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Effects of blended oils with different n-6/n-3 polyunsaturated fatty acid ratios on high-fat diet-induced metabolic disorders and hepatic steatosis in rats

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Abstract

This study investigated the effects of blended oils with different n-6/n-3 polyunsaturated fatty acid (PUFA) ratios on the metabolic disorders and hepatic steatosis in high-fat diet-fed rats. The 1:1 group had significantly lower serum low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol (HDL-C), and lipoprotein lipase (LPL) levels than those of the Lard group. The 5:1 group had significantly lower serum LDL-C, LPL, and adiponectin (ADP) than those in the Lard group. Serum LDL-C, angiotensin II (Ang II), endothelin-1, LPL and ADP levels in the 1:1 and 5:1 groups were significantly lower than those in the normal control group. The 5:1 group had significantly lower serum ADP, Ang II, LPL and resistin levels than those in the 20:1 group. The 20:1 group had significantly higher serum TC, LDL-C, HDL-C, nitric oxide (NO), 3-nitrotyrosine, free fatty acid and LPL levels than those in the 1:1 ratio group. Blended oils with a low n-6/n-3 PUFA ratio improved metabolic disorders and hepatic steatosis by regulating lipid metabolism, adipokines, endothelial cell function, and liver lipid metabolism. Conversely, a high n-6/n-3 PUFA ratio had adverse effects on metabolic profiles in high-fat diet-fed rats.

Keywords: n-6 fatty acids; n-3 fatty acids; saturated fatty acids; metabolic disorder; hepatic steatosis.

Practical Application: Blended oils with low n-6/n-3 PUFA ratios improve high-fat diet-induced metabolic disorders and hepatic steatosis in rats.

1 Introduction

Reducing the amount of saturated fatty acids (SFAs) by substituting polyunsaturated fatty acids (PUFAs) is an important strategy for dietary recommendations to reduce the risk of cardiovascular disease (Lenighan et al., 2019; Lovegrove, 2020). Linoleic acid (LA) and α-linolenic acid (ALA) are n-6 and n-3 PUFAs, respectively, which are present in high amounts in some vegetable oils (such as Linseed oil, perilla seed oil, etc.) (Chen et al., 2022; Russo, 2009). Both LA and ALA are essential fatty acids and their ratios play an important role in promoting health and preventing diseases (Mukhametov et al., 2022). Both n-3 and n-6 PUFAs have beneficial effects, but many metabolic disorders are adverse consequences of excess n-6 PUFAs and unbalanced intake of n-3 and n-6 PUFAs (Lands, 2012). N-3 PUFA and their derived metabolites have great potential for treating metabolic disorders, including direct effects on hepatocytes, adipocytes, and endothelial cells (Duan et al., 2021). ALA has anti-metabolic syndrome, anti-inflammatory, and antioxidant properties, and its therapeutic effects are dose-dependent (Yuan et al., 2022). ALA could also improve the structure of intestinal flora and promote the adhesion of intestinal probiotics to colonic cells (Liu et al., 2022). Partially substituting of ALA for LA could improve dyslipidemia, liver oxidative stress, and inflammation induced by a high-fructose diet in rats (Sakamuri et al., 2020). Thus, the PUFA/SFA and n-6/n-3 PUFA (LA/ALA) ratios are important indices to evaluate the nutritional value of food (Chen & Liu, 2020).

Optimal dietary fat intake might include a low intake of SFAs and n-6 fatty acids and a moderate intake of n-3 fatty acids (Lorgeril & Salen, 2012). The n-6/n-3 ratio was closer to 1:1 in early human diets, whereas the n-6/n-3 ratio is closer to 20:1 in western diets. The high n-6/n-3 ratio might be related to the pathogenesis of many chronic diseases, such as cardiovascular disease and inflammatory disease (Simopoulos, 2008). Our previous study showed that a low n-6/n-3 ratio could improve lipid metabolism, inflammation, and oxidative stress in rats (Yang et al., 2016). The pathogenesis of non-alcoholic fatty liver disease (NAFLD) is a complex process of metabolic abnormalities, involving hepatic fat accumulation, oxidative stress, chronic inflammation, lipid metabolism disorders, and the effects of adipokines such as adiponectin, leptin and resistin (Petrescu et al., 2022). Maintaining an optimally low n-6/n-3 PUFA ratio is associated with preventing the occurrence and development of NAFLD (Videla et al., 2022).

However, the benefits of replacing SFAs with PUFAs remain controversial and doubtful (Astrup et al., 2020; Astrup et al., 2021; Harcombe, 2019). This study was performed to investigate the effects of replacing SFA-rich lard with blended oils with different n-6/n-3 ratios on the metabolic disorders and hepatic steatosis in high-fat diet-fed rats.

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2 Materials and methods

2.1 Animal and diets

Male Sprague–Dawley (SD) rats (140-160 g) were purchased from Zhejiang Experimental Animal Center, and housed individually under a 12 h light/dark cycle (at 20-24 °C and 50%-60% humidity). The rats had free access to water and food. Rats were acclimatized for 8 days before the experiment. Food intakes and body weight (g) during the 12-week period were similar among the five groups. The experimental protocol was approved by the Ethics Committee on Animal Care and Use at Southeast University (NO.20130218). Animal care and experimental procedures were performed in accordance with the principles of European Directive 2010/63/EU.

Rats were randomly divided into five groups (ten rats per group; Figure 1), as follows: normal control group (NC); high-fat 1:1 (n-6/n-3 PUFA ratio) group; high-fat 5:1 (n-6/n-3 PUFA ratio) group; high-fat 20:1 (n-6/n-3 PUFA ratio) group; and high-fat lard group (Lard).

The NC group was fed AIN-76M synthetic feed formula. The NC diet (energy content: 3.6 kcal/g) contained 60.9% carbohydrate, 15.9% fat, and 23.2% protein (energy % kcal). The proportion of macronutrients was adjusted on the basis of the NC diet, and the contents of fat, cholesterol, and bile salts were increased to prepare high-fat lard diets or high-fat diets with different n-6/n-3 ratios; the oil for these diets was provided by blending edible oils with different fatty acid compositions. All high-fat diets had similar energy content (4.0 kcal/g), containing 47.1% carbohydrate, and 33.5% fat and 19.4% protein (energy % kcal). The diet ingredients and compositions are shown in Table 1.

2.2 Blood processing

Blood was collected after fasting overnight at 0, 4, 6, 10, and 12 weeks. At the end of the experiment, the animals were anesthetized with sodium pentothal and sacrificed by decapitation. The blood samples were centrifuged at 3000 × *g* for 10 minutes at 4 °C, and the serum samples were stored at –80 °C.

2.3 Blood biochemical analyses

Serum total cholesterol (TC), total triglycerides (TGs), Low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glucose (GLU), resistin, adiponectin (ADP), visfatin, leptin, free fatty acids (FFAs), lipoprotein lipase (LPL), gamma glutamyl transferase (GGT), endothelin-1 (ET-1), vascular endothelial growth factor A (VEGF-A), angiotensin II (Ang II), nitric oxide (NO), and 3-nitrotyrosine (3-NT) levels were measured. TG, TC, HDL-C, and LDL-C were determined using the enzyme method. The GLU level was determined using the glucose oxidase method. Resistin, visfatin, ADP, leptin, FFA, LPL, GGT, ET-1, VEGF-A, Ang II, NO, and 3-NT levels were determined using ELISA kits (JRDUN BIOTECH, Shanghai, China).

2.4 Pathological examination

At the end of the experiment, the liver was removed and fixed in a 10% formalin solution, which was followed by routine sampling, dehydration, paraffin embedding sectioning (4-μm thick sections), and hematoxylin and eosin staining. The section was examined under a light microscope to check for the presence of lesions.

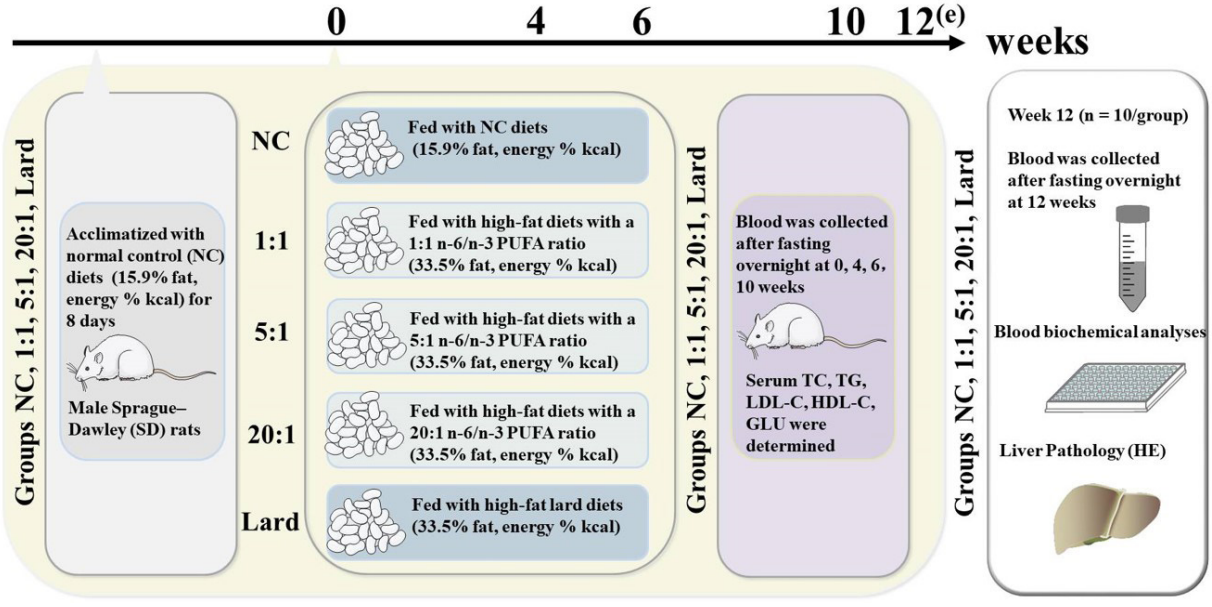

Figure 1. Experimental design. Groups NC, 1:1, 5:1, 20:1 and Lard were acclimatized with normal control (NC) diets for 8 days before the experiment. After acclimatizing 8 days, rats received NC diets (Group NC), high-fat diets with a 1:1 n-6/n-3 PUFA ratio (Group 1:1), high-fat diets with a 5:1 n-6/n-3 PUFA ratio (Group 5:1), high-fat diets with a 20:1 n-6/n-3 ratio (Group 20:1), or high-fat lard diets (Group Lard). Blood was collected after fasting overnight at 0, 4, 6, 10 and 12 weeks. Rats were euthanized at the end of week 12. PUFA: polyunsaturated fatty acid; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; GLU: glucose; HE: hematoxylin and eosin; e: euthanasia.

Table 1. Ingredients and compositions of the diets fed to rats (g/kg).

Ingredients of the diet (g/kg)		NC	n-6/n-3 PUFA ratio			Lard
			1:1	5:1	20:1	
Casein		230.0	215.0	215.0	215.0	215.0
Cornstarch		295.0	258.0	258.0	258.0	258.0
Sucrose		310.0	265.0	265.0	265.0	265.0
cellulose		50.0	50.0	50.0	50.0	50.0
Oils	Total	70.0	150.0	150.0	150.0	150.0
	Corn oil	0.0	28.8	71.0	86.2	0.0
	Linseed oil	0.0	44.7	13.8	2.7	0.0
	Olive oil	0.0	24.7	16.1	13.0	0.0
	butter	0.0	51.8	49.1	48.1	0.0
	lard	0.0	0.0	0.0	0.0	150.0
	Soybean oil	70.0	0.0	0.0	0.0	0.00
cholesterol		0.00	15.0	15.0	15.0	15.0
cholate		0.00	2.0	2.0	2.0	2.0
Mineral mixture		30.0	30.0	30.0	30.0	30.0
Vitamin mixture		10.0	10.0	10.0	10.0	10.0
DL- methionine		3.0	3.0	3.0	3.0	3.0
Choline		2.0	2.0	2.0	2.0	2.0
Energy (kcal/g)		3.6	4.0	4.0	4.0	4.0
∑SFA		10.9	46.4	47.2	47.4	65.7
∑MUFA		15.6	46.7	47.8	48.2	56.2
∑PUFA		41.4	50.2	48.5	47.8	20.7
SFA:MUFA:PUFA		1.0:1.4:3.8	1.0:1.0:1.1	1.0:1.0:1.0	1.0:1.0:1.0	1.0:0.8:0.3
∑n-6 PUFA		37.3	24.3	40.0	45.6	18.4
∑n-3 PUFA		5.1	25.9	8.5	2.2	2.3
n-6/n-3 ratio		7.3	0.9	4.7	20.7	8.0

SFA: saturated fatty acid(s); MUFA: monounsaturated fatty acid(s); PUFA: polyunsaturated fatty acid(s); NC: normal control.

2.5 Data analysis

The data were expressed as the mean ± SD, and the data were graphed using GraphPad Prism5 (GraphPad software, San Diego, CA, USA). SPSS17.0 statistical software (IBM, Inc., Armonk, NY, USA) was used for repeated measures analysis of variance (ANOVA) and one-way ANOVA. The least significant difference test was used for pairwise comparison between groups. Statistical significance was set as $P < 0.05$.

3 Results

3.1 Effects of different n-6/n-3 PUFA ratios on glucose and lipid metabolism in rats

The serum TG concentrations in the 1:1 n-6/n-3 ratio and NC groups were significantly lower than those in the Lard group ($P < 0.05$; Figure 2A). The serum TC (Figure 2B) and LDL-C (Figure 2D) concentrations in the 1:1 n-6/n-3 ratio groups were significantly lower than those in the 20:1 n-6/n-3 ratio and Lard groups ($P < 0.05$). Additionally, the 5:1 n-6/n-3 ratio group had significantly lower serum LDL-C levels than those in the Lard group ($P < 0.05$; Figure 2D). The 1:1 n-6/n-3 ratio group showed the lowest HDL-C concentration among the five groups ($P < 0.05$; Figure 2C).

3.2 Effects of different n-6/n-3 PUFA ratios on serum adipocytokines in rats

The serum resistin concentrations in the 1:1, 5:1 and 20:1 n-6/n-3 ratio groups were significantly higher than those in the Lard group ($P < 0.05$) but significantly lower than those the NC group ($P < 0.05$; Figure 3A). Serum visfatin levels in the 1:1 and 20:1 n-6/n-3 ratio groups were significantly higher than those in the 5:1 n-6/n-3 ratio and Lard groups ($P < 0.05$; Figure 3B). The three n-6/n-3 ratio groups showed higher leptin levels than those in the Lard and NC groups ($P < 0.05$; Figure 3C). The 1:1 and 5:1 n-6/n-3 ratio groups had lower ADP levels than those in the 20:1 n-6/n-3 ratio, NC and Lard groups ($P < 0.05$; Figure 3D).

3.3 Effects of different n-6/n-3 PUFA ratios on endothelial cell function in rats

Serum ET-1 (Figure 4A) and Ang II (Figure 4C) levels in the 1:1 and 5:1 ratio groups were lower than those in the NC groups ($P < 0.05$). The 1:1 n-6/n-3 ratio had a significantly lower serum NO level than that in the NC, 20:1 ratio, and Lard groups ($P < 0.05$; Figure 4B). Additionally, there was no significant difference in serum VEGF-A levels among the different n-6/n-3 ratio groups ($P > 0.05$), whereas the NC group had a higher serum VEGF-A level than that in the other groups ($P < 0.05$; Figure 4D). The serum FFA level in the 1:1 n-6/n-3 ratio and Lard groups was significantly lower than that in the 20:1 n-6/n-3 ratio and NC groups ($P < 0.05$; Figure 4E). Serum NT levels in the 1:1 n-6/n-3 ratio group were significantly lower than those in the 20:1 n-6/n-3 ratio and NC groups ($P < 0.05$; Figure 4F).

3.4 Effects of different n-6/n-3 PUFA ratios on serum enzyme activity in rats

Although there was no significant difference, the serum GGT levels in the NC group tended to increase compared

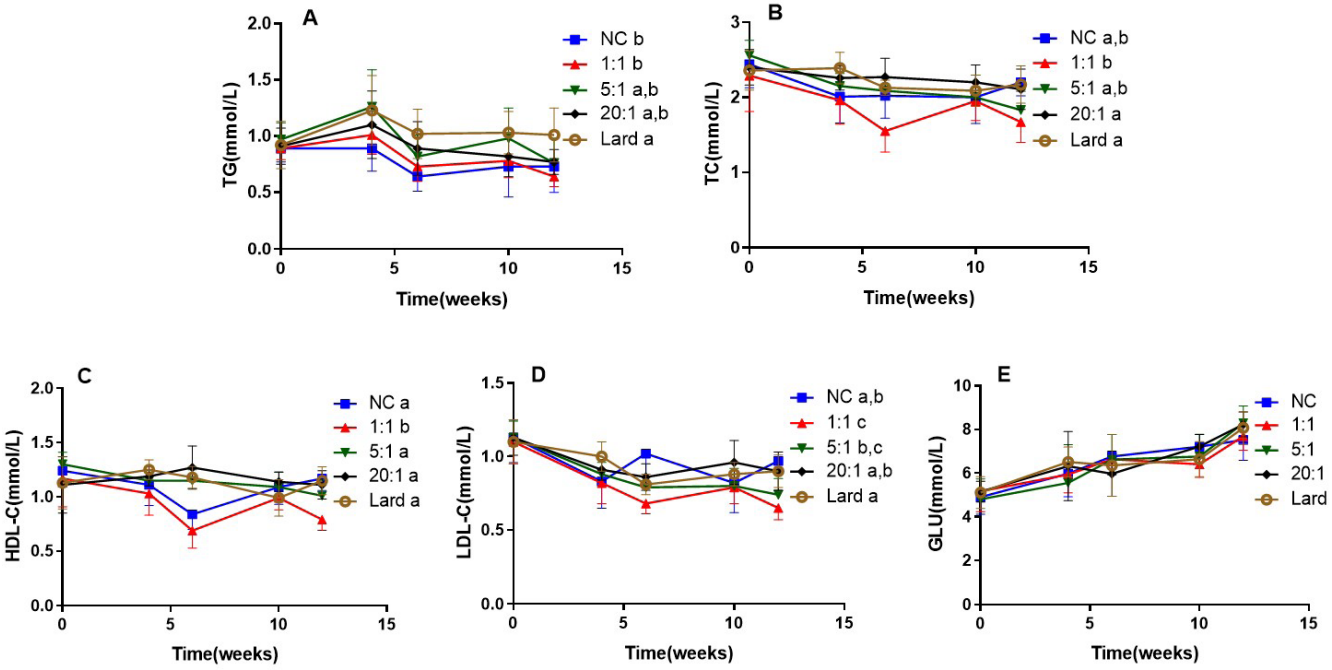

Figure 2. Effects of diets with different n-6/n-3 PUFA ratios on glucolipid metabolism in rats (Data presented as mean ± SD, n = 10). a-c: Mean values with different letters are significantly different (meal effect: $P < 0.05$, Repeated measures ANOVA).

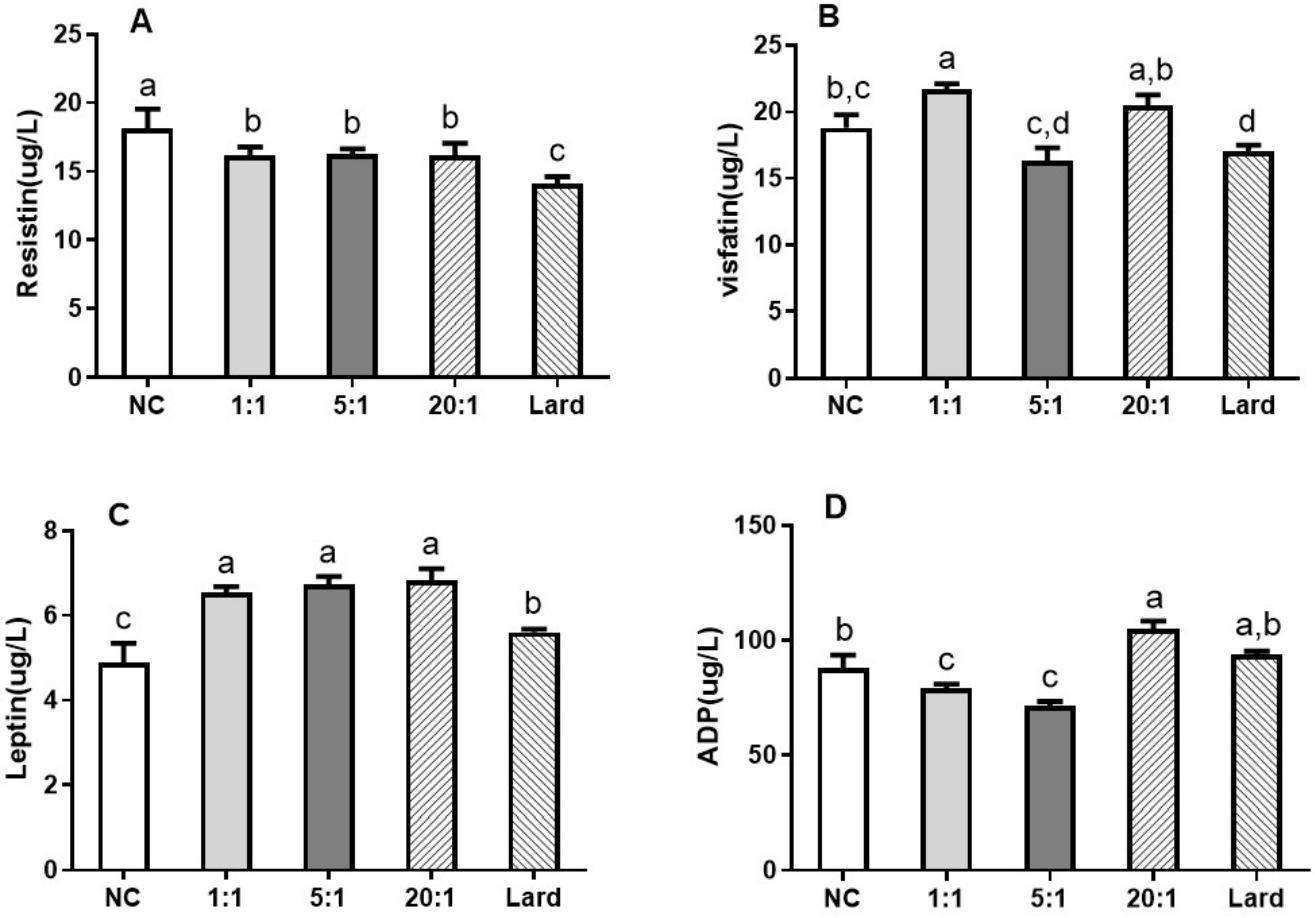

Figure 3. Effects of diets with different n-6/n-3 PUFA ratios on adipocytokines in rats (Data presented as mean ± SD, n = 10). a-d: Mean values with different letters are significantly different ($P < 0.05$).

with those in the 1:1, 5:1, 20:1, and Lard groups (Figure 5A). The 1:1 and 5:1 n-6/n-3 ratio groups showed significantly lower LPL levels than those in the 20:1 PUFA ratio, NC, and Lard groups ($P < 0.05$; Figure 5B).

3.5 Effects of different n-6/n-3 PUFA ratios on hepatic steatosis in rats

In this study, the liver lesions in rats were mainly hepatic cell lipidosis. There were two types of lipidosis, which were the vesicle type and bullae type, and most of them were the vesicle type (Figure 6B-6E). The hepatic lobule structure was clear, and no obvious lipid change, eosinophilic degeneration, or necrosis was observed in the liver cells in the NC group (Figure 6A). There was no inflammatory cell infiltration in the hepatic lobule in the NC group (Figure 6A). The other groups showed moderate lipidosis, and the lipidosis type was similar (Figure 6B-6E). In the 1:1 n-6/n-3 PUFAs ratio group (Figure 6B), the main liver lesions were lipid drops vacuolation to varying degrees in the liver cell cytoplasm. The type of lipid change was either the vesicle-dominated or the mixed vesicle type, and there was no fibrous tissue hyperplasia in the portal tract in the n-6/n-3 PUFA ratio 5:1 group. The degree of hepatocyte lipid change in the n-6/n-3 PUFA ratio 5:1 group (Figure 6C) was lower than that in the 1:1 group (Figure 6B). There was no necrosis in liver cells, and no inflammation of hepatic lobules or portal ducts in the 5:1 group (Figure 6C). The degree of hepatocyte lipid change in the Lard group (Figure 6E) was similar to that in the 5:1 group (Figure 6C), which showed moderate or severe hepatocyte lipidosis. The 20:1 n-6/n-3 PUFA ratio group (Figure 6D) showed a degree of hepatocyte lipid change that was between the 5:1 and 1:1 groups.

4 Discussion

Dietary guidelines around the world recommend reducing saturated fat and increasing monounsaturated fatty acid (MUFA)

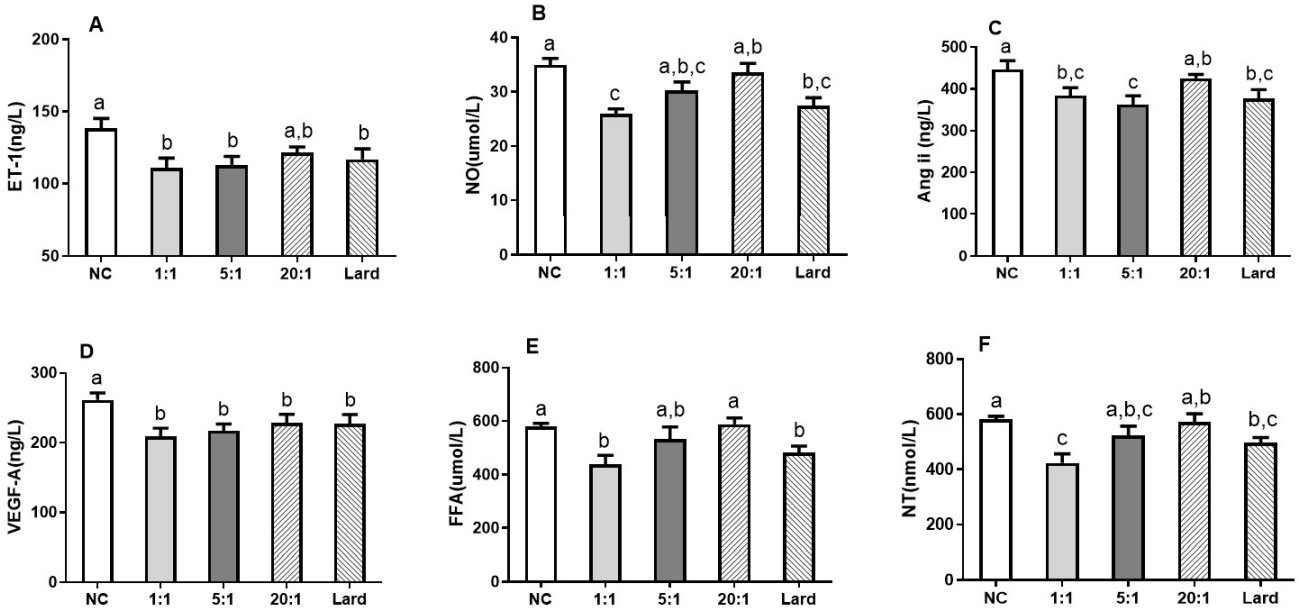

Figure 4. Effects of diets with different n-6/n-3 PUFA ratios on endothelial function in rats (Data presented as mean ± SD, n = 10). a-c: Mean values with different letters are significantly different ($P < 0.05$).

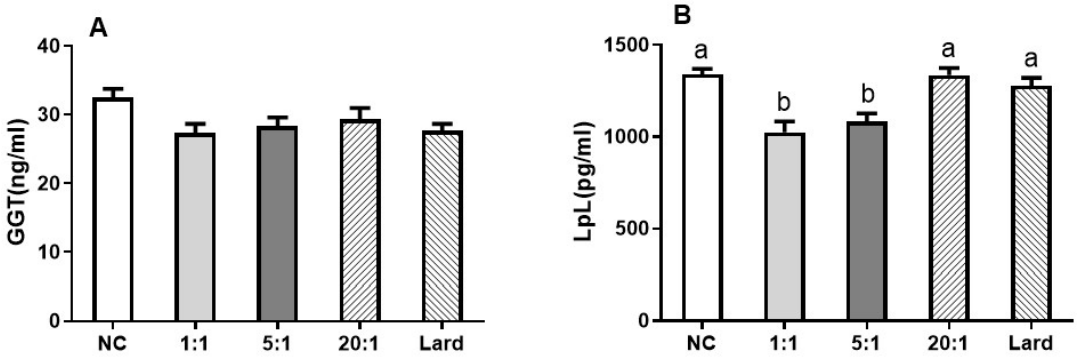

Figure 5. Effects of diets with different n-6/n-3 PUFA ratios on serum enzyme activity and oxidative stress in rats (Data presented as mean ± SD, n = 10). a-b: Mean values with different letters are significantly different (P < 0.05).

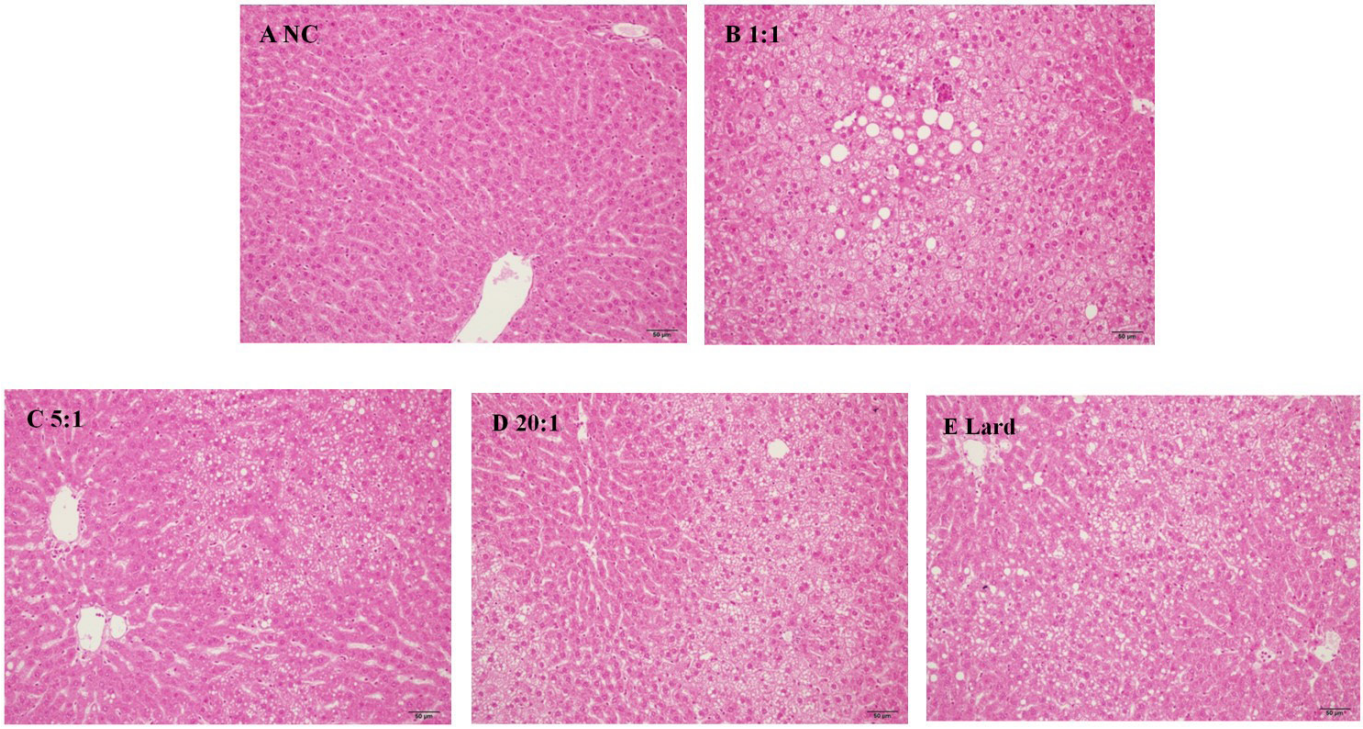

Figure 6. Effects of diets with different n-6/n-3 PUFA ratios on hepatic steatosis (HE staining × 200).

and PUFA intake (McLean et al., 2015). It is recommended to replace some SFAs with PUFAs to reduce the negative effects of high-fat diet on health (Schwingshackl et al., 2021). However, limiting the consumption of SFAs or replacing them with PUFA remains controversial (Astrup et al., 2021). One view speculates that PUFAs are predisposed to lipid peroxidation, which can lead to inflammation-related diseases such as atherosclerosis, and SFAs appear to be less harmful than PUFAs (Lawrence, 2021). We speculate that the proportion of different PUFA types that replace SFA may influence the observed health effects. Excessive n-6 PUFA intake and n-3 PUFA deficiency are important dietary factors that disturb the balance of antagonistic metabolic functions (Mariamenatu & Abdu, 2021). Therefore, the present study was performed to investigate the effects of replacing SFA-rich lard in high-fat diets with blended oils with different n-6/n-3 PUFA ratios on the health of rats.

Lipid metabolism disorders can lead to serious diseases such as cardiovascular disease and fatty liver (Chen et al., 2019). Replacing saturated fat with unsaturated fats had a beneficial effect on lipid biomarkers (Vafeiadou et al., 2015). SFAs could increase LDL-C, which is a major risk factor for cardiovascular disease, and partial replacement of SFAs by PUFAs could reduce LDL-C (Kris-Etherton & Krauss, 2020). As expected, the present study showed that the 1:1 and NC groups had reduced serum TG levels compared with that in the Lard group, and the 1:1 group had lower serum LDL-C and TC concentrations than those in the 20:1 and Lard groups. However, the HDL-C level was lowest in the 1:1 group compared with that in the other groups. Additionally, the 5:1 group showed lower serum LDL-C levels compared with those in the Lard group. The present study also demonstrated that the effects of different n-6/n-3 PUFA ratios on lipid metabolism showed a trend that varied over time in rats. Consistent with our findings, one previous study reported that a low n-6/n-3 PUFA ratio had a beneficial effect on lipid metabolism, and this effect could last for a long time (Li et al., 2021). Another study reported that the blending oil with a low n-6/n-3 PUFA ratio of 6:1 could improve lipid metabolism, inflammation, and oxidative stress in obese rats (Uriho et al., 2019). Our study indicated that replacing of SFAs with PUFAs with different n-6/n-3 PUFA ratios might affect lipid metabolism.

LPL is a key enzyme that metabolizes circulating TGs into FFAs and has a beneficial effect on plasma HDL, whereas the non-catalytic function of LPL is thought to promote atherosclerosis (Li et al., 2014). Elevated circulating FFA levels are associated with increased cardiovascular risk, which may be related to the increased oxidative stress in endothelial cells that is caused by FFAs (Wang et al., 2019). In the present study, the 1:1 and 5:1 groups had lower serum LPL levels than those in the 20:1, Lard, and NC groups, and the 1:1 and Lard groups had lower serum FFA concentrations compared with those in the 20:1 and NC groups. Halfen et al. (2016) found that a low n-6/n-3 PUFA diet was more effective in reducing blood TG and FFA levels in Wistar rats compared with those of high n-6/n-3 PUFA diets. Chang et al. (2014) reported that replacing SFAs in a high-fat diet with n-3 PUFAs reduced aortic LPL levels in $LDLR^{-/-}$ mice. Thus, our results suggested that low n-6/n-3PUFA ratios instead of SFAs might improve the risk of cardiovascular disease by decreasing serum LPL and FFA levels.

Adipokines dysregulation may be associated with inflammation and dysregulation of both lipid and liver metabolism (Kamada et al., 2008; Ouchi, 2016). Resistin can

induce vascular inflammation, lipid accumulation, and plaque vulnerability (Zhou et al., 2021). Visfatin could cause endothelial dysfunction by increasing inflammatory and adhesion molecule expression (Dakroub et al., 2021). Leptin is involved in energy homeostasis and lipid metabolism, and it is associated with the control of body weight, satiety, energy expenditure, and hepatic lipid degeneration (Jiménez-Cortegana et al., 2021; Mendoza-Herrera et al., 2021). ADP is thought to have insulin sensitization, anti-inflammatory, and cardiovascular protection properties (Matsuzawa et al., 2004). However, circulating ADP levels were an unexpected predictor of increased morbidity and mortality (Francischetti et al., 2020). In the present study, the different n-6/n-3 groups had significantly lower resistin levels than those in the NC groups, but they were higher than those in the Lard group. The 5:1 and Lard groups had significantly lower visfatin level than those in the 1:1 and 20:1 groups. Although there was no significant difference in the serum leptin levels among different n-6/n-3 PUFA ratio groups, all the n-6/n-3 groups had higher leptin levels than those of the NC and Lard groups. Serum ADP levels in the 1:1 and 5:1 groups were lower than those of the 20:1, NC, and Lard groups. Voon et al. (2021) reported that a high-fat diet supplemented with different proportions of LA (< 1%, 2.8%, 5.8%, or 9.7%) had no effects on visfatin and leptin levels. Another study reported that different n-6/n-3 PUFA ratio diets did not affect plasma leptin levels in piglets (Nguyen et al., 2020). Sundaram et al. (2016) reported that a high-fat diet reduced serum resistin concentrations in mice, whereas n-3 and n-6 PUFAs had no effect on leptin and resistin production at dietary levels (Sundaram et al., 2016). Nelson et al. (2007) reported that ALA (n-3 PUFA) supplementation for 8 weeks reduced ADP levels among healthy individuals (Nelson et al., 2007). Thus, our results suggested that replacing SFA with PUFA could affect adipokine secretion, which was affected by the dietary n-6/n-3 PUFA ratios.

Endothelial dysfunction is key events in the development and pathophysiology of atherosclerosis, and molecules associated with endothelial dysfunction are potential biomarkers and therapeutic targets for the prevention of atherosclerosis (Medina-Leyte et al., 2021). Ang II mediates the direct physiological effects of vasoconstriction and blood pressure regulation, and it is associated with inflammation, endothelial dysfunction, and atherosclerosis (Mehta & Griendling, 2007). ET-1 is considered to be an important factor in the development of vascular dysfunction and cardiovascular diseases (Böhm & Pernow, 2007). VEGF is a proinflammatory molecule that links inflammation and chronic diseases (Gupta et al., 2018). In this study, the 1:1 and 5:1 groups had significantly lower serum Ang II and ET-1 levels than those of the NC group. Additionally, the 5:1 group had significantly lower Ang II levels than those of the 20:1 group. Serum VEGF-A levels tended to increase in the high n-6/n-3 groups compared with those in the low n-6/n-3 groups. Yang et al. (2019) reported that n-3 PUFAs play an antihypertensive role by reducing plasma Ang II in hypertensive patients (Yang et al., 2019). Costantini et al. (2019) found that supplementation of ALA-rich chia seeds significantly reduced ET-1 levels in spontaneously hypertensive rats (Costantini et al., 2019). Bork et al. reported that a low LA:ALA ratio played an anti-inflammatory role by reducing VEGF production in tumor necrosis factor-α-stimulated endothelial cells (Bork et al., 2019). The present study suggested that low n-6/n-3 ratios might be beneficial for improving endothelial dysfunction.

Nitrosative stress was shown to be closely related to cardiovascular and other diseases (Yoon et al., 2021). During nitrosative stress, NO and superoxide react to produce the peroxynitrite anion ($ONOO^-$), which nitrates proteins and other biomolecules and generates 3-NT (Wang et al., 2021). 3-NT is a well-established stable biomarker of oxidative stress and a good predictor of disease progression (Campolo et al., 2020). The 1:1 group had significantly lower NO and NT concentrations than those in the NC and 20:1 groups. Consistent with our results, a previous study indicated that LA exposure increased NO and NT levels in vascular endothelial cells (Saraswathi et al., 2004). Reddy et al. found that a balanced n-6/n-3 diet could reduce NO levels in the colon tissues of adult rats compared with those of n-3 or n-6 PUFA-rich diets (Reddy & Naidu, 2016). In this study, the NC group had a higher level of nitrosative stress. This may be a result of enhanced oxidative stress that is caused by a high PUFA:SFA ratio diet because PUFAs are susceptible to lipid peroxidation (Zhong et al., 2019). We speculated that a low n-6/n-3 PUFA ratio could reduce the level of nitrosative stress and oxidative stress.

Hepatic steatosis is the initial stage of NAFLD, which is a metabolic disorder characterized by ectopic accumulation of lipids in liver cells (Barrios-Maya et al., 2022). N-3 PUFAs played an important role in regulating fat accumulation and fat elimination in the liver, and the disorder of n-6/n-3 PUFA ratio in the liver affected the histological pattern by regulating the amount of liver lipids (El-Badry et al., 2007). In the present study, the degree of hepatocyte lipid change in the 5:1 n-6/n-3 groups was smaller than that in the 1:1 group. Additionally, the degree of hepatocyte lipid change in the 20:1 n-6/n-3 group was between the 5:1 and 1:1 groups. In agreement with our findings, a previous study reported that low n-6/n-3 (2:1 and 5:1) ratios prevented NAFLD that was caused by a high-fat diet in rats (Jeyapal et al., 2018). Oxidative stress and inflammation contribute to the progression of hepatic steatosis to non-alcoholic steatohepatitis (NASH) (Li et al., 2022). GGT is used as an early marker of subclinical inflammation and oxidative stress, and it is also commonly used as a marker of liver dysfunction (Whitfield, 2001; Yamada et al., 2006). Although no significant difference was observed in the present study, the serum GGT levels in the high n-6/n-3 groups tended to increase compared with those of the low n-6/n-3 groups. Ketsa et al. reported that different n-6/n-3 PUFA ratios (7:1 and 9:1) did not affect the serum GGT level in rats (Ketsa & Marchenko, 2014). However, n-3 PUFAs could reduce serum GGT activity in patients with metabolic syndrome and NAFLD (Šmíd et al., 2022). Han et al. also found that ALA-rich Chia seed oil improved high-fat diet-induced hepatic steatosis and reduced hepatic lipid deposition in mice (Han et al., 2020). The results of the present study suggest that an appropriate n-6/n-3 PUFA ratio might be beneficial to improve liver steatosis induced by high fat diets.

The present study demonstrated that replacing SFA-rich lard with low n-6/n-3 PUFA ratio blended oils in a high-fat diet improved the health effects compared with those of the high-SFA lard diet. However, the underlying mechanism needs further

study, and the role of adipose tissue, liver, and gut microbiota in this process should be further investigated. The results of the present study were obtained in high-fat diet-fed rats, and the SFA:MUFA:PUFA ratio in blended oils was close to 1:1:1. Given the health benefits of MUFA, further studies are required to determine the interaction between MUFA intake and different n-6/n-3 ratios with appropriate PUFA/SFA ratios.

5 Conclusion

The results of the present study demonstrated that blending oils with low n-6/n-3 PUFA ratios in high-fat diets had beneficial effects on metabolic disorders in rats compared with the high-SFA lard diet. However, blended oils with a high n-6/n-3 ratio had negative effects on metabolic disorders and hepatic steatosis in rats compared with those of the blended oils with low n-6/n-3 PUFA ratios. The present study also showed that high PUFA levels had adverse effects on metabolic profiles in rats even at low fat levels. Our results indicated that the effects of replacing SFA-rich lard with PUFA-rich blended oils on high-fat diet-induced metabolic disorders and hepatic steatosis were influenced by the dietary n-6/n-3 PUFA ratios. Overall, our study suggested that the effects of the n-6/n-3 ratio in PUFAs on metabolic profiles should be considered when replacing SFAs with PUFAs. The present study also suggested that a high dietary n-6/n-3 PUFA ratio might have adverse effects on metabolic profiles in high-fat diet-fed rats.

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