Lard-Blended Vegetable Oil Diet Alleviates Metabolic Syndrome in Mice

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Research

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Abstract

**Background:** Our previous work has suggested that the balance fatty acid diet, blending lard with soybean oil, could reduce adipose tissue accumulation by decreasing adipogenesis and lipogenesis while increasing the hydrolysis of triglycerides. The aim of present study is to investigate whether the fat reducing function of balanced fatty acid diet extended to sunflower oil, and explore its effects on liver lipids metabolism and insulin resistance.

**Methods:** 50 mice were divided into 5 groups, fed with lard, sunflower oil, soybean oil, mixture of lard and sunflower oil, and mixture of lard and soybean oil for 12 weeks separately.

**Results:** Results showed that a mixture of lard and vegetable oil (sunflower oil and soybean oil), particularly a mixture of lard and soybean oil decreased body weight, body fat rate, liver triglyceride level compared to lard, sunflower oil, and soybean oil, a mixed oil also decreased serum triglyceride and free fatty acid levels compared to lard diet. Further analysis indicated that activation of the AMPK pathway contributes to these observed phenotypes, and co-upregulated of glucagon and GLP-1 in mice fed with mixture of lard and soybean oil may contribute to improved lipids metabolism.

**Conclusion:** Moderate lard intake—blended lard with vegetable oil especially soybean oil—has the potential to alleviate metabolic syndrome via activating AMPK pathway and co-upregulated of glucagon and GLP-1.

1. Introduction

Since 1950s, SFA was regard negative for lipid metabolism, consumption of SFA be correlated with cardiovascular disease \(^1\). Thus, energy derived from fat limited range from 20–30% with saturated fatty acid (SFA) limited to less than 10% in American and Chinese dietary guideline from the first edition \(^2\).

In recent years, the consumption of saturated fatty acid (SFA) and their replacement with unsaturated fatty acids (UFA) have triggered a growing number of disputes \(^3\)\(^4\). Most recent meta-analyses of randomized trials and observational studies found no beneficial effects of reducing SFA intake on cardiovascular disease, and instead found protective effects against stroke \(^5\). These contradictions result may contribute to neglect of possible maximal extent, over the maximal extent, has adverse effects on individual, but under the premise of not exceeding the maximum, decreasing consumption of SFA, even replace SFA with UFA may be not good for health. For majority of the American population, consumption of SFA far exceed recommendation \(^6\)\(^–\)\(^8\), it is necessary to reduce the intake of SFA. For most of Chinese, SFA energy is less than 10% \(^9\), it is worth exploring whether it is more healthy to replace SFA with UFA.

Lard primarily comprises of SFA and monounsaturated fatty acid (MUFA). Soybean oil and sunflower oil are rich in polyunsaturated fatty acid (PUFA); soybean oil has a higher n-3 PUFA content than sunflower oil \(^10\). Soybean oil and sunflower oil is widely consumed all over the world. Previous studies have mostly focused on fatty acids or a single oil such as animal-derived fat and vegetable oils, or a mixture of
various vegetable oils $^{11-13}$. According to dietary guideline, SFA should limited to 10%, and acceptable ranges of n-3 PUFA and n-6 PUFA are 0.5–2.0% and 2.5–10%, respectively $^{14}$. In addition, previous studies used diets containing fat energy of up to 40%, even reaching up to 60%, which all simulated the fat consumption in the Mediterranean and America that range from 27–48% $^{15-17}$. However, in the Eastern diet, particularly the Chinese diet, fat energy is usually lower than 40%. Some studies even reported a fat energy content of 22% in 1992, and 32.9% in 2012 $^{18}$ both in China, while a 27% fat energy in west India $^{19}$. In our previous study, we mixed lard with vegetable oil to make SFA: MUFA: PUFA close to 1:1:1, we have found that lard blended with soybean oil has anti-obesity effects under 25% fat energy by decreasing adipogenesis and lipogenesis while increasing the hydrolysis of triglycerides in adipose tissues $^{20}$, but the effects disappeared under 35% fat energy $^{21}$. In the present study, we aimed to investigate whether the anti-obesity effect of balanced fatty acid diet extended to sunflower oil under 30% fat energy, and explore its effects on liver lipids metabolism and serum hormones.

2. Materials And Methods

2.1. Animals, diet and experimental design

50 six-weeks-old male C57BL/6J mice were purchased from the Hunan Silaike Laboratory Animal Co., Ltd. (Changsha, China). The mice were housed in collective polypropylene cages inside an isolated room with controlled temperature (22±2°C) and humidity (65% ± 5%), and a 12-h light/dark cycle. They received an ad libitum supply of water and feeds/diet during the entire experimental period. After one week of acclimatization, they were randomly divided into five groups, 2 mice per cage, and fed with lard, sunflower oil (SFO), mixture of lard and sunflower oil (L-SFO), soybean oil (SBO), and mixture of lard and soybean oil (L-SBO) each group for 12 weeks. The compositions of the diets are listed in Supplementary Table S1. After the experiment, all mice were weighed, then anesthetized with isoflurane, collected blood, and euthanatized by cervical dislocation. All the experimental protocols were approved by the Ethics Committee of Hunan Agricultural University, China (No. 43321543).

2.2. Sample collection and preparation

Blood samples were collected overnight at 4°C through the retro-orbital plexus of the mice. The sera were separated by centrifugation at 3500g for 10 min at 4°C and were then immediately stored at −80°C until analyses. The liver, epididymal and perirenal adipose tissues were collected and weighed. The liver and epididymal adipose tissues were cut into five parts and washed with a physiologic saline solution. The left lobe of the liver was fixed in optimal cutting temperature compound, one part of was epididymal adipose tissues fixed in 10% neutral buffered formalin, whereas the remaining parts were stored at -80°C immediately until analyses.

2.3. Biochemical determinations

Glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), UREA, serum triglyceride (TG), and total cholesterol (TC) were directly assayed using their respective kits (Mindray,
Shenzhen, China) on Mindray Biochemical Analyzer BS-190 (Mindray, Shenzhen, China). Serum free fatty acid (FFA) and liver TG were measured using kits purchased from Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, China).

### 2.4. Histological analysis

Epididymal adipose tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histological analyses. The sections (6-mm thick) of the left lateral lobe of the frozen liver were stained with Oil Red O (Sigma, USA) for 20 min, and counterstained with hematoxylin for 1 min. The stained areas were observed using Olympus photomicroscope (Olympus Inc., Tokyo, Japan) at a magnification of 400× for epididymal adipose tissue, while at 200× for the liver.

### 2.5. Western blot analysis

Proteins were extracted from liver by lysis and homogenization using radio-immunoprecipitation assay buffer (Beijing Solarbio Science & Technology Co. Ltd. Beijing China) at 4°C. The total protein concentrations were measured by the bicinchoninic acid (BCA) method using a protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Proteins were separated using 10% sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. The membranes were blocked using a buffer composed of 5% non-fat dry milk and tris-buffered saline tween 20 (TBST) for 1 h at 4°C and were incubated at 4°C overnight with appropriate antibodies, including hormone sensitive lipase (HSL; Cell Signaling Technology, Inc. USA), Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK; Proteintech, Inc. USA), phosphorylated AMPK Thr 172 (p-AMPK; Proteintech, Inc. USA), carnitine palmitoyl transferase 1 (CPT-1; Proteintech, Inc. USA), and β-actin (Proteintech, Inc. USA). The membranes were washed with TBST, incubated with anti-mouse or anti-rabbit horseradish peroxidase-conjugated secondary antibodies for 1 h at 25°C, washed with TBST buffer, and finally incubated with an enhanced chemiluminescence labeling kit (ECL; Nanjing KeyGen Biotech. Co. Ltd., China). The intensities of the bands were quantified by AlphaEase FC software (Alpha Innotech Co., USA).

### 2.6. Measurement of serum hormones and liver enzyme related lipids metabolism

Serum adiponectin was tested by using ELISA assay kits purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). Serum glucagon-like peptide 1 (GLP-1), plasminogen activator inhibitor-1 (PAI-1), resistin, and glucagon were measured according to the manufacturer’s instructions using Bio-Plex Pro Mouse Diabetes assay (Bio-Rad life medicine Co. Ltd., California, USA).

Carnitine Palmitoyltransterase-1 (CPT-1), Fatty Acid Synthase (FAS), and Hormone Sensitive Lipase (HSL) Elisa Kit purchased from Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, China).

### 2.7. Statistical analysis
The results were expressed as the mean ± standard error of the mean (SEM). The statistical significance of the mean differences among the groups was carried out via one-way analysis of variance (ANOVA) and least significant difference test. A p value < 0.05 was considered statistically significant. Graphical data presentations were created using GraphPad Prism version 7 (Graph Pad Software, San Diego, CA, USA).

3. Results

3.1. Fatty acid composition in diets

As shown in Table 1, the dominant fatty acids in lard were saturated fatty acids, composed mainly of palmitic acid (C16:0) and stearic acid (C18:0), and monounsaturated fatty acid, mainly oleic acid (C18:1). The dominant fatty acid in both SFO and SBO was linoleic acid (C18:2 and C18:1, respectively). However, SBO has higher linoleic acid (C18:3) content compared with SFO and lard. L-SFO and L-SBO have balanced fatty acid compositions of SFA: MUFA: PUFA approximately to 1:1:1.
| Fatty acids | % total fatty acids |
|------------|---------------------|
| C4:0       |                     |
| C6:0       |                     |
| C8:0       |                     |
| C10:0      | 0.08               |
| C11:0      | 0.07               |
| C12:0      | 0.07               |
| C13:0      | 1.49               |
| C14:0      | 0.07               |
| C14:1      | 0.06               |
| C15:0      | 0.06               |
| C15:1      | 0.83               |
| C16:0      | 28.80              |
| C16:1      | 1.52               |
| C17:0      | 0.83               |
| C17:1      | 20.50              |
| C18:0      | 0.11               |
| C18:1n9t  | 33.60              |
| C18:1n9c  | 10.90              |
| C18:2n6t  | 0.27               |
| C18:2n6c  | 0.68               |
| C20:0      | 0.27               |
| C18:3n6   | 0.68               |
| C20:1      | 0.49               |
| C18:3n3   | 0.49               |
| C21:0      | 0.49               |
|               | Lard | SFO | L-SFO | SBO  | L-SBO |
|---------------|------|-----|-------|------|-------|
| C20:2         | 0.45 |     | 0.25  |      | 0.21  |
| C22:0         | 0.67 |     | 0.30  | 0.43 | 0.23  |
| C20:3n6       |      |     |       |      |       |
| C22:1n9       |      |     |       | 0.10 | 0.05  |
| C20:3n3       |      |     |       |      |       |
| C20:4n6       | 0.22 |     | 0.12  |      | 0.10  |
| C23:0         |      |     |       |      |       |
| C22:2         |      |     |       |      |       |
| C24:0         |      |     |       | 0.07 | 0.09  | 0.05  |
| C20:5n3       |      |     |       |      |       |
| C24:1         |      |     |       |      |       |
| C22:6n3       |      |     |       |      |       |
| SFA           | 52.10| 10.90| 33.57 | 15.90| 32.60 |
| MUFA          | 35.80| 27.47| 32.05 | 24.58| 29.74 |
| PUFA          | 11.61| 61.57| 34.09 | 59.45| 37.44 |
| n6/n3         | 22.69| 878.57| 40.41 | 8.51 | 9.40  |
Table S1
Composition of the diets (g/kg)

| Ingredient(g)     | Lard | SFO | L-SFO | SBO | L-SBO |
|-------------------|------|-----|-------|-----|-------|
| Lard % (g/g*100%) | 310  | 310 | 310   | 310 | 310   |
| Corn starch       | 310  | 310 | 310   | 310 | 310   |
| Wheat bran        | 280  | 280 | 280   | 280 | 280   |
| Soybean meal      | 180  | 180 | 180   | 180 | 180   |
| Fish meal         | 100  | 100 | 100   | 100 | 100   |
| Brewer yeast      | 20   | 20  | 20    | 20  | 20    |
| Premix            | 40   | 40  | 40    | 40  | 40    |
| Sunflower oil     | 0    | 70  | 31.5  | 0   | 0     |
| Soybean oil       | 0    | 0   | 0     | 70  | 37.8  |
| Lard              | 70   | 0   | 38.5  | 0   | 32.2  |
| Total energy (kcal/kg) | 3543 | 3543 | 3543 | 3543 | 3543 |
| Energy from fat (%) | 30   | 30  | 30    | 30  | 30    |

The different diets were prepared in accordance to the guidelines of Laboratory Animals Nutrients for Formula Feeds [GB 14924.3-2010] of China.

The fatty acid profiles were identified using Agilent Gas Chromatograph 7890A in accordance to the GB 5009.168-2016, fatty acid test in food. Blank space means the fatty acid was undetectable.

3.2. Mixture of lard and vegetable oil reduced body weight

No significant difference in the initial body weight was observed among the five groups (Fig. 1. A), at the end of the experiment, body weight of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups (p<0.05), body weight of the L-SBO group was lower than that of Lard (p<0.05) and SBO groups (p<0.05), and there was little difference of body weight between L-SFO and L-SBO (Fig. 1. B). During the 12 weeks of the experiment, the average food intake and energy intake among groups were no significant difference (Fig. 1. C, D), this result indicated that the change of body weight was not contribute to food intake.

3.3. Mixture of lard and vegetable oil reduced body fat accumulation

White adipose tissue weight including epididymal fat weight and perirenal fat weight were measured. The result showed that epididymal fat weight of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups, epididymal fat weight of the L-SBO group was lower than that of Lard (p<0.05) and SBO
groups (p<0.05) (Fig. 2. A). Similarly, perirenal fat weight of the L-SFO group was lower than that of Lard and SFO groups, perirenal fat weight of the L-SBO group was lower than that of Lard (p<0.05) and SBO groups (p<0.05) (Fig. 2. B). Consistent with epididymal fat weight, body fat rate of the L-SFO group was lower than that in Lard (p<0.05) and SFO groups, body fat rate of the L-SBO group was lower than that in Lard (p<0.05) and SBO groups (p<0.05) (Fig. 2. C). In addition, it was observed that cross-sectional areas of adipocytes in L-SFO group and L-SBO groups were smaller than Lard, SFO, and SBO groups from the HE staining sections (Fig. 2. D). Between the two mixed oil groups, body fat accumulation of the L-SBO group showed a decreasing trend compared to the L-SFO group (Fig. 2. A-D).

3.4. Mixture of lard and vegetable oil reduced serum lipids accumulation

Serum TC, TG and FFA levels of Lard group were highest among groups, especially serum TG and FFA of Lard group showed significantly higher than other four groups (Fig. 3. A-C). Serum TC level of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups, serum TC level of the L-SBO group was lower than that of Lard (p<0.05) and SBO groups (p<0.05) (Fig. 2. A). Serum TG level of L-SFO and L-SBO groups were lower than that of SFO and SBO groups, respectively, but there was no significant difference (Fig. 2. B). Serum FFA level of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups (p<0.05), serum FFA level of the L-SBO group was lower than that of the Lard (p<0.05), but little change compared to the SBO group (Fig. 2. C). Serum GLU among groups no significant difference, but that of Lard and L-SBO groups showed a decreasing trend compared to other three groups (Fig. 2. D).

3.5. Mixture of lard and vegetable oil improved liver and kidney function

Liver weight of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups (p<0.05), liver weight of the L-SBO group was lower than that of Lard (p<0.05) and SBO groups (p<0.05) (Fig. 4. A). Similarly, ALT activity of the L-SFO group was lower than that of Lard (p<0.05) and SFO groups, ALT activity of the L-SBO group was lower than that of Lard and SBO groups (p<0.05) (Fig. 4. B). AST activity and UA content of five groups were no significant difference (Fig. 4. C, D). UREA level of the SBO group was significantly higher than that of other four groups (Fig. 4. E). This result illustrated that mixed oil could improve liver and kidney function compared to single oil.

3.6. Mixture of lard and vegetable oil reduced liver lipids accumulation

It was observed that Oil red O intensity in L-SFO group and L-SBO groups were less than Lard, SFO, and SBO groups from the Oil red O staining sections (Fig. 5. A). Liver TG also be tested, result showed that liver TG level of the L-SFO group was lower than that in Lard and SFO (p<0.05) groups, body fat rate of the L-SBO group was lower than that in Lard (p<0.05) and SBO groups (p<0.05) (Fig. 2. C) (Fig. 5. B).
3.7. Mixture of lard and vegetable oil activated AMPK pathway in liver

To further understand the effects of the different types of dietary oils on lipid metabolism, the protein expressions of HSL, CPT-1α, AMPK, p-AMPK, and FAS in the liver were analyzed. HSL protein expressions of SFO and SBO groups were the lowest among five groups, HSL protein expression of L-SFO group was 2.47 folds higher than that of SFO group, HSL protein expression of L-SBO group was 2.8 folds higher than that of SBO group (Fig. 6. A), ELISA analysis also showed that HSL activity of L-SBO group significantly higher than SBO group (Fig. 6. D). CPT-1α protein expression of L-SFO group was 1.87 and 1.55 folds higher than that of SFO and Lard groups, respectively, CPT-1α protein expression of L-SBO group was 1.96 and 2 folds higher than that of SBO and Lard groups, respectively (Fig. 6. B), ELISA analysis also showed that CPT-1α activity of L-SBO group significantly higher than SBO and Lard groups (Fig. 6. E). Ratio of p-AMPK/AMPK of L-SFO group was 2.02 and 2.26 folds higher than that of SFO and Lard groups, respectively, Ratio of p-AMPK/AMPK of L-SBO group was 1.91 and 1.66 folds higher than that of SBO and Lard groups, respectively (Fig. 6. C). There were no significantly changes of FAS activity among Lard, SBO, and L-SBO groups (Figure 6. F). This result showed that mixture of lard and vegetable oil (sunflower oil or soybean oil) could activate AMPK, increase HSL and CPT-1α protein expressions.

3.7 Mixture of lard and soybean oil co-upregulated GLP-1 and glucagon

There were no significant changes of resistin and adiponectin concentrations among five groups (Fig. 7.A, B). PAI-1 concentration of SFO group was higher than Lard (p<0.05), L-SFO, SBO (p<0.05), and L-SBO groups (p<0.05) (Fig. 7. C). GLP-1 and glucagon concentrations of L-SBO group were significantly higher than other four groups (Fig. 7. D, E), GLP-1 and glucagon concentrations of SFO and SBO groups were lowest among five groups (Fig. 7. D, E). Correlation heatmap analysis revealed that liver TG, serum TC, body fat rate, and body weight were significantly negative correlated with GLP-1 and glucagon, respectively, body weight and GLU were positive correlated with resistin (Fig. 7. F)

4. Discussion

Randomized trial and rodent experiments have shown that SFA rich diet induce body fat, serum lipids, and liver lipids accumulation. However, discordant results also be reported. Our present study also found that SFA rich diet (lard) induce body fat (p>0.05) and serum lipids (p<0.05) accumulation than PUFA rich diet (sunflower oil or soybean oil), but liver lipids accumulation higher in PUFA rich diet (sunflower oil (p>0.05) or soybean oil) than SFA rich diet (lard), especially in soybean oil diet. Deol et al. showed that soybean oil diet induces greater weight gain, adiposity, and fatty liver than coconut oil diet that rich in SFA. Liver damage caused by dietary cholesterol in mice was strongly enhanced by a high fat diet containing soybean oil, but not by a lard-based high fat diet, soybean oil-based diet enhanced cholesterol-induced mitochondrial damage and amplified the ensuing oxidative stress. Di Rienzi proved
that toxicity of soybean oil fatty acid inhibits growth of *Lactobacilli*, beneficial members of the small intestinal microbiota\(^{28}\). Jurgoński also found that cecal butyrate level higher in lard diet rats than soybean oil diet rats, benefit gut metabolism\(^{22}\). Studies showed that the abundance of short chain fatty acid bacteria such as *Bifidobacterium, Enterococcus* and *Allobaculum* increased with high lard diet\(^{29-31}\).

Thus, it is speculated that different fat/oil might exert different effects in different tissues, mix lard with vegetable oil (a balance fatty acid diet) benefit for lipids metabolism from multiple metabolic pathways. In the next step, we will explore effect of lard and soybean oil diet on gut microbiota and metabolite change in gut and liver.

Our previous study (under 25% fat energy) found that a mixture of lard and soybean oil could reduce body fat accumulation compared to lard or soybean oil\(^{20}\), it was also proved in present study (under 30% fat energy), and can be extended to sunflower oil. However, under 35% fat energy, the function cannot be observed\(^{21}\). Catta-Preta \textit{et al.} found that a lard diet can induce adipose mass generation compared with the sunflower oil diet and other vegetable oils\(^{25}\). In fact, most studies evaluated the nutritional benefits of dietary oils based on a high-fat diet model\(^{32,33}\). In these models, the energy obtained from fat is typically 40–60%. Part of studies with lower fat energy present inconsistent results with Catta-Preta, Bourgeois \textit{F et al.} showed that mice fed with various kinds of dietary fat/oil (43% energy from lipids), specifically lard, beef tallow, sunflower oil, and soybean oil, can induce obesity\(^{34}\). A high-fat diet with 42% energy for 12 weeks also showed that rats fed with lard and olive oil were the most obese, having no significant differences between these diets\(^{35}\). The contradictory results may be attributed to the different level of fat energy, but the mechanism worth further exploration.

In the present study, mixture of lard and vegetable oil (sunflower oil or soybean oil) activated AMPK compared to single oil diet, and mixture of lard and soybean oil increased serum GLP-1 and glucagon levels. AMPK plays a key role in regulating energy metabolism\(^{36}\). Liver AMPK can decrease the rate of hepatic lipogenesis. Its phosphorylation leads to the inhibition of fatty acid biosynthesis via phosphorylation of acetyl Co-A carboxylase (ACC), thus affecting malonyl-CoA content that synthesis catalyzed by ACC\(^{37}\). The activity of CPT-1\(\alpha\) can be regulated by malonyl-CoA\(^{38}\), besides, activation of AMPK also can induce HSL and inhibit FAS expression\(^{39}\). HSL is a key enzyme that catalyzes the rate-limiting step of adipose tissue lipolysis from TG to FFA\(^{40}\). A previous study showed that administration of a specific HSL inhibitor can reduce the serum FFA levels in mice, rats, and dogs, demonstrating its role \textit{in vivo}\(^{41}\). Thus, the expression of HSL is closely associated with FFA content. HSL protein expressions were higher in lard, L-SFO, and L-SBO groups, benefit serum TG lipolysis to FFA, however, serum FFA cannot be oxidized due to inactivation of the AMPK and lower expression of CPT-1\(\alpha\), it also explains why serum FFA levels in the mice fed with lard were significantly higher than those fed with other oils.

Interestingly, the mixture of lard and vegetable oil not only induced the expression of HSL protein but also activated AMPK pathway, thereby increasing the expression of CPT-1\(\alpha\) proteins. This, subsequently, reduced fatty acid synthesis, induced higher levels of CPT-1 proteins, and, ultimately, strengthened fatty acid oxidation, indicating that the energy expenditure was higher. Palmitoleic acid has been reported to
improve metabolic functions by increasing the AMPK phosphorylation in the fatty liver induced by high-fat-diet.\textsuperscript{42}

Fasted GLU levels among groups were no significant different, even though GLU of mice fed with lard and mixture of lard and soybean oil were lower than other three groups. Wang \textit{et al} showed that mice fed with high lard diet and high SBO diet (15% weight of oil) for 18 weeks showed no significant difference in terms of glucose level, although those fed with lard showed a decreasing glucose level.\textsuperscript{43} Other studies yielded similar results.\textsuperscript{44} PAI-1 is an important regulator of the fibrinolytic process. Correlation heatmap showed that PAI-1 had no significant correlated with phenotypes of ectopic fat accumulation and GLU, actually, there still have debate that whether PAI-1 could be a predictor of atherosclerotic.\textsuperscript{45} It was put forwarded that the contradiction may be due to neglecting the distinction between physiological and pathological conditions, PAI-1 level still within physiological conditions.\textsuperscript{46} The main source of glucagon is the pancreatic α-cell, while intestinal L-cells and neurons in the nucleus of the solitary tract are the principal producers of GLP-1, and it was reported therapeutic approaches to treating obesity based on glucagon/GLP-1 synergistic interaction.\textsuperscript{47} Glucagon's main action is related to glucose homeostasis, where glucagon stimulation of hepatic glucose production by increasing glycogenolysis and gluconeogenesis, and simultaneously inhibiting glycogen synthesis, serves to restore glucose levels in hypoglycemic states. Metabolic actions of glucagon are exerted through a unique receptor which is mainly expressed in liver. GLP-1 secreted following food intake, and its action including inhibits glucagon secretion, stimulates insulin release, and decreases hepatic gluconeogenesis.\textsuperscript{48} In recent years, it was point out that glucagon and GLP-1 play a role in brown fat thermogenesis without decrement of food intake, glucagon and GLP-1 induced oxygen consumption and body temperature.\textsuperscript{49} Dual agonists glucagon/GLP-1 induced a massive decrease in body weight in diet-induced obese mice.\textsuperscript{50–52} The co-upregulated of glucagon and GLP-1 in mice fed with mixture of lard and soybean oil may contribute to improved lipids metabolism.

5. Conclusions

In conclusion, the present study fully proved that a mixture of lard and sunflower oil or soybean oil (a balanced fatty acid diet) benefit to improve metabolic disorder in mice. Mixed oil activating AMPK pathway, promoting TG hydrolysis and FFA oxidation, and a mixture of lard and soybean oil also can co-upregulating GLP-1 and glucagon that benefit for lipids metabolism. Extending from those results, the present findings provide a caution and much worth consideration against the current of replacing SFA with UFA.

Declarations

6. Ethics statement
This study was performed in strict accordance with the European Community (Directive 2010/63/EU) for the care and use of laboratory animals. All the experimental protocols were approved by the Ethics Committee of Hunan Agricultural University, China (No. 43321543).

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. Circulation. 2016;133:187–225.
2. Harcombe Z. US dietary guidelines: is saturated fat a nutrient of concern? Br J Sports Med. 2019;53:1393–6.
3. Kris-Etherton PM, Krauss RM. Public health guidelines should recommend reducing saturated fat consumption as much as possible: YES. Am J Clin Nutr. 2020;112:13–8.
4. Krauss RM, Kris-Etherton PM. Public health guidelines should recommend reducing saturated fat consumption as much as possible: NO. Am J Clin Nutr. 2020;112:19–24.
5. Astrup A, Magkos F, Bier DM, Brenna JT, de Oliveira Otto MC, Hill JO, King JC, Mente A, Ordovas JM, Volek JS, Yusuf S, Krauss RM. Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;76:844–57.
6. Schönbach JK, Nusselder W, Lhachimi SK. Substituting polyunsaturated fat for saturated fat: A health impact assessment of a fat tax in seven European countries. PloS one. 2019;14:e0218464.
7. Elmadfa I, Kornsteiner M. Dietary fat intake–a global perspective. Ann Nutr Metab. 2009;54(Suppl 1):8–14.
8. Lichtenstein AH, Kennedy E, Barrier P, Danford D, Ernst ND, Grundy SM, Leveille GA, Van Horn L, Williams CL, Booth SL. Dietary fat consumption and health. Nutrition reviews. 1998;56:3–19. discussion S19-28.
9. Yan S, Liu X, Li X, Li X, Wang J, Wen L. Critique of the Chinese dietary guidelines on the consumption of cooking oils. Food science nutrition. 2021;9:583–5.
10. Badee G, Hidaka S. Growth performance, carcass characteristics, fatty acid composition and CLA concentrations of lambs fed diets supplemented with different oil sources. Animal science journal = Nihon chikusan Gakkaiho. 2014;85:118–26.
11. Pavlisova J, Bardova K, Stankova B, Tvrzicka E, Kopecky J, Rossmeisl M. Corn oil versus lard: Metabolic effects of omega-3 fatty acids in mice fed obesogenic diets with different fatty acid composition. Biochimie. 2016;124:150–62.

12. Bargut TC, Souza-Mello V, Mandarim-de-Lacerda CA, Aguila MB. Fish oil diet modulates epididymal and inguinal adipocyte metabolism in mice. Food function. 2016;7:1468–76.

13. Louwe MC, van der Hoom JW, van den Berg SA, Jukema JW, Romijn JA, van Dijk KW, Rensen PC, Smit JW, Steendijk P. Gender-dependent effects of high-fat lard diet on cardiac function in C57Bl/6J mice, Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme, 2012, 37, 214-224.

14. Czernichow S, Thomas D, Bruckert E. n-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations. Br J Nutr. 2010;104:788–96.

15. Beulen Y, Martinez-Gonzalez MA, van de Rest O, Salas-Salvado J, Sorli JV, Gomez-Gracia E, Fiol M, Estruch R, Santos-Lozano JM, Schroder H, Alonso-Gomez A, Serra-Majem L, Pinto X, Ros E, Becerra-Tomas N, Gonzalez JI, Fito M, Martinez JA, Gea A, Quality of Dietary Fat Intake and Body Weight and Obesity in a Mediterranean Population: Secondary Analyses within the PREDIMED Trial, Nutrients, 2018, 10.

16. Garcia-Meseguer MJ, Burriel FC, Garcia CV. and R. Serrano-Urrea, Adherence to Mediterranean diet in a Spanish university population. Appetite. 2014;78:156–64.

17. Julibert A, Bibiloni MDM, Bouzas C, Martinez-Gonzalez MA, Salas-Salvado J, Corella D, Zomeno MD, Romaguera D, Vioque J, Alonso-Gomez AM, Warnberg J, Martinez JA, Serra-Majem L, Estruch R, Tinahones FJ, Lapetra J, Pinto X, Lopez-Miranda J, Garcia-Molina L, Gaforio JJ, Matia-Martin P, Daimiel L, Martin-Sanchez V, Vidal J, Vazquez C, Ros E, Toledo E, Becerra-Tomas N, Portoles O, Perez-Vega KA, Fiol M, Torres-Collado L, Tojal-Sierra L, Carabano-Moral R, Abete I, Sanchez-Villegas A, Casas R, Bernal-Lopez MR, Santos-Lozano JM, Galera A, Ugarriza L, Ruiz-Canela M, Babio N, Coltell O, Schroder H, Konieczna J, Orozco-Beltran D, Sorto-Sanchez C, Eguaras S, Barrubes L, M. Fito and J. A. Tur, Total and Subtypes of Dietary Fat Intake and Its Association with Components of the Metabolic Syndrome in a Mediterranean Population at High Cardiovascular Risk, Nutrients, 2019, 11.

18. Ju L, Yu D, Fang H, Guo Q, Xu X, Li S, Zhao L. [Trends and food sources composition of energy, protein and fat in Chinese residents, 1992-2012], Wei sheng yan jiu =. Journal of hygiene research. 2018;47:689–704.

19. Aleksandrowicz L, Green R, Joy EJM, Harris F, Hillier J, Vetter SH, Smith P, Kulkarni B, Dangour AD, Haines A. Environmental impacts of dietary shifts in India: A modelling study using nationally-representative data. Environment international. 2019;126:207–15.

20. Wang J, Yan S, Xiao H, Zhou H, Liu S, Zeng Y, Liu B, Li R, Yuan Z, Wu J, Yi J, Razack YBS, Wen L. Anti-obesity effect of a traditional Chinese dietary habit-blending lard with vegetable oil while cooking. Scientific reports. 2017;7:14689.

21. Yan S, Zhou H, Liu S, Wang J, Zeng Y, Matias FB, Wen L. Differential effects of Chinese high-fat dietary habits on lipid metabolism: mechanisms and health implications. Lipids Health Dis.
22. Jurgoński A, Juśkiewicz J, Zduńczyk Z. A high-fat diet differentially affects the gut metabolism and blood lipids of rats depending on the type of dietary fat and carbohydrate. Nutrients. 2014;6:616–26.

23. Rosqvist F, Kullberg J, Ståhlman M, Cedernaes J, Heurling K, Johansson HE, Iggman D, Wilking H, Larsson A, Eriksson O, Johansson L, Straniero S, Rudling M, Antoni G, Lubberink M, Orho-Melander M, Borén J, Ahlström H. and U. Risérus, Overeating Saturated Fat Promotes Fatty Liver and Ceramides Compared With Polyunsaturated Fat: A Randomized Trial. J Clin Endocrinol Metab. 2019;104:6207–19.

24. Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson HE, Larsson A, Johansson L, Ahlström H, Arner P, Dahlman I, Risérus U. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. Diabetes. 2014;63:2356–68.

25. Catta-Preta M, Martins MA, Cunha Brunini TM, Mendes-Ribeiro AC, Mandarim-de-Lacerda CA, Aguila MB. Modulation of cytokines, resistin, and distribution of adipose tissue in C57BL/6 mice by different high-fat diets. Nutrition. 2012;28:212–9.

26. Deol P, Evans JR, Dahbhi J, Chellappa K, Han DS, Spindler S, Sladek FM. Soybean Oil Is More Obesogenic and Diabetogenic than Coconut Oil and Fructose in Mouse: Potential Role for the Liver. PloS one. 2015;10:e0132672.

27. Henkel J, Alfine E, Sain J, Jöhrens K, Weber D, Castro JP, König J, Stuhlmann C, Vahrenbrink M, Jonas W, Kleinridders A, Püschel GP. Soybean Oil-Derived Poly-Unsaturated Fatty Acids Enhance Liver Damage in NAFLD Induced by Dietary Cholesterol, Nutrients, 2018, 10.

28. Di Rienzi SC, Jacobson J, Kennedy EA, Bell ME, Shi Q, Waters JL, Lawrence P, Brenna JT, Britton RA, Walter J, Ley RE. Resilience of small intestinal beneficial bacteria to the toxicity of soybean oil fatty acids, eLife, 2018, 7.

29. Zhong H, Abdullah L, Deng M, Zhao J, Tang T, Liu H, Zhang, Feng F. Probiotic-fermented blueberry juice prevents obesity and hyperglycemia in high fat diet-fed mice in association with modulating the gut microbiota. Food function. 2020;11:9192–207.

30. Li S, Li J, Mao G, Wu T, Lin D, Hu Y, Ye X, Tian D, Chai W, Linhardt RJ, Chen S. Fucosylated chondroitin sulfate from Isostichopus badionotus alleviates metabolic syndromes and gut microbiota dysbiosis induced by high-fat and high-fructose diet. Int J Biol Macromol. 2019;124:377–88.

31. Sudun S, Liu C, Xiao C, Peng L, Liang X, He S, Zhao, Zhang G. Probiotic strains improve high-fat diet-induced hypercholesterolemia through modulating gut microbiota in ways different from atorvastatin. Food function. 2019;10:6098–109.

32. Sales RC, Medeiros PC, Spreafico F, de Velasco PC, Gonçalves FKA, Martin-Hernandez R, Mantilla-Escalante DC, Gil-Zamorano J, Peres WAF, de Souza SAL, Davalos A. and M. G. Tavares do Carmo, Olive Oil, Palm Oil, and Hybrid Palm Oil Distinctly Modulate Liver Transcriptome and Induce NAFLD in Mice Fed a High-Fat Diet, International journal of molecular sciences, 2018, 20.

33. Qu L, Liu Q, Zhang Q, Tuo X, Fan D, Deng J, Yang H. Kiwifruit seed oil prevents obesity by regulating inflammation, thermogenesis, and gut microbiota in high-fat diet-induced obese C57BL/6 mice. Food
34. Bourgeois F, Alexiu A, Lemonnier D. Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice. Br J Nutr. 1983;49:17–26.
35. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, Bollheimer LC. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol. 2006;36:485–501.
36. Wang Q, Liu S, Zhai A, Zhang B, Tian G. AMPK-Mediated Regulation of Lipid Metabolism by Phosphorylation. Biol Pharm Bull. 2018;41:985–93.
37. Hardie DG, Pan DA. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. Biochemical Society transactions. 2002;30:1064–70.
38. Viollet B, Foretz M, Guigas B, Hormann S, Dentin R, Bertrand L, Hue L, Andreelli F. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. J Physiol. 2006;574:41–53.
39. Nakamura MT, Yudell BE, Loor JJ. Regulation of energy metabolism by long-chain fatty acids. Progress in lipid research. 2014;53:124–44.
40. Lampidonis AD, Rogdakis E, Voutsinas GE, Stravopodis DJ. The resurgence of Hormone-Sensitive Lipase (HSL) in mammalian lipolysis. Gene. 2011;477:1–11.
41. Claus TH, Lowe DB, Liang Y, Salhanick AI, Lubeski CK, Yang L, Lemoine L, Zhu J, Clairmont KB. Specific inhibition of hormone-sensitive lipase improves lipid profile while reducing plasma glucose. J Pharmacol Exp Ther. 2005;315:1396–402.
42. de Souza CO, Teixeira AAS, Biondo LA, Lima Junior EA, Batatinha HAP. and J. C. Rosa Neto, Palmitoleic Acid Improves Metabolic Functions in Fatty Liver by PPARalpha-Dependent AMPK Activation. Journal of cellular physiology. 2017;232:2168–77.
43. Wang X, Cheng M, Zhao M, Ge A, Guo F, Zhang M, Yang Y, Liu L, Yang N. Differential effects of high-fat-diet rich in lard oil or soybean oil on osteopontin expression and inflammation of adipose tissue in diet-induced obese rats. Eur J Nutr. 2013;52:1181–9.
44. Pereira S, Breen DM, Naassan AE, Wang PY, Uchino H, Fantus IG, Carpenter AC, Gutierrez-Juarez R, Brindley DN, Lam TK, Giacca A. In vivo effects of polyunsaturated, monounsaturated, and saturated fatty acids on hepatic and peripheral insulin sensitivity. Metab Clin Exp. 2015;64:315–22.
45. Schäfer K, Konstantinides S. PAI-1 and vasculopathy: the debate continues. Journal of thrombosis haemostasis: JTH. 2004;2:13–5.
46. Altalhi R, Pechlivani N, Ajjan RA. PAI-1 in Diabetes: Pathophysiology and Role as a Therapeutic Target, International journal of molecular sciences, 2021, 22.
47. Sandoval DA, D’Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. Physiological reviews. 2015;95:513–48.
48. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153–65.
49. González-García I, Milbank E, Diéguez C, López M, Contreras C. Glucagon, GLP-1 and Thermogenesis, *International journal of molecular sciences*, 2019, 20.

50. Capozzi ME, DiMarchi RD, Tschöp MH, Finan B, Campbell JE. Targeting the Incretin/Glucagon System With Triagonists to Treat Diabetes. *Endocr Rev.* 2018;39:719–38.

51. Clemmensen C, Chabenne J, Finan B, Sullivan L, Fischer K, Küchler D, Sehrer L, Ograjsek T, Hofmann SM, Schriever SC, Pfluger PT, Pinkstaff J, Tschöp MH, Dimarchi R, Müller TD. GLP-1/glucagon coagonism restores leptin responsiveness in obese mice chronically maintained on an obesogenic diet. *Diabetes.* 2014;63:1422–7.

52. Kinoshita K, Ozaki N, Takagi Y, Murata Y, Oshida Y, Hayashi Y. Glucagon is essential for adaptive thermogenesis in brown adipose tissue. *Endocrinology.* 2014;155:3484–92.

**Figures**

![Figure 1](image)

**Figure 1**

Effects of different dietary fat/oil on body weight and food intake. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Initial body weight; (B) Final body weight; (C) Food intake; (D) Energy intake. Data were
expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.

Figure 2

Effects of different dietary fat/oil on body fat accumulation. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Epididymal fat weight; (B) Perirenal fat weight; (C) Body fat rate; (D) Sections of epididymal adipose tissue stained with hematoxylin and eosin. Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.
Figure 3

Effects of different dietary fat/oil on serum lipids and glucose. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Serum total cholesterol (TC); (B) Serum triglyceride (TG); (C) Serum free fatty acid (FFA); (D) Serum glucose (GLU). Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.
Figure 4

Effects of different dietary fat/oil on liver and kidney function. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Liver weight; (B) Alanine aminotransferase (ALT); (C) Aspartate amino transferase (AST); (D) Uric acid (UA); (E) Urea. Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.
Figure 5

Effects of different dietary fat/oil on liver lipids. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Liver TG; (B) Liver sections stained with Oil Red O. Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.

Figure 6

Effects of different dietary fat/oil on AMPK pathway related proteins. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) Liver hormone sensitive lipase (HSL) analyzed by Western blotting; (B) Liver carnitine palmitoyl transferase 1 (CPT-1α) analyzed by Western blotting; (C) Liver adenosine 5′-monophosphate (AMP)-activated protein kinase (p-AMPK)/AMPK analyzed by Western bloting; (D) Liver HSL analyzed by ELISA; (E) Liver CPT-1 analyzed by ELISA; (F) Liver FAS analyzed by ELISA. Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.
Figure 7

Effects of different dietary fat/oil on hormones. The treatment groups were lard, sunflower oil (SFO), lard blended with SFO (L-SFO), soybean oil (SBO), and lard blended with SBO (L-SBO). (A) resistin; (B) adiponectin; (C) plasminogen activator inhibitor-1 (PAI-1); (D) glucagon-like peptide 1 (GLP-1); (E) glucagon; (F) Pearson's correlation heatmap between phenotype and cytokines. Data were expressed as the mean ± standard error of the mean (SEM), different superscript letters (a,b,c) in a column are statistically significant (p<0.05) with each other.

Supplementary Files

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