg *et al.*, 2007).

After the analyses, low molecular weight metabolites were detected as chromatographic peaks in the total ion chromatograms. The ionic spectra of potential biomarkers were matched with the metabolite structures acquired from the Human Metabolome Databases (HMDB) (<http://www.hmdb.ca>). The identified metabolites with variable importance in the projection (VIP) (threshold > 1),

together with the P-value of the Student's *t*-test lower than 0.05, were selected as significant variables responsible for group separation. Finally, the identified potential metabolites were investigated for their metabolism pathways using databases of Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.kegg.jp/>).

### Statistical analysis

Statistical analysis was performed by one-way ANOVA and the LSD-*t* test using SPSS (version 20.0; Beijing Stats Data Mining Co., Ltd, China).  $P < 0.05$  was considered statistically significant.

## Results

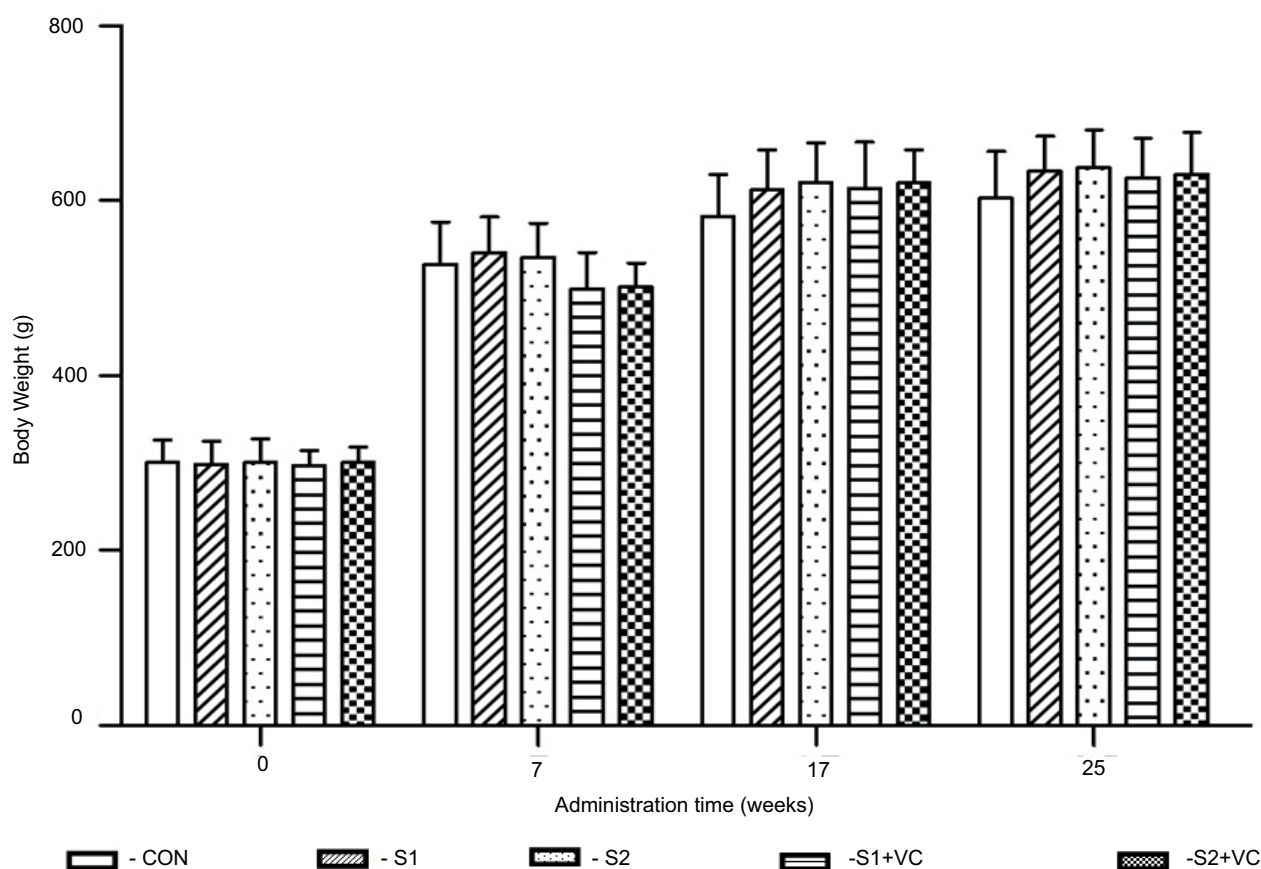
### Changes in the body weights (BW) of rats after treatment with Chinese sausage and VC

The body weights (BWs) of rats in the control and each treatment increased consistently, as observed at 0 w

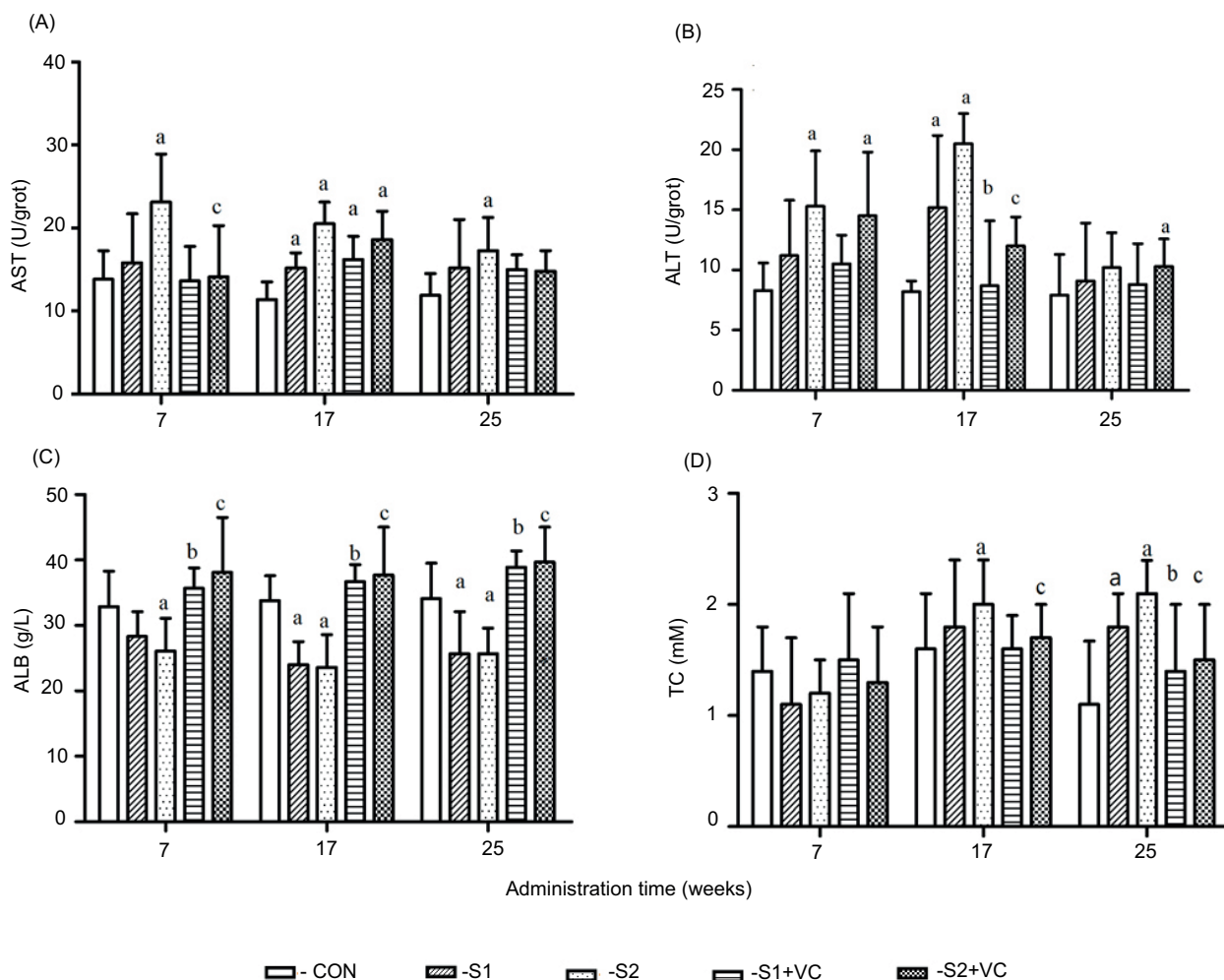
through 25 w, shown in Figure 1, that is there was no remarkable change in the BWs of rats in each experimental group as compared with the control group. Meanwhile, the amount of feed and water consumed in each treatment did not show any statistically significant difference from that in the control group (data not shown).

### Clinical chemistry

As shown in Figure 2, AST and ALT activities were markedly increased in Groups S1 and S2 as compared with the control group; however, in the groups with both VC and sausage (S1 + VC and S2 + VC), AST and ALT activities were not statistically different from that in the control group. Meanwhile, the ALT activities in the two VC-supplemented groups were significantly lower than that in the sausage (S1 and S2) groups ( $P < 0.05$ ) (Figure 2B). At the mid- and late-term of feeding experiment (17 and 25 weeks, respectively), the serum albumin (ALB) level in Groups S1 and S2 decreased significantly, while the serum total cholesterol (TC) increased, as compared with the control group. Nevertheless, the ALB levels in



**Figure 1.** The body weights of rats in each group along with the experimental process (0w through 25w). Data are expressed as means  $\pm$  SD. CON, control group, rats were fed with AIN- 93G diet; S1 and S2 group, rats were fed with sausage diet I (1 part Chinese sausage to 5.5 parts certified rat chow) and II (1 part Chinese sausage to 2.7 parts certified rat chow), respectively; S1 + VC group and S2 + VC group, rats were fed with 0.25 mg/mL VC in drinking water in addition to sausage diet I and II, respectively.



**Figure 2.** Values of serum biochemical parameters (reflecting hepatic damage) of rats treated with Chinese sausage and VC. Data are means  $\pm$  SD. Panels A, B, C and D represent the data of serum AST, ALT, ALB and TC, respectively. Statistical significance ( $P < 0.05$ ) was observed as compared with the CON group<sup>a</sup>, S1 group<sup>b</sup>, and S2 group<sup>c</sup>.

VC + S1 and VC + S2 groups were significantly higher than in the S1 and S2 groups, respectively, but with no statistically significant difference from the control group (Figure 2C). Moreover, at 25 weeks, the TC levels in S1 + VC and S2 + VC groups were significantly lower than that in the S1 and S2 groups, respectively (Figure 2D).

### Levels of serum MDA and several anti-oxidative enzymes or substance in rats exposed to sausage and VC

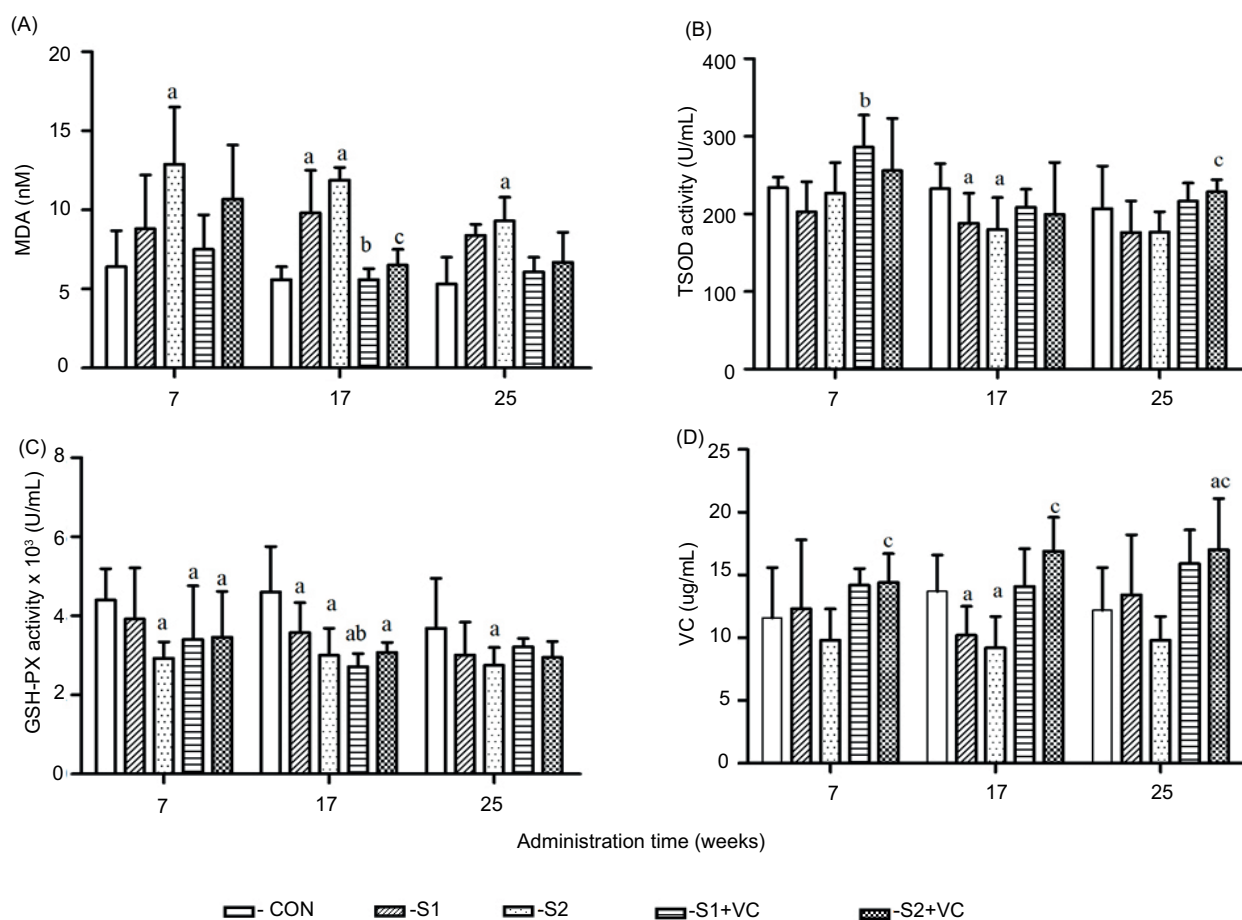
The levels of serum MDA, T-SOD activity, GSH-PX activity and VC in rats under various treatments are shown in Figure 3. The levels of MDA in S1 and S2 groups were both increased as compared with the control group, with statistical significance in S2 group; in the meantime, the values in S1 + VC and S2 + VC groups were not statistically different from that in the control group, as shown in Figure 3A. Moreover, the activities of

T-SOD and GSH-PX, and the level of VC in S1 and S2 groups were reduced as compared with the control group (Figures 3B–3D). However, the T-SOD activities in S1 + VC and S2 + VC groups were not statistically different from the control group, and the VC levels in these two groups were even statistically higher than in the control group. Supplementation of VC did not seem to influence the activity of GSH-PX, except that at a later stage (25 weeks) its activity in S1 + VC and S2 + VC groups gradually recovered to a level near to that in the control group (Figures 3B–3D).

### GC–MS analysis

#### GC–MS fingerprinting and multivariate analysis

All samples were analysed by GC–MS in full-scan mode to obtain serum metabolic profiles containing as many compounds as possible. In this study, the stability and



**Figure 3.** Levels of serum MDA, T-SOD activity, GSH-PX activity and VC (panels A, B, C and D, respectively) in rats exposed to sausage and VC. Values are means  $\pm$  SD, and statistical significance ( $P < 0.05$ ) was observed as compared with the control group<sup>a</sup>, S1 group<sup>b</sup>, and S2 group<sup>c</sup>.

reproducibility of the retention times and the intensity of the chromatographic peaks in the QC turned out to be standard (Figure 4), which indicated that the entire analysing process, including the sample pretreatment and the GC–MS system, was stable and reliable.

As shown in Figure 5 (I and II), the PCA plots for the two groups fed with Chinese sausage and the other two groups with both sausage and VC did not clearly deviate from that of the control group in the 7th week (panel a); however, at Week 25 they became separated from the control (panel b). Using PLS-DA score, the plots for Group S1 + VC were separated significantly from the S1 group (panels c and d) at Weeks 7 and 25, so were Group S2 + VC from Group S2 (panels e and f).

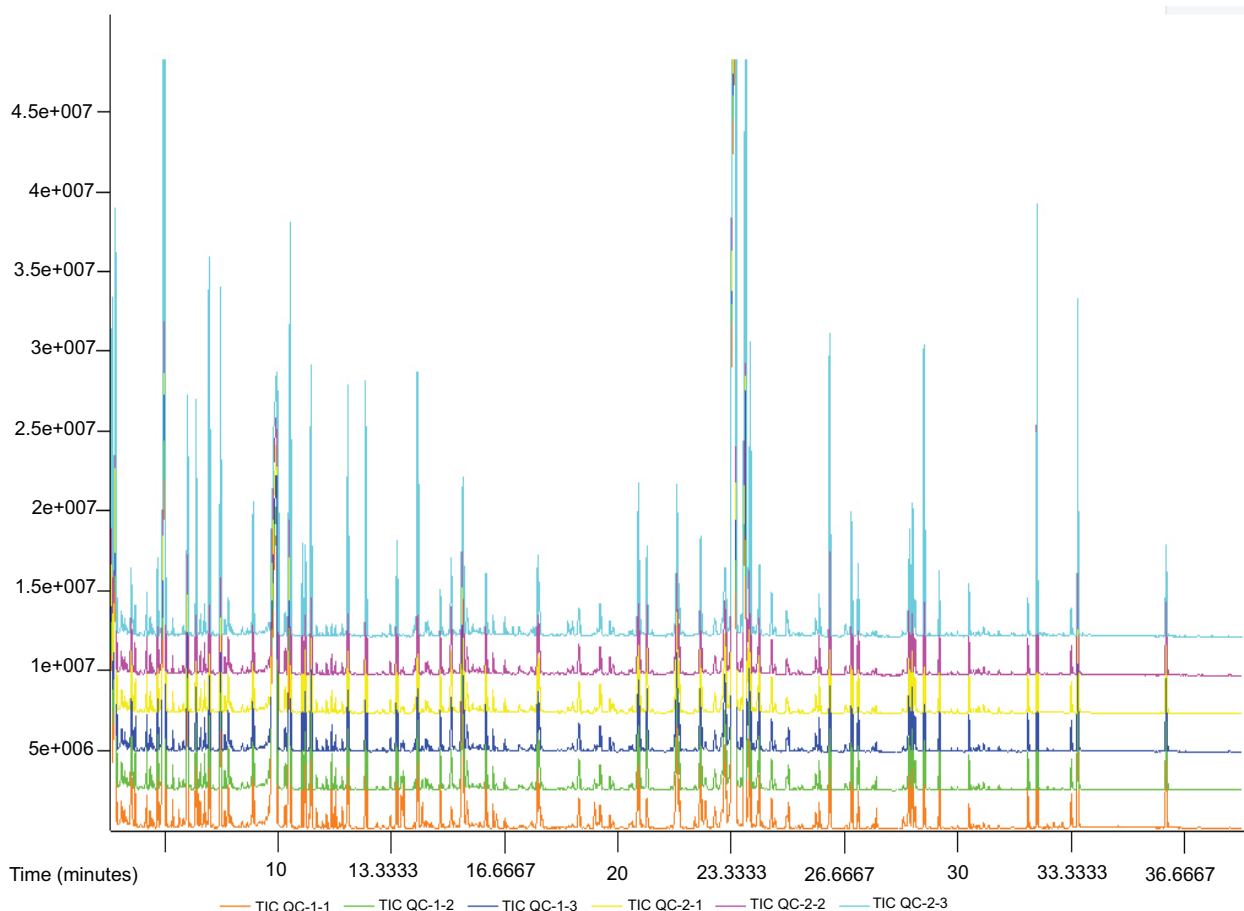
#### Differential metabolites in serum of rats fed with sausage/VC

Supplementation of sausage-treated rats with VC led to a change in serum metabolites, characterised by altered levels of 12 metabolites as compared with the groups treated with sausage alone. As indicated in Table 2, the levels of seven metabolites were decreased in the VC

supplemented groups, which included allantoic acid, uracil, thymine, cysteinylglycine, L-cysteine, tyrosine and linoleic acid, while five other metabolites, that is, 2-hydroxybutanoic acid, isoleucine,  $\alpha$ -ketoglutaric acid, glutamine and cumic acid, were notably increased.

## Discussion

In this study, while the BWs of rats were not changed by the intake of Chinese sausage (even for long term), the biochemical parameters indicating hepatic damage, that is, AST and ALT activities, were clearly elevated; moreover, these effects were significantly blocked or reduced by VC supplementation. Our previous studies suggest that the liver toxicity of Chinese sausage is primarily caused by NDMA (contaminated in sausages) (Qiu *et al.*, 2017). This study further confirms that VC is effective in relieving the hepatotoxicity of Chinese sausage. Our results are in accordance to previous observations that VC can reduce the hepatic toxicity (indicated by the activities of serum AST and ALT) of other hepatotoxicants, such as



**Figure 4.** The quality control (QC) of total ion flow chromatography (I) in GC-MS.

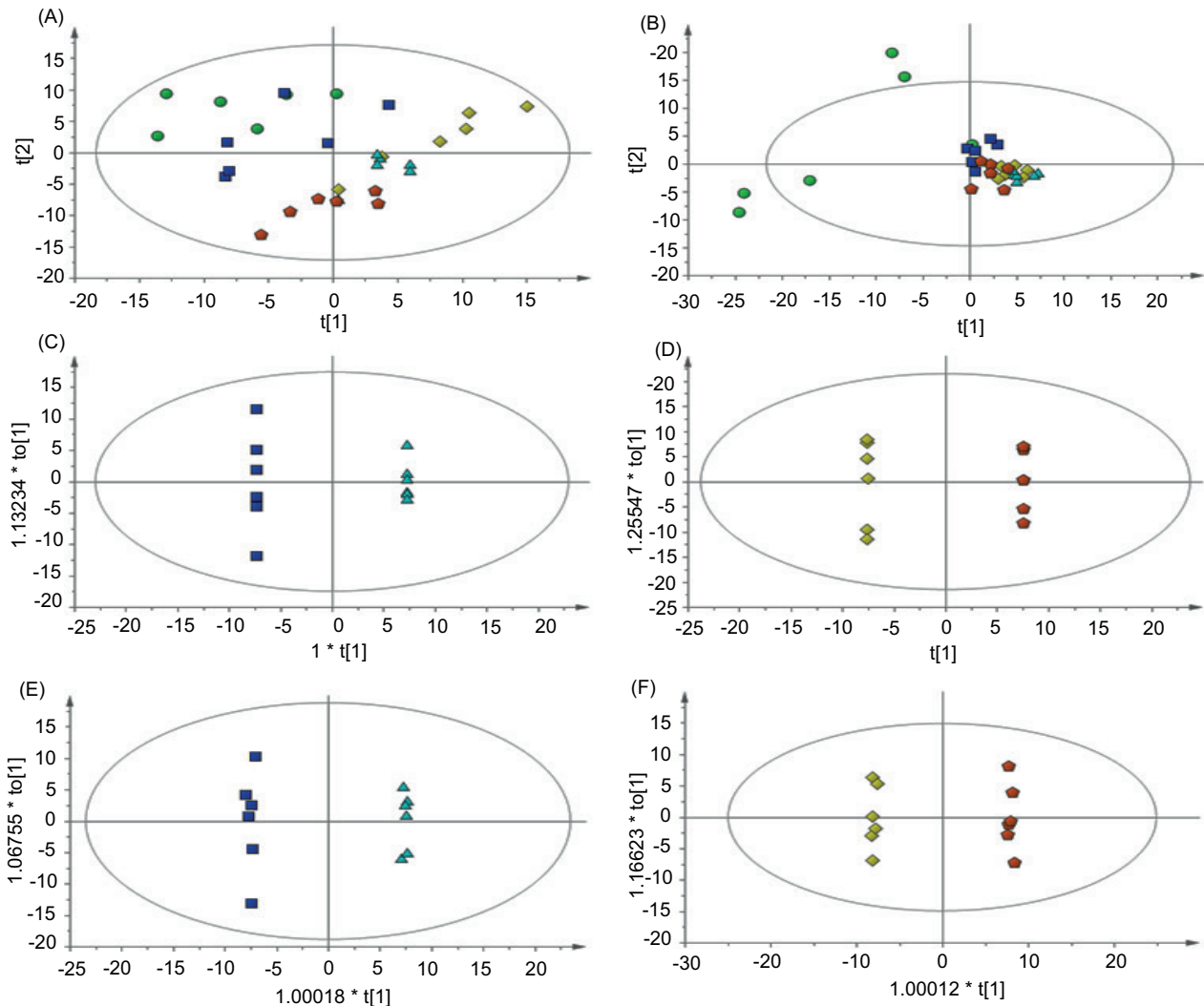
carbon tetrachloride, cypermethrin and some heavy metals in rats (Adikwu and Deo, 2013; Fu *et al.*, 2021), where the antioxidant property of VC is supposed to be contributive to its protective effect.

In this study, the serum level of ALB was decreased by sausage treatment, however, it was significantly recovered by VC supplementation. ALB is the most abundant serum protein, and its decreased levels are indicative of disturbed protein synthesis. ALB is mainly synthesised by hepatocytes, thus its level is an important index for the evaluation of liver function. In case of liver damage or malnutrition, the level of serum ALB may decrease (Qin *et al.*, 2019). Our results may suggest that VC supplementation is effective for maintaining a normal level of ALB in serum by protecting the hepatocytes. Moreover, long-term intake of sausage elevated the level of serum TC in rats, while VC supplementation abolished this effect. The influence of VC may be related to its participation in various hydroxylation reactions, as required in the metabolism of steroids, thus VC may promote the conversion of cholesterol to cholic acid, corticosteroids and sex hormones, and in

this way serum cholesterol is consumed with its serum level decreased.

As a primary oxidative product of peroxidised polyunsaturated fatty acids, MDA is an important indicator of lipid peroxidation (Abaukaka *et al.*, 2020). In this study, MDA was elevated in rats after intake of the sausage, which can be prevented by VC supplementation; in the meantime, the activities of serum reactive oxygen species (ROS) scavengers, that is, T-SOD and GSH-PX, were enhanced while the serum MDA was lowered, by VC supplementation. As previously reported, elevation in ROS scavengers by VC supplementation contributes significantly to alleviation of lipid peroxidation (Esmaeilzadeh *et al.*, 2020; Kauffmann *et al.*, 2021), which could be initiated by NDMA, a potent hepatotoxicant contained in Chinese sausages.

In the metabolomics study, serum cysteinylglycine (Cys-Gly) and L-cysteine (Cys) levels were notably decreased in rats exposed to both sausage and VC as compared with those exposed to sausage alone (Table 2). Cys-Gly is a metabolite of glutathione (GSH) and also serves as



**Figure 5.** The plots scoring of PCA (I) and PLS-DA (II) by multivariate analysis. Rats were treated with sausage/VC for 7 weeks (A, C, D) or 25 weeks (B, E, F) ( $n = 6$  per group). Green circles, blue quadrates, yellow rhombs, light blue triangles and orange pentagons represent CON, S1, S2, S1 + VC and S2 + VC groups, respectively.

a precursor to Cys (Kumar *et al.*, 2021; Wu *et al.*, 2004). Cys and Cys-Gly play an important role in GSH-directed antioxidant activity and sulfur exchange between amino acids, such as the conversion from Cys to taurine. A change in Cys/Cys-Gly level may alter the activity of the antioxidant system (Kumar *et al.*, 2022) and the metabolism of some biologically active substances in the body (Kumar *et al.*, 2023). Combined with the changes in the serum biochemical indicators, our study suggests that supplementation of VC may directly enhance the reducing property of GSH rather than increasing its level.

In this study, serum isoleucine (Ile) was decreased in sausage-treated rats, however, coexposure to VC recovered Ile level significantly. On the other hand, serum  $\alpha$ -ketoglutaric acid (AKG) in rats was elevated by sausage

exposure, but was reduced by coexposure to VC. Ile plays an important role in protein biosynthesis as an essential amino acid with a branched chain (backbone), and in energy metabolism as well. The recovery of serum Ile from its sausage-decreased level by VC supplementation appears to be in accordance to the concurrent increase in ALB, an important indicator of protein nutrition. AKG participates in tricarboxylic acid (TCA) cycle (via transformation to succinyl-CoA and acetyl-CoA, major intermediates in the TCA cycle) (Gu *et al.*, 2019). In the meantime, AKG is also an intermediate in the metabolism of several amino acids (including glutamic acid, tyrosine, and phenylalanine), and a substrate of transaminases such as AST and ALT. Therefore, the increase in serum AKG level in rats exposed to sausage alone seems consistent with the observed increase in the serum AST/

Table 2. Changes in levels of some metabolites in serum of rats treated with sausage or Vitamin C.

No.	Metabolites	RT* (min)	Treatment for 7 weeks				Treatment for 25 weeks					
			S1 vs CON	S2 vs CON	S1+ VC vs S1	S2+VC vs S2	S1 vs CON	S2 vs CON	S1+VC vs S1	S2+VC vs S2		
1	2-Hydroxybutanoic acid	6.0	↔	↔	↑	↑	↔	↔	↔	↔	↔	↔
2	Allantoic acid	8.8	↑	↑	↓	↓	↑	↑	↑	↑	↓	↓
3	Isoleucine	10.6	↓	↓	↑	↑	↓	↓	↓	↓	↑	↑
4	Uracil	11.6	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
5	Thymine	12.2	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
6	Cysteinyglycine	13.6	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
7	L-cysteine	16.2	↑	↑	↓	↓	↑	↑	↑	↑	↓	↓
8	α-Ketoglutaric acid	16.7	↑	↑	↓	↓	↑	↑	↑	↑	↓	↓
9	Tyrosine	20.6	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
10	Glutamine	20.9	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
11	Cumic acid	22.2	↓	↓	↑	↑	↓	↓	↓	↓	↑	↑
12	Linoleic acid	28.6	↓	↓	↓	↓	↓	↓	↓	↓	↑	↑

\*RT: retention time; ↑/↓: values were increased or decreased in the absence of statistical significance (P > 0.05); ↑/↓: levels increased or decreased with P < 0.05; ↑↑/↓↓: levels increased or decreased with P < 0.01.

ALT activities; moreover, its decrease by VC supplementation is also in accordance to the concurrent decrease in AST/ALT activities.

Linoleic acid (LA) is an polyunsaturated and essential fatty acid with important physiological functions. Reduction in LA levels may result in hepatic steatosis and lipid metabolism disorders (Alarcon-Gil *et al.*, 2022). In this study, LA was decreased in response to sausage exposure, while it got recovered to some extent following supplementation of VC. This is consistent with the concurrent increase of serum TC by sausage exposure, and its reduction in response to VC supplementation. This effect of VC may be related to the recovered level of LA, which enhances the catabolism of lipid (particularly cholesterol) (Center *et al.*, 1995; Nava Lauson *et al.*, 2023).

## Conclusions

This study demonstrates a protective effect of VC on long-term Chinese sausage intake-induced hepatic damage, by enhancing the antioxidant system and improving energy/amino acid metabolism. GC-MS-based metabolomic study suggests that some changes in the levels of metabolites, such as Cys, Cys-Gly, Ile, AKG and LA, may help explain the blocking or alleviating influence of VC on the Chinese sausage-induced hepatic damage, as reflected by some serum biochemical parameters.

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## Conflicts of interest statement

The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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