Insulin Response to Glucose-6-Phosphate Dehydrogenase Activity is Elevated in Rats Fed Diets Low in Polyunsaturated Fatty Acids

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Summary Glucose-6-phosphate dehydrogenase (G6PD) activity in the rat liver was elevated when the animals were fed a diet that contained 5% coconut oil or 1% corn oil plus 4% palmitic acid, in comparison with that of rats fed 5% corn oil. G6PD activity correlated inversely with the PUFA/SFA ratio of total liver phospholipid as well as the ratio of plasma membrane phospholipid. Elevation of G6PD activity was slightly affected by dietary protein. Serum insulin levels were apparently not influenced by dietary fats, and were not correlated with G6PD activity. Insulin dose-response to G6PD activity was augmented in primary cultured hepatocytes isolated from rats fed a diet with 1% corn oil plus 4% palmitic acid in comparison with those fed a diet with 5% corn oil. These findings indicate that augment in insulin dose-response to G6PD and elevation of its activity shown in rats fed diets low in polyunsaturated fatty acid are associated with lowering the PUFA/SFA ratio in plasma membrane phospholipid.

Key Word insulin, glucose-6-phosphate dehydrogenase, polyunsaturated fatty acid, phospholipid, PUFA/SFA ratio, plasma membrane phospholipid, rat liver, cultured hepatocytes, insulin dose-response, fat type

Glucose-6-phosphate dehydrogenase (d-glucose 6-phosphate: NADP oxidoreductase, EC 1.1.1.49; G6PD) plays a role in providing NADPH for lipogenesis, and G6PD activity is altered by complex interactions of diet and hormone, including insulin. Liver G6PD synthesis increased when starved rats were fed on a high carbohydrate diet (1,2), and the stimulation of G6PD activity was ascribed to the increase in mRNA of G6PD at a pretranslational level (3). In rat liver, G6PD activity was increased by a high dose of insulin (4), while G6PD synthesis was suppressed by glucagon (5). In contrast, glucagon was without effect in primary

Abbreviations: PUFA/SFA, polyunsaturated fatty acid/saturated fatty acid; G6PD, glucose-6-phosphate dehydrogenase.
cultured hepatocytes (6, 7). The elevation by insulin was attributed to increases in mRNA of G6PD and synthesis (7, 8).

In addition to carbohydrate and hormones, dietary fat also affected insulin action by altering insulin binding capacity for the receptor (9). However, effects of fat on insulin varied with cell species and type of fat ingested. Feeding rats on a diet rich in linoleic acid induced higher insulin binding to epididymal fat cells than did a diet high in saturated fatty acids, without changing in serum insulin levels (10, 11). Hepatocytes isolated from BHE rats fed menhaden oil had a greater affinity for insulin than did those fed hydrogenated coconut oil (12). When hepatoma cells were cultured in medium enriched with linoleic acid, the number of insulin receptors decreased (13). Reconstituted turkey erythrocyte membrane was shown to have a greater affinity for insulin in the saturated lipid environment than in the unsaturated lipid environment (14). The number of insulin receptors of erythrocytes decreased when rabbits were fed a diet high in saturated fatty acid (15). All these studies indicated that fats affect insulin action in a various manner. To elucidate type of dietary fat which regulates insulin action in the liver and thus G6PD activity, we fed rats various fat diets, and G6PD activity was assayed along with analysis of fatty acid constituents of phospholipid. Insulin response to G6PD activity was determined with primary cultured hepatocytes isolated from rats fed fat diets differing in PUFA/SFA ratios.

MATERIALS AND METHODS

Rats and diets. Weanling male Wistar rats (Charles River, Japan; body weight: 55±3 g) were housed in individual cages and maintained in a temperature-controlled room (20–22°C) with a controlled 12-h cycle of light and dark. In experiment 1, rats were fed 10% soybean protein diets, each of which contained 5, 10, or 20% of corn or coconut oil as shown in Table 1. In experiment 2, diets consisted of each of 20% soybean protein and casein, and contained 5% corn oil or

| Table 1. Composition of the diets. |
|-----------------------------------|
| Exp. 1                            |
| Soybean protein                   |
| 5% fat                           |
| 100                               |
| 10% fat                          |
| 100                               |
| 20% fat                          |
| 100                               |
| Casein                           |
| —                                |
| Fat                              |
| 50                               |
| 100                              |
| 200                              |
| Palmitic acid                    |
| —                                |
| —                                |
| —                                |
| Mineral mixture,1 50; vitamin mixture,1 10; choline chloride, 1.5; cellulose powder,2 20; and dextrin to 1 kg. |

All ingredients are given in g/kg diet. 1Mineral and vitamin mixtures of Harper's formula. 2Cellulose powder were purchased from Oriental Yeast Co. Ltd., Tokyo.

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1% corn oil plus 4% palmitic acid. Corn oil and palmitic acid were purchased from Katayama Chemical Ind. Co. Ltd., Japan, and coconut oil (Miyoshi Oil, Japan) were purchased commercially. Fatty acid composition of fats is shown in Table 2. Soybean protein isolate (SPI), kindly provided by Fuji Seiyu Co. Ltd., Osaka, Japan, and casein (NRC, U.S.A.) were used as protein sources. To the rats for cell culture, 20% protein diets of experimental 2 and a laboratory chow diet of CE-2, Japan Clea Co., Tokyo, were used. Diets and drinking water were given ad libitum.

**Enzyme assay.** Rats were sacrificed at 0800 to 1000 h under anesthetization with diethylether. The livers were homogenized in 5 vol. of the homogenizing buffer, 0.25 M sucrose solution containing 5 mM Tris-HCl buffer, pH 7.4, and 0.1 mM EDTA. The homogenates were centrifuged at 104,000 × g for 60 min, and the upper cytosol fraction was used for determination of G6PD activity by the method of Lee (16). The reaction mixture contained 0.1 M Tris-HCl, pH 8.0, 0.1 mM glucose 6-phosphate, and 1 mM NADP. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of glucose 6-phosphate per minute at 25°C. Protein was determined by the method of Lowry et al. (17), and enzyme activity was expressed as mU/mg protein.

**Insulin.** Serum was separated from blood taken from the femoral artery, and insulin concentrations in the serum were determined using an enzyme immunoassay with insulin-EIA test (Wako Pure Chemicals, Osaka, Japan). Insulin levels in the serum were expressed as mU/liter, as compared with human insulin as a standard.

| Fatty acid | Experiment 1 | Experiment 2 |
|-----------|--------------|--------------|
|           | Corn oil | Coconut oil | Corn oil plus palmitic acid<sup>2</sup> |
| 8:0<sup>1</sup> | 0.3 | 4.7 | — |
| 10:0 | 0.3 | 6.0 | — |
| 12:0 | 1.6 | 52.6 | 0.4 |
| 14:0 | 0.4 | 19.2 | 1.0 |
| 16:0 | 10.4 | 8.4 | 81.9 |
| 18:0 | 1.8 | 2.2 | 0.6 |
| 18:1 | 28.1 | 5.1 | 5.1 |
| 18:2 | 53.4 | 1.4 | 9.8 |
| 18:3 | 1.9 | — | 0.4 |
| PUFA | 55.3 | 1.4 | 10.2 |
| MUFA | 28.1 | 5.1 | 5.1 |
| SFA | 14.8 | 93.1 | 83.9 |
| P/S | 3.74 | 0.02 | 0.12 |

<sup>1</sup>Designations used: number of carbons in chain followed by number of double bonds. MUFA, monounsaturated fatty acid. <sup>2</sup>Corn oil plus palmitic acid mixture: 1 g corn oil added to 4 g palmitic acid. Composition of fatty acid (%) of palmitic acid commercially obtained: 14:0 = 1.1, 16:0 = 98.0, and 18:0 = 0.7.
Cell culture. Parenchymal cells were isolated by in situ perfusion of the liver with collagenase according to Seglen's method modified by Tanaka et al. (18). The cells were plated at $2 \times 10^6$ cells/60 mm plastic dish in Williams medium E (WE medium) containing 5% newborn calf serum and were cultured as monolayers at 37°C in 95% air and 5% CO$_2$. After a 1-day culture, the cells were incubated for 48 h in WE medium containing $10^{-6}$M of dexamethasone, 0.1 μg/ml aprotinin, 30 μg/ml kanamycin, and insulin at $10^{-10} - 10^{-6}$M.

Lipids. A portion of the liver (0.5 g), isolated hepatocytes (50–100 μg protein) or plasma membrane was homogenized in chloroform/methanol (2/1 = v/v). Plasma membrane was prepared from the liver homogenates by sucrose discontinuous gradients (19). Lipids were extracted by the method of Folch et al. (20). Phospholipid was separated by thin-layer chromatography on a silica gel 60 F$_{254}$ plate with fluorescent indicator (Merck, Germany). Methylation of fatty acids of phospholipid was performed with a borofluorate-methanol mixture (21). Fatty acids were analyzed by Shimadzu gas chromatograph equipped with a 5% Shinchrom E 71 column (3.2 mm × 3.1 m). Triglyceride concentration in the liver was assayed with Triglyceride-Test (Wako Pure Chemicals, Osaka, Japan) principally based on Fletcher’s method (22).

Chemicals. Glucose 6-phosphate was purchased from Boehringer Mannheim GmbH, and NADP was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Other chemicals were of reagent grade.

Statistical analysis. The data were analyzed by Duncan's multiple range test or paired test using ANOVA, and differences associated with $p < 0.05$ were considered statistically significant.

RESULTS

After feeding rats the various diets for 3 weeks, growth rate, triglyceride content in the liver, G6PD activity, and serum insulin levels were determined (Table 3). Growth rate, expressed as body weight gain, was reduced in 20% fat diets in comparison with each of 5 and 10% fat diets, respectively. Liver triglyceride content varied between fats; the content elevated significantly in a 20% corn oil diet, but increase was slight in 20% coconut oil diet, in comparison with respective 5% fat diet. G6PD activity differed greatly between fat diets, regardless of fat levels in diets, body weight gain, liver triglyceride content, or serum insulin levels. Compared with corn oil diets, G6PD activity was elevated greatly in the case of all the coconut oil diets.

Fatty acid composition in liver phospholipid of the rats fed 5% corn and coconut oil diets is shown in Table 4. Increases in n-9 fatty acids, palmitoic and oleic acids, and in n-3 fatty acid, docosahexaenoic acid, and decreases in n-6 fatty acids, linoleic and docosatetraenoic acids, were seen in cases of coconut oil diets. There was no significant decrease in eicosatetraenoic acid in coconut oil diet, in comparison with corn oil diet; this might be due to short duration of feedings.
Table 3. Effects of dietary fats on liver triglyceride and glucose-6-phosphate dehydrogenase activity and serum insulin levels.

| Dietary fat | Body weight gain (g/3 weeks) | Liver (g) | Triglyceride (mg/g liver) | G6PD activity (mU/mg protein) | Insulin (mU/liter serum) |
|------------|-----------------------------|-----------|--------------------------|------------------------------|--------------------------|
| Corn oil   |                             |           |                          |                              |                          |
| 5%         | 45.7±8.8 a                  | 4.4±0.3 a | 25.1±4.2 a               | 62±10 a                      | 33.4±12.5 a^1            |
| 10%        | 40.2±1.5 a                  | 4.3±0.4 ac| 26.6±5.1 a               | 65±7 a                       | —                        |
| 20%        | 25.5±5.2 b                  | 3.5±0.4 bc| 48.2±6.4 b               | 45±8 a                       | —                        |
| Coconut oil|                             |           |                          |                              |                          |
| 5%         | 40.2±6.7 a                  | 4.5±0.4 a | 18.4±2.8 ac              | 132±25 c                     | 25.9±13.8 a^1            |
| 10%        | 30.2±8.1 a                  | 4.2±0.5 ac| 28.2±4.8 a               | 143±32 c                     | —                        |
| 20%        | 22.3±5.7 b                  | 3.9±0.4 bc| 34.2±8.3 ad              | 140±36 c                     | —                        |

Values are M±SD of 8 rats in each group. Weanling rats were fed corn or coconut oil diets described in Table 1 as experiment 1 diets for 3 weeks. ^1 Insulin was determined with 5 rats. Different superscript letters in the same vertical lane denote significant differences between groups at p<0.01.

Table 4. Fatty acid composition of phospholipid in rat livers.

| Fatty acid | Corn oil | Coconut oil |
|------------|----------|-------------|
| 14:0       | 0.3±0.0  | 1.0±0.1*    |
| 16:0       | 20.4±1.1 | 20.7±1.5   |
| 16:1 (n-9) | 1.2±0.1  | 2.6±0.0*    |
| 18:0       | 22.0±0.3 | 23.5±0.7*   |
| 18:1 (n-9) | 6.5±0.3  | 11.6±0.6*   |
| 18:2 (n-6) | 12.8±0.8 | 6.9±0.1*    |
| 20:2 (n-6) | 0.7±0.2  | 1.8±0.5*    |
| 20:3 (n-6) | 1.7±0.2  | 1.3±0.1     |
| 20:4 (n-6) | 20.5±0.6 | 19.6±1.2   |
| 22:4 (n-6) | 2.0±0.3  | 0.5±0.1*    |
| 22:5 (n-6) | 6.3±0.1  | 1.9±0.7*    |
| 22:6       | 4.2±0.2  | 6.8±0.0*    |

P/S 1.15 0.84

Values (percent of the total fatty acids) are M±SD of 4 rats fed a 10% soybean with 5% respective fat diet of experiment 1 in Table 3. Fatty acids more than 0.5% were described in this table, but calculation for P/S ratio was made with all detected fatty acids more than 0.1%. ^* Statistically different in a coconut oil diet from a corn oil diet at p<0.05.

PUFA/SFA ratio in liver phospholipid was decreased in a coconut oil group, in which G6PD activity was elevated. PUFA/SFA ratios in 10 and 20% fat diets were similar to respective 5% fat diet. PUFA/SFA ratios of 10% corn and coconut oil diets were 1.28, and 0.92, and those of 20% corn and coconut oil diets were 1.29, and 1.00, respectively (data not shown in Table 4). There was an inverse correlation between G6PD activity and PUFA/SFA ratios of liver phospholipid.
Fig. 1. Relationship between G6PD activity and PUFA/SFA in phospholipid of rat liver. Rats were fed on each diet of 5, 10, and 20% of corn and coconut oils, and G6PD activity and fatty acid composition of phospholipid in livers were analyzed respectively. ○, 5% corn oil; △, 10% corn oil; ●, 20% corn oil; ◯, 5% coconut oil; ▲, 10% coconut oil; ▲, 20% coconut oil.

among all the groups (Fig. 1).

In the second experimental diet, PUFA/SFA ratio of corn oil plus palmitic acid was at 0.81, the value being reduced to that of coconut oil, and G6PD activity was greatly elevated by palmitic acid fortification in either soybean protein or casein diet (Table 5). In contrast to the enzyme activity, growth rate and triglyceride content were not significantly affected by adding palmitic acid to corn oil. After a diet of 1% corn oil plus 4% palmitic acid, fatty acid composition in phospholipid of the liver changed similar to that of coconut oil feeding; oleic acid increased and linoleic acid decreased. However, palmitic acid was increased from 20.5 to 23.5% by fortifying palmitic acid, and PUFA/SFA in liver phospholipid of rats fed a 10%

Table 5. Effects of dietary fats on liver glucose-6-phosphate dehydrogenase activity.

| Diet | Body weight gain (g/3 weeks) | Liver (g) | Triglyceride (mg/g liver) | G6PD activity (mU/mg protein) | Insulin (mU/liter serum) |
|------|-----------------------------|-----------|--------------------------|-------------------------------|-------------------------|
| 20% SPI A | 128.4±15.2 a | 6.8±0.9 a | 7.4±1.5 a | 40±8 a | 21.2±6.4 a |
|   | B | 135.1±14.6 a | 6.4±0.7 a | 6.9±1.2 a | 114±34 b | 26.1±8.5 a |
| 20% casein A | 141.3±12.1 a | 7.2±0.9 a | 6.8±1.1 a | 61±15 a | 28.6±7.6 a |
|   | B | 149.5±12.3 a | 6.8±0.6 a | 7.1±1.3 a | 188±28 c | 26.6±6.4 a |

Values are M±SD of 8 rats. To each of a 20% SPI (soybean protein isolate) or casein diet, a fat of A (5% corn oil) or B (1% corn oil plus 4% palmitic acid) was added, and rats were fed respective diet for 3 weeks. Different superscript letters in the same vertical lane denote significant differences between groups at p<0.01.

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Table 6. Fatty acid composition of phospholipid of liver plasma membrane prepared from rats fed 5% corn oil or 1% corn oil plus 4% palmitic acid diets.

| Fatty acid | 20% soybean protein | 20% casein |
|-----------|---------------------|------------|
|           | 5% corn oil | 1% corn oil +4% palmitic acid | 5% corn oil | 1% corn oil +4% palmitic acid |
| 14:0      | 1.5       | 1.4       | 1.9       | 1.6       |
| 16:0      | 18.5      | 19.4      | 20.7      | 19.4      |
| 16:1 (n-9)| 1.4       | 2.8*      | 1.0       | 3.0*      |
| 18:0      | 19.3      | 18.7      | 20.2      | 20.3      |
| 18:1 (n-9)| 7.9       | 11.8*     | 9.2       | 12.5*     |
| 18:2 (n-6)| 13.7      | 7.8*      | 7.7       | 5.9*      |
| 20:3 (n-9)| 1.5       | 1.1       | 0.9       | 1.5       |
| 20:4 (n-6)| 24.2      | 25.5      | 26.0      | 24.3      |
| 20:5 (n-3)| 0.9       | 0.9       | 0.4       | 1.0       |
| 22:3      | 1.6       | 0.6*      | 1.0       | 0.5*      |
| 22:4 (n-6)| 3.8       | 3.0       | 6.7       | 4.7       |
| 22:5 (n-3)| 0.9       | 0.6       | 0.2       | 0.2       |
| 22:6      | 4.2       | 5.9*      | 3.5       | 3.6       |

P/S  1.29  1.15  1.11  1.01

Values are mean of each fatty acid of the liver plasma membrane phospholipid prepared from each dietary group of 3 rats described in Table 5. Others are the same as in Table 4. *Significantly different at least p<0.05 between corn oil and corn oil plus palmitic acid in each protein diet.

soybean protein diet decreased to 0.81 (data not shown).

In experiment 2, plasma membrane was fractionated from liver homogenates and its fatty acid composition of phospholipid was analyzed. The composition pattern was nearly the same as that of the whole liver (Table 6). A slight difference of the composition was observed between soybean protein and casein. In 10% casein and soybean protein diets, PUFA/SFA ratios of liver plasma membrane of rats on 5% corn oil diets were 1.32 and 1.23, and were 1.08 and 1.11 on 1% corn oil plus 4% palmitic acid, respectively (data not shown). At 20% protein, PUFA/SFA ratio of phospholipid of plasma membrane was slightly higher in soybean protein than in casein either in a corn oil or in palmitic acid-fortified diet and G6PD activity was slightly high in casein diets (Tables 5 and 6). On a palmitic acid-fortified diet, G6PD activity increased similarly to the rats on a coconut oil diet in which saturated fatty acid and medium chain fatty acid were highly contained. There was an inverse relationship between G6PD activity and PUFA/SFA ratio in phospholipid of liver plasma membrane prepared from rats fed diets of soybean protein and casein, which contained 5% corn oil or 1% corn oil plus 4% palmitic acid, respectively (Fig. 2). In the case of the palmitic acid-fortified diets, polyunsaturated fatty acid decreased and saturated fatty acid increased in phospholipid of plasma membrane as did those of the whole liver. Serum insulin levels did
Fig. 2. Relationship between G6PD activity and PUFA/SFA in phospholipid of liver plasma membrane. Rats were fed on 5% corn oil or 1% corn oil plus 4% palmitic acid added to each 20% casein and soybean protein diet. ○, 5% corn oil in casein; □, 1% corn oil plus 4% palmitic acid in casein; ■, 5% corn oil in soybean protein; ◇, 1% corn oil plus 4% palmitic acid in soybean protein diet.

Fig. 3. Insulin dose-response to G6PD activity of primary cultured hepatocytes. Hepatocytes were prepared from rats which were fed respective diet for 3 weeks. ○, 5% corn oil in casein; □, 1% corn oil plus 4% palmitic acid in casein; ■, 5% corn oil in soybean protein; ◇, 1% corn oil plus 4% palmitic acid in soybean protein; ▲, laboratory chow diet. Error bar denotes ±SD of the mean of 3 animals. *p<0.05, **p<0.01.
not statistically differ by fat types, and did not directly relate with liver G6PD activity.

In primary cultured hepatocytes, insulin dose-response to G6PD activity was greatly augmented in hepatocytes isolated from rats fed a 1% corn oil plus 4% palmitic acid diet in comparison with those fed a 5% corn oil diet, regardless of the type of dietary protein, casein and soybean protein (Fig. 3). Insulin dose-response of hepatocytes prepared from rats fed 5% corn oil was similar to that of the hepatocytes from rats fed a laboratory chow diet.

DISCUSSION

Liver G6PD activity was regulated by amount of carbohydrate intake (1). In this experiment, effects of fats on enzyme activity were studied with different type of 10 and 20% protein levels. G6PD activity was slightly high in 10% soybean protein diet (76.95% dextrin) of experiment 1, in comparison with that of 20% soybean protein diet (66.95% of dextrin) of experiment 2. However, G6PD activity was affected predominantly by type of fats. By feeding rats on coconut oil (experiment 1) or corn oil fortified with palmitic acid (experiment 2), liver G6PD activity increased greatly in comparison with feeding corn oil regardless of dietary fat levels or protein type.

In coconut oil, medium chain fatty acids were contained in high proportion, while these fatty acids were contained only in small quantities in a palmitic acid-fortified diet. When rats were fed on a palm oil diet, in which oleic acid was highly contained at 42%, PUFA/SFA ratio in liver phospholipid was at 1.05 (1.03–1.07), and G6PD activity increased as well as that of rats fed a coconut oil diet (data not shown). On the other hand, G6PD activity of rats fed a 5% soybean oil (7% linolenic acid (n-3)) was nearly the same as those of the rats fed a corn oil diet (data not shown). It was reported that polyunsaturated fats were lacking in specificity for diminishing effects on elevating G6PD activity when fasted rats were refed fat diets (23). The present findings together with the previous report indicate that an elevating effect of dietary fat on G6PD activity is attributable neither to the medium chain fatty acids, oleic acid (n-9), nor to a specific polyunsaturated fat, but to high content of saturated fatty acids and low content of polyunsaturated fatty acids which result in lowering the PUFA/SFA ratio of phospholipid in liver plasma membrane and augment insulin response to G6PD activity.

Hepatocytes isolated from rats fed menhaden oil showed a greater affinity for insulin than hepatocytes from rats fed hydrogenated coconut oil (12), and the present results of a lowering effect of polyunsaturated fatty acids on insulin response seem to be inconsistent. In adipocytes from BHE rats, dietary beef tallow oil enhanced glucose metabolism (24), and insulin stimulation of glucose metabolism occurred in rats when safflower oil was replaced with edible tallow oil (25). These findings of insulin stimulation by saturated fats were consistent with the present results of elevation of insulin response by high saturated fat diets.

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By addition of 0.1 mM of linoleic acid or arachidonic acid to WE medium, G6PD-mRNA synthesis was suppressed in primary cultured hepatocytes, while it was affected only slightly by palmitic acid (26). Suppressing effect of polyunsaturated fatty acids in mRNA synthesis may be explained by the present findings to be due to lowering insulin response by elevating the PUFA/SFA in membrane phospholipid. In our experiment, fatty acid composition of phospholipid of cultured hepatocytes changed only slightly during incubation for 3 days in WE medium, in which 0.1 μM of methyl linoleate was contained (data not shown). However, at exceedingly high concentration of fatty acids added to the medium in the above experiment (26), fatty acid composition in the hepatocytes could be affected and could reflect upon PUFA/SFA ratio of membrane phospholipid, though a direct stimulating effect of fatty acids on mRNA synthesis is undeniable.

In contrast to elevation of G6PD activity, which provides NADPH for lipid synthesis, liver triglyceride was not increased in rats fed high saturated fatty acid diets. However, triglyceride content in the liver was lower in 20% protein diets than that of each 10% protein diet regardless of fat type (Tables 3 and 5). Thus, dietary protein levels are more responsible for accumulation of triglyceride in the liver than oil types that associated with NADPH synthesis.

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