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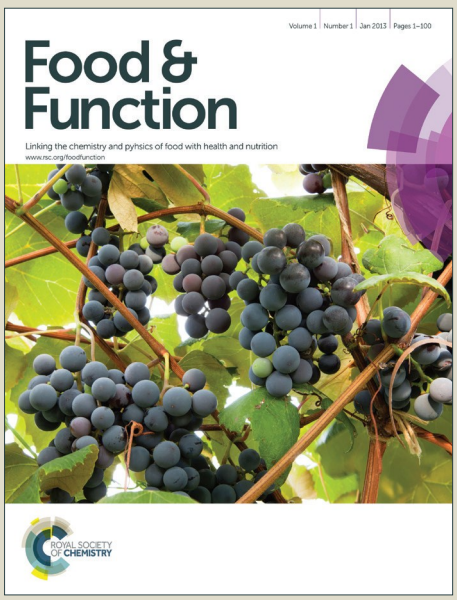

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Anti-steatotic and anti-inflammatory roles of syringic acid in high-fat diet-induced obese mice

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Abstract

This study examined the effects of syringic acid (SA) on obese diet-induced hepatic dysfunction. Mice were fed high-fat diet (HFD) with or without SA (0.05%, wt/wt) for 16 weeks. SA reduced body weight, visceral fat mass, serum levels of leptin, TNFα, IFNγ, IL-6 and MCP-1, insulin resistance, hepatic lipid content, droplets and early fibrosis, whereas it elevated circulation of adiponectin. SA down-regulated lipogenic genes (*Cidea*, *Pparγ*, *Srebp-1c*, *Srebp-2*, *Hmgcr, Fasn*) and inflammatory genes (*Tlr4*, *Myd88*, *NF-κB*, *Tnfα*, *Il6*), whereas it up-regulated fatty acid oxidation genes (*Pparα*, *Acsl*, *Cpt1*, *Cpt2*) in the liver. SA also decreased hepatic lipogenic enzyme activities and elevated fatty acid oxidation enzyme activities relative to the HFD group. These findings suggested that dietary SA possesses anti-obesity, anti-inflammatory and anti-steatotic effects via regulation of lipid metabolic and inflammatory genes. SA is likely to be a new natural therapeutic agent for obesity or non-alcoholic liver disease.

Keywords: inflammation, liver, obesity, syringic acid, steatosis

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance and adipose tissue distribution, which are risk factors for nonalcoholic steatohepatitis (NASH), diabetes and associated pathologies.[1] Although the pathogenesis of NAFLD is varied and complex, the traditional two-hit theory of its development is widely accepted.[2] The first hit refers to the accumulation of triglyceride (TG) in hepatocytes that results from an imbalanced hepatocellular lipid metabolism. This is followed by the second hit, which is an increase in inflammatory mediators such as cytokines, chemokines and adipocytokines that causes hepatocellular injury, inflammation and fibrosis.[3] Many researchers have used natural compound intervention to treat or prevent obesity or NAFLD.

Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid, SA) (Fig. 1) is a major benzoic acid derivative of edible plants and fruits[4] that possesses antimicrobial and anti-DNA oxidation properties.[5-7] SA has been shown to inhibit diabetic cataracts in both *in vitro* lens cultures and *in vivo* rat models.[8] Recently, SA isolated from *Tamarix aucheriana* was shown to be an antimitogenic and chemo-sensitizing agent for human colorectal cancer.[9] Itoh *et al.*[10] reported that SA could act as an immunomodulator in mice with ConA-induced liver injury and has protective effects in mice based on a CCl_4-treatment model. Therefore, SA might be a promising oral agent for the prevention of liver disease; however, the anti-obesity or anti-steatotic effects of SA are still not known.

Excessive consumption of high-fat diet (HFD) contributes to onset insulin resistance and hepatosteatosis, which are predictive biomarkers for NAFLD.[11] Hepatosteatosis represents an excessive accumulation of fat that surpasses the energy combustion capability of fatty acid oxidation systems.[12] C57BL/6J mice are a useful model for examining changes that occur in body composition that predispose toward future metabolic disorders, including NAFLD.[11]

Therefore, we examined the effects of SA on obesity, hepatosteatosis and inflammation in diet-induced obese (DIO) C57BL/6J mice.

2. Materials and methods

2.1. Animals and diets

The present study was performed according to the national and institutional guidelines and approved by the Sunchon National University Institutional Animal Care and Use Committee (SCNU IACUC-2013-11). A total of 24 four-week-old C57BL/6J male mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and individually housed at a controlled temperature (22 ± 2°C) under a 12-hr light-dark cycle. After a one week adaptation period, the mice were randomly divided into three groups and fed a normal diet (NC, n = 8), high-fat and high-cholesterol diet (HFD, n=8, 20% fat and 1% cholesterol) or HFD with 0.05% SA (HFD-SA, n=8, 0.5 g/kg diet, Sigma, St. Louis, MO, USA) for 16 weeks. At the end of the experimental period, the mice were anesthetized with ether after withholding food for 12-hr. Blood samples were then taken from the inferior vena cava for serum biomarker analysis, after which the liver and adipose tissues were removed, rinsed with a physiological saline solution and stored immediately at -70°C until analysis.

2.2. Blood glucose, intraperitoneal glucose tolerances test (IPGTT), serum insulin level and homeostasis model assessment of insulin resistance (HOMA-IR)

At week 8, 12 and 16, the 6-hr fasting glucose levels were measured in tail vein blood using a glucometer (GlucoDr SuperSensor, Allmedicus, Korea).[13] Following a 6-hr fast at week 16, mice were injected intraperitoneally with glucose (1 g/kg body weight), and blood glucose levels were then determined from tail vein blood collected 0, 30, 60, and 120 min

after glucose injection. The results were presented as the area under the curve (AUC). Insulin (Crystal Chem, Downers Grove, IL, USA) levels were determined using a quantitative sandwich enzyme immunoassay kit. The HOMA-IR was calculated according to the homeostasis of assessment as previously described: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5.[14]

2.3. Serum adipokine, cytokine and chemokine levels

Adiponectin and leptin (R&D System, Minneapolis, MN, USA) levels were determined using a quantitative sandwich enzyme immunoassay kit. Tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) levels were determined using a multi detection kit and a Luminex 200 lamp system (Bio-Rad, Hercules, CA, USA).

2.4. Serum lipid profiles and hepatic lipid analyses

The serum total cholesterol (TC), HDL-cholesterol (HDL-C), triylglyceride (TG) (Asan Diagnostics, Seoul, Korea) and free fatty acid (FFA) (Shinyang Diagnostics, Seoul, Korea) concentrations were determined using commercial kits. The amount of low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation as follows: LDL-C = TC– HDL-C–(TG/5).[15] The hepatic lipid was extracted as previously described,[16] after which the cholesterol and TG contents were analyzed with the same enzymatic kit that was used for the plasma analysis.

2.5. Morphology of liver and adipose tissues

The liver and epididymal adipose tissues were removed and fixed in buffer solution containing 10% formalin, after which the fixed tissues were paraffin-embedded and 4 μm

sections were prepared and stained with hematoxylin and eosin (H & E) or Masson's trichrome. To further confirm hepatic lipid droplet accumulation, frozen sections were stained with Oil Red O solution. The stained area was then viewed using a microscope at 200× magnification.

2.6. Liver damage biomarkers

To assess liver damage, serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were analyzed using automated chemistry analyzer (Fuji Dri-Chem 3500; Fujifilm, Tokyo, Japan). Ten μL of serum was deposited on each AST-PIII and ALT- PIII slide for whole analyzer's process and then we obtained the results according to the manufacturer's procedures.

2.7. Hepatic lipid metabolic enzyme activities

The phosphatidate phosphohydrolase (PAP) activity was determined using the method developed by Walton and Possmayer.[17] Fatty acid synthase (FAS) activity was determined using a spectrophotometric assay that measured the malonyl-CoA-dependent oxidation of NADPH according to the method described by Nepokroeff *et al.*[18] with slight modification. The carnitine palmitoyltransferase (CPT) was assayed spectrophotometrically by measuring the release of CoA-SH from palmitoyl-CoA using the general thiol reagent 5,5′-dithiobis (2-nitrobenzoate) as described by Bieber *et al.*[19] with slight modifications. Fatty acid β-oxidation (β-oxidation) activity was measured spectrophotometrically by monitoring the reduction of NAD^+ to NADH in the presence of palmitoyl-CoA using the method described by Lazarow[20] with slight modification.

2. 8. RNA isolation and quantitative real-time PCR analysis

The liver or adipose tissue was homogenized in Trizol reagent (Invitrogen Life Technologies, Grand Island, NY, USA), after which total RNA was isolated according to the manufacturer's specifications. DNase digestion was used to remove DNA contamination, after which the RNA was re-precipitated in ethanol to ensure that there was no phenol contamination. For quality control, RNA purity and integrity were evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The total RNA (1 μg) was then reverse-transcribed into cDNA using a QuantiTect® reverse transcription kit (Qiagen, Hilden, Germany). Next, mRNA expression was quantified by real-time quantitative PCR using a SYBR green PCR kit (Qiagen) and the CFX96TM real-time system (Bio-Rad, Hercules, CA, USA). The sequences of the primers are shown in the supplementary data. The cycle thresholds were determined based on the SYBR green emission intensity during the exponential phase. The fold changes were determined using the $2^{-\Delta\Delta Ct}$ method.[21] In addition, transcripts of *Gapdh* were amplified from the samples to assure normalized real-time quantitative RT-PCR detection.

2. 9. Statistical analysis

All data are presented as the mean ± SE. Statistically significant differences between groups were identified by one-way analysis of variance (ANOVA) using SPSS version 21 (Chicago, IL) with Duncan's multiple-range post hoc test. Values were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. SA reduced body weight, visceral fat and insulin resistance in DIO mice

SA led to a significant decrease in body weight and food efficiency ratio without suppressing food intake compared to the HFD group (Fig 2A). Histological staining of the epididymal adipose tissue showed that adipocyte size was dramatically increased in the HFD group by 2.9-fold compared to the NC group, but that it decreased in response to SA supplementation (25%) relative to the HFD group (Fig. 2B & C). The weights of epididymal and perirenal adipose tissue in the HFD group were also elevated compared to the NC group, but these weights were significantly lower in the SA group (16% and 34%, respectively) than in the HFD group (Fig 2C). Thus, SA significantly reduced HFD-induced visceral fat mass.

Obesity is one of risk factors of insulin resistance; therefore, we confirmed that HFD induced hyperglycemia and hyperinsulinemia, which results in insulin resistance by evidenced as HOMA-IR (Fig. 3A-C). However, SA effectively lowered serum g1ucose and insulin levels and improved glucose intolerance and insulin resistance (Fig. 3A-D).

The serum leptin content and its mRNA expression in adipose tissue were reduced, whereas those of adiponectin were increased by SA supplementation (Fig. 4A-D).

3.2. SA inhibited inflammatory response in DIO mice

To determine the effects of SA on inflammatory response, we measured the serum cytokine and chemokine levels. Pro-inflammatory markers, serum TNFα, IFNγ, IL-6 and MCP-1 levels were significantly increased in the HFD group compared to the NC group (Fig. 5A); however, SA lowered the levels. SA also significantly down-regulated expression of inflammatory genes, such *Tlr4*, *Myd88*, *NF-κB*, *Tnfα* and *Il6* in the liver, which were increased in DIO mice (Fig. 5B). Thus, SA suppressed long-term HFD-induced inflammation through inhibition of the TLR4 pathway. Furthermore, trichrome staining revealed that SA

prevents early progression of fibrosis in DIO mice (Fig. 6A).

3. 3. SA protects hepatic damage by suppressing hepatosteatosis in DIO mice

The activity of serum ALT, a hepatic lipotoxicity marker, was significantly increased by obesity; however, SA protected against hepatotoxicity (Table 1). Additionally, SA supplementation reduced hepatic lipid accumulation (TG 44%, FFA 9% and cholesterol 44%) and lipid droplets (H & E and Oil Red O stains) compared to the HFD group (Fig. 6A-D). Serum TG and LDL-C contents were significantly lowered and HTR was increased by SA supplementation (Table 1). To understand the lipid-lowering effects of SA, we measured lipid metabolic gene expression in the liver. SA significantly down-regulated lipogenic gene levels, such *Cidea*, *Pparγ*, *Srebp-1c*, *Srebp-2*, *Fasn* and *Hmgcr*, whereas it up-regulated fatty acid oxidation genes, such as *Pparα*, *Acsl*, *Cpt1* and *Cpt2* compared to the HFD group (Fig. 7 & 8). SA also decreased FAS and PAP activity and increased fatty acid β-oxidation and CPT activities compared to the HFD group (Fig. 9A-D).

4. Discussion

This study demonstrated that dietary SA effectively prevented obesogenic diet-induced weight gain, adiposity, insulin resistance, dyslipidemia and hepatosteatosis. Obesity has been associated with NAFLD in many animal models[22,23] and the imbalanced production of pro- and anti-inflammatory adipokines secreted from visceral fat contributes to NAFLD.[24] Two key adipokines are leptin and adiponectin. The former acts as a profibrogenic molecule, but the latter has strong antifibrotic properties.[25] In cases of NAFLD associated with obesity, serum leptin is increased and adiponectin is decreased.[26] We also confirmed that long-term HFD induced hyperleptinemia and hypoadioponectinemia; however, SA significantly

lowered the serum leptin level and elevated adiponectin level, which is consistent with the changes in their gene expression in visceral adipose tissue. Hypoadiponectinemia is more likely to be responsible for obesity-related NAFLD progression, fibrosis and potential for treatment.[27] SA can modulate serum leptin and adiponectin levels by regulating their gene expression in adipose tissue of DIO mice.

Adiponectin is also known to have insulin-sensitizing and anti-inflammatory roles for liver diseases.[28] Therefore, we determined the effects of SA on insulin resistance and insulin sensitivity using HOMA-IR and GTT, which are commonly employed to measure whole-body and peripheral insulin resistance and insulin sensitivity. Our results revealed that SA significantly reduced serum glucose (from week 8) and insulin (from week 12) levels in DIO mice, which resulted in improved insulin resistance as evidenced by IPGTT and HOMA-IR. It has been reported that high insulin levels are positively correlated with the development of NAFLD in nondiabetic humans.[29] Steneberg *et al.*[30] also suggested that diet-induced hyperinsulinemia is an early and potent inducer of hepatosteatosis, insulin resistance and dysglycemia. Peripheral insulin resistance may cause hepatosteatosis by elevating the plasma levels of fatty acid, glucose and insulin, which stimulates lipid synthesis and impairs β-oxidation.[31] Our results showed that HOMA-IR, an insulin resistant marker, is positively correlated with hepatic lipid contents (TG: r=0.692, cholesterol: r=0.684, p <0.001) and SA dramatically decreased hepatic levels of TG (44%) and cholesterol (44%) as well as FFA content and lipid droplets compared with the HFD group.

Hepatic TG storage during obesity is achieved through a complex program coordinated by transcription regulators, including *Serbp-1c*[32] and *Pparγ*.[33] Hepatic *Pparγ* expression is increased in obese mice and human NAFLD[34,35] and hepatic overexpression of *Pparγ* provokes fatty liver development in mice.[36-38] Insulin is known to stimulate hepatic *Serbp-1c*

expression, which promotes lipogenesis in the liver that subsequently contributes to fatty liver development in response to an energy-rich diet.[39,40] In the present study, the gene expression of *Serbp-1c* and *Pparγ* was increased in DIO mice compared with the normal diet fed mice; however, SA significantly down-regulated these genes and their downstream *Fasn*, which resulted in decreased FAS and PAP activities. In this study, SA also significantly down-regulated hepatic gene expression of *Cidea* and the cholesterol biosynthesis genes, *Srebp-2* and *Hmgcr*, which was increased by HFD. *Cidea* plays a key role in the formation of lipid droplets[41] and is known to be controlled by *Pparγ*.[42] Jinno *et al*.[41] reported that *Cidea* gene expression is modulated in parallel with *Pparγ* gene expression and closely related to the progression of hapatosteatosis with elevation of ALT, a serum marker of liver injury. In the present study, SA inhibited mRNA expression of *Pparγ* and *Cidea* while decreasing the serum ALT levels induced by HFD. SA also reduced hepatic and serum cholesterol contents compared with the HFD group. The rate-limiting step in the cholesterol biosynthesis pathway is the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) into mevalonate by HMG-CoA reductase,[43] which is regulated by *Srebp-2*.[44] Hepatic *Srebp-2* up-regulation parallels the severity of liver disease in animal and human NAFLD.[45-47] Therefore, *Srebf-2* is an ideal candidate for modulating the genetic susceptibility to NAFLD and NASH.[48] In the current study, hepatic *Srebp-2* and *Hmgcr* mRNAs were significantly down-regulated in DIO mice treated with SA, which resulted in inhibition of cholesterol biosynthesis. On the other hand, SA up-regulated fatty acid oxidation genes such as *Pparα*, *Acsl*, *Cpt1* and *Cpt2*. *Ascl* is induced by *Pparα* and plays an important role in pathways of long-chain fatty acid mitochondrial and peroxisomal β-oxidation,[49] while *Cpt1* and *-2*, the mitochondrial gateway for fatty acid entry into the matrix in outer and inner membrane, respectively, are the main controllers of hepatic β-oxidation.[50] Thus, SA markedly ameliorated hepatic steatosis by

stimulation of fatty acid oxidation via *Pparα* and suppression of lipid synthesis through *Pparγ-Srebp-1c*, as well as reduced cholesterol accumulation by inhibiting *Srebp-2*.

It has become apparent that obesity is linked to a state of chronic inflammation in tissues such as liver, adipose tissue and skeletal muscle.[51] Therefore, we evaluated changes in serum cytokine and chemokine levels. The results showed that long-term HFD elevated serum TNFα, IL-6 and MCP-1 levels; however, SA reversed them. Dysregulation of the expression of adipokines caused by adipocyte hypertrophy and dysfunction has been linked to chronic inflammation and insulin resistance.[3] Hypertrophic adipocytes secrete various pro-inflammatory cytokines such as TNFα, IL-6 and IL1β, which can down-regulate the insulin sensitivity of organs including the liver and skeletal muscle.[52] Hypertrophic adipocytes also secrete MCP-1, which promotes the infiltration of monocytes into adipose tissue, where they differentiate into macrophages and promote inflammation via the secretion of pro-inflammatory cytokines.[3] IL-6 concentrations are elevated in obesity and patients with type 2 diabetes[51] and contributes to obesity-induced insulin resistance.[53] Another cardinal feature of obesity and NAFLD is hepatic inflammation.[54] Similar to its effects on adipose tissue, obesity induced hepatic inflammation, as indicated by increased production of pro-inflammatory cytokines and by activation of the NF-κB pathway.[54] We also showed that hepatic TG level was positively correlated with inflammatory genes (*NF-κB*: r=0.576, p <0.01, *Tnfα*: r=0.526, p <0.05, *Il6*: r=0.433, p <0.05). SA significantly down-regulated gene expression of *NF-κB*, *Tnfα* and *Il6* in the liver. Inhibition of *NF-κB* explains how medicinal plants reduce downstream induction of cytokines expression during liver fibrosis and suggests a common mechanism between their bioactive compounds.[55] *NF-κB* is a transcription factor implicated in the regulation of a wide range of genes related to apoptosis, inflammation and immune response. Expression of many chemokines, cytokines and other inflammatory mediators,

including *Nos2*, *Cox2*, *Mip-2*, *Mcp-1*, *Il12* and *Tnfα*, is under control of *NF-κB* activation.[55] *NF-κB* related to TLRs, with stimulation of *Tlr4* leading to activation of *NF-κB* through up-regulation of the myeloid differentiation primary response 88 (*Myd88*).[56] In consequence, the *Tlr4-NF-κB* signaling pathway appears as a potential therapeutic target to suppress inflammation in liver fibrosis. In addition to its role in obesity, *Tlr4* was identified as a critical effector in the development of NAFLD.[57] Based on these findings, *Tlr4* could be considered as a critical node between obesity and NAFLD; therefore, we examined the effects of SA on the *Tlr4-Myd88* pathway in DIO mice. SA significantly down-regulated hepatic *Tlr4* and *Myd88* gene expression increased in response to HFD. Thus SA decreased the hepatic gene expression of pro-inflammatory cytokines, including *Tnfα* and *Il6* via down-regulation of the *Tlr4-Myd88* pathways.

In conclusion, obesity-induced hepatosteatosis and inflammation were reduced by SA supplementation via a mechanism involving down-regulation of lipid synthesis, up-regulation of fatty acid oxidation and reduction of inflammation in the liver. Our results suggest that SA is a promising complementary supplement that can ameliorate NALD or obesity.

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Figure legends

Fig. 1. Chemical structure of syringic acid.

Fig. 2. Effect of syringic acid supplementation on body weight changes, food intake, FER (A), adipocyte morphology (B) and adipocyte size and visceral fat weights (C) in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; FER, food efficiency ratio.

Fig. 3. Effect of syringic acid supplementation on serum glucose (A) and insulin (B) levels, HOMA-IR (C) and IPGTT (D) in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [ab]Means not sharing a common letter are significantly different among groups ($p < 0.05$). NC, normal diet fed group; HFD, high-fat fed group; HFD-SA; high-fat plus 0.05% syringic acid fed group; HOMA-IR, homeostasis model assessment of insulin resistance; IPGTT, intraperitoneal glucose tolerance test.

Fig. 4. Effect of syringic acid supplementation on serum leptin and adiponectin levels and their mRNA expression in adipose tissue in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [ab]Means not sharing a common letter are significantly different among groups ($p < 0.05$). mRNA level of each group was normalized to *Gapdh* and

expressed relative to the corresponding amount in the NC group. NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; *Adipoq*, adiponectin.

Fig. 5. Effect of syringic acid supplementation on serum cytokine and chemokine levels (A) and hepatic inflammatory gene expression (B) in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). mRNA level of each group was normalized to *Gapdh* and expressed relative to the corresponding amount in the NC group. NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; TNFα (*Tnfα*) tumor necrosis factor α; IFNγ, interferon γ, IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; *Tlr4*, toll-like receptor 4; *Myd88*, myeloid differentiation primary response gene 88; *NF-κB*, nuclear factor kappa-light-chain-enhancer of activated B cells.

Fig. 6. Effect of syringic acid supplementation on hepatic histology (A) and lipid levels (B-D) in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; TG, triglyceride; FFA, free fatty acid.

Fig. 7. Effect of syringic acid supplementation on hepatic lipogenic gene expression in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). mRNA level of each group was normalized to *Gapdh* and expressed relative to the corresponding amount in the NC group. NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; *Cidea*, cell death-inducing DFFA-like effector α; *Pparγ,* peroxisome proliferator-activated

receptor γ; *Fasn,* fatty acid synthase; *Srebp-1c,* sterol regulatory element-binding transcription factor 1; *Srebp-2,* sterol regulatory element-binding protein 2; *Hmgcr,* HMG-CoA reductase.

Fig. 8. Effect of syringic acid supplementation on hepatic fatty acid oxidation gene expression in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). mRNA level of each group was normalized to *Gapdh* and expressed relative to the corresponding amount in the NC group. NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; *Pparα,* peroxisome proliferator-activated receptor alpha; *Acsl,* acly-CoA synthetase long-chain family member 1; *Cpt1,* carnitine palmitoyltransferase 1; *Cpt2,* carnitine palmitoyltransferase 2.

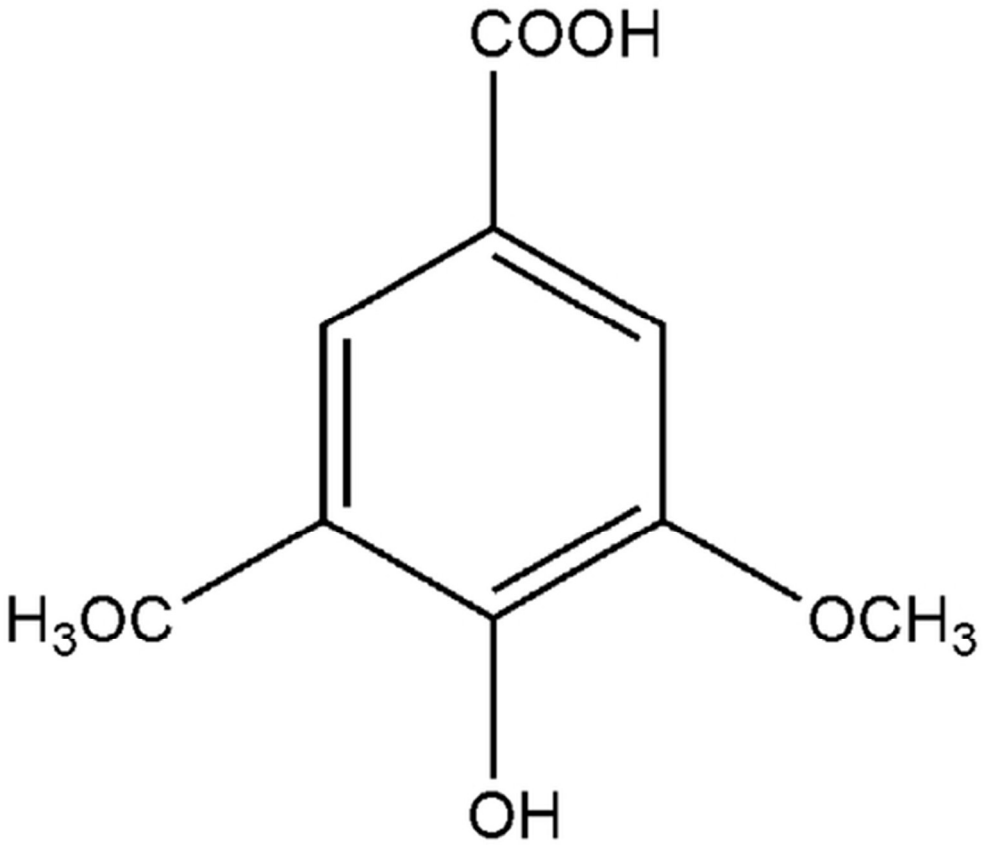
Fig. 9. Effect of syringic acid supplementation on hepatic lipid metabolic enzyme activities in high-fat diet-induced obese mice. Values are expressed as the means ± S.E. (n = 8). [abc]Means not sharing a common letter are significantly different among groups ($p < 0.05$). NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group; FAS, fatty acid synthase; PAP, phosphatidate phosphatase; CPT, carnitine palmitoyltransferase.

Table 1. Effects of syringic acid supplementation on serum AST, ALT and lipid levels in high-fat diet-induced obese mice

	NC	HF	HF-SA
AST (U/L)	**41.50±2.36^{a}**	**67.67±5.48^{b}**	**56.00±2.74^{b}**
ALT (U/L)	**14.33±1.78^{a}**	**53.67±4.81^{c}**	**32.00±2.07^{b}**
Triglyceride (mmol/L)	**0.63±0.03^{a}**	**0.76±0.03^{b}**	**0.67±0.03^{a}**
Free fatty acid (mmol/L)	**1.00±0.01**	**1.14±0.07**	**0.99±0.01**
Total-cholesterol (mmol/L)	**3.19±0.18^{a}**	**3.88±0.15^{b}**	**3.65±0.12^{b}**
LDL-C (mmol/L)	**0.63±0.14^{a}**	**1.41±0.18^{b}**	**0.79±0.21^{a}**
HDL-C (mmol/L)	**2.33±0.25**	**2.47±0.07**	**2.91±0.20**
HTR (%)	**74.96±2.67^{b}**	**62.77±2.81^{a}**	**80.79±5.40^{b}**

Values are mean±S.E.^{abc}Mean in the same row not sharing a common superscript are significantly different between group (p <0.05).

NC, normal diet fed group; HFD, high-fat fed group; HFD-SA, high-fat plus 0.05% syringic acid fed group.



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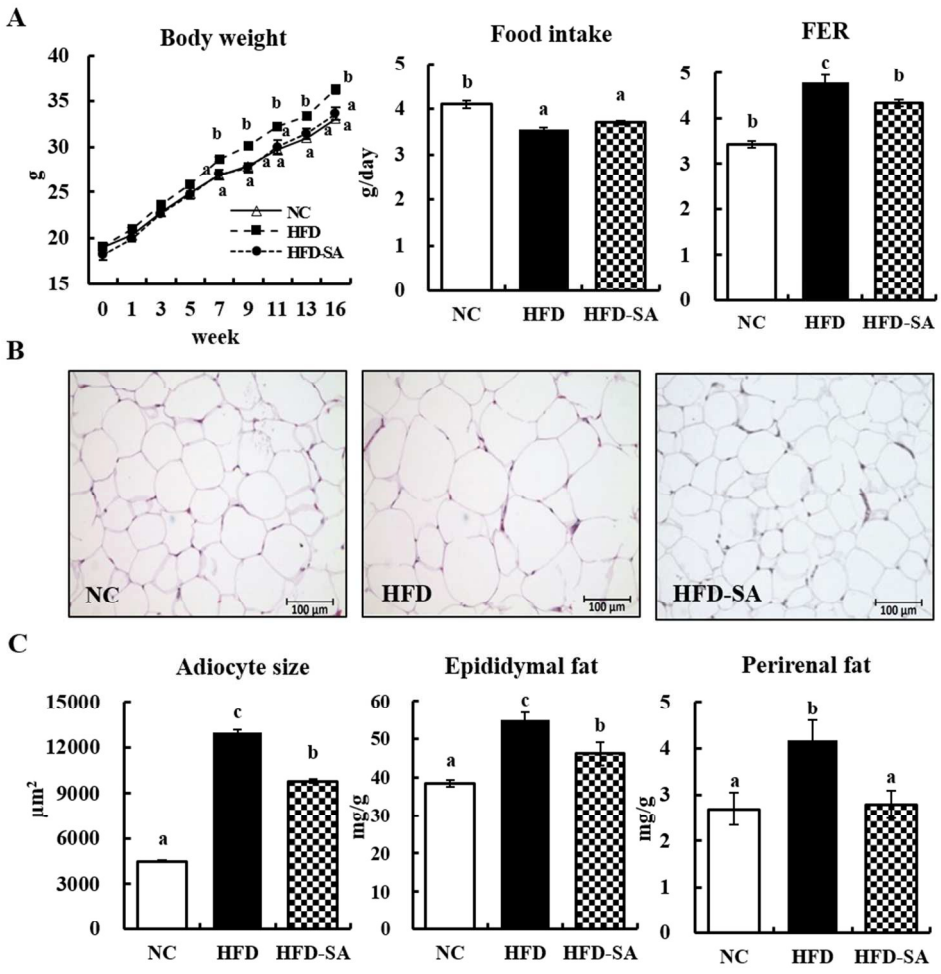

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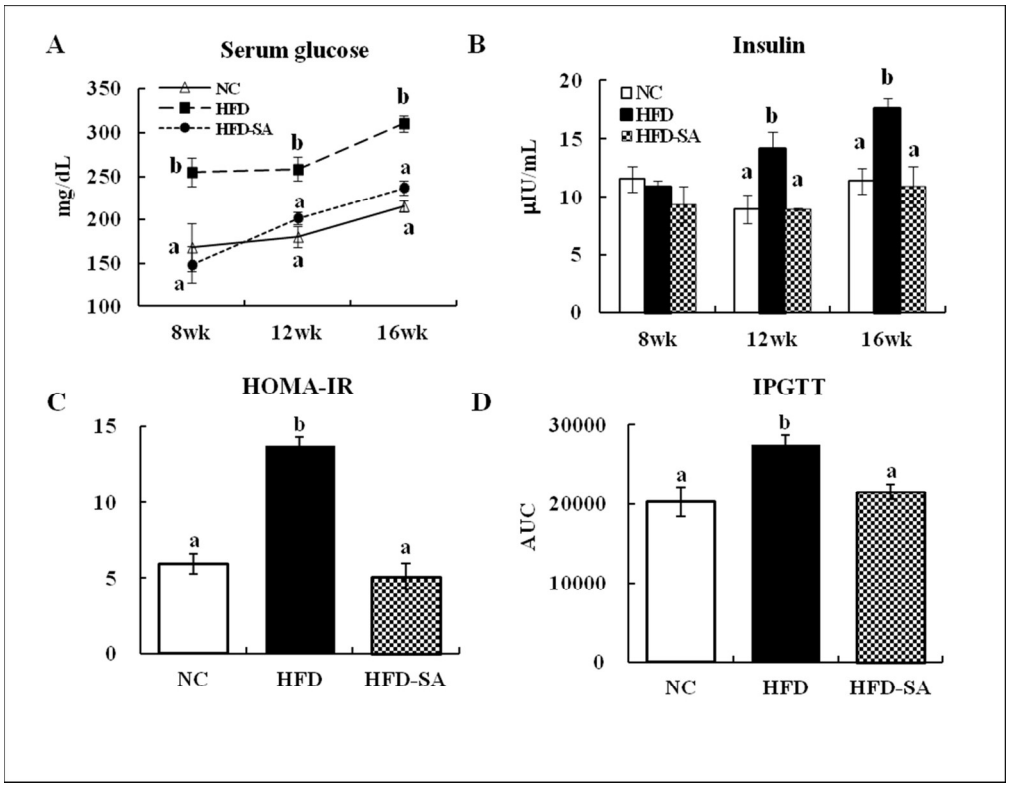

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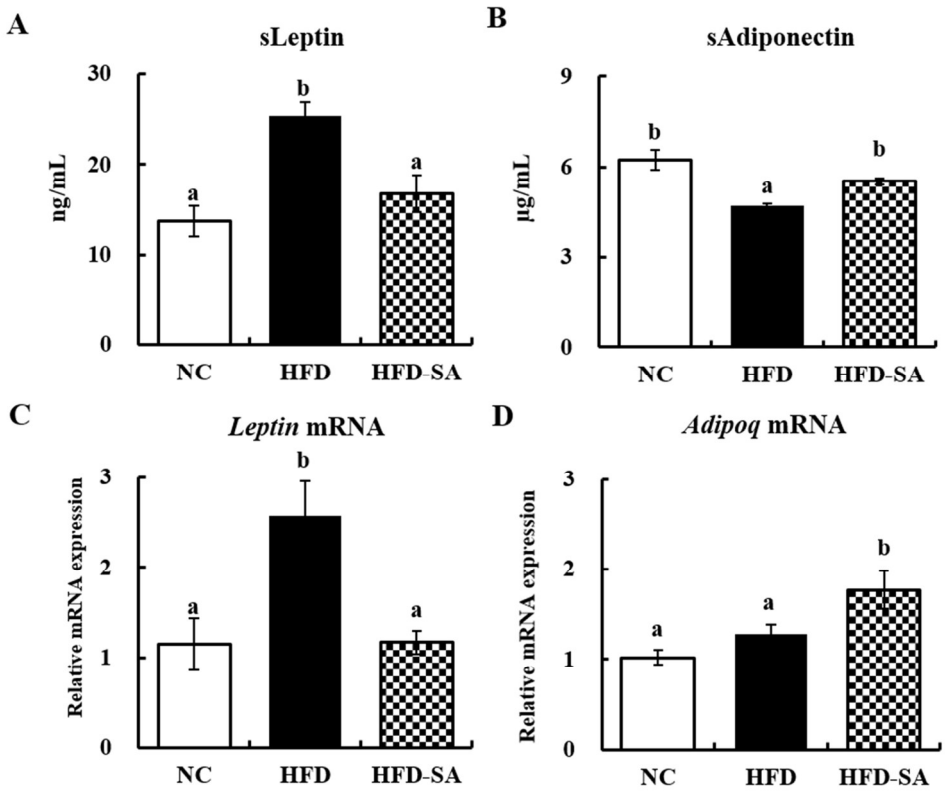

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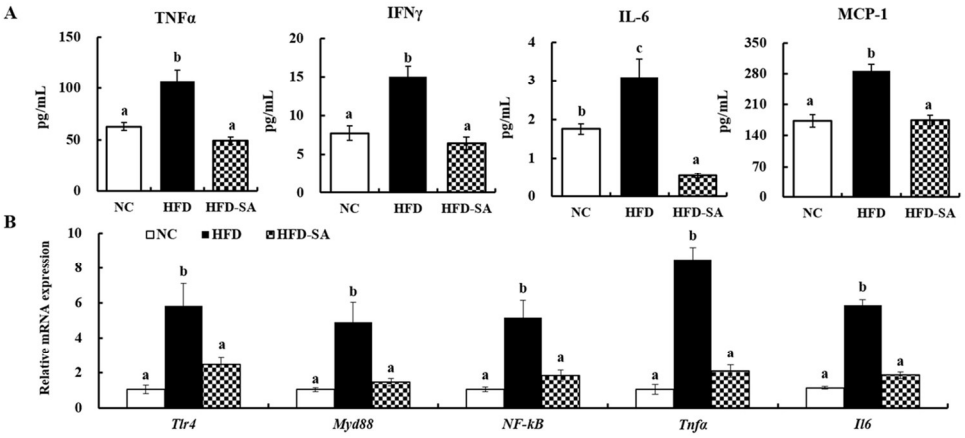

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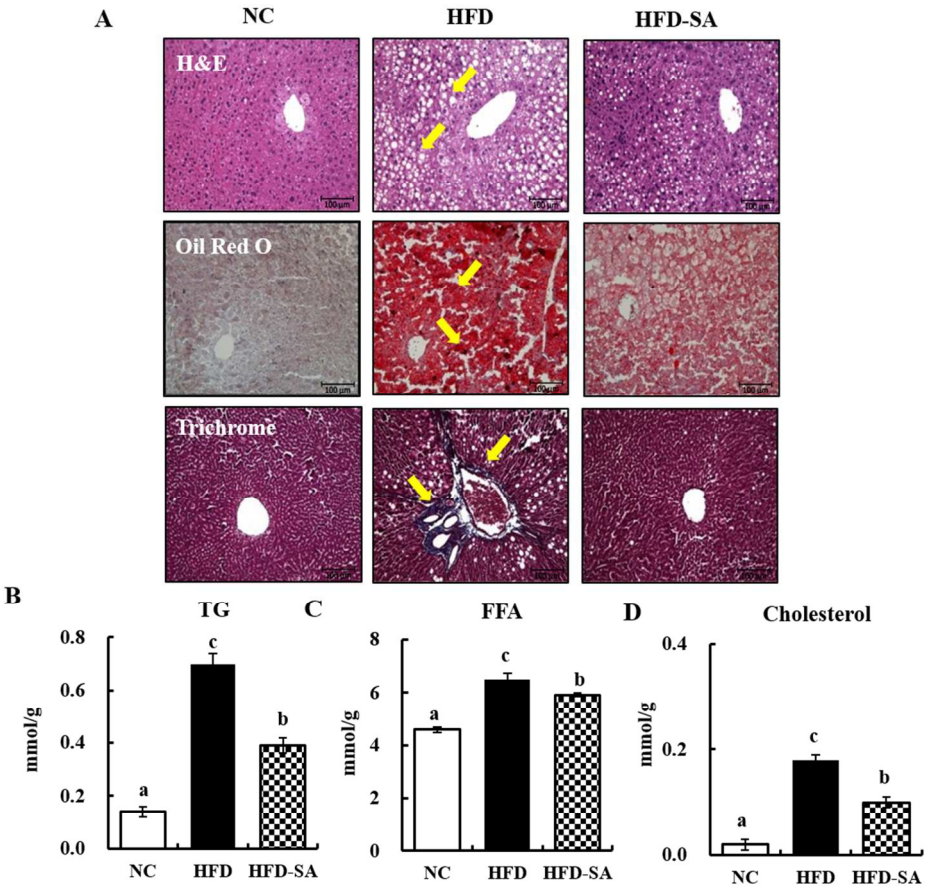

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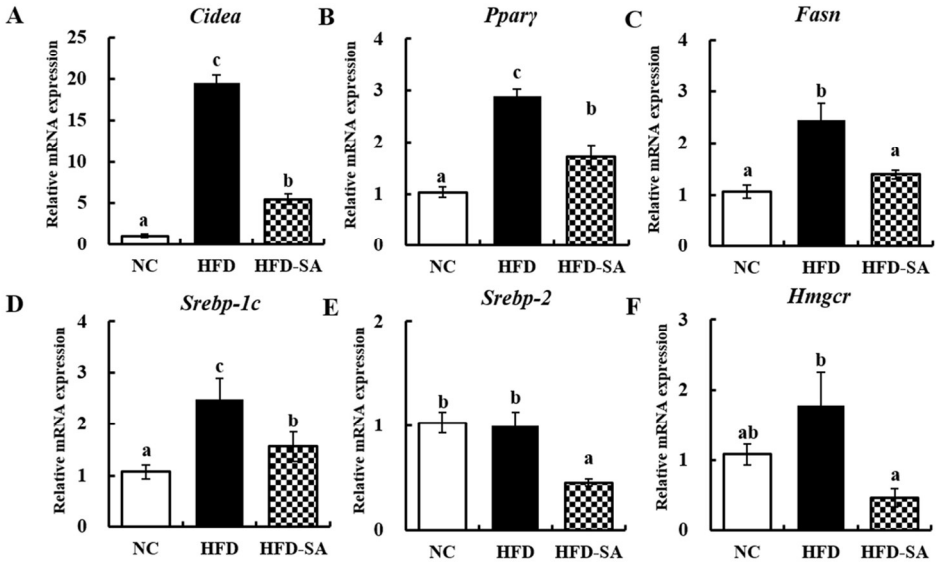

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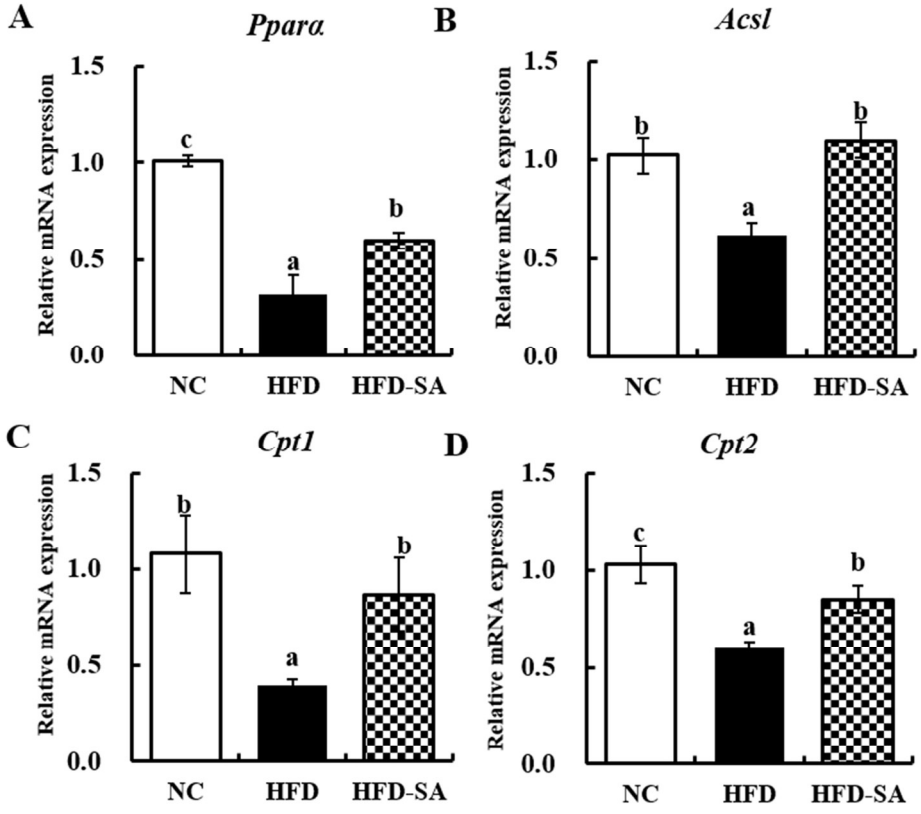

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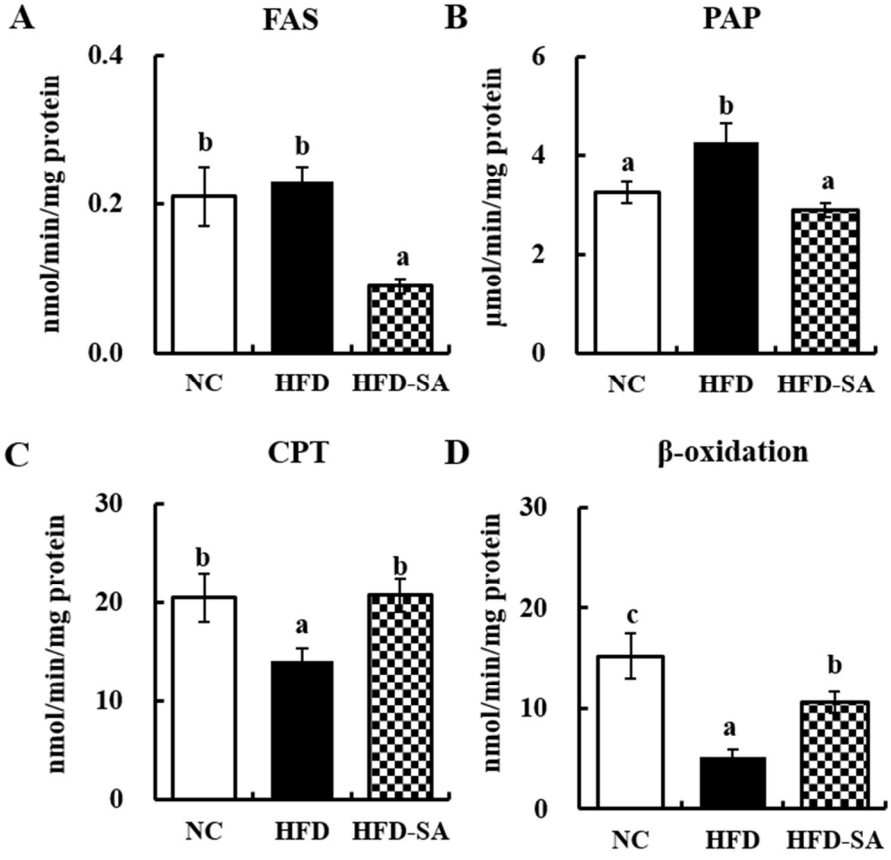

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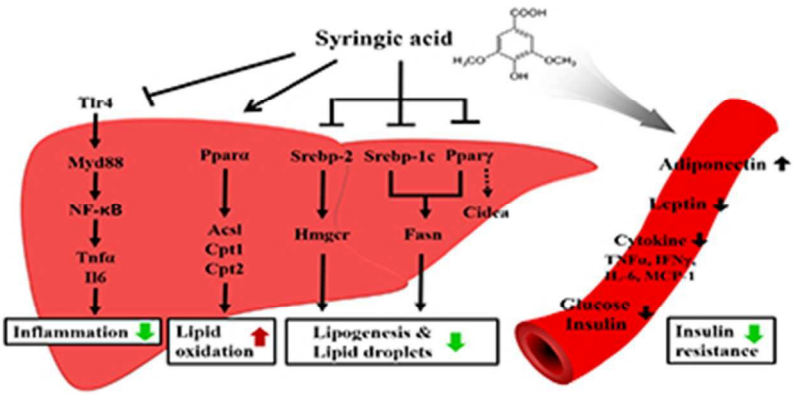

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