Journal of Exploratory Research in Pharmacology **2024** vol. 9(3) | 135–144
DOI: 10.14218/JERP.2024.00017

Original Article

Taurine Reduces Atherosclerotic Plaque Area and Stability in Mice

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Received: April 18, 2024 | **Revised:** August 14, 2024 | **Accepted:** September 27, 2024 | **Published online:** September 25, 2024

Abstract

Background and objectives: Previous studies suggest that taurine supplementation may attenuate atherosclerosis by reducing lipid levels. However, energy drinks containing taurine have been shown to increase blood pressure, a key risk factor for atherosclerosis. Thus, the role of taurine in atherosclerosis remains controversial. This study aimed to investigate the effect of taurine on the development of atherosclerotic plaques.

Methods: Plasma taurine levels were measured in 105 patients with varying degrees of coronary heart disease and in 40 healthy individuals using 1,2-$^{13}C_2$-taurine-based ultra-performance liquid chromatography-tandem quadrupole mass spectrometry (UPLC-QQQ-MS/MS). Apolipoprotein E knockout (ApoE$^{-/-}$) C57BL/6J mice, fed a high-fat diet and subjected to left carotid artery ligation with cannula insertion, received taurine or saline for four consecutive days. Healthy control mice were fed a normal chow diet and underwent a sham operation. Serum taurine levels, lipid indicators, and arterial histology in the individual mice were examined.

Results: Plasma taurine levels were significantly higher in patients with acute myocardial infarction (4.04 ± 0.24 μg/mL) compared to healthy controls (3.52 ± 0.22 μg/mL). Taurine treatment significantly decreased plaque areas in the carotid artery, reduced Masson's Trichrome staining, and lowered the ratio of anti-α-SMA to anti-CD68 staining in ApoE$^{-/-}$ mice. Additionally, taurine treatment increased the levels of matrix metalloproteinase 2 in the cultured vascular endothelial cells *in vitro*.

Conclusions: These findings suggest that taurine supplementation may reduce both the size and stability of atherosclerotic plaques. Therefore, dietary taurine supplements should be used with caution.

Introduction

Atherosclerosis is a chronic, progressive inflammatory disorder affecting large and medium-sized arteries,[1] which can lead to myocardial infarction, ischemic cardiomyopathy, strokes, and peripheral arterial disease.[2] In recent years, atherosclerosis has become a significant global health threat, particularly with the aging population.[3] Together, myocardial infarction and stroke are the leading causes of death, accounting for 31% of fatalities in America and a similar percentage worldwide.[4] Since the 21st century, the incidence of atherosclerosis in China has increased significantly. Various risk factors contribute to the development of atherosclerosis, including hyperlipidemia, arterial hypertension, and diabetes.[5] This condition involves plaque formation, comprising various cells, lipids, and tissue debris in the vascular intima,[6] where the arteries narrow and harden,[7] leading to blood flow restriction or blockage.[3] Recent studies have demonstrated that vascular smooth muscle cells are crucial for the occurrence and progression of atherosclerosis, as well as in plaque stability.[8] During lesion growth,

Keywords: Taurine; Atherosclerosis; Atherosclerotic plaques; ApoE knockout mice; Coronary heart disease; UPLC-MS; Matrix metalloproteinase 2.

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How to cite this article: Wei MG, Ying A, Huang FQ, Wang FX, Alolga RN, Ma GX, *et al*. Taurine Reduces Atherosclerotic Plaque Area and Stability in Mice. *J Explor Res Pharmacol* 2024;9(3):135–144. doi: 10.14218/JERP.2024.00017.

smooth muscle cells in the media transition from a contractile to a proliferative state and migrate into the intima. Over time, these intimal smooth muscle cells secrete an extracellular matrix primarily composed of collagen, forming a protective fibrous cap that helps prevent rupture.[9] Therefore, developing therapies to stabilize atherosclerotic plaques is vital for preventing plaque rupture and its harmful consequences.

Taurine is a vital nutrient found abundantly in human organs such as the heart, brain, and liver.[10] Although taurine can be synthesized in the body, most is obtained through diet.[11] It has been widely used as a dietary supplement to manage cardiovascular diseases and type 2 diabetes.[12,13] However, studies have shown contradictory effects of taurine on blood pressure. While clinical administration of taurine at 1.6 g/day for 12 weeks has been found to lower blood pressure in patients with hypertension,[14] combining taurine with caffeine in energy drinks may lead to acute blood pressure elevation.[15] Hypertension can promote atherosclerosis by increasing vessel contractility and causing endothelial injuries, which leads to the formation of atherosclerotic plaques.[16] Hence, the role of taurine in the development of atherosclerosis remains largely unclear.

To investigate the effects of taurine on atherosclerosis, we conducted a targeted quantitative analysis of plasma taurine levels in 105 patients with varying degrees of coronary heart disease using ultra-performance liquid chromatography-tandem quadrupole mass spectrometry (UPLC-QQQ-MS/MS). We also induced plaque formation by carotid artery ligation and cannulation in apolipoprotein E knockout ($ApoE^{-/-}$) mice and studied the impact of taurine administration on plaque size, stability, and related indicators. We found that plasma taurine levels in patients with acute myocardial infarction were significantly higher than those in healthy cohorts. Taurine administration reduced plaque size and stability in the mouse model of atherosclerosis. Accordingly, taurine may diminish atherosclerotic plaque formation and stability in mice.

Materials and methods

Human specimens

Plasma samples were obtained from patients with varying degrees of coronary heart disease at the Sir Run Run Shaw Hospital of Nanjing Medical University, in compliance with a human research protocol approved by their institutional review board, between January 2017 and April 2018. Upon collection, the samples were aliquoted into 120 µL tubes and immediately stored at –80 °C. The plasma samples were categorized into four groups based on the patients' conditions: normal coronary artery (NCA, n = 40), stable angina (SA, n = 36), unstable angina (UA, n = 46), and acute myocardial infarction (AMI, n = 23).[17] Detailed information regarding the sample donors is presented in Table S1.

Sample preparation

Plasma taurine was extracted using methanol/acetonitrile (1:1) (Merck), containing 0.15 µg/mL of 1,2-$^{13}C_2$-taurine (Cambridge Isotope Laboratories). After centrifugation at 16,200 *g* for 10 m, 150 µL of the supernatant was transferred to a new EP tube and dried under a gentle stream of nitrogen at room temperature. The residue was then dissolved in 150 µL of 20% acetonitrile and centrifuged at 16,200 *g* for 10 m at 4°C. Subsequently, 60 µL of the supernatant was transferred to an MS vial for analysis. The quality control (QC) samples were prepared as a mixture of the collected plasma, consistent with the aforementioned method for other samples. QC samples were run after every 12 injections.

UPLC-QQQ-MS/MS analysis

Taurine quantification was conducted using an Agilent 6470 Triple Quadrupole LC-MS/MS system (Agilent Technologies). Chromatographic separation was performed on a Waters ACQUITY UPLC BEH Amide (2.1 × 100 mm, 1.7 µm) column. The mobile phase consisted of water with 10 mM ammonium acetate (A) and 90% acetonitrile with 10 mM ammonium acetate (B). The elution gradient was as follows: 95% B at 0–1 m, 95–80% B at 1–2.5 m, 80–50% B at 2.5–3.5 m, 50–95% B at 3.5–4 m, and 95% B until 10 m. The sample volume injected was 1 µL, with a flow rate of 0.4 mL/min and a column temperature maintained at 45 °C.

The electrospray ionization source operated in negative ion mode, with MS parameters set as follows: fragment voltage at 120 V, nebulizer gas at 45 psig, capillary voltage at 3,500 V, drying gas flow rate at 10 L/min, and temperature at 300 °C. Multiple reaction monitoring transitions were performed at *m/z* 124→80 for taurine and *m/z* 126→80 for 1,2-$^{13}C_2$-taurine.[18]

Method validation

The linearity of taurine quantification was validated using the isotope internal standard method. Briefly, a series of taurine concentrations and 5 µL of 0.15 µg/mL 1,2-$^{13}C_2$-taurine were added to 50 µL of the QC sample, and the extracted taurine was analyzed. The standard curve equation was $y = 0.9327x + 0.076$ ($r^2 = 0.9996$, weight factor: $1/x^2$). The relative standard deviation for inter-day precision was 0.83%. The extraction recovery for spiked high, moderate, and low taurine was 91.8%, 91.6%, and 94.4%, respectively, with a relative standard deviation of 1.7%. The matrix effect for spiked high, moderate, and low taurine was 352.1%, 405.4%, and 399.5%, respectively, with a relative standard deviation of 7.6%. The limit of detection (S/N > 3) and limit of quantification (S/N > 10) were 0.3 ng/mL and 2 ng/mL, respectively.

Mice

Male $ApoE^{-/-}$ C57BL/6J mice were obtained from Changzhou Cavens Laboratory Animal Co. Ltd and housed at the Animal Center of China Pharmaceutical University on a 12 h/12 h light/dark cycle with free access to food and water *ad libitum*. Normal chow was purchased from the Animal Center of China Pharmaceutical University, and a high-fat diet (60 kcal% fat) was prepared and provided by the Institute of Laboratory Animals, Chinese Academy of Medical Sciences. All animals were allowed to acclimate for seven days prior to experiments. All animal experiments were conducted in accordance with regulations on experimental animal welfare and were approved by the Ethics Committee of Animal Experiments of China Pharmaceutical University (No. 2022-05-031).

Animal model

Male $ApoE^{-/-}$ mice (nine weeks old) were randomized into three groups: Normal chow diet (NCD) + Sham, high-fat diet (HFD) + Ligation and Cuff, and HFD + Ligation and Cuff + Taurine (n = 10 per group). The mice in the latter two groups were fed a high-fat diet for four weeks and subjected to left carotid artery ligation, while the NCD + Sham group received a normal chow diet and a sham operation. A PE-50 tube was then cannulated into the artery below the ligation site.[19,20] Mice in the HFD + Ligation and Cuff + Taurine group were administered 50 mg/kg taurine (T103829, Aladdin) by gavage daily for four consecutive days.[21–23] Four days later, all mice were anesthetized with 1% pentobarbital sodium, and the common carotid artery at the surgical site was isolated and

immersed in 4% paraformaldehyde (KeyGEN BioTECH). After one day of fixation, the tissues were embedded in paraffin and subjected to immunohistochemistry, hematoxylin & eosin (H&E) staining, and collagen staining.

Histological analysis

After fixation in paraformaldehyde for one day, the tissues were embedded in paraffin and sectioned. The 4 μm sections were stained with Hematoxylin and Eosin or Masson's Trichrome, following the manufacturer's instructions (Solarbio). Furthermore, some sections were dewaxed, rehydrated, and blocked with 5% fat-free dry milk in tris-borate-sodium-tween-20 (TBST), then incubated overnight at 4 °C with anti-α-SMA (Beyotime) or anti-CD68 (Beyotime).[24] After being washed with phosphate-buffered saline, the sections were treated with goat anti-rabbit IgG and counterstained with hematoxylin. All results were captured using a slide scanner (NanoZoomer 2.0RS), and protein expression levels were analyzed using ImageJ software.

ELISA assays

Plasma samples were collected from 10 mice with atherosclerosis (fed a high-fat diet for 12 weeks) and from 114 patients with coronary heart disease. Plasma taurine concentrations were quantified using ELISA kits according to the manufacturer's instructions (Jiancheng). Absorbance was measured at 450 nm, and taurine concentrations were calculated from the standard curves.

Cell culture

Human umbilical vein endothelial cells and rat smooth muscle cells were obtained from the American Type Culture Collection and maintained at 37 °C with 5% CO_2 in DMEM (KeyGEN BioTECH) containing 4.5 g/L D-glucose and supplemented with 10% FBS (Gibco).

RNA extraction and qRT-PCR

Total RNA was extracted from human umbilical vein endothelial cells using TRIzol reagent (Invitrogen) and reverse-transcribed into cDNA using the HiScript QRT Super Mix (Invitrogen) following the manufacturer's protocols. The relative levels of target gene mRNA transcripts were quantified by qRT-PCR on the Roche LightCycler 96 system using Fast SYBR Green Master Mix (Roche). Expression levels were normalized to *18s* rRNA. Primer sequences are as follows:

- Human *18s* rRNA, Forward: 5′-CGGCTACCACATCCAAGGAA-3′;
- Human *18s* rRNA, Reverse: 5′-GCTGGAATTACCGCGGCT-3′;
- Human matrix metalloproteinase (*MMP)-2*, Forward: 5′-TACAGGATCATTGGCTACACACC-3′;
- Human *MMP-2*, Reverse: 5′-GGTCACATCGCTCCAGACT-3′;
- Human *MMP-9*, Forward: 5′-TGTACCGCTATGGTTACACTCG-3′;
- Human *MMP-9*, Reverse: 5′-GGCAGGGACAGTTGCTTCT-3′.

Quantifications of total bile acid, total cholesterol, and low-density lipoprotein

Serum levels of total bile acid, total cholesterol, and low-density lipoprotein were measured using commercial kits, according to the manufacturer's instructions (Jiancheng).

Cell cycle flow cytometry analysis

Rat smooth muscle cells in the logarithmic phase were treated with serum-free medium containing 1 μmol/L angiotensin II or angiotensin II + 20 mmol/L taurine for 24 h. The cells were harvested, and cell cycle analysis was performed using flow cytometry kits, following the manufacturer's instructions (Beyotime). Data were analyzed using ModFitLT.

Western-blot analysis

Cells from each group were harvested and lysed in buffer containing protein inhibitors. After determining protein concentrations, 30 μg of each sample was separated by SDS-PAGE on 12% gels and transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). Membranes were blocked with 5% fat-free dry milk in TBST and incubated with primary antibodies overnight at 4 °C. After being washed, the bound antibodies were detected with diluted HRP-conjugated secondary antibodies and visualized using a chemiluminescence imaging system (Tanon). The primary antibodies included mouse monoclonal anti-P53, rabbit monoclonal anti-P21, mouse monoclonal anti-MDM2, rabbit monoclonal anti-CDC2, and rabbit monoclonal anti-β-Actin (Cell Signaling Technology).

Statistical analysis

Data are presented as mean ± standard error of the mean. Statistical analysis between two groups was made using a two-tailed Student's t-test. Statistical analysis between three or more groups was made using analysis of variance. Spearman correlation coefficients were used to assess differences among the four groups, and the U test was used for paired samples. Statistical significance was defined as $p < 0.05$. Data were analyzed using GraphPad Prism version 7.0.

Results

Higher levels of plasma taurine are detected in patients with acute myocardial infarction

To investigate the association between plasma taurine levels and coronary heart disease as well as atherosclerosis, we quantitatively analyzed plasma taurine levels in patients with NCA, SA, UA, and AMI using UPLC-QQQ-MS/MS. The results showed that plasma taurine levels in the AMI group were significantly higher than those in the NCA and SA groups, while the UA group displayed an upward trend (Fig. 1a). Additionally, we measured plasma taurine levels in 114 patients using ELISA and found that plasma taurine was significantly elevated in those with acute coronary syndrome (UA + AMI) (Fig. 1b). These findings were consistent with the results from taurine-targeted quantitative analysis. Clearly, higher plasma taurine levels were detected in patients with acute coronary syndrome, a late stage of atherosclerosis, which may be due to taurine's protective effects on the myocardium. However, it remains unclear whether the increased plasma taurine concentrations have a harmful or protective impact on atherosclerotic plaques and vessel walls.

Taurine treatment reduces plaque areas and stability in mice

Next, we investigated the effects of taurine on atherosclerosis *in vivo* using $ApoE^{-/-}$ mice fed with HFD for 12 weeks. Compared to NCA mice, HFD significantly elevated plasma taurine levels in atherosclerotic mice (Fig. 2a), consistent with the results from taurine-targeted quantitative analysis. To accelerate the development of larger arterial plaques, we induced carotid artery ligation and cannulation in the mice and fed them HFD for four weeks

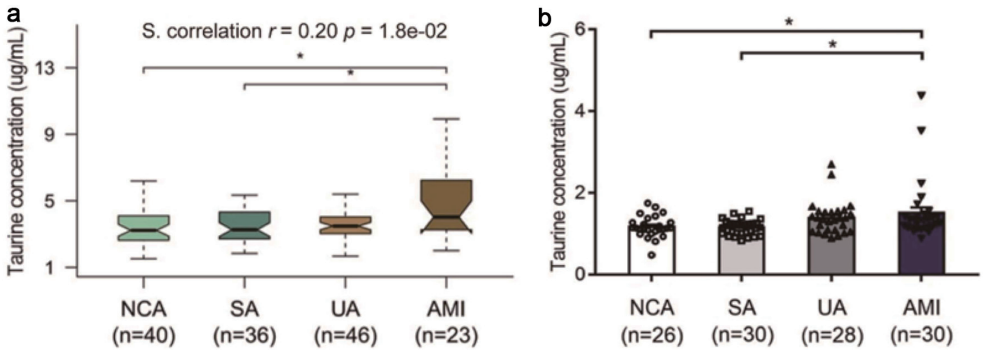

Fig. 1. Targeted quantitative analysis of plasma taurine levels in patients with varying degrees of coronary heart disease. (a) Taurine quantification in 145 plasma samples. The Spearman correlation coefficient was used to test differences among the four groups of samples, and the U test was used to perform statistical analyses on paired samples. (Standard curve: y = 0.9327x + 0.076, r^2 = 0.9996); (b) Detection of taurine levels in 114 plasma samples using an ELISA kit. *p < 0.05. AMI, acute myocardial infarction; NCA, normal coronary artery; S. correlation, Spearman correlation; SA, stable angina; UA, unstable angina.

(Fig. 2b). Histological analysis revealed that neointimal hyperplasia progressed gradually over 30 days after ligation, and arterial plaques expanded rapidly within four days post-cannulation, with most atherosclerotic mice having plaque areas of over 90% (Fig. 2c). Taurine was administered to the mice for four days, and H&E staining of the left carotid artery revealed that higher doses of taurine significantly reduced plaque areas (Fig. 2b, d, e). Similarly, Masson's Trichrome staining of the left carotid artery revealed that taurine administration also significantly reduced the proportion of collagen in plaques (Fig. 2d, f). These findings suggest that taurine treatment decreased plaque stability, potentially increasing the risk of plaque rupture.

Taurine treatment decreases plaque stability by reducing the ratios of smooth muscle cells to macrophages

Under normal physiological conditions, the tunica media is composed of vascular smooth muscle cells, which help maintain the elasticity of blood vessels. Atherosclerotic plaques initially form in the tunica intima of the vessel wall. In the early stages of plaque formation, vascular smooth muscle cells migrate from the tunica media to the tunica intima, where their secreted extracellular matrix encapsulates lipoproteins, promoting plaque expansion. Smooth muscle cells contain large amounts of α-SMA, making the expression levels of α-SMA a reflection of the smooth muscle cell content within plaques. Immunohistochemistry revealed that the content of anti-α-SMA staining in plaques was significantly higher in the HFD + Ligation and Cuff group compared to the NCD + Sham group, but lower in the HFD + Ligation and Cuff + Taurine group (Fig. 3a, b). This suggests that taurine treatment may reduce the smooth muscle cell content in plaques, potentially leading to thrombolysis and a reduction in plaque area. Additionally, taurine did not affect the number of smooth muscle cells in the tunica media but did reduce the content of abnormally proliferating smooth muscle cells in the tunica intima, indicating that taurine might not impair vascular elasticity (Fig. 3e, f). Macrophages are crucial for plaque development, as they phagocytose low density lipoprotein to form lipid-rich foam cells, which make up the core of the plaque. CD68 antigen serves as a biomarker for macrophages within plaques. Our results indicated that macrophage content in the HFD + Ligation and Cuff + Taurine group was not significantly different from the HFD + Ligation and Cuff group, suggesting that taurine does not reduce macrophage content in the plaques (Fig. 3a, c). The ratio of smooth muscle cells to macrophages is an indicator of plaque stability, with a higher ratio suggesting more stable plaques. The ratio of anti-α-SMA to anti-CD68 staining in the HFD + Ligation and Cuff + Taurine group was evidently lower than in the HFD + Ligation and Cuff group, indicating that taurine decreased plaque stability and increased the possibility of plaque rupture, despite reducing plaque area (Fig. 3d).

Taurine induces MMP-2 expression in vitro

MMP-2 and MMP-9, are key enzymes secreted by vascular endothelial cells that degrade collagen within plaques. Clinical studies have shown that MMP-2 and MMP-9 levels are significantly elevated at the rupture sites of carotid plaques, indicating a strong correlation between these enzymes and plaque rupture as well as fibrous cap thinning. We investigated the effect of taurine on MMP expression in endothelial cells and found that taurine treatment significantly increased MMP-2 mRNA levels but had no effect on MMP-9 levels in umbilical vein endothelial cells. This suggests that taurine may reduce collagen content in plaques primarily by increasing MMP-2 expression (Fig. 4a, b).

Taurine reduces the neointima area in carotid arteries without affecting elastic fibers

Atheroma formation results in a significant expansion of the tunica intima and media, with an increase in media area indicating the stretching of elastic fibers, which impairs the elasticity of the vessel wall. This, in turn, increases the risk of hypertension, thrombosis, or rupture under high blood flow. However, after four days of taurine treatment, no significant change in the media area was observed (Fig. 5a–c). Consequently, the ratios of carotid intima areas to media areas were significantly reduced (Fig. 5d). Moreover, the outer circumference of blood vessels in both the HFD + Ligation and Cuff group and the HFD + Ligation and Cuff + Taurine group was similar to that of the NCD + Sham group (Fig. 5e), indicating that taurine treatment did not alter the length of elastic fibers in the intima and media. Therefore, taurine treatment did not reverse elastic fiber stretching or affect the length of these fibers.

Taurine treatment does not improve cholesterol metabolism in atherosclerotic mice

Hyperlipidemia is one of the primary causes of atherosclerosis. While adding 0.5% taurine to the drinking water for 14 weeks significantly reduced plaque areas in atherosclerotic rabbits, taurine

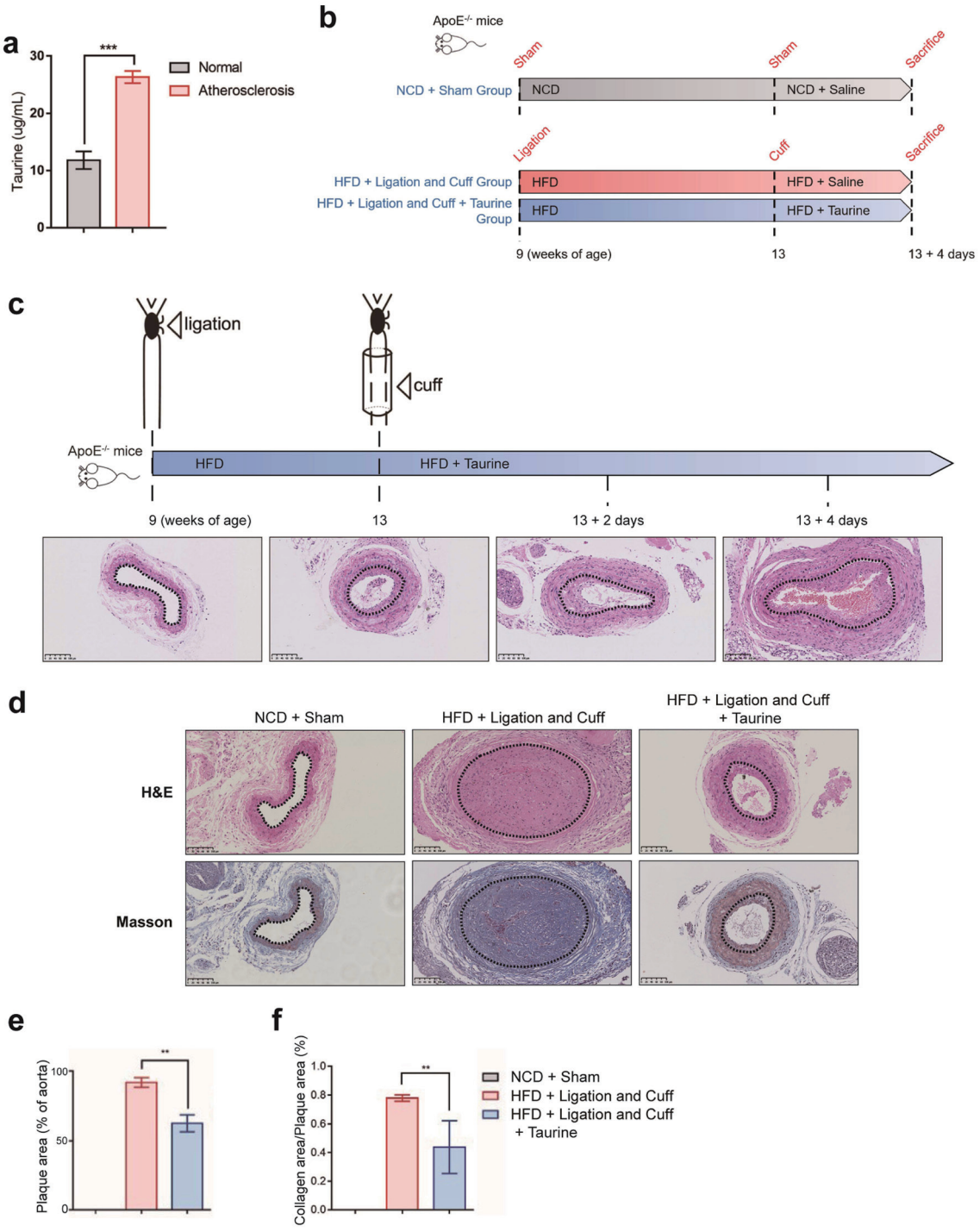

Fig. 2. Effects of taurine on plaque formation and stability in atherosclerotic mice. (a) Detection of plasma taurine levels in atherosclerotic mice using ELISA; (b) Experimental procedure for the mouse model of atherosclerosis; (c) Comparison of arterial plaques in model mice at different stages of atherosclerosis; (d–f) Effects of taurine on plaque areas and collagen content in atherosclerotic mice (n = 6). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Scale bar = 100 μm. HFD, high fat diet; H&E, hematoxylin & eosin; NCD, normal chow diet.

treatment for four weeks in atherosclerotic mice reduced plaque size but did not improve lipid metabolism. In our study, taurine treatment for four days did not significantly decrease plasma levels of total bile acid (TBA), total cholesterol (T-CHO), or low density lipoprotein-cholesterol (LDL-C) in atherosclerotic mice (Fig. 6a–c). This suggests that the short-term reduction in plaque areas observed with taurine treatment may not be due to improvements in cholesterol metabolism. Instead, taurine may act on atherosclerosis

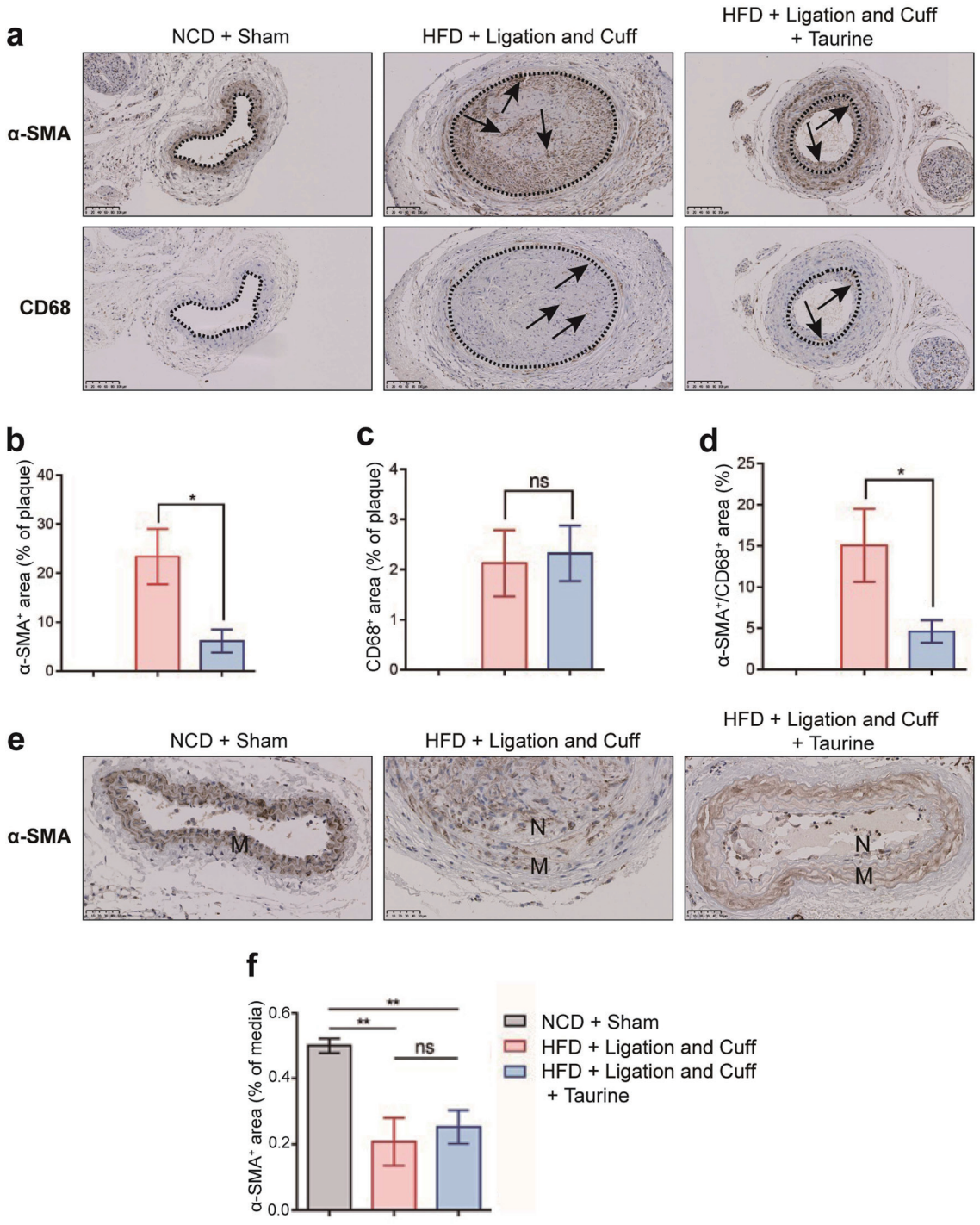

Fig. 3. Effects of taurine on smooth muscle cells and macrophages in the carotid arteries of atherosclerotic mice (n = 6). (a–d) Effects of taurine on the proportion of smooth muscle cells and macrophages in plaques of atherosclerotic mice; (e–f) Effects of taurine on carotid media smooth muscle cells in atherosclerotic model mice. M: media area; N: intima area. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Scale bar = 100 μm. HFD, high-fat diet; NCD, normal chow diet.

rotic plaques through other mechanisms.

Discussion

Previous studies have reported that long-term taurine treatment can improve atherosclerosis through its hypolipidemic effects.[25–27] However, in our study, we did not observe significant changes in plasma cholesterol metabolism indicators, such as TBA, T-CHO, and LDL-C, after four days of taurine treatment in mice. This suggests that short-term taurine treatment may not be suf-

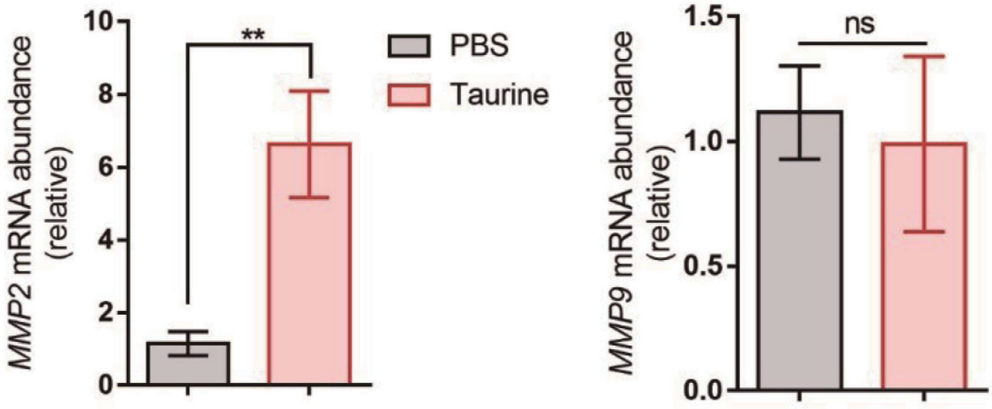

Fig. 4. Effects of taurine on the expression of metalloproteinases in vascular endothelial cells (n = 6). (a) *MMP-2* relative gene expression in endothelial cells after taurine administration; (b) *MMP-9* relative gene expression in endothelial cells after taurine administration. **$p < 0.01$. MMP-2, matrix metallopeptidase 2; MMP-9, matrix metallopeptidase 9.

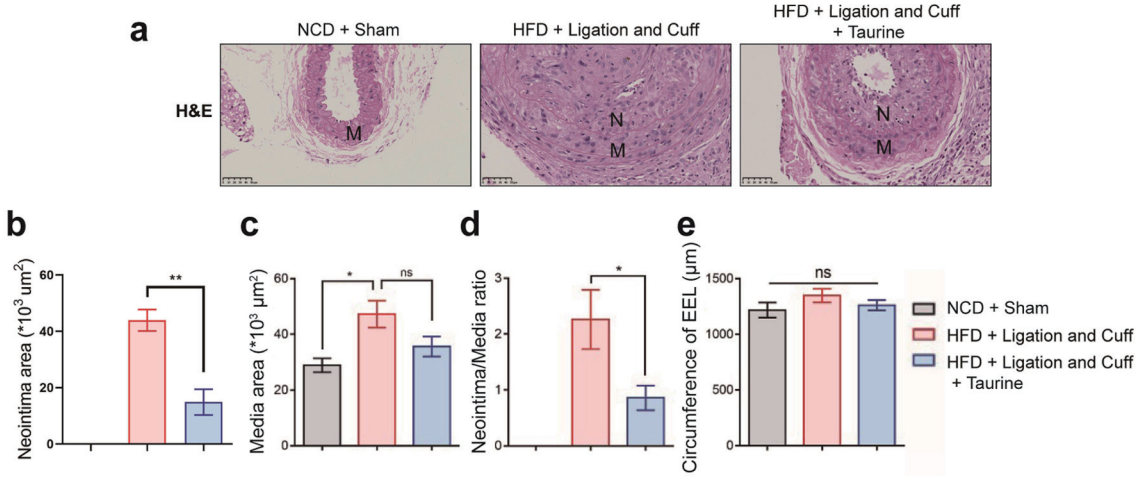

Fig. 5. Effects of taurine on carotid intima-media ratio and outer circumference in mice (n = 6). (a) HE-stained carotid intima and media; (b) Neointima areas; (c) Media areas; (d) Ratios of intima-media areas; (e) Outer circumference of the carotid artery. *$p < 0.05$; **$p < 0.01$. Scale bar = 100 μm. EEL, external elastic lamina; HFD, high-fat diet; NCD, normal chow diet.

ficient to improve hypercholesterolemia in this mouse model of atherosclerosis. Therefore, the mechanism by which short-term taurine treatment improves atherosclerosis may act through other pathways. Taurine has been reported to mediate the reduction of carotid plaques.[28,29] Consistently, our study found that high-dose, short-term taurine treatment significantly reduced carotid plaque

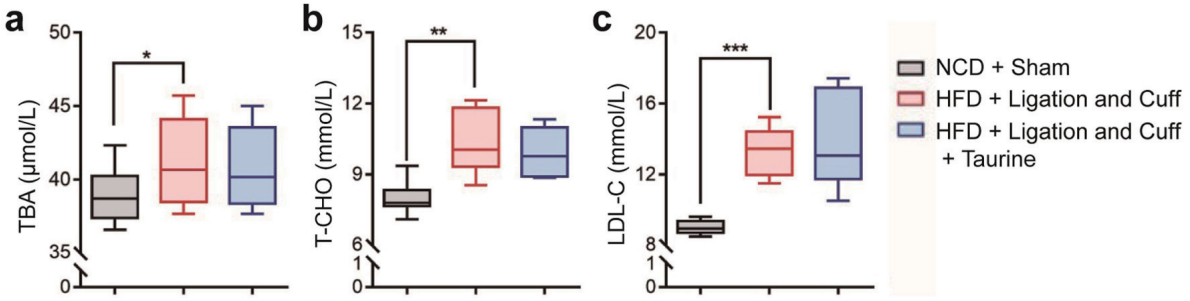

Fig. 6. Effects of taurine on plasma lipid metabolism indicators in atherosclerotic mice (n = 6). (a) Plasma total bile acid levels; (b) Plasma total cholesterol levels; (c) Plasma low-density lipoprotein levels. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. HFD, high-fat diet; LDL-C, low density lipoprotein-cholesterol; NCD, normal chow diet; TBA, total bile acid; T-CHO, total cholesterol.

areas in atherosclerotic mice.

Assessing plaque vulnerability is crucial to preventing the harmful consequences of cardiovascular disease. Previous reports have shown that taurine may inhibit the rupture of atherosclerotic lesions.[29,30] However, our study revealed that taurine treatment significantly reduced plaque stability in mice, which could increase the risk of plaque rupture. Supporting this, taurine treatment enhanced the expression of MMP-2 in vascular endothelial cells *in vitro*, which can reduce the collagen content in plaques. However, we did not find that taurine treatment significantly altered MMP-9 expression in vascular endothelial cells, expanding on previous reports that taurine inhibits MMP-9 activation in the glomerular basement membrane. Additionally, we only verified the mRNA transcripts, not the protein levels, of MMP-2 and MMP-9 in our experiment. As previous reports have shown, when plaques are unstable, protein levels of MMP-2 and MMP-9 are elevated.[31,32] Since we have already confirmed that taurine increases plaque vulnerability, we hypothesize that the protein levels of MMP-2 and MMP-9 would also be upregulated after taurine treatment, though this has not yet been experimentally validated. Furthermore, long-term taurine treatment may stabilize artery plaques. In ApoE$^{-/-}$ mice fed a high-fat diet for 16 weeks, with 2% (w/v) taurine in drinking water for six weeks, taurine alleviated plaque vulnerability, as shown by quantification of the proportion of collagen, CD68, and α-actin.[28] Therefore, we speculate that in the high-fat diet plus carotid artery ligation and cannula model used in our research, prolonging the taurine treatment period may reduce the risk of plaque rupture. This may be because the long-term taurine treatment lowers cholesterol levels.[33] As cholesterol is the lipid core of plaques, reducing its levels may inhibit plaque growth. The fibrous cap on the plaque surface would then stop thinning, and the risk of plaque rupture would decrease.

Accumulating evidence from clinical investigations suggests that long-term taurine supplementation may decrease the risk of cardiovascular diseases,[26,34–39] possibly due to its thrombolytic effect.[40] However, it is known that urokinase, a natural clot-busting drug, binds to its receptor on macrophages, potentially increasing the likelihood of plaque rupture.[41] Based on this, we speculate that taurine may also contribute to increased plaque rupture vulnerability. Moreover, we observed that taurine treatment induced cell cycle arrest in the G/M phase in smooth muscle cells, possibly through its effects on p53 (Fig. S1a–c). This may explain why taurine reduces the smooth muscle cell content in plaques, thus lowering the ratios of smooth muscle cells to macrophages and decreasing plaque stability.

Our study found that plasma taurine levels in patients with acute myocardial infarction were significantly higher than in those with normal coronary function or stable angina. This may reflect the myocardial protective effect of compensatory taurine increase. Additionally, we observed a negative correlation between taurine levels and age in patients with UA and AMI, suggesting that taurine's potential to improve acute coronary syndromes may be greater in younger patients. Collectively, clinicians should monitor vascular smooth muscle cells closely when using taurine as a dietary supplement, as excessive administration may reduce smooth muscle cells and increase the risk of plaque rupture.

Limitations

First, this study only explored the impact of short-term administration of taurine on atherosclerotic plaques. Long-term supplementation of taurine, which would better reflect the situation of people consuming energy drinks in daily life, is recommended in future studies. Second, oil red O staining is lack in our study as a direct evidence to determine the plaque area. Third, carotid artery sections should display the longitudinal rather than the cross-sectional view of the blood vessel so that the size of the plaque and the thickness of the fibrous cap can be directly displayed and quantified. Forth, this study did not identify the targets and downstream pathways by which taurine reduces plaque area and stability.

Future directions

Although the present study found that short-term taurine treatment significantly reduced plaque area and stability in atherosclerotic mice, which may increase plaque vulnerability to rupture, further investigations are needed to elucidate the mechanisms by which taurine affects both plaque area and stability. Additionally, the potential risks of taurine in reducing atherosclerotic plaque formation in patients should be further explored.

Conclusions

In this study, we utilized a targeted analytical strategy and biological approaches to investigate the role of taurine in atherosclerosis development. Based on our quantification of taurine, we found that plasma taurine concentrations in patients with coronary heart disease were significantly higher than those in the NCA group. Consistently higher levels of plasma taurine were detected in atherosclerotic mice. Furthermore, taurine administration reduced plaque area and stability in atherosclerotic mice, which may increase the vulnerability of atherosclerotic plaques to rupture.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare no potential conflict of interest.

Author contributions

Research conception (AP, GXM), experiment performance (AY, FXW), figure preparation and manuscript writing (MGW, FQH), and manuscript review (AP, GXM, RNA). All authors approved the manuscript for publication.

Ethical statement

Plasma samples from patients were obtained from the Sir Run Run Shaw Hospital of Nanjing Medical University in compliance with the human research protocol approved by the institutional review board. The study adhered to the guidelines outlined in the 1975 Declaration of Helsinki. Informed consent was obtained from all participants. All animal experiments were performed in accordance with the regulations on experimental animal welfare and were approved by the Ethics Committee of Animal Experiments of China Pharmaceutical University (No. 2022-05-031).

Data sharing statement

No additional data are available.

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