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Sodium sulfite promotes the assembly and secretion of very low-density lipoprotein in HL-7702 hepatocytes

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ABSTRACT

This study investigated the effects of Na_2SO_3 on the fat metabolism in human normal diploid HL-7702 (referred as L-02) hepatocytes. After 24 h and 48 h, treatment with different concentrations of Na_2SO_3, the intra and extra-hepatocellular triglyceride (TG) contents of L-02 were determined using chemical-enzymatic method. The contents of very low-density lipoprotein (VLDL) and apolipoprotein B100 (apoB100) in the culture supernatants were determined using enzyme-linked immunosorbent assay (ELISA). Western blot was applied to detect the expressions of fatty acid oxidation and fat synthesis related proteins, VLDL assembly and secretion in L-02 cells.

Results: Na_2SO_3 treatment (10 mM, 24 h/48 h) significantly increased the intra TG level in the hepatocytes. Different concentrations of Na_2SO_3 increased the extra-hepatocellular TG content. After 24 h exposure, the extracellular VLDL levels and secretions of apoB100 in 0.1–10 mM Na_2SO_3 groups were significantly higher than that of the negative control ($P < 0.05$). Meanwhile, the expression of CPT1 and SREBP1 protein were significantly reduced by Na_2SO_3. MTP and TGH protein expressions were significantly elevated in each Na_2SO_3 treatment group. The expression level of LDLR in hepatocytes was reduced by Na_2SO_3.

Conclusion: Na_2SO_3 exposure may promote the hepatocellular VLDL assembly and secretion, through increasing of MTP and TGH expressions and inhibiting the uptake of extracelluar VLDL.

1. Introduction

Na_2SO_3 is the main residue in traditional food and pharmaceutical products after sulfur fumigation. It is widely used as an additive in various dried fruits (such as pistachio nuts, preserved fruit, etc.), pharmaceutical products and alcoholic drinks [1,2]. Sulfur dioxide can be transformed to Na_2SO_3 after inhalation via respiratory tract; eventually this compound enters other organs including the liver and kidney via the blood circulation [3].

However, extensive studies have reported that Na_2SO_3 is able to damage the respiratory, cardiovascular and nervous systems [4–6]. For example, respiratory system diseases caused by Na_2SO_3 including asthma [8–10], chronic mucous hypersecretion, cough, rhintis, bronchitis [11,12] and even lung cancer [7]. Na_2SO_3 exposure can also increase the mortality of cardiovascular disease [13] and the risk of acute stroke [14]. Na_2SO_3 can impair nervous system by causing synaptic injury, spatial learning and memory impairment and an increase in reactive oxygen species and a decrease in ATP in Neuro-2a and PC12 cells [5,6,15]. However, It is reported that deficiency of sulphite oxidase could lead to mental retardation, neurological symptoms such as spastic quadriplegia, and early death [16,17]. In particular, Na_2SO_3 is more dangerous to sensitive individuals, Vally et al. reported that topical, oral or parenteral Na_2SO_3 exposure induces a range of adverse clinical effects, ranging from dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhoea to life-threatening anaphylactic and asthmatic reactions in sensitive individuals [18].

Although different organs have different reactivities to Na_2SO_3, but they also share some similar mechanisms to Na_2SO_3 injury. For example, Na_2SO_3 exposure can change the redox state in liver, kidney and nervous system [19–21]. Some studies have reported that mice might become hyperlipidemic after exposure to sulfur dioxide, and triglyceride (TG) content in hepatocytes was increased [22,23], Bai and Meng. suggested that Na_2SO_3 may cause metabolic disorders in mice hepatocytes.The molecular mechanism of Na_2SO_3 influence on fat metabolic disorders in human liver remains unclear. Because both extrahepatic and intrahepatic

Abbreviation: TG, triglyceride; VLDL, very low-density lipoprotein; apoB100, apolipoprotein B100; ELISA, enzyme-linked immunosorbent assay; CPT1, carnitine palmitoyl transferase 1; SREBP1, sterol regulatory element binding protein-1; MTP, microsomal triglyceride transfer protein; TGH, triglyceride hydrolase; Arf1, ADP-ribosylation factor-1; COP I, coated protein I; LDLR, low-density lipoprotein receptor; NAFLD, Nonalcoholic fatty liver disease.

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Table 1
Primary antibodies used in western blot.

Primary antibody	Company	Catalogue #	Dilution
Rabbit anti-human MTP	Abcam	Ab63467	1:1000
Rabbit anti-human LDLR	Abcam	Ab52818	1:1000
Rabbit anti-human COP β	Abcam	Ab2899	1:1000
Rabbit anti-human TGH	Abcam	Ab68190	1:3000
Rabbit anti-human Arf1	Epitomics	#1635-1	1:2000
Rabbit anti-human CPT1a	Protein Tech, China	15184-1-AP	1:1000
Rabbit anti-human SREBP1c	Protein Tech, China	14088-1-AP	1:1000
Mouse anti-human β-actin	Wuhan Boster Biological Technology Co., Ltd., China	BM0005	1:1000

Table 2
Secondary antibodies used in western blot.

Secondary antibody	Company	Catalogue #	Dilution
Goat anti-rabbit IgG	Wuhan Boster Biological Technology Co., Ltd., China	BA1050	1:4000
Goat anti-mouse IgG	Wuhan Boster Biological Technology Co., Ltd., China	BA1055	1:4000

factors cause fat metabolic disorders [28], Nonalcoholic fatty liver disease (NAFLD) is now threatening growing number of people both in developed and developing countries [24,26,27]. Our study targets on the intrahepatic factors include abnormalities in fat synthesis and fatty acid decomposition of the hepatocytes, as well as abnormal assembly and secretion of very low-density lipoprotein (VLDL) [29]. The most studied fat synthesis regulation protein is sterol regulatory element binding protein-1(SREBP1), which chiefly regulates the synthesis of fatty acids [30] and the key enzyme of fatty acid decomposition is carnitine palmitoyl transferase 1(CPT1b) in liver [31]. The VLDL assembly and secretion related proteins includes MTP, TGH, Arf1 and COP I, etc. [32,34–36]. In the present study, in vitro experiments were used to investigate the changes of above intrahepatic factors associated with fat metabolism after treating L-02 hepatocytes with different concentrations of Na_2SO_3.

2. Materials and methods

2.1. Materials

The normal human diploid hepatocytes (HL-7702; referred as L-02) were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, China. Fetal calf serum and high glucose Dulbecco's modified Eagle's medium (DMEM) were from Hyclone. Na_2SO_3, oleic acid, and tryptase were bought from Sigma. Prestained protein marker was obtained from Fermentas, Western blot substrate was from Thermo Scientific, human very low-density lipoprotein (VLDL) (ESK5997) and human apolipoprotein B100 (apoB100) (ESK5994) test kit were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China. TG assay Kit (GPO-POD method) were bought from Applygen Technologies Inc., China.

The primary and secondary antibodies used in this study is listed in Tables 1 and 2.

2.2. Culture and treatment of cells

L-02 cells were cultured in high glucose DMEM containing 10% calf serum in an incubator at 5% CO_2 and 37 °C. The medium was replaced every two-three days. Cells were digested and passaged with 0.25% trypsin. After achieving 80% adhesion, they were selected for the experiments. Seven treatment groups were set up as the followings: one negative control group (complete medium), four Na_2SO_3 treatment groups, and two positive control groups (1 mM oleic acid group and 10 mM carbon tetrachloride group). The concentrations in the Na_2SO_3 treatment groups were configured as 10, 2.5, 0.5, and 0.1 mM according to previous report [15,37]. Cells were routinely seeded onto a six-well culture plate, each group in two wells. The extracellular supernatants after exposure for 24 and 48 h were collected for detecting VLDL and apoB100 contents in the supernatant, and intracellular proteins were collected for protein expression analysis. All the experiments were repeated thrice for statistic analysis.

2.3. Detection of intra-hepatocellular TG level

Detection of TG in hepatocytes was performed as reference with minor adjustment [38]. The cells were routinely seeded onto a 6-well culture plate. After treatment the supernatants were removed for further protein analysis. The cell pellets were washed with 2 ml PBS. They were dispersed with 400 μl of PBS/10 mM EDTA, pH 7.4. 200 μl of cell suspension was centrifuged and lysed for protein measurement. 200 μl of cell suspension was added to glass tubes with 2 ml of isopropanol–hexane–water (IHW) for organic extraction, vortexed and incubated with aluminum foil for 30 min. Then 500 μl of hexane–diethyl ether (1:1) was added to each tube. Samples were vortexed, incubated and covered with aluminum foil for 10 min at room temperature. 1 ml of water was added to separate phases by vortexing. Another incubation covered with aluminum foil until phases separate (~20 min) at room temperature. The organic phase (900 μl) was pipetted into a new glass tube for nitrogen evaporation using a 96-well format manifold at 50 °C. Then 10 μl water and 190 μl of TG reagent were added to each tube according to the instruction. The tubes were vortexed and covered with parafilm and incubated for 30 min at 37 °C with shaking at 100 rpm. Finally 150 μl of TG reagent with samples was dispensed to a flat-bottom 96-well plastic microplate to determine absorbance at 550 nm.

2.4. Determination of extra-hepatocellular TG content

The cells were routinely seeded onto a 24-well culture plate, each treatment group in two wells. The supernatants were collected after exposure for 24 and 48 h, and the TG was extracted using organic extraction method similar to intracellular TG detection, evaporated by using a 96-pin microplate format gas manifold [38]. 1 ml of supernatant was used for extraction instead of 200 μl of cell suspension. The TG level was determined according to the instructions provided along with the kit, and the experiment was repeated 4 times.

2.5. Detection of secretions of VLDL and apoB100 of hepatocytes treated with Na_2SO_3

VLDL and apoB100 in the supernatant was detected according to the instructions provided with the kit.

2.6. Detection of protein expression

The intracellular proteins were collected using the loading buffer after exposure for 24 and 48 h, and quantified by using trichloroacetic acid method. A loading amount of 25–50 μg protein sample was collected for sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 10 or 15% separating gel and 5% spacer gel. Separated proteins were transferred electronically to nitrocellulose

Table 3
Effect of Na_2SO_3 treatment on the TG level of L-02 hepatocytes ($n = 3$, $\bar{x} \pm s$).

Group	TG (mmol g^{-1})	
	24 h	48 h
Negative control	1.2218 ± 0.5272	1.4837 ± 0.1236
0.1 mM Na_2SO_3	1.2609 ± 0.4914	1.3167 ± 0.2209
0.5 mM Na_2SO_3	1.5099 ± 0.3047	1.9047 ± 0.5721
2.5 mM Na_2SO_3	1.8227 ± 0.4524	2.1050 ± 0.3275
10.0 mM Na_2SO_3	2.4713 ± 0.7691*	6.3060 ± 0.8734*
1.0 mM OA	3.0940 ± 0.7968*	3.0130 ± 0.6844*
10.0 mM CCl_4	3.2353 ± 1.1168*	1.8090 ± 0.5705

Cells were treated with Na_2SO_3 (as described in Section 2), the lipids in the cells were extracted with hexane-isopropanol-diethyl ether, and quantified.
* $P < 0.05$ when compared to negative control.

membrane. Membrane was blocked with 5% defatted milk powder at room temperature for 1 h. Blocked membranes were incubated with corresponding first antibody (CPT1a 1:1000, SREBP1c 1:1000, MTP 1:1000, TGH 1:3000, Arf1 1:2000, COP β 1:1000, LDLR 1:1000, β-actin 1:1000) in a 4 °C shaker overnight. Later the membranes were shaken and rinsed thrice with phosphate-buffered saline with Tween 20 (PBST) at room temperature. Secondary antibody coupled with horse radish peroxidase (goat anti-rabbit 1:4000 and goat anti-mouse 1:4000) was added and incubated at room temperature for 1 h. The protein expression was detected using enhanced chemiluminescence. β-actin was detected for loading control of same amount of total protein. Films were developed, the optical density in hybridization maps was semiquantitatively analyzed using Image-Pro Plus 6.0 software.

2.7. Statistical analysis

Statistical analyses were performed using SPSS17.0 software, and the results were expressed as mean ± standard error. One-factor analysis of variance was used to compare the mean values of various groups. Multiple comparisons between groups were performed using Dunnett's t test. A difference with $P < 0.05$ was considered statistically significant.

3. Results

3.1. Na_2SO_3 has no significant effects on intra-hepatocellular TG level of human L-02

Na_2SO_3 could increase lipid droplets in mouse [22], herein we examined its effects on the TG level in human hepatocytes. The present results showed that TG level in hepatocytes treated by 0.1 mM–2.5 mM Na_2SO_3 was not significantly increased, but the TG level in hepatocytes was increased significantly by 10 mM Na_2SO_3 24 h/48 h exposure, accompanied by cell death (Table 3).

3.2. Na_2SO_3 increases extra-hepatocellular TG level of human L-02

TG content in extra-hepatocytes was detected by GPO-POD method. The present results showed that TG level in the supernatant of hepatocytes was significantly increased when treated by 0.1mM–10 mM Na_2SO_3, (*$P < 0.05$). As control settings, OA or CCl_4 treatment also increased the TG content in the supernatant (Table 4). It means that Na_2SO_3 can increase the secretion of TG from hepatocytes.

3.3. Na_2SO_3 promotes secretion of VLDL in L-02 hepatocytes

As illustrated in Table 5, after 24 h exposure to Na_2SO_3, VLDL content in the supernatant of each Na_2SO_3 group was significantly

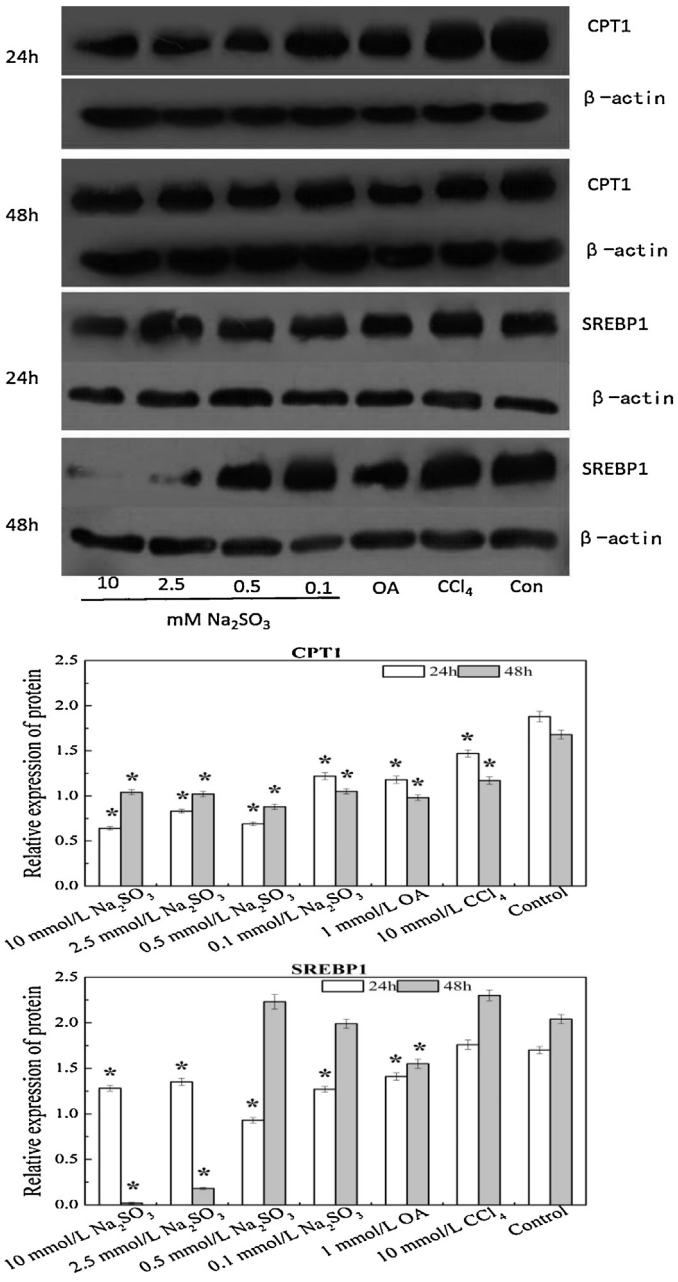

Fig. 1. Effect of Na_2SO_3 treatment on protein expression levels of CPT1 and SREBP1 in L-02 hepatocytes. Cells were treated with Na_2SO_3 (as described in Section 2), proteins were collected, equal total protein levels were loaded, and levels were analyzed using western blot. The expression of CPT1 and SREBP1 was significantly reduced.

Table 4
Effect of Na_2SO_3 treatment on the secretion of TG from L-02 hepatocytes ($n = 4$, $\bar{x} \pm s$).

Group	TG(mmol/L)	
	24 h	48 h
Negative control	0.1203 ± 0.0354	0.5245 ± 0.0333
0.1 mM Na_2SO_3	0.5557 ± 0.1606*	0.6326 ± 0.1369*
0.5 mM Na_2SO_3	0.6477 ± 0.1084*	0.5881 ± 0.0633*
2.5 mM Na_2SO_3	0.5991 ± 0.1107*	0.6311 ± 0.0707*
10.0 mM Na_2SO_3	0.6399 ± 0.0759*	0.7501 ± 0.0990*
1.0 mM OA	0.4586 ± 0.0453*	1.0816 ± 0.0111*
10.0 mM CCl_4	0.7163 ± 0.0879*	0.7027 ± 0.0948*

Cells were treated with Na_2SO_3 (as described in Section 2), the lipids in the supernatants were extracted with hexane-isopropanol-diethyl ether, and quantified.
* $P < 0.05$ when compared to negative control.

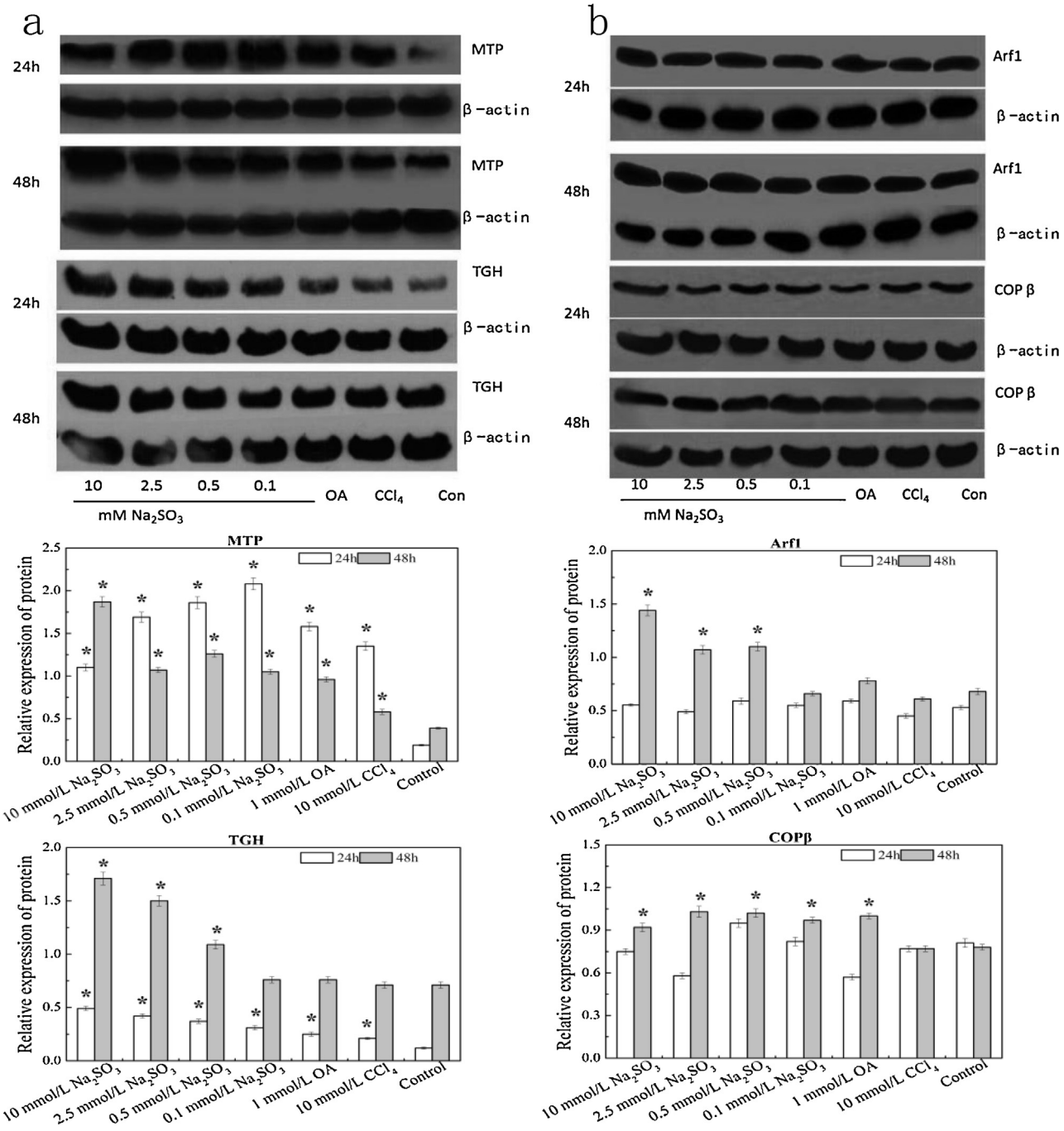

Fig. 2. Effect of Na_2SO_3 treatment on protein expression levels of VLDL assembly-related proteins (MTP and TGH) and transportation-related proteins (Arf1 and COP β) in L-02 hepatocytes. Cells were treated with Na_2SO_3 (as described in Section 2), proteins were collected, equal total protein levels were loaded by detection of β-actin as a reference, and levels were analyzed using western blot. The expressions of MTP and TGH in each Na_2SO_3 group and positive control were significantly increased after 24/48 h exposure. Arf1 and COP β were increased after 48 h exposure.

increased as compared with the negative control ($P < 0.05$). This result suggests that Na_2SO_3 promotes the secretion of VLDL in hepatocytes. However, after exposure to Na_2SO_3 for 48 h, the VLDL content in each treatment group did not significantly change as compared with the negative control.

3.4. Na_2SO_3 promotes the secretion of apoB100 in L-02 hepatocytes

After exposure to Na_2SO_3 for 24 or 48 h, the apoB100 contents in the supernatant of extracellular medium were significantly increased as compared with the negative control group ($P < 0.05$). This reflected that in a dose dependent manner, Na_2SO_3 can promote the secretion of apoB100 in hepatocytes.

3.5. Na_2SO_3 suppresses the expressions of CPT1 and SREBP1

CPT1 is a key rate-limiting enzyme for fatty acid decomposition in hepatocytes, and SREBP1 is an important transcription factor that promotes the expression of fat synthesis related genes. After exposure to Na_2SO_3 for 24 h and 48 h, the expressions of CPT1 protein in 0.1–10 mM Na_2SO_3 treatment groups were significantly reduced.

Table 5
Effect of Na_2SO_3 treatment on the secretion of VLDL from L-02 hepatocytes ($n = 3$, $\bar{x} \pm s$).

Group	VLDL(μmol/L)	
	24 h	48 h
Negative control	242.08 ± 3.52	239.00 ± 7.05
0.1 mM Na_2SO_3	278.23 ± 6.01**	287.97 ± 14.21
0.5 mM Na_2SO_3	296.44 ± 2.47*	289.77 ± 29.08
2.5 mM Na_2SO_3	276.44 ± 33.09*	256.44 ± 25.48
10.0 mM Na_2SO_3	263.10 ± 10.24*	235.92 ± 32.31
1.0 mM OA	300.54 ± 12.66*	271.82 ± 36.20
10.0 mM CCl_4	245.40 ± 15.46	237.46 ± 20.16

Cells were treated with Na_2SO_3 (as described in Section 2), the supernatants were collected and VLDL contents were quantified.
* $P < 0.05$ when compared to negative control.

This suggests that Na_2SO_3 exposure can suppress β-oxidation of hepatic fatty acids, which is conducive to the synthesis of TG. After exposure for 24 h, the expression of SREBP1 was decreased by Na_2SO_3; while after 48 h exposure, the expressions of SREBP1 were significantly decreased in the 2.5–10 mM Na_2SO_3 groups. This similar responsiveness as OA treatment indicates that hepatocytes may counteract the risk of increased synthesis of TG.

3.6. Na_2SO_3 promotes the expression of TG transfer protein in L-02 hepatocytes

MTP is responsible for the assembly of TG and apoB100 to generate primary VLDL. TGH is responsible for hydrolysis and re-esterification of TG in endochylema of hepatocytes, followed by assembly with apoB100 to generate VLDL. At least 70% of the TG in the hepatocytes enters VLDL through this route. The initial products of VLDL are transported into the Golgi apparatus with the effects of Arf1 and COP I after their generation in the endoplasmic reticulum, followed by further processing for secretion. After exposure to Na_2SO_3 for 24 and 48 h, the expressions of MTP and TGH in each Na_2SO_3 group and positive control were significantly increased (Fig. 2a). The expressions of Arf1 and COP β protein were increased significantly after exposure to Na_2SO_3 for 48 h (Fig. 2b). The above results indicated that Na_2SO_3 may promote the expressions of MTP and TGH proteins, and in the same time upregulate Arf1 and COP β proteins for more VLDL secretion.

3.7. Na_2SO_3 inhibits the expression of LDLR protein in L-02 hepatocytes

The LDLR in the liver is primarily responsible for removal of LDL from the circulation. It has been speculated that LDLR affects plasma apoB containing lipoprotein (Blp) metabolism by uptake LDL from the plasma by apoB100 and apoE related pathway. It is also responsible for the uptake of VLDL in vitro. Similar to positive control (OA and CCl_4 groups), Na_2SO_3 experimental groups have significant lower LDLR expression than the negative control (Fig. 3). That means that Na_2SO_3 may inhibit the uptake of VLDL/LDL from the supernatant of L-02.

4. Discussion

Na_2SO_3 is a common reductive food additive, but it is also a metabolic derivative of the air pollutant sulfur dioxide in the body. Previous animal experiments have found that inhalation of sulfur dioxide could increase hepatic lipid droplets and TG level and increase the plasmic TG content in mouse liver [22,23]. We hypothesized that exposure to Na_2SO_3 may cause metabolic disorder of human liver, thereby lead to NAFLD by inhibition the secretion of VLDL. At present, NAFLD is a burgeoning health problem affecting

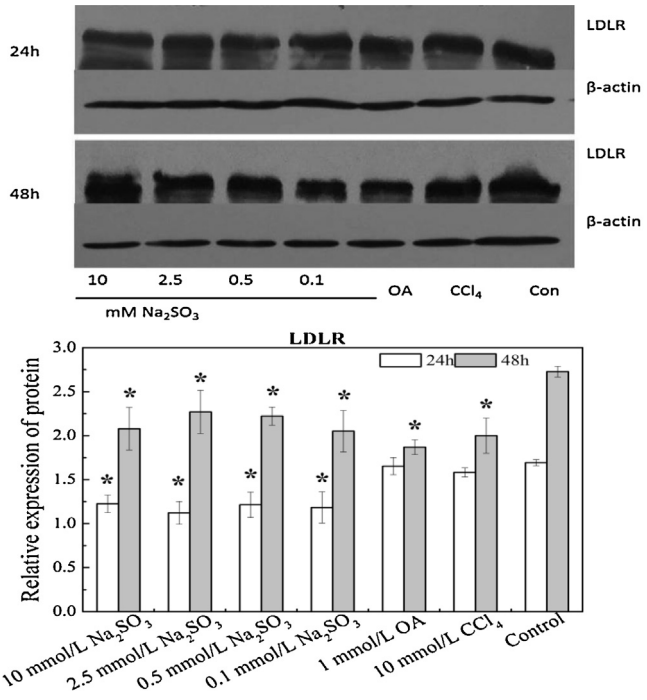

Fig. 3. Effect of Na_2SO_3 treatment on protein expression level of LDLR.
Cells were treated with Na_2SO_3 (as described in Section 2), proteins were collected, equal total protein levels were loaded by detection of β-actin as a reference, and levels were analyzed using western blot. LDLR expression was inhibited by Na_2SO_3 treatment for 24/48 h (similar as OA and CCl_4).

one-third of adults and an increasing number of children in developed countries. The NAFLD begins with the aberrant accumulation of triglyceride in the liver. Some individuals elicits an inflammatory response that can progress to cirrhosis or liver cancer [24–27].

In this study, both intra- and extra-hepatocellular TG contents from L-02 were examined after exposure to Na_2SO_3. The results showed that Na_2SO_3 has no direct effect on TG content in the human liver cells. At a higher concentration (10 mM), Na_2SO_3 significantly increased the TG content and cell death (75% for 24 h, 50% for 48 h, data not shown) (Table 3). But, Na_2SO_3 promotes the secretion and exocytosis of TG (Table 4). Our data suggest that Na_2SO_3 may not induce NAFLD directly. We also did not find the inhibition of VLDL secretion, on the contrary, the findings of the present study revealed that Na_2SO_3 was able to promote the secretions of VLDL and apoB100 (Tables 5 and 6).

It was reported that the fat content in the hepatocytes is controlled by the decomposition of the fatty acid and synthetic rate of TG [29]. CPT1 is the key rate-limiting enzyme for the decomposition of fatty acid in hepatocytes. Suppression of CPT1 activity might lead to increased TG synthesis, thereby cause TG accumulation [31,39]. Our data showed that the expression of CPT1 was significantly inhibited by Na_2SO_3 exposure (Fig. 1), suggesting that Na_2SO_3 has the potential to increase the TG content in the hepatocytes. Interestingly, we did not find significant increase of TG in cells. Probably the inhibition of CPT1 is not strong enough or other counteract factors are involved in the TG processing. In this study, the expression of SREBP1c was markedly reduced after exposure to Na_2SO_3 similar to 1 mM OA treatment (Fig. 1) [40]. SREBP1c mainly regulates the synthesis of fatty acids by enhancing transcription of the genes encoding acetyl CoA carboxylase, fatty acid synthase, stearoyl CoA desaturase-1 [41,42]. Our results indicated that Na_2SO_3 may increase the synthesis of TG through the inhibition of CPT1. But hepatocytes can mobilize their protective mechanism to response to this possible adverse effect under mild threat. However, since in vitro experiments can only reflect the short-term

Table 6
Effect of Na_2SO_3 treatment on the secretion of apoB100 from L-02 hepatocytes ($n = 3$, $\bar{x} \pm s$).

Group	apoB100(ng/ml)	
	24 h	48 h
Negative control	135.13 ± 3.33	143.59 ± 13.44
0.1 mM Na_2SO_3	155.90 ± 6.63*	171.03 ± 3.62*
0.5 mM Na_2SO_3	177.95 ± 8.75*	197.69 ± 2.47*
2.5 mM Na_2SO_3	199.49 ± 2.35*	240.26 ± 3.33*
10.0 mM Na_2SO_3	235.64 ± 3.95*	255.13 ± 2.96*
1.0 mM OA	201.54 ± 5.81*	230.00 ± 5.40*
10.0 mM CCl_4	231.79 ± 5.46*	263.33 ± 4.62*

Cells were treated with Na_2SO_3 (as described in Section 2), the supernatants were collected, and apoB100 contents were quantified.
* $P < 0.05$ when compared to negative control.

effect of a toxicant on the body or cells, further in-depth studies are required to investigate long-term effect Na_2SO_3 exposure.

In addition to fatty acid decomposition and fat synthesis, VLDL secretion is a key factor that affects the TG level in the hepatocytes [43,28,29]. This study investigated the assembly and secretion of VLDL of L-02 hepatocytes after exposure to Na_2SO_3. After 24 h exposure, the VLDL content in the supernatant was significantly increased (Table 5). This result is consistent with our previous results using protein hybridization (data not shown), and further proved that Na_2SO_3 is prone to promote the secretion of VLDL. Meanwhile, it was also found that exposure to Na_2SO_3 could promote the secretion of apoB100 (Table 6).

In normal physiological condition, TG should be effectively and properly assembled and transported before VLDL secretion. This complex process requires many important protein molecules. Our study mainly focused on four types of commonly recognized proteins, that is MTP, TGH, Arf1 and COPI. Initially, MTP was discovered to catalyze the transportation of neutral lipids, and serves as an essential molecule for the assembly and maturation of lipoprotein VLDL, which contains abundant apoB100 [29,44,45]. Studies have found that inhibition of MTP activity by chemical inhibitor might lead to failure of VLDL assembly [46], while the secretion of VLDL might be elevated with increased expression of MTP [31]. In this study, the expressions of MTP in the hepatocytes were significantly increased after exposure to Na_2SO_3 (Fig. 2), thereby we speculate that MTP is associated with increased secretions of VLDL (Table 5) and apoB100(Table 6) in the hepatocytes. TGH is involved in the hydrolysis and re-esterification of TG in the VLDL assembly [34]. More than 70% of the TG in the hepatocytes can be evacuated after hydrolysis and re-esterification. An elevated TGH expression in the hepatocytes may enhance its mobilization and the re-esterification [47,48]. On the contrary, inhibition of TGH activity in the hepatocytes may lead to limited mobilization of TG and reduced secretion of VLDL [33]. Our study found that the expression of TGH was significantly increased after exposure to Na_2SO_3 suggesting that TGH alternation is associated with the increased Na_2SO_3-related secretions of VLDL and apoB100. The effects of Na_2SO_3 on the expressions of Arf1 and COP β proteins were also studied. Arf1 is an important molecule for sorting and transporting proteins from the endoplasmic reticulum to the Golgi apparatus. Arf1 can also facilitate the functions of mitochondria and endoplasmic reticulum [49,50]. Arf1 is necessary for the movement of special secretory vesicles VLDL precursor from the endoplasmic reticulum to cis-face of the Golgi apparatus, where an overexpression of Arf1 might promote the secretion of VLDL [35]. COP β is one of COPI' s subunits. This experiment showed that Na_2SO_3 promotes the expression of Arf1 and COPβ, suggesting that they are associated with the promoting effect of Na_2SO_3 on secretions of apoB100.

We observed increased VLDL content in the supernatant after Na_2SO_3 exposure. LDLR is transcriptionally regulated by the sterol regulatory element binding protein 2 (SREBP-2). LDLR is also post-translationally regulated by proprotein convertase subtilisin/kexin-type 9 (PCSK9) and inducible degrader of the LDLR (IDOL) [51–55]. We found that Na_2SO_3 decreased the expression of LDLR, it means that it can inhibit the uptake of VLDL in the supernatant. It may also contribute to the increase of VLDL and apoB100 in the supernatant.

In summary, this study indicated that exposure to Na_2SO_3 has little effects on the fat accumulation in human hepatocytes. However, Na_2SO_3 may promote the secretions of VLDL and apoB100, and increase the expressions of MTP and TGH. As these proteins are related to VLDL assembly, our results indicate that Na_2SO_3 may promote the secretions of VLDL and apoB100 through MTP and TGH pathway. This study also suggests that exposure of Na_2SO_3 may decrease the expression to LDLR which may contribute to the increase of VLDL secretion.

Conflict of interest

The authors declare that there are no conflicts of interest.

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