British Journal of Nutrition (2024), 131, 1342–1351 doi:10.1017/S0007114523002969

Preventive effects of caffeine on nicotine plus high-fat diet-induced hepatic steatosis and gain weight: a possible explanation for why obese smokers with high coffee consumption tend to be leaner

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(Submitted 6 June 2023 – Final revision received 30 November 2023 – Accepted 20 December 2023 – First published online 27 December 2023)

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a prevalent liver disorder, affecting approximately 25 % of the population. Coffee-drinking obese smokers exhibit lower body weights and decreased NAFLD rates, but the reasons behind this remain unclear. Additionally, the effect of nicotine, the main component of tobacco, on the development of NAFLD is still controversial. Our study aimed to explore the possible reasons that drinking coffee could alleviate NAFLD and gain weight and identify the real role of nicotine in NAFLD of obese smokers. A NAFLD model in mice was induced by administering nicotine and a high-fat diet (HFD). We recorded changes in body weight and daily food intake, measured the weights of the liver and visceral fat, and observed liver and adipose tissue histopathology. Lipid levels, liver function, liver malondialdehyde (MDA), superoxide dismutase (SOD), serum inflammatory cytokine levels and the expression of hepatic genes involved in lipid metabolism were determined. Our results demonstrated that nicotine exacerbated the development of NAFLD and caffeine had a hepatoprotective effect on NAFLD. The administration of caffeine could ameliorate nicotine-plus-HFD-induced NAFLD by reducing lipid accumulation, regulating hepatic lipid metabolism, alleviating oxidative stress, attenuating inflammatory response and restoring hepatic functions. These results might explain why obese smokers with high coffee consumption exhibit the lower incidence rate of NAFLD and tend to be leaner. It is essential to emphasise that the detrimental impact of smoking on health is multifaceted. Smoking cessation remains the sole practical and effective strategy for averting the tobacco-related complications and reducing the risk of mortality.

Keywords: **Non-alcoholic fatty liver: Caffeine: Nicotine: Inflammation: Oxidative stress**

Non-alcoholic fatty liver disease (NAFLD) is a prevalent and rapidly growing liver disorder, characterised by the abnormal accumulation of lipids in hepatocytes and the development of insulin resistance. As a metabolic organ, the liver functions in maintaining overall physiological balance. However, NAFLD could disrupt liver function, resulting in symptoms such as oxidative stress, inflammatory responses, lipid metabolism disorders and dyslipidemia[1]. Epidemiological analysis revealed NAFLD affected approximately 25 % of the global population, with the incidence of non-alcoholic steatohepatitis projected to increase by 56 % by the year 2030[2]. Moreover, NAFLD has rapidly emerged as the leading cause of hepatocellular carcinoma worldwide[3].

Obesity and smoking are widely regarded as two risk factors for NAFLD[4]. Clinical studies have shown that the incidence of NAFLD was higher in obese than in non-obese populations[5,6]. Further, there is a strong correlation between obesity and smoking behaviour[7,8]. Obesity combined with smoking could exacerbate NAFLD through oxidative stress, inflammatory responses, abnormal hepatic lipid metabolism and apoptosis, indicating that obese smokers were susceptible to NAFLD[9,10]. Nicotine is one of the main components of tobacco. Long-term exposure to nicotine affected the sensitivity of nicotinic acetylcholine receptor $\alpha 7$ and aggravated NAFLD[11]. However, nicotine can also improve hepatic steatosis, inflammation and endoplasmic reticulum stress in rats with NAFLD[12,13]. The authentic role of nicotine in NAFLD remains unclear.

Caffeine is the key functional ingredient in coffee, tea and soft drinks. Due to the increasing popularity of coffee in recent years, many people have developed the habit of drinking coffee daily[14]. Epidemiologically speaking, the habit of drinking

Abbreviations: HFD, high-fat diet; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; SAL, saline; SOD, superoxide dismutase; TC, total cholesterol.

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coffee demonstrated a beneficial impact on NAFLD patients: regular coffee drinkers had a significantly lower risk of NAFLD than non-coffee drinkers[15–18]. Caffeine could reduce lipid accumulation, inhibit hepatic lipogenesis and promote lipid catabolism, thereby improving NAFLD induced by high-fat diet (HFD) and preventing obesity-related complications[19–23].

Compared with the general population, health risk factors such as smoking are significantly elevated among the obese. For this specific group – obese smokers, individuals with a daily coffee habit seem to exhibit lower body weights and a reduced incidence of NAFLD. Our study sought to elucidate the actual influence of nicotine on NAFLD and offered a potential explanation for why obese smokers who regularly consume coffee tend to have a lower incidence of NAFLD and tend to be leaner.

Materials and methods

Animals

The 6-week-old specific pathogen free male C57BL/6J male mice (initial body weight 20 ± 2g) were provided by Changzhou Cavins Laboratory Animal Co., Ltd (production licence number: SCXK (Su) 2021–0013), and the quality certificate number is 202227787. The animals were kept in the Animal Experimental Center of Jiangnan University. The ambient temperature was controlled at 25 ± 2°C, the relative humidity was maintained at 50–70 %, and the light conditions of alternating dark and light for 12 h were guaranteed. The mice drank purified water freely and changed the padding at least once a week to keep dry and clean. Animal experiments in this chapter were carried out with the approval of the Ethics Committee of the Animal Experiment Center of Jiangnan University (ethics no.: JN. No 20230215c0400701[016]). Animal experiments and operations are conducted following the guiding principles of animal protection to ensure the welfare of experimental animals.

Experimental protocol

The animals were fed adaptively for a week, and no abnormal condition was detected before the experiment was started. Mice were divided into four groups with six mice in each group. Each mouse was injected intraperitoneally once a day (0·1 ml/10 g): mice in the normal group were injected intraperitoneally with normal saline (SAL) and received a chow diet; the HFD + SAL group was intraperitoneally injected with normal SAL and received a HFD; the HFD + nicotine (NIC) group was intraperitoneally injected with an injection dose of 1·5 mg/kg nicotine solution and received a HFD; and the HFD + NIC + caffeine (CAF) group was intraperitoneally injected with an injection dose of 1·5 mg/kg nicotine and 20mg/kg caffeine and received a HFD. In this experiment, nicotine tartrate (purity ≥ 98 %) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd, caffeine (purity ≥ 98 %) was purchased from Shanghai Chunyou Biotechnology Co., Ltd, mouse maintenance feed 1010001 was purchased from Synergia Bioengineering Co., Ltd and mouse HFD (60 kcal %fat) D12492 was supplied by Research Diets, USA. The experiment lasted for 16 weeks. On the day before death, the diets were taken away and the mice were allowed for water. The output of isoflurane was adjusted to 300–500 ml/min, and the induced concentration was adjusted to 3 %–4 %. The mice were completely anesthetised, and the serum was obtained after cardiac blood collection at room temperature for centrifugation. The serum was stored in the refrigerator at −80°C for subsequent determination.

Body weight, food intake, liver weight, lipid weight and liver appearance

The weights were measured at 08:00 every Monday. The weight of diets was weighed before each diet addition, and the rest of diets were weighed at the next diet addition to obtain the average food intake. After the mice were killed, liver tissue, epididymal lipid, perirenal lipid and brown lipid were taken. The residual blood on the surface was rinsed with precooled normal SAL. The surface moisture was carefully absorbed with absorbent paper:

$$\text{Liver weight index} = \frac{\text{Liver wet weight}}{\text{body weight}} \times 100\% \quad (1\text{-}1)$$

$$\text{Visceral lipid index} = \frac{\text{Wet weight of visceral lipid}}{\text{body weight}} \times 100\% \quad (1\text{-}2)$$

Haematoxylin–eosin staining of liver tissue and epididymal adipose tissue

The tissues were clipped for the appropriate size and fixed with 4 % paraformaldehyde. The tissues were successively dehydrated with different concentrations of ethanol. After dehydration, the tissue was soaked in xylene until it was completely transparent. Next, the tissues were embedded in paraffin wax and cut into slices in 5 μm. Then the slices were placed in different concentrations of xylene and ethanol and stained them with hematoxylin and eosin.

Oil Red O staining of liver tissue

The liver tissues were embedded in the optimal cutting temperature compound freezing embedding agent. The liver tissues were cut into 5 μm thick slices with a frozen microtome and stained with the Oil Red O dye kit (Shanghai Biyuntian Biotechnology Co., Ltd).

Determination of blood lipid and transaminase

The contents of total triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum were determined according to the instructions of the kits. All kits were purchased from Nanjing Jiancheng Bioengineering Research Institute Co., Ltd.

Measurement of liver total triglyceride, total cholesterol and oxidative stress

The tissues were taken and precooled normal SAL at 1:10 (w/v), and pick beads were added to make 10 % liver homogenate by

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homogenizer (48T, Eppendorf). The supernatant fluid was obtained after centrifugation at 2500 rpm for 10 min. The contents of TG, TC, malondialdehyde (MDA) and superoxide dismutase (SOD) in the liver were determined according to the instructions of the kits. Total TG and TC kits were purchased from Nanjing Jiancheng Bioengineering Research Institute Co., Ltd, and MDA kit and SOD kit were purchased from Beijing Soleberg Technology Co., Ltd.

Inflammatory cytokine assay

The levels of serum IL-1β, IL-6, TNF-α and IL-10 were determined according to the ELISA kit instructions (Xiamen Huijia Biotechnology Co., LTD).

Liver total RNA extraction and real-time quantitative PCR

Total RNA was extracted from liver tissue using a Trizol solution. Then RNA concentrations were measured using NanoDrop one ultramicro spectrophotometer (Thermo Fisher Technology Co., Ltd). The reverse transcription system and reaction setting conditions were determined according to the instructions of the kit, and the cDNA was obtained by reverse transcription of RNA. The cDNA was diluted ten times as a template, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) fluorescence signal was used as an internal reference to detect the Ct values of each template and the gene expression was calculated by $\Delta\Delta$Ct method. RNA Isolation Reagent, RT SuperMix perfect reverse transcription kit and SYBR qPCR Master Mix Fluorescent DNA binding Dye were purchased from Nanjing Noweizan Biotechnology Co., Ltd. This procedure was carried out using a real-time fluorescence quantitative PCR instrument (Bio-Rad Corporation). The sequences of PCR primers are shown in Table 1.

Statistical analysis

GraphPad 9.0 software was used for analysis, and all data were shown as mean and standard deviation. The Shapiro–Wilk test was used to analyse the normal distribution. If the data conformed to the normal distribution and had the same/similar variance homogeneity, one-way ANOVA was used; if the data conformed to the normal distribution but had different variances homogeneity, Brown–Forsythe and Welch's ANOVA tests were used. Then, the Tukey test was used for *post hoc* testing of all experimental groups. Bar graphs labelled with different letters (a–c) are significantly different ($P < 0{\cdot}05$).

Results

Caffeine reduced weight gain and lipid accumulation in mice treated with nicotine plus a high-fat diet

To investigate the influence of caffeine on the weight in obese population, we examined the weight gain and food intake in HFD mice administered with nicotine for 16 weeks. During 16 weeks, the HFD group revealed the fastest growth in weight, but the weight gain of the HFD + NIC and HFD + NIC + CAF groups was significantly inhibited. The results demonstrated that caffeine and nicotine can effectively control weight gain (Fig. 1(a)). Food intake can greatly influence the weight of people in daily life. We found that the food intake of normal mice was higher than other groups (Fig. 1(b)), as HFD can supply more energy and make mice feel more satiety. The food intake of the HFD + NIC + CAF group remained lowest (Fig. 1(b)), showing the effects of nicotine and caffeine on decreasing food intake although differences were not statistically significant.

Apart from the weight gain and food intake, we further assessed lipid accumulation *in vivo*. HFD mice had the highest liver weight and liver:body ratio (Fig. 1(c) and (d)), indicating lipid accumulation in HFD mice. Compared with the HFD group, the liver weight of the HFD + NIC and HFD + NIC + CAF groups were much lower (Fig. 1(c)), but the liver:body ratio of the three groups had no significant difference (Fig. 1(d)). In terms of visceral fat, the HFD group showed the highest levels of visceral fat and visceral fat index (Fig. 1(e) and (f)). However, other groups represented lower visceral fat accumulation, and the effects of nicotine combined with caffeine on decreasing visceral fat were best (Fig. 1(e) and (f)), indicating both nicotine and caffeine can reduce visceral fat.

Caffeine attenuated the development of hepatic lipid accumulation and steatosis

To demonstrate the effect of caffeine on tissues more intuitively, we employed histological examination to exhibit changes in liver and epididymis lipids in each group. The normal liver presented reddish brown, with a smooth and delicate surface and sharp edges. However, the liver volume of mice in the HFD and HFD + NIC groups obviously expanded compared with the normal group. In addition, these livers had rounded edges, a yellow colour and a greasy sensation. Notably, the liver of mice in the HFD + NIC group showed a deeper yellow colour than that in the HFD group, indicating nicotine plus HFD aggravated hepatic lipid accumulation. The liver volume of mice in the

Table 1. The sequence of the primers for reverse transcription-polymerase chain reaction (RT-PCR)

Genes	Forward primer sequence (5'-3')	Reverse primer sequence (3'-5')
FAS	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
ACC	CTCCCGATTCATAATTGGGTCTG	TCGACCTTGTTTTACTAGGTGC
SREBP-1c	TGACCCGGCTATTCCGTGA	CTGGGCTGAGCAATACAGTTC
PPARα	AACATCGAGTGTCGAATATGTGG	CCGAATAGTTCGCCGAAAGAA
CPT1	TGGCATCATCACTGGTGTGTT	GTCTAGGGTCCGATTGATCTTTG
CD36	ATGGGCTGTGATCGGAACTG	TTTGCCACGTCATCTGGGTTT
GAPDH	AGGCCGGTGCTGAGTATGTC	TGCCTGCTTCACCACCTTCT

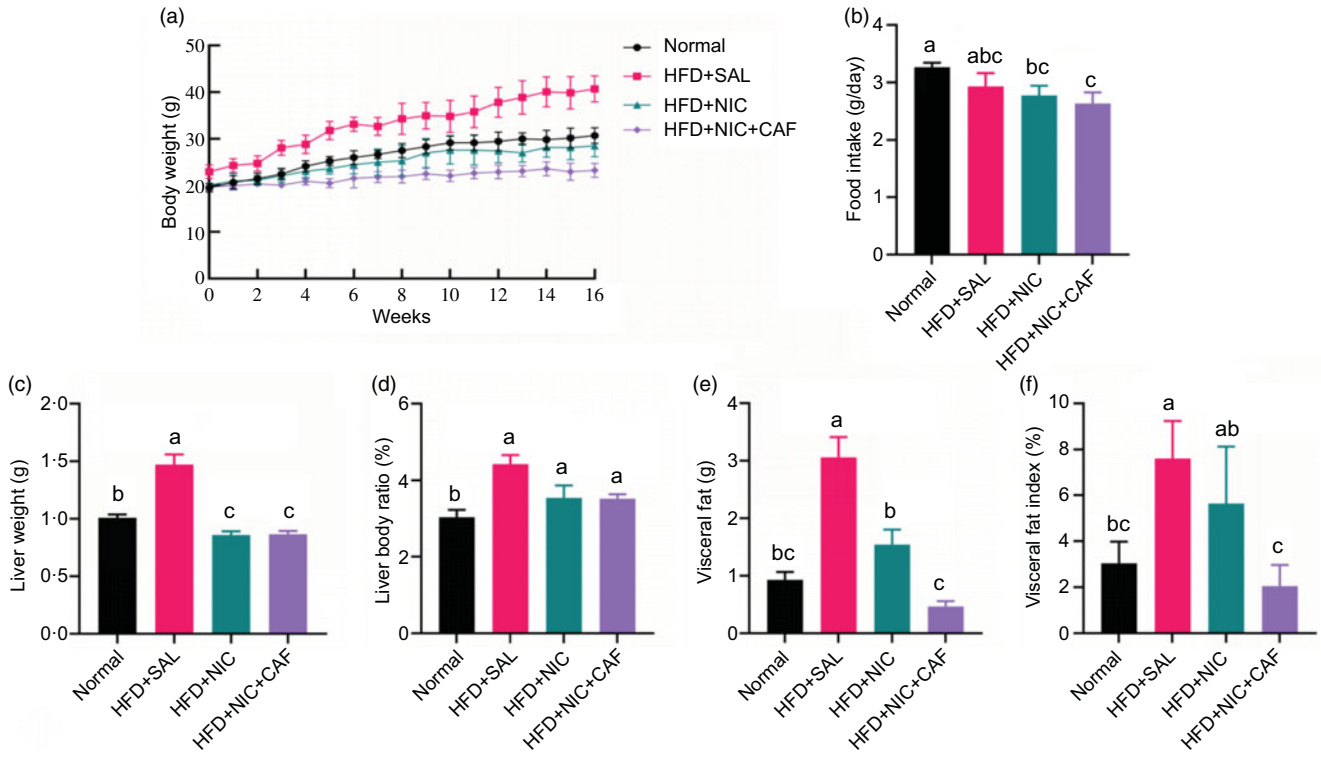

Fig. 1. The beneficial effect of caffeine on weight gain and lipid accumulation *in vivo*. (a) Body weight changes of mice in each group in 16 weeks. (b) Food intake of mice in each group. (c), (d) Liver weight and liver:body ratio showed the situations of hepatic lipid accumulation. (e), (f) Visceral fat and visceral fat index presented peripheral fat accumulation. HFD, high-fat diet; SAL, saline.

HFD + NIC + CAF group had no significant decrease, but the surface of the liver showed a deep red colour and no greasy sensation, confirming caffeine can effectively alleviate the development of NAFLD (Fig. 2(a)). Next, we examined the conditions of hepatocytes by haematoxylin–eosin staining. In the normal group, the morphology of hepatocytes was regular, with the circular centred nucleus and uniform distribution. In the HFD and HFD + NIC groups, cells presented larger volume, many vacuoles in the cytoplasm, and displaced nuclei. Moreover, hepatocytes in the HFD + NIC group noticeably swelled and were severely damaged, which was a pathological marker of NAFLD. After caffeine intervention, the lipid accumulation in hepatocytes decreased, indicated by the decrease in the size and number of vacuoles (Fig. 2(b)). The results of haematoxylin–eosin staining showed that caffeine can effectively alleviate the hepatic steatosis induced by nicotine plus HFD. Moreover, the results of Oil Red O staining showed lipid accumulation in hepatocytes directly. In the normal group, the hepatocytes were densely and regularly arranged, without obvious red lipid droplets. In contrast, a large number of lipid droplets were observed in the HFD and HFD + NIC groups, indicating that HFD successfully induced the NAFLD model. Further, the HFD + NIC group showed much more red lipid droplets compared with other groups. However, caffeine significantly reduced red lipid droplets, indicating the ability of caffeine to degrade hepatic lipids, consistent with the results of haematoxylin–eosin staining (Fig. 2(c)).

Epididymal fat is a typical representative of visceral lipids. Therefore, we also performed haematoxylin–eosin staining on the epididymis fat of mice to detect the accumulation of visceral lipids. Adipocytes of the epididymis in the normal group were smaller and arranged neatly, while those of the HFD and HFD + NIC groups became much bigger. Compared with the HFD + NIC group, adipocytes in the HFD + NIC + CAF group were significantly smaller, indicating that caffeine improved the accumulation of free fatty acids *in vivo* (Fig. 2(d)).

Caffeine restored hepatic functions impaired by nicotine plus high-fat diet

Serum alanine aminotransferase and aspartate aminotransferase are markers for evaluating hepatocyte damage. The results indicated that nicotine combined with HFD significantly increased the levels of alanine aminotransferase and aspartate aminotransferase, while caffeine effectively decreased the levels of two markers (Fig. 3(a) and (b)), confirming caffeine was able to alleviate hepatocyte damage and restore liver functions. In addition, the levels of blood lipid indicators were also evaluated. In the HFD + NIC group, the levels of serum TG and TC increased markedly, while the administration of caffeine could reverse this trend (Fig. 3(c) and (d)). Serum HDL-c and LDL-c are related to endogenous cholesterol transport and atherosclerosis. The content of serum HDL-c significantly decreased in the HFD + NIC group and increased in the

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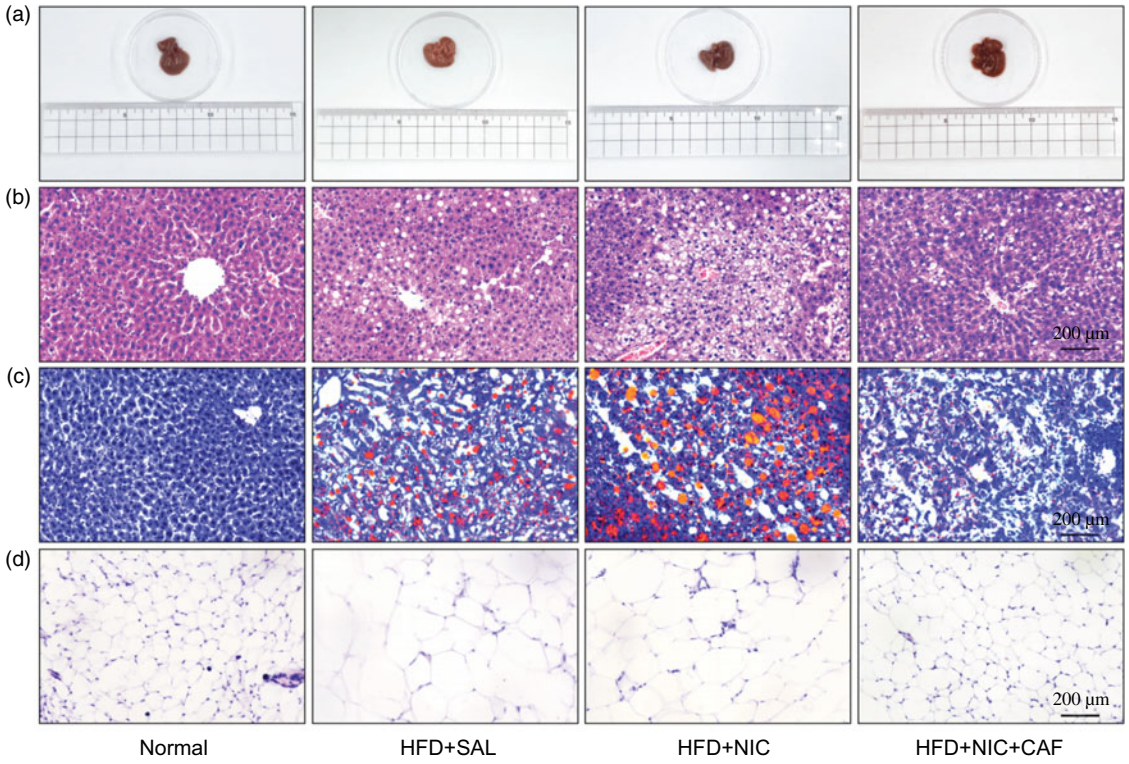

Fig. 2. Histological examination showed caffeine alleviated hepatic lipid accumulation and steatosis, indicated by appearance, haematoxylin–eosin staining, Oil Red O staining of the liver and haematoxylin–eosin staining of epididymis fat. HFD, high-fat diet; SAL, saline.

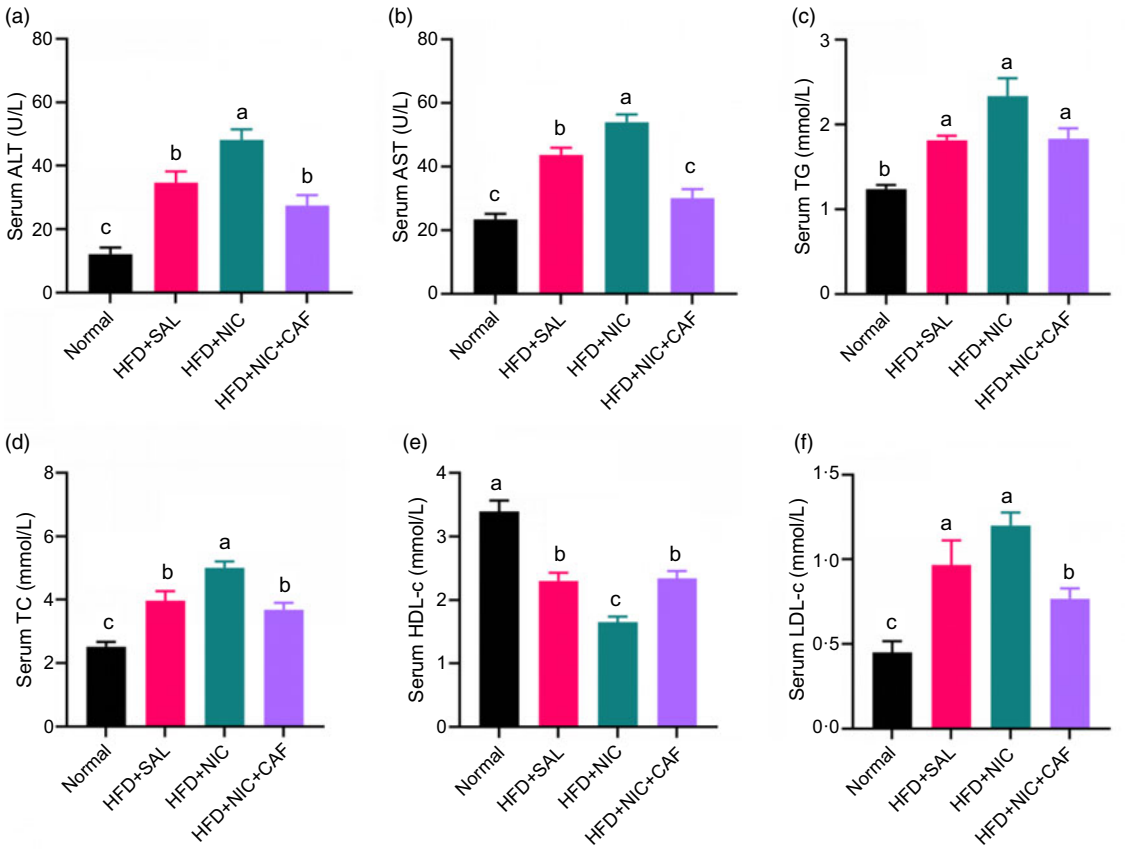

Fig. 3. The influence of caffeine in restoring hepatic functions impaired by nicotine plus HFD. (a), (b) Serum alanine aminotransferase and aspartate aminotransferase showed the degree of hepatocyte damage. (c)–(f) Serum TG, TC, HDL-c and LDL-c presented liver functions in synthesising and transporting lipids. HFD, high-fat diet; SAL, saline; TC, total cholesterol.

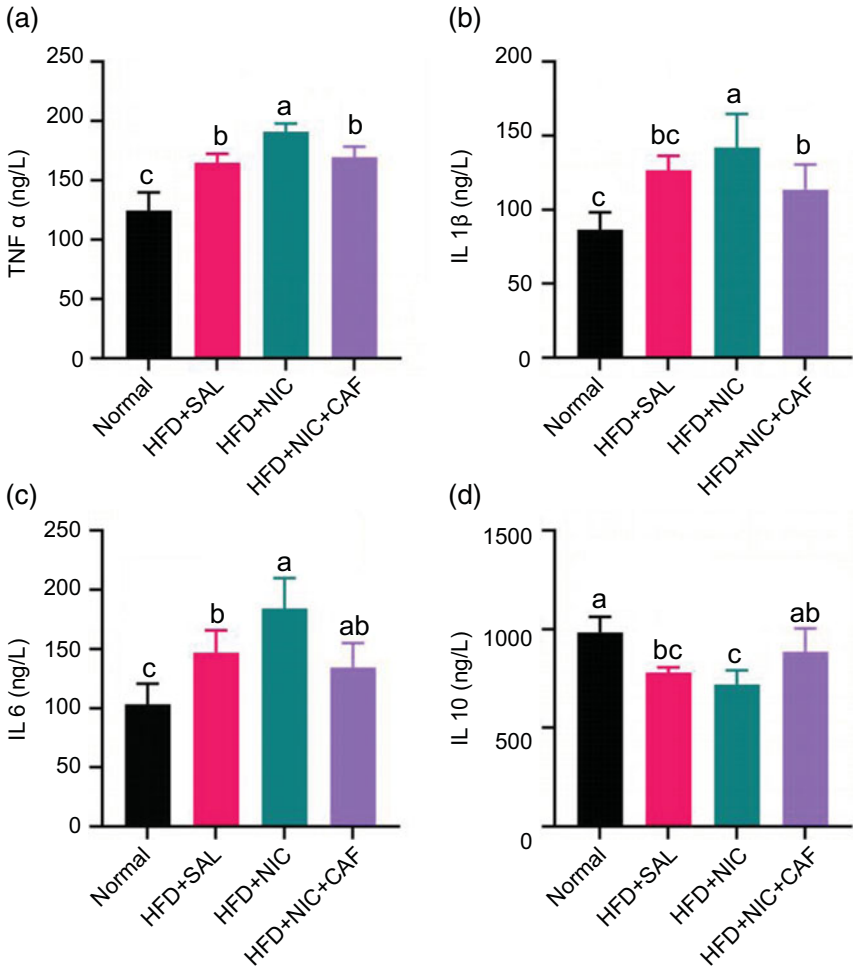

Fig. 4. Caffeine maintained levels of multiple inflammatory cytokines exacerbated by nicotine in HFD mice. Pro-inflammatory cytokines (a) TNF-α, (b) IL-1β, (c) IL-6 and anti-inflammatory cytokines. (d) IL-10 reflected the inflammatory response of mice in each group. HFD, high-fat diet. SAL, saline.

HFD + NIC + CAF group due to the caffeine intervention. In contrast, the results of serum LDL-c showed the opposite trend (Fig. 3(e) and (f)).

Caffeine ameliorated inflammatory response exacerbated by nicotine in HFD mice

Studies showed that HFD-induced hepatic steatosis was closely linked to liver inflammation[24]. To explore the effects of caffeine on mice with nicotine plus HFD, multiple inflammatory cytokines were tested. The pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) increased to the highest levels in the HFD + NIC group and decreased markedly in the HFD + NIC + CAF group (Fig. 4(a)–(c)). However, the anti-inflammatory cytokines (IL-10) showed no significant difference in the three groups (Fig. 4(d)). These results illustrated that caffeine had anti-inflammatory effects, while nicotine tended to induce an inflammatory response.

Caffeine improved hepatic lipid metabolism and oxidative stress aggravated by nicotine combined with high-fat diet

Besides inflammatory response, we also examined situations of hepatic lipid metabolism and oxidative stress in the liver. We found that nicotine plus HFD aggravated the lipid metabolism in the liver, as the HFD + NIC group presented the highest liver TG and liver TC (Fig. 5(a) and (b)). In contrast, the administration of caffeine could effectively rescue hepatic lipid metabolism, as evidenced by lower levels of two indicators in the HFD + NIC + CAF group (Fig. 5(a) and (b)).

Moreover, MDA and SOD reflected the degree of oxidative stress in hepatocytes. This study revealed that MDA showed the highest content in the HFD + NIC group, and that was much lower in the HFD + NIC + CAF group (Fig. 5(c) and (d)). In addition, the results of SOD presented opposite trends (Fig. 5(c) and (d)), but there was no statistical significance in liver SOD, which may be caused by individual differences in mice. Overall, caffeine was able to inhibit the production of lipid peroxidation MDA to reduce oxidative stress.

Caffeine balanced hepatic lipid metabolism via regulating the expression of related genes

To explore the mechanism of caffeine improving hepatic lipid metabolism in depth, we detected the expression of related genes in lipid metabolism via RT-PCR. acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and sterol

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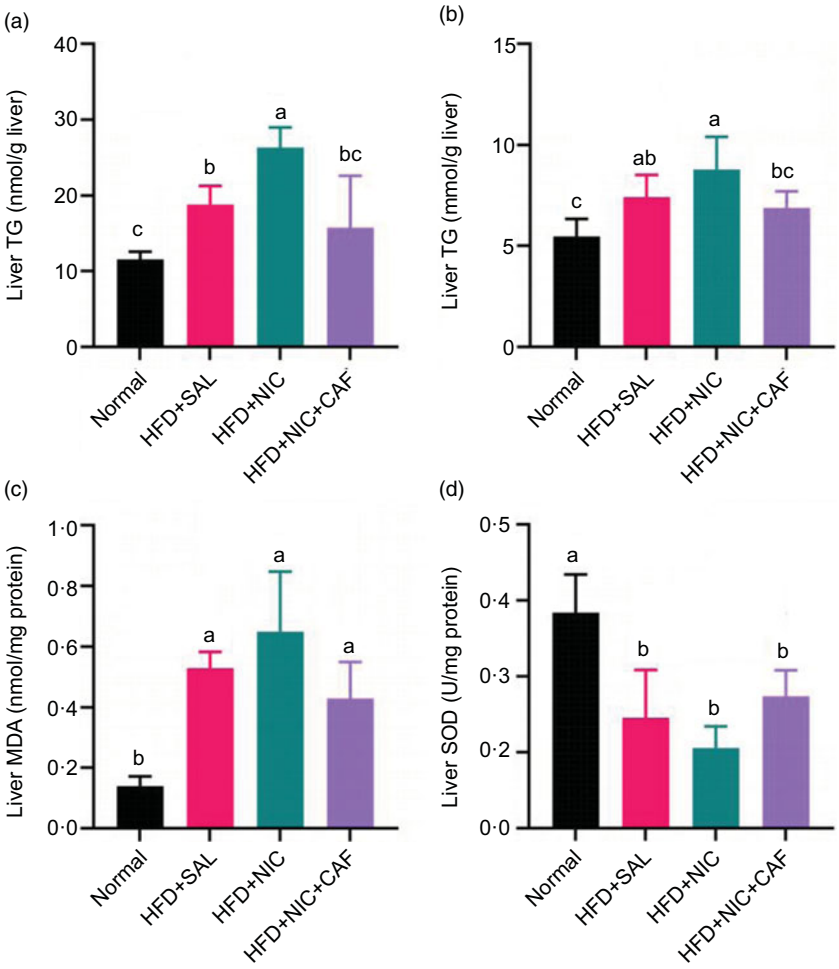

Fig. 5. Four liver biochemical indicators proved the active role of caffeine in lipid metabolism and oxidative stress aggravated by nicotine combined with HFD. (a)–(b) Liver TG and liver TC displayed the metabolism of hepatic lipids. (c)–(d) Liver MDA and liver SOD indicated the level of oxidative stress in the liver. HFD, high-fat diet; MDA, malondialdehyde; SAL, saline; SOD, superoxide dismutase.

regulatory element-binding protein-1 (SREBP-1c) were responsible for regulating liposynthesis. The results demonstrated that nicotine could dramatically up-regulate the expression of genes in liposynthesis, while caffeine significantly down-regulated the expression of these genes (Fig. 6(a)–(c)).

Besides, cluster of differentiation 36 (CD36), PPAR-α and carnitine palmitoyltransferase 1 (CPT-1) can regulate hepatic lipid lipolysis. For PPAR-α and CPT-1, caffeine significantly up-regulated the expression of these genes, while nicotine showed completely reverse effects (Fig. 6(e) and (f)). However, both caffeine and nicotine were able to down-regulate the expression of CD36 (Fig. 6(d)).

Discussion

In recent years, changes in the global dietary structure have brought both new threats and therapeutic hopes to human health. Excessive intake of fats and carbohydrates led to a quarter of the global population suffering from NAFLD[25]. Obesity and smoking were considered risk factors for NAFLD[26,27]. HFD or nicotine can further exacerbate hepatocyte apoptosis and promote the development of NAFLD[28]. Obese smokers were more likely to suffer from NAFLD. Caffeine, the main ingredient of coffee, was reported to have a protective effect on NAFLD, with good application prospects[29,30]. Moreover, caffeine was proven to relieve NAFLD induced by HFD[20], but it is still unknown whether caffeine can alleviate the development of NAFLD caused by HFD plus nicotine. In our study, a 20 mg/kg dose of caffeine and a 1·5 mg/kg dose of nicotine were provided, which was equivalent to 2–3 cups of high-concentration coffee and twenty cigarettes by an adult every day. This model may simulate the real state of obese smokers in daily life.

Recent research has shown that nicotine and caffeine can effectively reduce weight gain[31,32]. In our study, we confirmed their significant impact on weight reduction, with an additive effect. Both nicotine and caffeine were found to suppress food intake, supporting previous studies[33,34]. However, apart from normal mice, there were no significant differences in food intake among the groups, suggesting that weight change is unrelated to food intake. Instead, nicotine and caffeine primarily affect *in vivo*

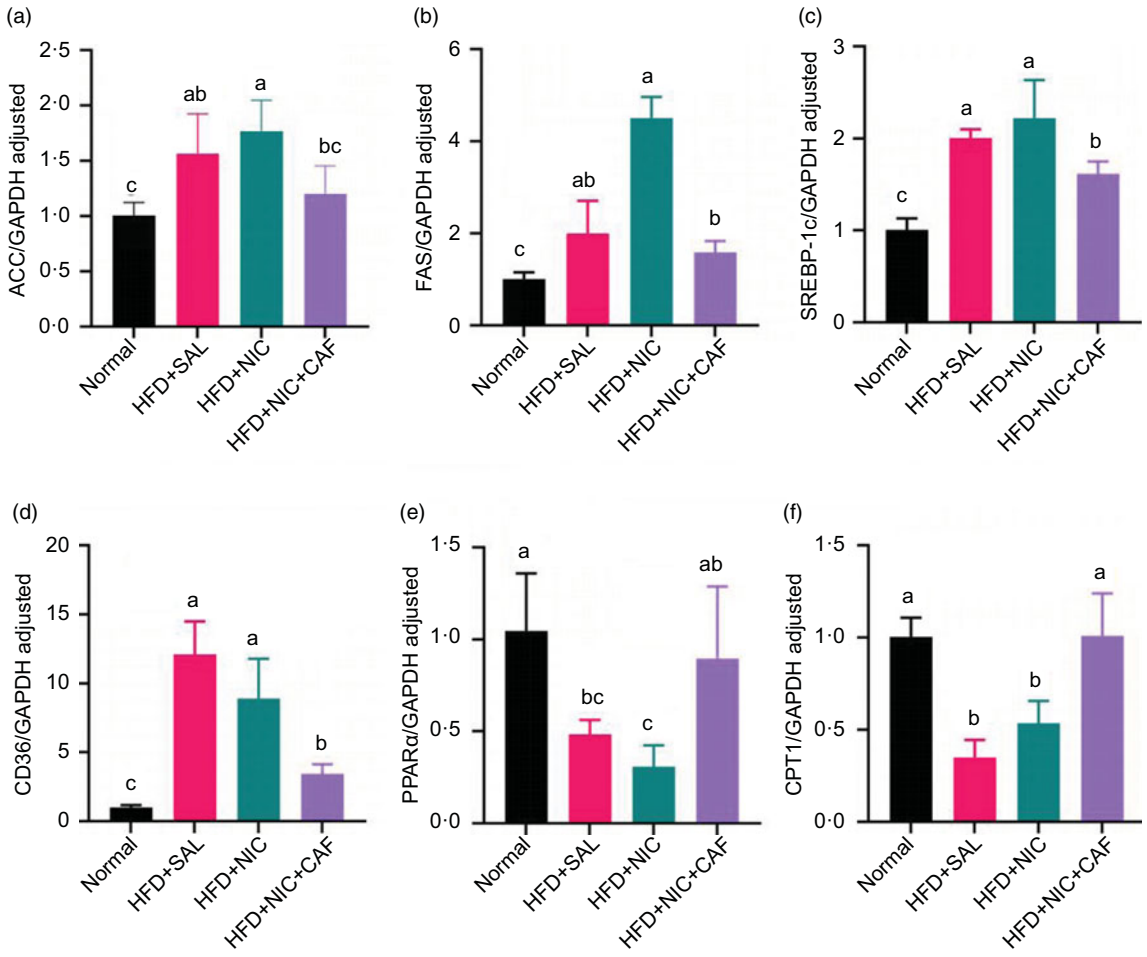

Fig. 6. The regulatory effect of caffeine on hepatic lipid metabolism genes. Genes of liposynthesis: (a) ACC, (b) FAS, (c) SREBP-1c and lipolysis, (d) CD36, (e) PPAR-α, and (f) CPT-1 revealed the mechanism of hepatic lipid metabolism. HFD, high-fat diet; SAL, saline; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SREBP-1c, sterol regulatory element-binding protein-1; CD36, cluster of differentiation 36; PPAR-α, peroxisome proliferator-activated receptor alpha; CPT-1, carnitine palmitoyltransferase 1.

metabolism to limit weight gain. Moreover, our study demonstrated that caffeine effectively reduces lipid accumulation and restores disrupted lipid metabolism caused by nicotine and HFD. Additionally, caffeine alone inhibits the increase in the liver:body ratio, while nicotine induces hepatocyte damage, leading to decreased liver weight. Liver biochemical indicators and histological examination confirmed the worsening effect of nicotine on NAFLD and the protective role of caffeine against hepatic lipid accumulation induced by nicotine plus HFD.

Liver lipid metabolism dysfunction is the main cause of NAFLD[35]. Serum TG, TC, HDL-cholesterol and LDL-cholesterol levels were assessed as common lipid indicators. Excess TG content exceeding 5 % of liver weight indicates NAFLD[36]. Cholesterol transport occurs primarily in the liver, with cholesterol esters forming serum HDL and LDL[37]. HDL aids in cholesterol homoeostasis, preventing arteriosclerosis[38], while LDL increases the risk of atherosclerosis[39]. Caffeine effectively improved blood lipid levels induced by nicotine and HFD, indicating its potential to restore liver lipid disorder in NAFLD. Caffeine also down-regulates lipogenesis genes, inhibiting lipid accumulation and relieving NAFLD. Furthermore, caffeine promotes liver lipid catabolism by up-regulating PPAR α and CPT-1 expression. The potential mechanism may involve the Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and epidermal growth factor receptor (EGFR) signalling pathways[40–42], although further experimental verification is required.

NAFLD development involves lipid metabolism, inflammation and oxidative stress. HFD-induced hepatic lipid accumulation triggers an inflammatory response, characterised by pro-inflammatory cytokine release and macrophage activation[43]. Excessive lipid decomposition generates free fatty acids, causing lipotoxicity and hepatocyte apoptosis[44]. Inflammatory cytokines like IL-1β, IL-6, TNF-α and IL-10 contribute to hepatic steatosis and inflammation[45]. Caffeine alleviates the inflammatory response by inhibiting pro-inflammatory cytokine generation and promoting anti-inflammatory cytokines. Oxidative stress, an imbalance between antioxidants and oxidants, also influences NAFLD[46]. Caffeine reduces oxidative stress by scavenging free radicals and decreasing lipid peroxidation (MDA). It inhibits hepatic oxidative stress and NAFLD development. These findings align with previous studies[47], highlighting the potential of caffeine in preventing NAFLD through its anti-inflammatory and antioxidant properties.

Conclusion

Taken together, our study indicated that nicotine aggravated hepatic steatosis and NAFLD. In contrast, caffeine could effectively attenuate nicotine plus HFD-induced NAFLD by regulating lipid metabolism, suppressing inflammatory responses and oxidative stress. These findings may provide insight into the reduced incidence of NAFLD and the relatively leaner profile observed in obese smokers who consume high amounts of coffee. However, it is essential to emphasise that the harm resulting from smoking extends well beyond hepatic steatosis. Smoking is intricately associated with the development of various cancers, cardiovascular diseases and metabolic-related disorders, resulting in significantly elevated mortality risks. Smoking cessation represents the only practical and effective strategy for averting a multitude of tobacco-related complications and reducing the risk of premature mortality.

Acknowledgements

This work was supported by the Young project of the Wuxi Health Committee (no. Q202102).

W. X. and N. L. conceived and designed the study. X. L. and W. X. performed the experimental part. X. M., X. L. and W. X. contributed to data analysis and supervision. N. L., X. M. and X. T. wrote the paper. G. Y. and W. X. reviewed and edited the manuscript. W. X. is the corresponding author. All authors have read and approved the final manuscript.

The authors have no conflict of interest to declare.

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