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Green tea and selenium-enriched green tea ameliorates non-alcoholic fatty liver disease through peripheral 5-hydroxytryptamine signals in high-fat diet-fed mice

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Abstract

Green tea and selenium (Se) improve non-alcoholic fatty liver disease (NAFLD). However, studies on the effect of green tea and Se-enriched green tea on NAFLD are limited. C57BL/6 mice were divided into high-fat diet (HFD), HFD+regular green tea (T), and HFD+Se-enriched green tea (SeT) groups after 12 weeks of feeding with HFD. HFD feeding was continued, and the mice in the HFD+T and HFD+SeT groups drank corresponding tea solution for another 12 weeks. The control (CON) group was given normal diet. At the end of the experiment, serum, liver, fat, and intestinal tract were collected. Results showed that both tea interventions decreased body and fat weight. Histological analysis showed that both tea interventions alleviated steatosis, which is supported by the changes in lipid profiles and lipogenic pathways. Tea interventions significantly increased superoxide dismutase, glutathione peroxidase, and catalase levels; and decreased TNF-α, IL-1β, IL-6, and malondialdehyde contents. HFD significantly increased total bile acid in the intestinal contents and liver, duodenal 5-hydroxytryptamine (5-HT) level and tryptophan hydroxylase gene expression, and hepatic 5-HT, 5-HT receptor 2A, monoamine oxidase-A (MAO-A), and H_2O_2; all of them reversed by the tea interventions. Furthermore, the improved oxidative stress, inflammatory response, duodenal 5-HT, and hepatic MAO-A were more pronounced in the HFD+SeT group than in the HFD+T group. Our findings revealed that green tea ameliorates NAFLD through peripheral 5-HT signals in HFD fed mice.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by hepatic steatosis in hepatic parenchymal cells which occurs in the absence of alcohol abuse. NAFLD starts from simple steatosis, which may progress to non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis, and hepatocellular carcinoma in a subset of patients. NAFLD has become a serious public health problem globally, affecting more than half a billion people worldwide (Xia *et al.,* 2019). Despite intense research efforts invested in recent years, pharmacological treatment options for NAFLD are limited. However, emerging evidence suggests that lifestyle modifications (*e.g.,* weight loss/maintenance, increased physical activity/exercise, and dietary changes) may be useful in preventing or delaying NAFLD progression (Nseir *et al.,* 2014).

Tea is the second most widely consumed beverage worldwide after water (Yu *et al.,* 2018). Green tea contains abundant polyphenols such as catechins, flavonoids, and flavonols. Evidence from animal studies suggests that consumption of green tea may have favourable effects on obesity, type 2 diabetes, and NAFLD, possibly through the increased energy expenditure, lipid-lowering effects, antioxidant effects, and anti-inflammatory effects, as well as improved insulin sensitivity and gut health (Yu *et al.,* 2017; Zhou *et al.,* 2019). The effects of green tea in NAFLD patients have been investigated in a few clinical studies with small samples. Green tea

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tablets or tea catechins reduce fat accumulation and liver inflammation in Iranian and Japanese NAFLD patients, respectively (Fukuzawa *et al.,* 2014; Tabatabaee *et al.,* 2017). However, a cross-sectional study in China showed that green tea consumption is not significantly associated with the prevalence of NAFLD after adjustment of confounding factors (Xia *et al.,* 2019). A recent meta-analysis revealed that green tea consumption has a beneficial effect on liver enzymes in patients with NAFLD, while for healthy individuals, a small but significant detrimental effect was observed (Mahmoodi *et al.,* 2020).

Selenium (Se)-enriched green tea is becoming popular in China because consumers believe that this type of tea has stronger bioactivity than regular green tea (Hu *et al.,* 2013). Dietary Se is absorbed in the small intestine and then metabolised to selenide in the liver, which is subsequently used for the synthesis of selenoproteins such as selenoprotein P (SePP) and glutathione peroxidase (GPx). Selenoproteins play a crucial antioxidant role linked with NAFLD (Polyzos *et al.,* 2019). Theoretically, Se-enriched green tea may have a higher anti-metabolic syndrome activity than regular green tea. However, the combinational effect of Se and green tea does not always confer additional benefits. Some reported an adverse effect or U-shaped curve of Se supplementation on NAFLD (Yang *et al.,* 2016).

5-hydroxytryptamine (5-HT, serotonin) is a monoamine neurotransmitter from the essential amino acid tryptophan. 5-HT synthesis is regulated by the availability of tryptophan and the activity of tryptophan hydroxylase (TPH) (Choi *et al.,* 2020). TPH has two distinct isoforms: TPH1 in peripheral tissues, and TPH2 in neurons of the central and enteric nervous systems. Since 5-HT cannot cross the blood-brain barrier, central and peripheral 5-HT systems are functionally separated (Berger *et al.,* 2009). Most peripheral 5-HT is synthesised by TPH1 in enterochromaffin (EC) cells in the gut, and exerts physiological function depending on 5-HT receptors (5-HTRs) upon entering systemic circulation (Choi *et al.,* 2020). Emerging evidence suggests that peripheral 5-HT is a factor that enhances nutrient absorption and storage. In brief, certain nutrients are modulated by the gut microbiome production of short-chain fatty acids, which increase TPH1 and 5-HT syntheses in EC cells, and subsequently elevate peripheral 5-HT concentrations (Yano *et al.,* 2015). Animal studies indicated that fructose-rich diet or fat-rich diet increases peripheral 5-HT level, which leads to liver steatosis (Choi *et al.,* 2018). 5-HT can stimulate *de novo* lipogenesis (DNL) and inhibit lipolysis in the liver and white adipose tissue (WAT) (Choi *et al.,* 2018). Furthermore, peripheral 5-HT signalling suppression can improve hepatic lipid droplet accumulation and liver steatosis (Choi *et al.,* 2018).

Currently, the benefits of green tea and Se in regulating NAFLD focus on the improvement of lipids, inflammatory response, and oxidative stress (Polyzos *et al.,* 2019). Therefore, the present work aimed to investigate the potential beneficial effects of green tea and Se on NAFLD through peripheral 5-HT signals. Whether Se enrichment in green tea provides any additional benefit on NAFLD was also investigated by comparing the effect of green tea and Se-enriched green tea in mice fed with high-fat diet (HFD).

Materials and methods

Preparation of tea and selenium-enriched tea solution

Green tea and Se-enriched green tea were purchased from Baguilingyun Tea Group Co. Ltd., Guangdong, China. Six grams of tea was infused in 300 mL of boiling water for 4 min. Cold supernatant fraction (tea solution) was used in the experiment. The Se contents of tea leaves and tea solutions ($n = 3$) were determined according to China's National Food Safety Standard (determination of selenium in food).

Animal and diets

A total of 42 male C57BL/6 mice aged four weeks were purchased from Shanghai Laboratory Animal Company, Shanghai, China. The mice were housed in individual cages under a 12 h light/dark cycle (off at 8 pm) at a constant room temperature of 23 ± 2°C, and relative humidity of 60%. After one week of acclimatisation, 34 mice were fed with HFD (D12492, 60% kcal fat, 20% kcal protein) from Research Diets Inc. (New Brunswick, NJ, USA). After 12 weeks, the mice were divided into three groups as follows: HFD group ($n = 10$), HFD+tea (T) group ($n = 12$), and HFD+Selenium-enriched tea (SeT) group ($n = 12$). HFD feeding was continued, and the mice in the HFD+T group and HFD+SeT group drank regular and Se-enriched green tea solution for another 12 weeks, respectively. Eight mice fed with a normal diet (D12450J, 10% kcal fat, 20% kcal protein) from the start of the experiment were assigned to a control (CON) group. The amount of protein (lactic casein and cystine) per kcal were similar between HFD and CON diets. The mice in the HFD and CON group drank distilled water. Drinking

solutions and water were changed every two days. The mice were given free access to corresponding foods and drinks. Body weight and food intake were monitored weekly throughout the experiment. All the procedures were performed according to the guidelines in the Care and Use of Animals approved by Soochow University Animal Welfare Committee (No. 201811A114).

Sample collection at autopsy

At the end of the experiment, mice were deprived of food for 12 h, and sacrificed. Blood samples were collected from the retrobulbar vein. The serum was separated by centrifugation at 3,500 *g* for 10 min at 4°C, and stored at -80°C. The liver, subcutaneous WAT, and visceral (including perirenal and epididymal) WAT were dissected, and the WAT ratio (total WAT weight/body weight) was calculated. Portions of the liver and fat were fixed immediately in 10% formalin for histological observation. The remaining organs and intestinal contents from the colon and cecum were collected and immediately stored at -80°C.

Histological examination of liver and fat

The formalin-fixed liver and fat tissues were embedded in paraffin, and cut into 6 μm slices. The sections were deparaffinised in xylene, and rehydrated by serially washing with decreasing ethanol concentration. The sections were then performed with haematoxylin-eosin (HE) staining, and mounted in a xylene-based mounting media. Oil Red O staining in liver tissue was performed on the formalin-fixed section to investigate hepatic lipid droplets following a routine procedure (Tan *et al.,* 2017). The samples were observed and photographed under a microscope (IX3-AN, Olympus, Japan). The cell size of adipocytes was quantified using Image J software. NAFLD activity score (NAS) was calculated by a pathologist who was blinded to the treatment group using previously described criteria; steatosis, hepatocyte ballooning, and lobular inflammation fibrosis, and miscellaneous features (Kleiner *et al.,* 2005)

Determination of lipid profiles and liver function

Serum triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), aspartate transaminases (AST), and alanine transaminase (ALT) were analysed using the blood biochemistry analyser (Ci8200, Abbott Laboratories, Abbott Park, IL, USA). Approximately, 10% of the hepatic homogenate was prepared in ice-cold normal saline, and centrifuged at 12,000 *g* and 4°C twice, for 5 min, each time. Their supernatants were obtained to determine the hepatic TG, TC, and free fatty acid (FFAs) using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of oxidative stress and inflammatory adipokines

The Iiver was centrifuged twice at 12,000 *g* and 4°C for 5 min. The supernatants were used to determine the SePP, GPx, superoxide dismutase (SOD), catalase (CAT, the velocity method), TNF-α, IL-1β, and IL-6 by an enzyme-linked immunosorbent assay kit (ELISA, KeJing Biological Technology Co. Ltd., Jiangsu, China). The total antioxidant capacity (T-AOC) and malondialdehyde (MDA) concentration were also measured using commercial kits (Shanghai Biyuntian Biological Co. Ltd., Shanghai, China) according to the manufacturer's instructions.

Analysis of peripheral 5-HT/5-HTR2A signalling

The total bile acid (TBA) in the Iiver and intestinal content from the colon and cecum were measured by commercial kits (KeJing Biological Technology Co. Ltd., Jiangsu, China). Duodenal and liver 5-HT, 5-HTR2A, and monoamine oxidase-A (MAO-A) were measured using an ELISA kit (KeJing Biological Technology Co. Ltd., Jiangsu, China). 5-HT is mainly degraded to 5-hydroxyindolic acid and reactive oxygen species, primarily H_2O_2 by MAO-A (Billett, 2004). H_2O_2 levels in the liver were determined using commercial kits (Comin Biotechnology Co., ltd. Suzhou, China) according to the manufacturer's instructions.

Detection of gene expression involved in lipogenesis

The total RNA was extracted from the liver and duodenum using the TRIZOL reagent (Tiangen Biotech Co. Ltd., Beijing, China). The concentration and purity of the isolated RNA were determined by measuring its optical density at 260 and 280 nm. Complementary DNA was synthesised from the total RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc. Waltham, MA, USA). The qPCR primers were designed using PrimerBank, and the sequences are shown in Table 1. The levels of mRNAs were quantified on a QuantStudio 6 Flex system (Thermo Fisher Scientific Inc., Waltham, MA, USA) using the ChamQ Universal SYBR green PCR master mix (Vazyme Biotech Co., Ltd, Nanjing, China).

Statistical analysis

All data were analysed using SPSS version

Table 1. Primer sequences of genes used for the quantification of mRNAs by Q-PCR.

Gene	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
GAPDH	TGTCCCCGCCTATACAATGG	CCTTGGTGATAGACAGGCTACTG
SREBP1c	CAAGGCCATCGACTACATCCG	CACCACTTCGGGTTTCATGC
ACCα	GATGAACCATCTCCGTTGGC	GACCCAATTATGAATCGGGAGTG
FAS	AGGTGGTGATAGCCGGTATGT	TGGGTAATCCATAGAGCCCAG
GPAT1	CTTGGCCGATGTAAACACACC	CTTCCGGCTCATAAGGCTCTC
PPARγ	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
MOGAT1	TGTCTTGTCAAAACGCAGGAT	ACAACGGGAAACAGAACCAGA

19.0 (SPSS Inc., Chicago, IL, USA), and expressed as mean ± standard deviation (SD). Differences between groups were determined using one-way ANOVA followed by the least significant difference *post hoc* multiple comparisons (LSD, if homogeneity of variance was satisfied) or Tamhane's T2 (if homogeneity of variance was not satisfied). The difference was considered statistically significant when $p < 0.05$.

Results

Selenium content of tea

Se contents in the tea leaves were 2.92 ± 0.29 mg/kg for Se-enriched tea, and 0.14 ± 0.02 mg/kg for regular tea. Despite the different rate of dissolution between Se-enriched tea and regular tea, the Se level was still significantly higher in Se-enriched tea solution (10.36 ± 1.26 μg/L) than in regular tea solution (0.99 ± 0.71 μg/L).

Effects of tea on body weight and fat content

The HFD fed mice were significantly heavier than normal diet-fed mice (33.30 ± 3.22 vs. 28.58 ± 2.29 g). The body weight among three HFD-fed groups was comparable at the beginning of the intervention, and the increments of body weight were lower in the HFD+T and HFD+SeT groups than in the HFD group during the experiments (Figure 1A). At the end of experiments, body weight was significantly lower in HFD+T (37.96 ± 5.87 g) and HFD+SeT (38.02 ± 6.30 g) groups than in the HFD group (43.42 ± 6.08 g) (Figure 1B). The mice in the HFD group had higher subcutaneous fat weight, visceral fat weight, and WAT ratio when compared with those in the CON group; these adiposity indices decreased in HFD+T and HFD+SeT groups, and the reduction in visceral fat weight was statistically significant (Table 2).

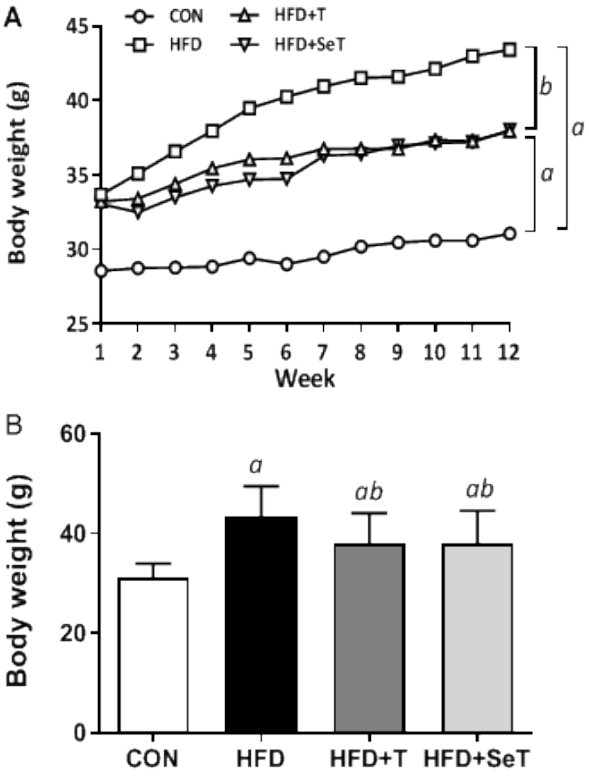

Figure 1. Effects of tea on body weight during the experiments (A), and the final body weight (B) among four groups during the experiments. Both tea interventions decreased the body weight. Values are mean ± SD (n = 8 - 12). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group.

Effects of tea on adipocyte size and hepatic steatosis

Histological analysis of adipose tissue showed an increase in cell volume in the HFD group. The enlargement of adipocytes was reversed in the HFD+T and HFD+SeT groups (Figure 2A). Quantitatively, cell sizes of the subcutaneous and visceral WAT were significantly smaller in the HFD+T and HFD+SeT groups than in the HFD group, thus suggesting the alleviation of lipid

Table 2. Effects of tea on adiposity, hepatic, and serum lipid profiles.

	CON	HFD	HFD+T	HFD+SeT
Fat				
Subcutaneous fat (g)	0.64 ± 0.22	2.03 ± 0.81[a]	1.28 ± 0.70	1.61 ± 1.03
Viscus fat (g)	0.57 ± 0.13	3.06 ± 0.67[a]	2.11 ± 0.82[ab]	2.22 ± 1.15[ab]
WAT ratio (%)	4.18 ± 0.87	12.04 ± 2.32[a]	8.78 ± 3.17[a]	10.10 ± 4.40[a]
Serum				
TG (mmol/L)	2.25 ± 0.25	2.83 ± 0.15[a]	1.92 ± 0.22[a]	1.86 ± 0.26[a]
TC (mmol/L)	3.16 ± 0.37	5.49 ± 0.88[a]	4.17 ± 0.94[ab]	4.65 ± 0.85[ab]
LDL-C (mmol/L)	0.43 ± 0.07	0.80 ± 0.14[a]	0.48 ± 0.16[ab]	0.63 ± 0.10[ab]
HDL-C (mmol/L)	2.49 ± 0.32	4.01 ± 0.65[a]	3.51 ± 0.68[a]	3.88 ± 0.60[a]
ALT (U/L)	29.91 ± 3.18	42.39 ± 10.84[a]	31.29 ± 2.79[b]	32.69 ± 9.74[b]
AST (U/L)	126.46 ± 12.36	142.86 ± 24.58	136.42 ± 16.63	133.91 ± 16.92
Liver				
TG (μmol/g)	55.39 ± 16.45	294.95 ± 129.31[a]	193.45 ± 84.54[ab]	213.32 ± 85.54[ab]
TC (μmol/g)	19.66 ± 7.30	28.62 ± 8.91[a]	18.09 ± 6.59[b]	13..62 ± 8.89[b]
FFAs (μmol/g)	8.31 ± 0.96	11.59 ± 1.19[a]	9.71 ± 1.26[ab]	9.45 ± 1.28[ab]

Values are mean ± SD (n = 8 - 12). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group. WAT = white adipose tissue; TG = triglyceride; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; ALT = alanine transaminase; AST = aspartate transaminases; and FFAs = free and fatty acid.

accumulation by both tea interventions (Figures 2B and 2C).

HE staining (Figure 3A) and Oil Red O staining (Figure 3B) showed that HFD feeding increased fat deposition and hepatocellular ballooning in the liver, which is confirmed by NAS. When compared with the HFD group (9.75 ± 0.50), NAS was significantly lower in the HFD+T (5.25 ± 2.22) and HFD+ SeT (6.25 ± 1.71) groups, thus suggesting the improvement of steatosis by both tea interventions (Figure 3C).

Effects of tea on lipid profile and liver function

As shown in Table 2, the HFD group showed hyperlipidaemia because the levels of serum TC, TG, and LDL-C were higher than those in the CON group. Similar to histological observation, the hepatic contents of TG, TC, and FFAs increased in the HFD group. Following the tea and Se-enriched tea interventions, serum TC and LDL-C levels, and hepatic TG, TC, and FFAs levels significantly decreased, but without significant differences between the HFD+T and HFD+SeT groups. It was also observed that the increase in serum ALT by HFD was reversed by both tea interventions, thus suggesting the improvement of damaged liver function.

Effects of tea on lipogenic pathways in the liver

To further elucidate the effects of tea on lipogenic pathways, the hepatic mRNA expression on DNL pathways such as FAS and ACCα was measured, as well as their upstream transcript factors SREBP1-c, PPARγ, and gene expression of GPAT1 and MOGAT1, which catalyse the step of TG biosynthesis (Figures 4A - 4F). When compared with the CON group, HFD feeding markedly and significantly up-regulated the gene expression mentioned above. Following tea interventions, the gene expression levels of FAS, SREBP1-c, and PPARγ were significantly down-regulated (Figures 4A, 4C, and 4D). A significant decrease in ACCα expression was observed in the HFD+T group (Figure 4B). Similarly, the hepatic mRNA expression levels of GPAT1 and MOGAT1 were also significantly down-regulated in the HFD+T and HFD+SeT groups (Figures 4E and 4F).

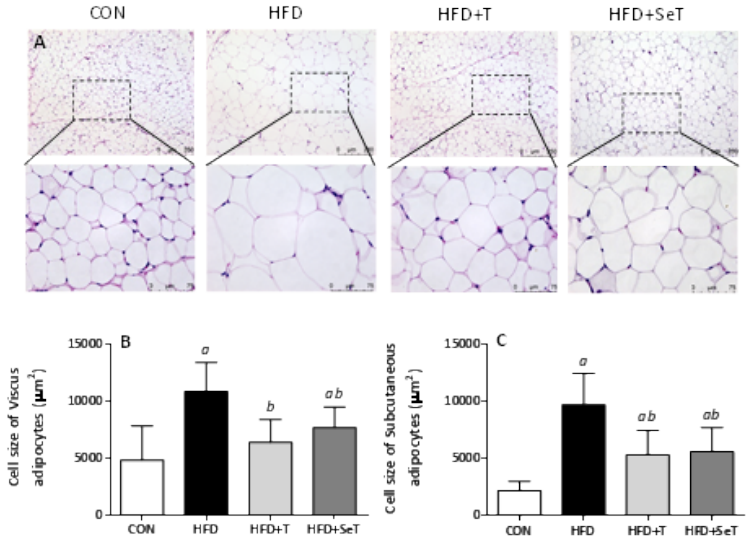

Figure 2. Effects of tea on subcutaneous adipose histology examined by HE (A) (upper, 100×; lower, 400×), cell size of viscus adipocytes (B), and subcutaneous adipocytes (C). Tea interventions decreased cell sizes of the subcutaneous and visceral adipocytes. Values are mean ± SD (n = 4). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group.

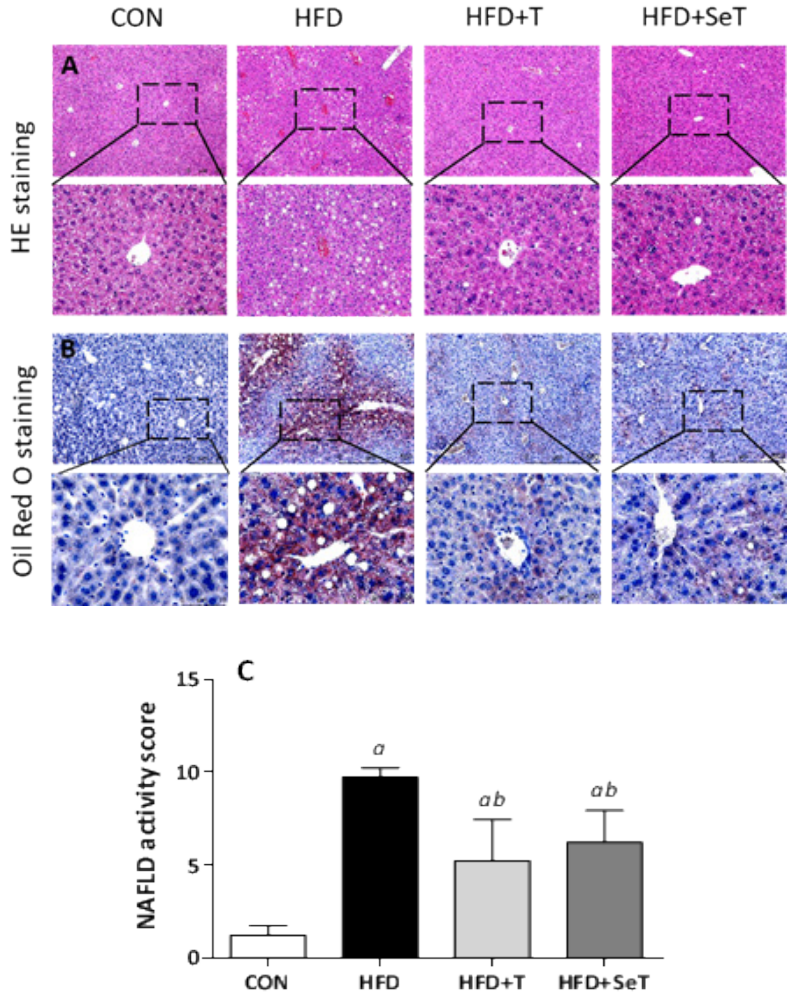

Figure 3. Effects of tea on hepatic histology examined by HE (A), Oil Red O (B) staining (upper, 100×; lower, 400×), and NAFLD activity score (C). Both tea interventions decreased NAFLD activity score. Values are mean ± SD (n = 4). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group.

Effects of tea on oxidative stress and inflammation in the liver

As shown in Table 3, HFD feeding impaired oxidative stress as manifested by decreased SOD, GPx, and CAT levels; and increased MDA level in the liver. Both tea interventions reversed SOD, GPx, CAT, and MDA levels. Furthermore, hepatic SOD, GPx, and CAT were significantly higher in the HFD+SeT group than in the HFD+T group. Hepatic T-AOC was also significantly higher in the HFD+SeT group than in the HFD group. When compared with the CON group, SePP significantly decreased in the HFD group. However, the increase in SePP by tea and Se-enriched tea interventions did not reach statistical significance. On the other hand, hepatic TNF-α, IL-1β, and IL-6 levels were significantly higher in the HFD group than in the CON group. Both tea treatments significantly decreased TNF-α, IL-1β, and IL-6 levels, and the magnitude of reductions was greater in the HFD+SeT group than in the HFD+T group (Table 3).

Effects of tea on peripheral 5-HT signalling

When compared with the CON group, the TBA in the intestinal content, 5-HT level, and TPH1 gene expression in the duodenum were significantly higher in the HFD group. Both tea interventions significantly decreased their levels. When compared with the HFD group, duodenal 5-HT decreased by 35.79% and 43.28% in the HFD+T and HFD+SeT groups, respectively. Duodenal 5-HT was significantly lower in the HFD+SeT group than in the HFD+T group (Figures 5A, 5B, and 5C). Similarly, the TBA, 5-HT, and 5-HTR2A in the liver increased by HFD was reversed by the tea interventions; however, no significant differences were observed in the HFD+T and HFD+SeT groups (Figure 5D, 5E, and 5F). We found that HFD feeding increased hepatic MAO-A and H_2O_2. Both tea treatments decreased MAO-A; however, the magnitude of MAO-A reduction was greater in the HFD+SeT group than in the HFD+T. H_2O_2 was also significantly lower in the HFD+SeT group than in the HFD group (Figures 5G and 5H).

Discussion

Tea-drinking has been considered a health-promoting habit by publics and academics. In the present work, both tea interventions decreased body weight and visceral fat weight, and improved dyslipidaemia and steatosis. Both tea interventions improved oxidative stress and inflammatory response, and decreased duodenal and hepatic 5-HT levels. Se-enriched tea appeared to be more potent than regular tea. Tea interventions consistently regulated TBA secretion, duodenal TPH1 gene expression, and hepatic 5-HTR2A level.

Animal studies have provided evidence on the association of green tea extract (GTE) and tea components with the symptoms of NAFLD (Zhou *et al.,* 2019). Bruno *et al.* (2008) found that GTE intervention for six weeks decreased body weight gain and protected against NAFLD without affecting

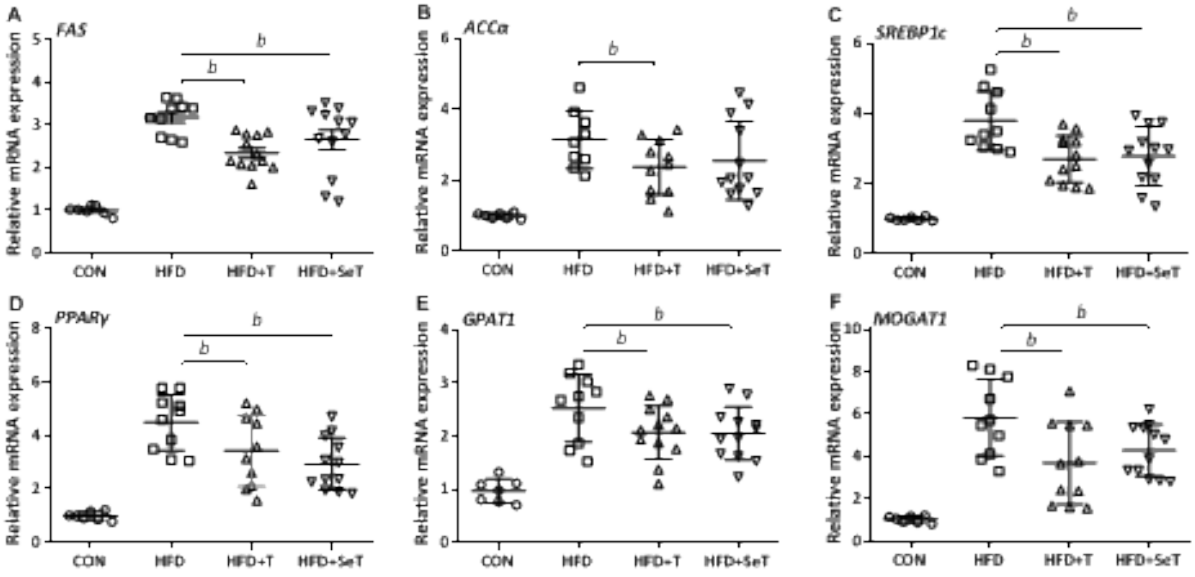

Figure 4. Effects of tea on the expression of genes involved in lipogenesis in liver. All gene expressions were significantly higher in the HFD and treatment groups than in the CON group (not indicated in the figure). Tea interventions down-regulated the expression of these genes. Values are expressed as mean ± SD (n = 8 - 12). [b] $p < 0.05$ versus HFD group.

Table 3. Effects of tea on oxidative stress and inflammation in the liver.

	CON	HFD	HFD+T	HFD+SeT
Oxidative stress				
SOD (U/mg)	3.39 ± 0.24	2.17 ± 0.22[a]	2.49 ± 0.23[ab]	2.93 ± 0.23[abc]
GPx (U/mg)	7.44 ± 0.39	5.56 ± 0.37[a]	5.88 ± 0.41[ab]	6.44 ± 0.38[abc]
CAT (U/mg)	0.76 ± 0.07	0.49 ± 0.08[a]	0.57 ± 0.06[ab]	0.65 ± 0.06[abc]
T-AOC (μmol/gprot)	37.73 ± 6.61	34.57 ± 12.02[a]	45.38 ± 16.80	50.55 ± 12.93[b]
MDA (μmol/gprot)	0.56 ± 0.14	0.88 ± 0.24[a]	0.56 ± 0.19[b]	0.43 ± 0.19[b]
SePP (μg/gprot)	0.95 ± 0.06	0.73 ± 0.12[a]	0.83 ± 0.17	0.84 ± 0.26
Inflammation				
TNF-α (ng/g)	2.24 ± 0.41	4.67 ± 0.56[a]	4.08 ± 0.41[ab]	3.42 ± 0.19[abc]
IL-1β (ng/g)	0.42 ± 0.07	1.00 ± 0.10[a]	0.80 ± 0.07[ab]	0.65 ± 0.07[abc]
IL-6 (ng/g)	0.61 ± 0.08	1.27 ± 0.14[a]	1.01 ± 0.07[ab]	0.86 ± 0.09[abc]

Values are mean ± SD (n = 8 - 12). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group, and [c] $p < 0.05$ versus HFD+T group. SOD = superoxide dismutase; GPx = glutathione peroxidase; CAT = catalase; T-AOC = total antioxidant capacity; MDA = malondialdehyde; SePP = Selenoprotein P; TNF-α = tumour necrosis factor-α; IL-1β = interleukin-1β; and IL-6 = interleukin-6.

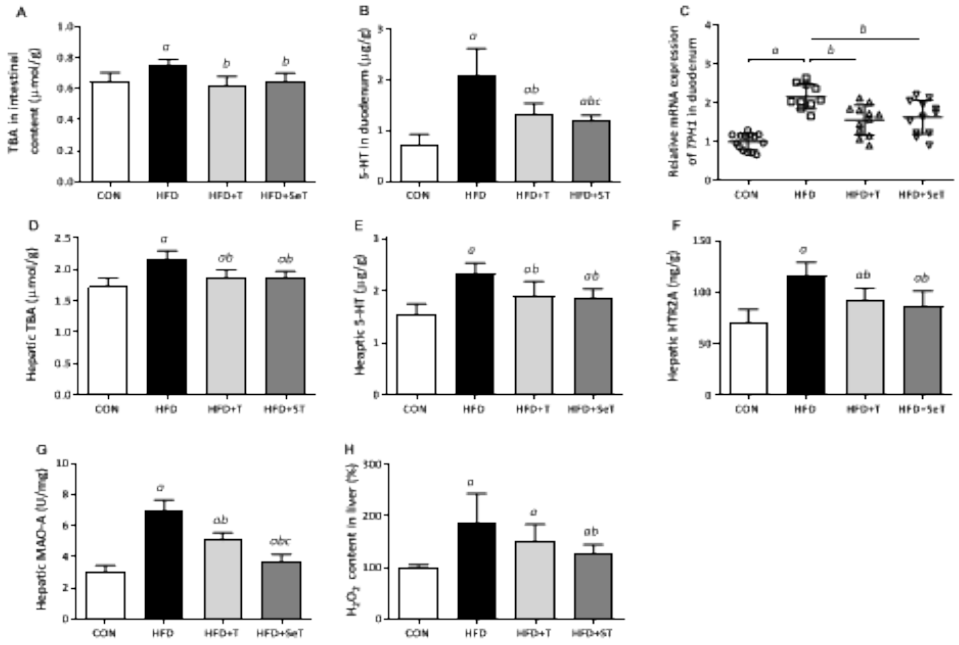

Figure 5. Effects of ST on peripheral 5-HT/5-HTR2A signalling. Tea treatments decreased TBA in intestinal content, 5-HT level, and TPH1 gene expression in the duodenum (A, B, C), and decreased TBA, 5-HT, and 5-HTR2A in the liver (D, E, F). Tea treatments also lowered hepatic MAO-A and H_2O_2 relative content (G, H). Values are mean ± SD (n = 8 - 12). [a] $p < 0.05$ versus CON group, [b] $p < 0.05$ versus HFD group, [c] $p < 0.05$ versus HFD+T group.

hepatic antioxidant status in ob/ob (obese) mice. Kuzu *et al.* (2008) found that (−)-epigallocatechin-3-gallate (EGCG), the richest catechins in green tea, reduced the development of HFD-induced NAFLD in rats, potentially through the improvement of lipid metabolism and antioxidant characteristics. A variety of rodent models (Santamarina *et al.,* 2015; Tan *et al.,* 2017; Huang *et al.,* 2018; Li *et al.,* 2018; Yu *et al.,* 2018) indicated that the beneficial effects of GTE and EGCG on NAFLD were attributed to the improvement of lipid and glucose metabolism, antioxidant, and anti-Inflammatory effects, or reduced endotoxin. However, relatively high doses of GTE, which did not reflect the amounts of green

tea consumed by humans through diet, was used in these animal studies. Karolczak *et al.* (2019) found that GTE supplementation reflecting habitual consumption of 5 - 8 cups of this beverage per day successfully reduced the amount of hepatic fat in rats fed with HFD. In the present work, we used tea drink, a common method of tea consumption, to investigate the action of green tea on HFD-induced NAFLD. Similar to most animal studies, our findings also exhibited that tea interventions markedly reduced lipid droplets through their lipid-lowering, anti-oxidant, and anti-inflammation properties. Despite the benefits of green tea, liver injury by GTE should be taken into consideration. In the study of Hirsch *et al.* (2016), 1% GTE (polyphenon 60), which is comparable to the doses consumed by humans, was mixed in powder diets. Fatty liver disease was induced in mice fed with diet containing 1% cholesterol and 0.5% cholate (HCD). Unexpectedly, additional GTE supplementation for six weeks exacerbated hepatic steatosis, oxidative stress, inflammatory response, and altered bile acid synthesis in mice with fatty liver disease (Hirsch *et al.,* 2016). The possible reason for this phenomenon may be due to low fat content (16% kcal fat) and additional cholate in that HCD diet. Cholate is often used to induce liver injury with serious hypercholesterolemia, which is not like HFD-induced NAFLD (Ling *et al.,* 2019).

Dietary absorption and DNL are the two main approaches for liver lipid accumulation. In the present work, we measured hepatic mRNA expression in the DNL pathway. FAS catalyses *de novo* synthesis in the cytosol, whereas ACCα catalyses the rate-determining step in the biosynthesis of long-chain fatty acids. SREBP-1c plays an important role in FAS and ACCα regulation. In ACC-deficient livers, SREBP-1c activation induces the expression of GPAT1, which catalyses the first committed step in TG synthesis in the liver (Kim *et al.,* 2017). PPARγ also plays a dominant role in lipid synthesis and storage. Liver-specific PPARγ knockdown dramatically reduces MOGAT1 expression (Greenstein *et al.,* 2017), while MOGAT1 expression is increased in the livers of mice with hepatic steatosis (Soufi *et al.,* 2014). We confirmed that tea and Se-enriched tea markedly decreased fat accumulation in liver and adipose tissue, as displayed by the decreased mRNA suppression of SREBP-1c, PPARγ, FAS, and ACCα involved in DNL, and GPAT1, and MOGAT1 related to TG synthesis. Our results are consistent with previous studies (Santamarina *et al.,* 2015; Seo *et al.,* 2017; Wu *et al.,* 2017; Torres *et al.,* 2019). In the study of Torres *et al.* (2019), GTE supplementation for 16 weeks protected against NAFLD development with lower NAS, and increased the expression of gene involved in triglyceride and fatty acid catabolisms (Torres *et al.,* 2019). Seo *et al.* (2017) found that fermented GTE intervention significantly down-regulated the mRNA expression of SREBP1c, ACC, and FAS in the WAT and liver of HFD-induced obese mice. At the protein level, GTE intervention significantly decreased the hepatic protein expression of ACC, FAS, and SREBP-1 in HFD-induced obese mice (Santamarina *et al.,* 2015). Interestingly, the inhibition of PPARγ and FAS expression at mRNA and protein levels by EGCG was also observed in 3T3-L1 cells (Wu *et al.,* 2017).

Although Se possesses strong bioactivity, scientific evidence that supports this possibility is insufficient. We did not observe the additional benefit of Se-enriched tea on body weight, fat accumulation, and lipogenesis. Gao *et al.* (2020) also demonstrated that Se-rich black tea, while ameliorating HFD-induced liver damage, was not better than regular black tea in preventing fat accumulation (Gao *et al.,* 2020). As expected, we found that both tea interventions improved oxidative stress and inflammatory response, which was more remarkable in Se-enriched tea. Most, albeit not all, experimental data show the favourable antioxidant effect of Se on hepatic steatosis and inflammation (Ren *et al.,* 2015; Han *et al.,* 2017; Yu *et al.,* 2018; Polyzos *et al.,* 2019). In the study of Yu *et al.* (2018), gavage with green tea or zinc (Zn) Se organic tea decreased hepatic necrosis and dropsy in rats fed with a high-sucrose high-fat diet. Importantly, the hepatosomatic index, a representative marker of hepatic injury, was significantly reduced in the Zn Se tea group, but not in green tea group. In the study of Ren *et al.* (2015), Se-containing tea polysaccharides reduced mouse hepatic injuries induced by high fructose water with a dose-dependent reduction of hepatic SOD and GPx activities. Se and SePP are associated with NAFLD (Polyzos *et al.,* 2019). In a mouse model of acute alcoholism, Se supplementation weakened liver damage with the increase of SePP, which involved the regulation of inflammatory cytokines such as TNF-α, IL-1β, and IL-10 (Zhang *et al.,* 2017). Unexpectedly, the increase in SePP by tea and Se-enriched interventions did not reach statistical significance in the present work. The possible explanations are that SePP is not sensitive enough for oxidative stress in this case, or the ELISA kit for SePP measurement had low specificity (Schomburg and Melander, 2019). Although Se-enriched green tea has higher antioxidant and

inflammation activities than regular green tea, more studies are needed to confirm the action of selenoprotein on NAFLD improvement.

The novelty of the present work was that we investigated the direct effects of green tea on NAFLD through peripheral 5-HT signals in HFD-fed mice. Although most 5-HT in the body is synthesised at the periphery, its biological roles have not been well elucidated (Choi *et al.,* 2020). Both previous and present studies demonstrated that HFD feeding increased TPH1 expression and tissue 5-HT level (Choi *et al.,* 2018). HFD-fed TPH1 gene knockout (GKO) mice are protected against hepatic steatosis (Oh *et al.,* 2015). Thus, it is plausible that 5-HT possibly regulates HFD-induced hepatic steatosis. Interestingly, the expression of genes involved in DNL and TG synthesis is down-regulated by TPH inhibitor for 5-HT depletion (Namkung *et al.,* 2018). In the present work, a decrease in 5-HT, 5-HTR2A, and TPH1 gene expression by green tea and Se-enriched green tea was accompanied by the down-regulation of genes in DNL and TG synthesis. Se-enriched tea significantly decreased duodenum 5-HT when compared with non-enriched tea. However, Seo *et al.* (2017) found that fermented GTE increased plasma 5-HT levels, although no corresponding changes in gut TPH1 expression was observed, possibly due to distinct components found in green tea and fermented green tea. For example, the EGCG content of fermented green tea is much lower than that of green tea (Seo *et al.,* 2017). The other possible explanation is that the plasma level of free 5-HT is very low because 5-HT, which is released from the gut, is stored in platelets or metabolised in the liver (Choi *et al.,* 2020). In general, 5-HT released from EC cells may activate serotonergic receptors, and be taken up by enterocytes through serotonin reuptake transporter (SERT). Increasing the expression of TPH1 and decreasing the expression of SERT can aggravate the symptoms of NAFLD (Zhang *et al.,* 2020). Although we did not determine SERT expression in the duodenum, the downstream of 5-HT was investigated. Tea intervention decreased MAO-A, an enzyme that degrades 5-HT into H_2O_2 (Torres *et al.,* 2019), following the decrease in 5-HT. Thus, the health benefit of green tea with and without Se enrichment on NAFLD was partially due to peripheral 5-HT signals.

Besides 5-HT, bile acids are essential mediators of gut-liver crosstalk (Ling *et al.,* 2019). Furthermore, the interactions of bile acids and 5-HT should be considered when investigating the effect of nutrients on NAFLD. Intraperitoneal 5-HT injection accelerated the turnover of bile acids (excretion, biosynthesis, and re-absorption) (Watanabe *et al.,* 2010). On the other hand, liver-secreted bile acids such as deoxycholic acid (DCA), which upregulate the expression of TPH1, play an important role in the regulation of 5-HT synthesis (Sun *et al.,* 2018). In the present work, we determined TBA excretion, and found that tea interventions decreased TBA in the liver and intestinal content. Ushiroda *et al.* (2019) observed that EGCG altered bile acid metabolism, and suppressed fatty liver disease in HFD-fed mice. Huang *et al.* (2018) found that EGCG decreased bile acid reabsorption, thus resulting in a decrease in lipid absorption in mice fed with a high-fat western-style diet. Yu *et al.* (2018) demonstrated a notable depression in serum TBA in the Zn Se tea group, but not in the green tea group. However, Hirsch *et al.* (2016) observed that GTE addition further increased serum TBA in mice with fatty liver disease.

The present work has several limitations. First, peripheral 5-HT signalling is a complex process. The association of 5-HT signals with lipogenic pathways cannot fully explain the role of green tea. Future studies should consider 5-HT as a treatment, tryptophan-free diet, or TPH1 GKO mice as a model to investigate the role of peripheral 5-HT signalling in green tea. Second, although tea intervention decreased TBA in the liver and intestinal content, we did not determine the species of bile acids. The relationship between conjugated and unconjugated bile acids is important in removing excess cholesterol and regulating lipid metabolism (Ling *et al.,* 2019). Furthermore, bile acids are released in the gut, where they can be further metabolised by the microbiota. Bile acids critically control gut microbiota. Unfortunately, gut microbiota, which is related to NAFLD, was not assessed in the present work.

Conclusions

In summary, green tea and Se-enriched green tea interventions reduced body weight and ameliorated NAFLD in mice, possibly by the regulation of lipogenic pathways and 5-HT signal pathway. There was no significant difference between green tea and Se-enriched green tea in inhibiting liver fat synthesis and reducing the size of white fat cells. However, Se-enriched green tea appeared to be more potent than regular green tea in improving oxidative stress and inflammatory response and decreasing duodenum 5-HT and hepatic MAO-A levels.

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