Effect of Dietary Lard Containing Higher α -Linolenic Acid on Plasma Triacylglycerol in Rats

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Abstract: It is well known that the consumption of n-3 polyunsaturated fatty acid (PUFA) decreases the plasma triacylglycerol (TAG) level. The technology of elevating the content of n-3 PUFAs in pig meat has already reached a practical level. In this study, the effects of dietary lard containing higher α -linolenic acid (LNA) on plasma TAG were compared with those of normal lard in rats. The rats were fed a diet containing either 10% normal lard or a high linolenic lard for 4 weeks. The plasma and liver TAG levels in the high linolenic lard group were significantly lower than those in the normal lard group. The activity of the fatty acid synthase (FAS) of the liver in the high linolenic lard group was significantly lower than that in the normal lard group. The contents of n-3 PUFAs in hepatic total lipid, TAG fraction, and the phospholipids (PLs) fraction increased in the high linolenic lard group. The results indicate that the high linolenic lard suppressed hepatic FAS activity compared with the control lard, resulting in a lower concentration of plasma TAG. These results also suggest that pig meat containing high LNA may be more nourishing than normal pig meat.

Key words: n-3 PUFA, lard, plasma triacylglycerol, rat

1 INTRODUCTION

It is well known that the consumption of n-3 polyunsaturated fatty acids (PUFAs), such as α -linolenic acid (LNA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) decrease the blood TAG level^{1,2}. It was found that the blood TAG concentration is an independent coronary heart disease risk factor³. Recent studies have shown that taking n-3 PUFAs in one's daily diet is very important for preventing coronary heart disease and diabetes^{4,5}. Fish is an effective dietary source of n-3 PUFAs. However, fish consumption is at a much lower level in current industrial countries than would be needed to obtain a sufficient amount of n-3 PUFAs, and it would be difficult to greatly change the dietary habits of persons in those countries. Western diets include a lot of fat from animal meats such as beef, pork, poultry, and lamb^{4,6-8}. Therefore, if the content of n-3 PUFAs in meat increases, the consumption of n-3 PUFAs would be increased.

Many studies to increase the content of n-3 PUFAs in

chicken, cow and pig have been attempted. In particular, it is thought that pig meat and poultry products will become a source of n-3 PUFAs in the near future⁹. Ruminant animals hydrogenate n-3 PUFA with microorganisms in their rumen, and a lower amount of PUFAs are incorporated into lipids than in single-stomached animals. Moreover, it has been pointed out that the smell of the meat in ruminant animals changes by the administering of feed containing long-chain PUFAs¹⁰⁻¹². On the other hand, the fatty acid composition of pig fat is more closely related to feed fatty acid composition than for other livestock species^{10,11,13}. Therefore, dietary PUFAs are readily deposited in pig fat¹⁴. For example, it was reported that the content of n-3 PUFAs in lard could be increased up to 12% by adjusting the administering period and amount¹⁵. These research studies showed that increasing the content of n-3 PUFAs in pig fat has already reached a practical level. However, to our knowledge, there are few reports evaluating the nutrients in high content n-3 PUFAs pig meat. The object of this

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study was to evaluate the effects of lard containing higher LNA on the TAG level of blood and the liver in rats.

2 EXPERIMENTAL

2.1 Animals and diets

All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Notification of the Prime Minister's Office in Japan). Four-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were individually housed in stainless steel wire cages. The temperature of the animal room was set at $23 \pm 1^\circ\text{C}$, with a humidity of $50 \pm 10\%$ and illumination from 08:00 to 20:00 h. Eight rats each were assigned to the control lard-fed and high linolenic lard-fed groups. Each group of rats was allowed free access to water and the experimental diet for 4 weeks. The composition of the experimental diets, based on the AIN-93G purified diet for rodents¹⁶⁾, is shown in **Table 1**. The dietary lipid level was 10% of the experimental diets. Control lard was purchased commercially (Snow Brand Milk Products Co., Ltd., Tokyo, Japan). High linolenic lard was extracted from donated pig meat (Itoham Foods Inc., Nishinomiya, Japan). The pig had been fed high α -linolenic feed that contained flax oil for 8 weeks. The TAG contents of these lard were fractionated by thin layer chromatography (PLC60; Merck KGaA, Darmstadt, Germany) with hexane, diethylether and acetic acid (80:20:1) (**Table 2**). The LNA contents of the control and high linolenic lard were 0.4 and 4.1%, respectively (**Table 3**).

At the end of the feeding period, the rats were killed under anesthesia after 7 h food deprivation. The blood was collected from the inferior vena cava using a syringe. Sodium citrate was used as an anticoagulant (final concentration 3.8 mg/ml), and plasma was separated by centrifugation. The liver, perirenal adipose tissue and epididymal adipose tissue were exposed and weighed. Plasma and tissues were stored at -80°C until use.

2.2 Plasma and liver lipids level

The plasma TAG concentration was determined by using a commercial Triglyceride E-test Wako assay kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The total lipids of the liver were extracted by the method of Folch *et al.*¹⁷⁾. The 0.02 ml of this extract were added a tube and removed of solvent. Then the sample was completely dissolved with enzymatic reagent and the liver TAG content was determined by using the Triglyceride E-test Wako assay kit. The levels of total plasma and HDL-cholesterol were determined by using the commercial Cholesterol E-test Wako and HDL Cholesterol E-test Wako assay kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively within 2 weeks after collection¹⁸⁾.

Table 1 Composition of the Experimental Diet.

	(g/100g diet)
Corn starch	15.0
Sucrose	42.298
Casein	20.0
Cellulose powder	5.0
AIN-93 mineral mixture	3.5
AIN-93 vitamin mixture	1.0
DL-methionine	3.0
Choline bitartrate	0.2
TBHQ	0.002
Fat	10.0

Table 2 Composition of the Experimental Lipids.

	Control lard	High linolenic lard
	(%)	
TAG	97.9	96.6
Cholesterol	0.1	0.8
Free Fatty Acid	0.8	1.0
Others	1.2	1.6

Table 3 Fatty Acid Composition of the Experimental Lipids.

	Control lard	High linolenic lard
	(g/100g)	
C16:0 ^a	25.2	23.7
C16:1	2.6	1.9
C18:0	14.6	13.7
C18:1 (n-9)	43.7	39.7
C18:2 (n-6)	9.4	12.5
C18:3 (n-3)	0.4	4.1
C20:3 (n-6)	0.1	0.1
C20:4 (n-6)	0.1	0.1
C20:3 (n-3)	0.0	0.5
C20:5 (n-3)	0.0	0.1
C22:6 (n-3)	0.0	0.1
Others	3.9	3.5

^aNumber of carbon atoms : number of double bonds.

2.3 Fatty acid synthase activity

Liver homogenates were prepared in 0.25 M sucrose solution. The homogenate was centrifuged at $500 \times g$ for 10 min at 4°C, and the supernatant was further centrifuged at $9000 \times g$ for 10 min at 4°C. The post-mitochondrial fraction was used for the fatty acid synthase activity (FAS) assay. The FAS activity was determined by the method of Kelly *et al.*¹⁹. The cytosolic protein content was measured by using a commercial Protein assay rapid kit WAKO (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

2.4 Fatty acid composition

Total lipids of the liver were extracted by the method of Folch *et al.*¹⁷. The extracted lipids were fractionated into TAG and PL by thin layer chromatography (PLC60; Merck KGaA, Darmstadt, Germany) with chloroform and methanol (9:1). For fatty acid analyses, these lipids were methylated with methanolic sulfuric acid. These lipids of fatty acid compositions (total, TAG and PL) were determined by gas-liquid chromatography (6898 series; Agilent Technologies, California, USA) with a capillary column (OMEGAWAX320; Supelco, Pennsylvania, USA).

2.5 Statistical analysis

Data were expressed as mean $\pm$ SEM. Significant differences between the diet groups were determined by the Student's t-test. Differences with a value of $P < 0.05$ were considered significant. A statistical software package (Toukei version 2, Esumi, Tokyo, Japan) was used for all of the statistical analyses.

3 RESULTS

3.1 Body weight, food intake, liver and adipose tissues

There were no significant differences in the body weight, food intake, liver and abdominal adipose tissues

weight between the two groups (Table 4).

3.2 Plasma TAG, total cholesterol and HDL-cholesterol

The plasma TAG in the high linolenic lard group was significantly lower than in the control lard group (Fig. 1). There was no significant difference in the concentration of plasma total cholesterol, while the HDL-cholesterol in the high linolenic lard group was significantly higher than in the control lard group (Fig. 2).

3.3 TAG content and FAS activity in liver

The TAG content of the liver was significantly lower in the high linolenic lard group than in the control lard group (Fig. 3). In addition, the FAS activity in the high linolenic lard group was significantly lower than in the control lard group (Fig. 4).

Fig. 1 Plasma TAG Concentrations in Rats Fed Control Lard or High Linolenic Lard for 4 Weeks. Values are means $\pm$ SE for 8 rats. Asterisks indicate difference from control lard group, * $P < 0.05$.

Table 4 Body Weight, Food Intake, Liver Weight, Epididymal Fat Pad and Perirenal Fat Pad in Rats Fed Control Lard or High Linolenic Lard for 4 Weeks.

	Control lard	High linolenic lard
Body weight (g)		
Initial	152.1 $\pm$ 2.3	151.7 $\pm$ 2.9
Final	361.5 $\pm$ 8.8	360.1 $\pm$ 5.1
Food intake (g/day)	21.3 $\pm$ 0.7	21.7 $\pm$ 0.5
Liver weight (g/100g body weight)	4.6 $\pm$ 0.1	4.5 $\pm$ 0.1
Epididymal fat pad (g/100g body weight)	2.6 $\pm$ 0.2	2.7 $\pm$ 0.2
Perirenal fat pad (g/100g body weight)	2.1 $\pm$ 0.2	2.1 $\pm$ 0.2

Each value was expressed as a mean $\pm$ SE for 8 rats.

Fig. 2 Plasma Total Cholesterol (A) and Plasma HDL Cholesterol (B) Concentrations in Rats Fed Control Lard or High Linolenic Lard for 4 Weeks. Values are means $\pm$ SE for 8 rats. Asterisks indicate difference from control lard group, $*P < 0.05$.

3.4 Fatty acid composition in liver

The fatty acid compositions of total lipids, TAG and PLs in the liver are presented in Table 5. In the total lipids, the proportions of C18:2, C18:3, C20:3, C20:5, C22:5 and C22:6 were higher in the high linolenic lard group than in the control lard group. The proportion of C18:1 in the high linolenic lard group was lower than in the control lard group.

In the TAG, the proportions of C18:2, C18:3, C20:5, C22:5 and C22:6 were significantly higher in the high linolenic lard group than in the control lard group. The proportions of C14:0 and C18:1 were significantly lower in the high linolenic lard group than in the control lard group.

In the PLs, the proportions of C18:2, C20:3, C20:5 and C22:6 were significantly higher in the high linolenic lard group than in the control lard group. The proportions of C18:1 and C20:4 were significantly lower in the high linolenic lard group than in the control lard group.

Fig. 3 Liver TAG Concentrations in Rats Fed Control Lard or High Linolenic Lard for 4 Weeks. Values are means $\pm$ SE for 8 rats. Asterisks indicate difference from control lard group, $*P < 0.05$.

Fig. 4 Fatty Acid Synthase Activity in Liver of Rats Fed Control Lard or High Linolenic Lard for 4 Weeks. Values are means $\pm$ SE for 8 rats. Asterisks indicate difference from control lard group, $*P < 0.05$.

4 DISCUSSION

This study demonstrated that the plasma TAG concentration in the high linolenic lard group was significantly lower than in the control lard group. Furthermore, the activity of hepatic FAS in the high linolenic lard group was lower than in the control lard group. These results indicated that the intake of high linolenic lard suppresses the activity of hepatic FAS, and this suppression may lead to a reduction of the plasma TAG concentration. The hepatic TAG concentration was significantly lower in the high linolenic lard group. The lower content of hepatic TAG in the high linolenic lard group did not contradict the result

Table 5 Fatty Acid Compositions (Total, TAG, and PL) in the Liver.

	Total lipid		TAG		PL	
	Control lard	High linolenic lard	Control lard	High linolenic lard	Control lard	High linolenic lard
	(g/100g)		(g/100g)		(g/100g)	
14:0 ^a	—	—	1.26 ± 0.05	1.08 ± 0.06*	—	—
16:0	27.19 ± 0.75	27.26 ± 0.65	34.78 ± 0.62	35.80 ± 0.56	16.45 ± 0.50	17.41 ± 0.68
16:1	4.11 ± 0.41	3.26 ± 0.50	6.05 ± 0.31	5.58 ± 0.26	0.98 ± 0.05	0.84 ± 0.04
18:0	12.48 ± 0.68	12.85 ± 0.60	2.61 ± 0.11	2.76 ± 0.05	34.40 ± 0.80	35.28 ± 0.90
18:1	36.71 ± 0.78	33.45 ± 0.99*	50.96 ± 0.57	47.83 ± 0.60**	11.73 ± 0.29	9.69 ± 0.30**
18:2 (n-6)	3.61 ± 0.21	5.85 ± 0.23**	2.78 ± 0.21	4.65 ± 0.29**	4.06 ± 0.18	6.33 ± 0.20**
18:3 (n-3)	0.00 ± 0.00	0.33 ± 0.04**	0.03 ± 0.02	0.40 ± 0.04**	0.00 ± 0.00	0.00 ± 0.00
20:3 (n-6)	0.34 ± 0.03	0.53 ± 0.05**	0.00 ± 0.00	0.01 ± 0.01	0.75 ± 0.04	1.20 ± 0.10**
20:4 (n-6)	11.59 ± 0.79	10.29 ± 0.54	0.69 ± 0.05	0.61 ± 0.05	25.83 ± 0.84	21.56 ± 1.17*
20:5 (n-3)	0.00 ± 0.00	0.40 ± 0.04**	0.00 ± 0.00	0.10 ± 0.02**	0.00 ± 0.00	0.53 ± 0.06**
22:5 (n-3)	0.00 ± 0.00	0.44 ± 0.03**	0.00 ± 0.00	0.13 ± 0.02**	0.28 ± 0.15	0.53 ± 0.04
22:6 (n-3)	2.34 ± 0.15	3.79 ± 0.24**	0.01 ± 0.01	0.26 ± 0.04**	3.13 ± 0.44	5.00 ± 0.46*

^aNumber of carbon atoms : number of double bonds. Each value was expressed as a mean ± SE for 8 rats. Asterisks indicate different from Control lard group, ***P* < 0.01, **P* < 0.05.

of the lower activity of hepatic FAS in the high linolenic lard group. Ikeda *et al.*²⁰⁾ reported that the serum TAG concentration was highly correlated with the hepatic FAS activity. Kim *et al.*²¹⁾ reported that the reduction of the plasma TAG concentration and hepatic FAS activity in rats administered LNA correlate with a low amount of FAS mRNA. PUFAs regulate hepatic lipogenesis through suppressing sterol regulatory element-binding protein (SREBP)-1c nuclear abundance accompanied with a comparable decrease in the transcription rate of hepatic FAS, and the decrease in SREBP-1 is accompanied by a comparable decrease in the transcription rate of hepatic FAS^{22,23)}. PUFAs activate peroxisome proliferator-activated receptor (PPAR α) by direct binding, leading to the induction of carnitine palmitoyl transferase which regulate hepatic fatty acid oxidation²²⁾. The lower concentration of plasma TAG in the high linolenic lard group may be related to the activation of β -oxidation through the PPAR α .

The high linolenic lard group diet contained higher proportion of linoleic fatty acid than control group diet. Ikeda *et al.*²⁰⁾ reported dietary linoleic fatty acid consequently increased TAG concentration of blood. It is probable that the lower content of plasma TAG concentration in the high linolenic lard group was influenced by LNA rather than linoleic fatty acid.

There were no differences in the plasma total cholesterol concentration between the high linolenic lard group and

control lard group. On the other hand, the HDL-cholesterol concentration of plasma in the high linolenic lard group was significantly higher than in the control lard group. High linolenic lard might cause the plasma HDL-cholesterol to increase.

The fatty acid compositions of total lipids, TAG and PLs in the liver were influenced by dietary lipid in this study. In the hepatic total lipid fatty acid composition, not only LNA but also the other n-3 PUFAs (EPA, docosapentaenoic acid, and DHA) were higher in the high linolenic lard group. These results show that part of the consumed LNA was metabolized to EPA, docosapentaenoic acid, and DHA in the liver. The TAG fraction also contained high amounts of LNA, EPA, docosapentaenoic acid and DHA in the high linolenic lard group compared with the control lard group. In both groups, LNA wasn't detected from the PL fraction of the liver lipids. However, the composition levels of EPA and DHA in the high linolenic lard group were higher than in the control lard group. These results suggest that DHA generated from LNA in the liver was incorporated into PL in the rats ingesting high linolenic lard. Farrell²⁴⁾ reported that the consumption of enriched n-3 PUFA egg increased the EPA, DHA, and total n-3 PUFAs concentration in the blood. Jiang *et al.*²⁵⁾ reported that the consumption of n-3 PUFA enriched yolks increased the LNA, EPA, and DHA in the liver lipids and liver PLs.

We used a lard extracted from pig's meat in this report.

The final object of our study is to clarify the nutritional characteristic of pig meat containing high amounts of LNA. Therefore, it is necessary to examine the influence of plasma TAG when administered with whole meat. It is also necessary to examine what the effective consumption level in humans would be when consuming meat containing high LNA. In humans, Oh *et al.*²⁶⁾ reported that the plasma TAG concentration was decreased by consuming a high dose n-3 PUFA egg.

5 CONCLUSION

The present study suggests that high linolenic lard suppressed hepatic FAS activity compared with the control lard in rats. As a result, high linolenic lard could decrease the concentration of plasma TAG. A western diet is characterized by a higher proportion of fat coming from meat rather than fish. The technology for producing high content n-3 PUFA has already reached a level of possible practical use. There is a possibility that the consumption of high LNA pork would increase if the effects on chronic disease prevention were clarified.

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