< Original treatise >

**Hen-egg suppresses fatty liver induced by sugar,
glucose or fructose.**

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease in the world. Excess fructose and sugar intake as well as high fat diet have been shown to be major risk factors for this disease. Chicken egg (egg) is a conventional food that contains every nutrient required for the growth of the chicken embryo. Eggs contain large amounts of important proteins and fat, a very small amount of carbohydrates, and all vitamins and minerals needed for development of the chick. A high sugar, glucose or fructose diet has been shown to cause fatty liver by inducing biosynthesis of glycerol and fatty acid from glucose or fructose. We analyzed the effects of eggs on the development of fatty liver induced by a high sugar, glucose or fructose diet. Feeding an egg-only diet for 4 weeks to adult C57BL/6 female mice did not induce fatty liver. In contrast, 2 to 4 weeks feeding of a sugar-, glucose- or fructose- only diet induced overt NAFLD with a large number of vacuoles observed in hepatocytes, associated with high serum level of ALT and AST. Adding boiled eggs or raw egg yolk in water to glucose- or fructose- only diet markedly suppressed NAFLD. One week raw egg yolk-only diet reversed the NAFLD caused by one week sugar-only diet. Serum ketone bodies and cholesterol levels were greatly increased in mice fed an egg-only diet. Adding raw egg yolk to sugar or changing to egg from sugar diet increased ketone bodies and cholesterol levels.

Key words: egg-only diet; mice; low carbohydrates; NAFLD; fructose; sugar

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease and has the prevalence of 25–30% worldwide including western countries, Japan and China¹⁻⁴⁾. The highest prevalence of NAFLD occurs in the Middle East and South America⁵⁾.

NAFLD is diagnosed histologically as accumulation of fat in >5 % of hepatocytes⁵⁾. Although the incidence of NAFLD ranges between 75 % –100 % in obese individuals⁶⁾, simple steatosis designated as non-alcoholic fatty liver (NAFL) is relatively benign. However, serious consequences of NAFLD is progression to non-alcoholic steatohepatitis (NASH), characterized by liver injury caused by hepatocyte ballooning, inflammation, and/or fibrosis. These changes may proceed to liver cirrhosis and hepatocellular carcinoma (HCC)⁷⁻⁹⁾. Another important point is that NAFLD has a close association with the metabolic syndrome and type 2 diabetes mellitus (T2DM)¹⁰⁾. The condition is also a significant independent risk factor for cardiovascular disease¹¹⁾. Although the pathogenesis of NASH from NAFL has not yet been determined, many scientists from basic and clinical field have carried out extensive research on the mechanism of the transitions from NAFL to NASH

and NASH to liver cirrhosis and HCC. However, pathogenesis of NAFLD is not simply caused by obesity. In this report we concentrate the pathogenesis of NAFLD.

NAFLD is thought to be initiated by deposition of fat in the liver and develops when the influx of lipids into the liver exceeds hepatic lipid disposal by fatty acid oxidation and production of very low-density lipoprotein (VLDL)^{12,13)}. The influx of lipids include fatty acids released from peripheral adipose tissue, dietary fatty acids from ingestion of fat as chylomicrons. There is currently interest in de novo lipogenesis (DNL), in which lipids are synthesized from carbohydrates. Intake of sucrose (a disaccharide of fructose and glucose) and high-fructose corn syrup (HFCS) has increased markedly recently, a dietary change that may lead to the development of metabolic syndrome with NAFLD^{14,15)}.

Chicken egg (egg) is a conventional food that contains every nutrient needed for the normal growth of the chicken embryo. Eggs contain large amounts of important proteins and fat, a very low amount of carbohydrates, and all the vitamins and minerals needed for chick development. However, excess eggs consumption has been suggested to be dangerous for health, because they contain large amount of lipid, and a high

fat diet is associated with NAFLD.

This study analyzed the effects of eggs on high sugar, glucose or fructose-induced NAFLD by feeding mice a range of diets, each containing only one of these nutrient.

2. Materials and methods

2-1. Mice

Five-week-old C57BL/6 (C57BL/6n; B6) mice were purchased from SLC Japan. They were maintained on a normal diet (ND; CLEA Rodent Diet CE-2) under 12-hour light and dark cycles and specific pathogen-free conditions in the Animal Research Facility at the Nagoya Women's University. Care of the mice was carried out according to institutional guidelines. All mice were housed with up to 5 mice per cage and ad libitum access to diet and tap water. The Animal Care and Use Committee of Nagoya Women's University approved the study protocol.

2-2. Mouse diets and feeding

The CLEA Rodent Diet CE-2 was the ND; it contains 884% water, 25.48% protein, 4.61% fat, and 61.07% carbohydrate and essential vitamins and minerals. The energy content of 100 g of CE-2 is 339.1 kcal (Chubu Kagaku, Japan). The sugar, glucose and fructose diet were dissolved in PBS. Hen eggs (eggs) obtained from firms in Aichi, Japan were used to

prepare the egg-only diet. Eggs were boiled and they were put into each cage. The nutritional analysis of the boiled eggs was performed at the Japan Food Research Laboratories (Nagoya, Japan) that demonstrated the eggs had a high water content (75.6 g/100 g), and 12.9 g/100 g protein, 9.6 g/100 g fat and 1.1 g/100 g carbohydrate and vitamins and minerals. This corresponded to 36.1% of calories (E) from protein, 60.8% E from lipid and 3.1% E from carbohydrates. Block sugar was obtained from a supermarket in Nagoya and D (+) glucose and D (–) fructose from Nakarai, Japan, Kyoto. The sugars were dissolved in water or PBS (+). MEM vitamins and MEM amino acids were purchased from Sigma-Aldrich.

2-3. Histological analysis

Liver, small intestine and other tissues was fixed in 4% paraformaldehyde buffered with PBS (pH 7.4) and blocked in paraffin. Paraffin-embedded 4- μ m sections were stained with hematoxylin for 5 minutes and with eosin for 3 minutes. In order to detect glycogen, liver sections were stained with PAS.

2-4. Biochemical analysis

Serum samples were obtained from the mice after 1 hour starvation. Serum glucose, ketone bodies, and the liver marker enzymes alanine transaminase

(ALT) and aspartate transaminase (AST) were measured by enzymatic colorimetric assays. Serum levels of ALT and AST were calculated by the measurement of pyruvate (555 nm) using WAKO assay kits (Wako Pure Chemicals, Osaka, Japan) and blood glucose by the LaboAssay™ Glucose kit (Wako Pure Chemicals). Ketone bodies, including total ketone bodies (T-KB) and 3-hydroxybutyrate (3-HB) were measured by AUTO WAKO Total Ketone Bodies and 3-HB, respectively (Wako Pure Chemicals). Total cholesterol, HDL cholesterol and triglycerides (TG) were measured using the Wako E test, and free fatty acids (FFA) (non-esterified fatty acids; NEFA) by the NEFA C test (Wako Pure Chemicals, Osaka, Japan). Wavelengths in the assay solution were measured using an xMark™ Microplate Absorbance Spectrophotometer (Bio Rad).

2-5. Statistical analyses

Data are expressed as means $\pm$ standard deviations (SD). Statistical comparisons were performed using Student t-test or a one-way analysis of variance (ANOVA) and Tukey-Kramer test. P-values < 0.05 were considered statistically significant.

3. Results

3-1. The mice fed only sugar without fat developed severe NAFLD within 1 week, whereas the mice fed only egg containing 60% E fat did not develop NAFLD.

As a preliminary experiment, we compared the mice fed only block sugars with mice fed only boiled egg freely for 4 weeks (Fig. 1a). We found that the body weights of the egg-only mice were increased slightly, whereas the body weights of the mice fed only block sugars decreased (Fig. 1b). The mice fed only block sugars looked smaller than the mice fed only boiled egg (Fig. 1c). Autopsy at 4 weeks showed the mice fed only boiled egg have abdominal fat, whereas the mice fed only block sugars have no abdominal fat (Fig. 1c). The levels of blood transaminases ALT and AST in mice fed this diet for 1 or 2 weeks were considerably higher at both these time points than in mice fed only eggs (Fig. 1d). These results suggested severe NAFLD had developed in the mice fed sugar-only diet. Unexpectedly, the serum glucose levels of mice fed only sugar was about half of those of the mice fed egg-only diet (Fig. 1e). As expected, the levels of serum ketone bodies (T-KB and 3-HB) of the mice fed only eggs were considerably higher than those of the mice fed sugar (Fig. 1f).

Figure 1:
Induction of NAFLD by block sugar but not egg-only diet.

(a) Five-week-old C57BL/6 (C57BL/6n; B6) female mice were fed freely for 4 weeks only block sugars or only boiled eggs. (b) Body weights of mice fed block sugar or eggs were measured at 1, 2, 3, and 4 weeks of feeding. (c) Photographs were taken before and after opening the abdomen at 2 or 4 weeks. (d) Liver tissues removed at 2 or 4 weeks were fixed in 4% paraformaldehyde and blocked in paraffin. Paraffin-embedded 4-μm sections were stained with hematoxylin and eosin and examined by Olympus microscopy. (e) Levels of the liver marker enzymes, alanine transaminase and aspartate transaminase, measured at 4 weeks. (f) Serum glucose levels were measured at 4 weeks. (g) Serum ketone bodies (T-KB and 3-HB) levels were measured at 4 weeks. The data are expressed as means $\pm$ standard deviations. Statistical comparisons were performed using the Student T-test. P-values < 0.05 were considered statistically significant. *p-values < 0.05 **p-values < 0.01.

3-2. Egg suppresses NAFLD induced by glucose or fructose-only diet.

We hypothesized that egg may suppress NAFLD and therefore investigated the effects of egg on the changes induced by the glucose or fructose only diet (Fig. 2a). In this experiment we used 30 % glucose or fructose dissolved in 1xPBS instead of water. The mice needed to drink these water-soluble diets, because they were provided neither water nor other diets. The body weights of the mice fed only glucose or fructose decreased, but increased in mice fed the glucose or fructose diet combined with egg. (Fig. 2b). At 2 weeks in the experiments, the mice fed only glucose or fructose looked small (Fig. 2c), and the liver of these

animals appeared whitish (Fig. 2c). The weights of the liver, spleen, thymus, lung, kidney and intestine of the mice fed only glucose or fructose were all lower than those of the mice fed only egg or CE-2. The weights of these organs of mice fed the egg and glucose or fructose diet were similar to those of the mice fed only egg or CE-2. (Table 1). Liver histology showed that many vacuoles appeared at 2 weeks in the liver of the mice fed glucose- or fructose-only diet. However, the liver of the mice with egg added to the glucose or fructose diet had an almost normal pattern, similar to that seen with CE-2 or egg only diet (Fig. 2d). In order to discriminate glycogen and TG deposition, we stained

Fig.2a

Fig.2b

Fig.2c

the liver sections using PAS method. Fine vacuoles in the liver stained by PAS indicate glycogen deposition. The liver of the mice fed only glucose was strongly stained with PAS, although

many big vacuoles remained. A similar finding was found in mice fed only fructose, although the staining intensity with PAS was lower. The liver of mice fed egg added to the glucose or fructose

Fig.2d

Fig.2e

Fig.2f

Fig.2g

Figure 2: Egg suppresses NAFLD induced by glucose or fructose-only diet.

(a) Five-week-old B6 female mice were fed for 2 weeks with 1) only 30% glucose, 2) only 30% fructose, 3) 30% glucose with boiled egg, 4) 30 % fructose with boiled egg, 5) boiled egg only, and 6) CE-2. (b) Body weights of each group were measured at 1 and 2 weeks (n=5) . (c) Photographs taken before and after opening the abdomen at 2 weeks. (d) Liver tissues removed at 2 or 4 weeks were fixed in 4% paraformaldehyde and blocked in paraffin. Paraffin-embedded 4-μm sections were stained with hematoxylin and eosin or PAS staining and examined by Olympus microscopy with pictures taken. (e) Serum glucose levels measured at 2 weeks. (f) Serum ketone bodies (T-KB and 3-HB) levels measured at 2 weeks. (g) Serum cholesterol levels were measured at 2 weeks. The data are expressed as means $\pm$ standard deviations. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. *P*-values <0.05 were considered statistically significant. **p*-values <0.05 ***p*-values <0.01.

Table 1

Tissue weight	30% Glucose	30% Fructose	30% Glucose + Egg	30%Fructose + Egg	Egg	CE-2
Liver	0.532 ± 0.050 ^a	0.654 ± 0.042 ^{c,e}	1.115 ± 0.160 ^{b,f}	1.008 ± 0.070 ^{b,d}	1.048 ± 0.056 ^{b,f}	1.003 ± 0.028 ^{b,d}
Heart	0.102 ± 0.008	0.080 ± 0.003 ^a	0.098 ± 0.011	0.134 ± 0.021 ^b	0.107 ± 0.005	0.107 ± 0.005
Lung	0.126 ± 0.011	0.130 ± 0.013	0.141 ± 0.010	0.178 ± 0.010	0.168 ± 0.025	0.153 ± 0.014
Kidney	0.176 ± 0.009 ^a	0.169 ± 0.011 ^a	0.237 ± 0.012 ^{b,c}	0.302 ± 0.020 ^{b,d}	0.316 ± 0.014 ^b	0.285 ± 0.010 ^b
Spleen	0.041 ± 0.004 ^a	0.025 ± 0.002 ^a	0.083 ± 0.009 ^b	0.085 ± 0.013 ^b	0.084 ± 0.005 ^b	0.078 ± 0.005 ^b
Stomach	0.131 ± 0.022 ^b	0.166 ± 0.016 ^b	0.163 ± 0.007 ^b	0.161 ± 0.015 ^b	0.158 ± 0.009 ^b	0.322 ± 0.030 ^a
Intestine	0.981 ± 0.082 ^b	0.992 ± 0.088 ^b	1.656 ± 0.130 ^a	1.839 ± 0.134 ^a	1.901 ± 0.145 ^a	2.014 ± 0.119 ^a
Thymus	0.038 ± 0.006 ^c	0.025 ± 0.005 ^{a,c,g}	0.062 ± 0.016	0.082 ± 0.010 ^b	0.066 ± 0.008 ^d	0.072 ± 0.008 ^h

^{a, b}*p* < 0.01, ^{c, d}*p* < 0.05, ^{e, f}*p* < 0.01, ^{g, h}*p* < 0.05

Five-week-old B6 female mice were fed for 2 weeks with 1) only 30 % glucose, 2) only 30 % fructose, 3) 30 % glucose with boiled egg, 4) 30% fructose with boiled egg, 5) boiled egg only, and 6) CE-2. Organ weights from each group of the mice were measured. Data are expressed as the means ± standard deviation. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. P-values < 0.05 were considered statistically significant. (a,b) (e,f) *p*-values < 0.01, (c, d) (g,h) *p*-values < 0.05.

diet was not stained with PAS, similar to that observed with the CE-2 or egg-only diet. These results indicate that the mice fed only glucose or fructose had a high level of liver cell damages caused by excessive accumulating of TG. The addition of egg reversed this liver cell damage caused by fat accumulation (Fig. 2d).

The serum glucose levels were also examined. The glucose levels in the egg-only diet were almost the same as that with the CE-2 diet, while levels in mice fed only glucose or fructose were lower than those fed egg-only or CE-2 diet. When eggs were added to the glucose or fructose diets, the serum glucose levels increased (Fig. 2e). T-KB and 3-HB levels were considerably higher in the mice fed egg-only than those

receiving the CE-2 or glucose-only diets. T-KB levels were also high in mice fed fructose-only (Fig. 2f). Although total cholesterol levels were almost the same in mice fed the glucose- or fructose-only diets as those of CE-2 die, total cholesterol level was much higher in egg-only mice than that in CE-2 mice. Adding eggs to the glucose or fructose diet increased the total cholesterol level (Fig. 2g).

2-3. Egg yolk added in glucose or fructose bottle suppresses fatty liver formation.

To support the metabolism of glucose or fructose we added vitamins to the bottles containing 30% glucose or fructose. One group of mice had raw egg yolk added to these diet, while had another

group amino acids added (Fig. 3a). At 2 weeks only the mice with the added egg yolk showed increase in weight, while the other group had a reduction in weights (Fig. 3b). At autopsy, every mouse had a whitish liver (Fig. 3c). The weight of every organ was higher in mice fed the diet supplemented with egg yolk than that measured in all the other groups (Table 2). However, the weight of organ/ body in each group

was not so different (Table 3). Liver histology showed many vacuoles in the hepatocytes of the mice fed glucose, fructose or sugar. However, the number of vacuoles was greatly reduced by the addition of egg yolk (Fig. 3d). The liver vacuoles of mice fed a sugar diet were stained strongly by PAS indicating storage of glycogen. Some vacuoles in the liver of glucose fed mice were stained, whereas vacuoles of the mice

Fig.3a

Fig.3b

Fig.3c

Fig.3d

Figure 3: Egg yolk added to the glucose or fructose bottle suppresses development fatty liver.

(a) Five-week-old B6 female mice were fed 1) 30% glucose with vitamins, 2) 30% fructose with vitamins, 3) 30% sugar with vitamins, 4) 30% sugar with vitamins and low egg yolk, 5) 30% sugar with vitamins and amino acids. (b) Body weights of each group were measured at 1 and 2 weeks (n=5). (c) Photographs taken before and after opening the abdomen at 2 weeks. (d) Liver tissues removed at 2 or 4 weeks were fixed in 4% paraformaldehyde and blocked in paraffin. Paraffin-embedded 4- μm sections were stained with hematoxylin and eosin or PAS staining. They were observed by Olympus microscopy and pictures were taken. (e) The levels of liver marker enzymes alanine transaminase and aspartate transaminase were measured at 2 weeks. (f) Serum glucoses were measured at 2 weeks. (g) Serum ketone bodies (T-KB and 3-HB) were measured at 2 weeks. Data are expressed as means $\pm$ standard deviations. Statistical comparisons were performed by a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. P-values < 0.05 were considered statistically significant. *p-values < 0.05 **p-values < 0.01.

Table 2

Tissue weight (g)	30% Glucose	30% Fructose	30% Sugar	30% Sugar+ egg	30% Sugar+ AA
Liver	0.702 ± 0.006 ^{b,c}	0.694 ± 0.020 ^{b,d}	0.839 ± 0.058 ^b	1.181 ± 0.074 ^a	0.797 ± 0.049 ^b
Heart	0.082 ± 0.004	0.074 ± 0.004	0.081 ± 0.003	0.086 ± 0.009	0.072 ± 0.003
Lung	0.183 ± 0.010	0.149 ± 0.006	0.126 ± 0.005 ^d	0.210 ± 0.047 ^c	0.129 ± 0.006 ^d
Kidney	0.180 ± 0.008 ^b	0.188 ± 0.005 ^b	0.181 ± 0.008 ^b	0.258 ± 0.019 ^a	0.185 ± 0.011 ^b
Spleen	0.050 ± 0.002 ^b	0.032 ± 0.005 ^b	0.041 ± 0.003 ^b	0.073 ± 0.008 ^a	0.037 ± 0.004 ^b
Stomach	0.142 ± 0.008	0.154 ± 0.016	0.147 ± 0.008	0.173 ± 0.017	0.136 ± 0.008
Intestine	0.832 ± 0.030 ^b	0.974 ± 0.084	0.827 ± 0.026 ^b	1.158 ± 0.132 ^a	0.913 ± 0.055
Thymus	0.022 ± 0.003 ^d	0.011 ± 0.003 ^b	0.021 ± 0.008 ^d	0.047 ± 0.011 ^{a,c}	0.020 ± 0.002 ^d

a, b $p < 0.01$, c, d $p < 0.05$

Five-week-old B6 female mice were fed 1) 30 % glucose with vitamins, 2) 30 % fructose with vitamins, 3) 30 % sugar with vitamins, 4) 30 % sugar with vitamins and low egg yolk, 5) 30 % sugar with vitamins and amino acids. Organ weights from each group of the mice were measured at 2 weeks. Data are expressed as the means ± standard deviation. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. P -values < 0.05 were considered statistically significant. (a, b) p -values < 0.01 , (c, d) p -values < 0.05 .

Table 3

Tissue weight (g/100g B.W)	30% Glucose	30% Fructose	30% Sugar	30% Sugar+ egg	30% Sugar+ AA
Liver	4.920 ± 0.211 ^c	5.183 ± 0.187	5.361 ± 0.384	5.593 ± 0.272	5.860 ± 0.563 ^d
Heart	0.571 ± 0.040 ^d	0.555 ± 0.043	0.521 ± 0.029	0.409 ± 0.033 ^c	0.612 ± 0.048 ^d
Lung	1.288 ± 0.122 ^c	1.109 ± 0.085	0.805 ± 0.068 ^d	1.001 ± 0.191	1.752 ± 0.069
Kidney	1.255 ± 0.038	1.401 ± 0.053	1.157 ± 0.118	1.227 ± 0.105	1.752 ± 0.132
Spleen	0.353 ± 0.024	0.240 ± 0.034	0.260 ± 0.021	0.352 ± 0.046	0.215 ± 0.031
Stomach	0.991 ± 0.040	1.153 ± 0.133	0.945 ± 0.069	0.830 ± 0.101	0.901 ± 0.141
Intestine	5.830 ± 0.324	7.291 ± 0.672	5.298 ± 0.295	5.527 ± 0.720	6.281 ± 0.635
Thymus	0.156 ± 0.024	0.084 ± 0.020	0.129 ± 0.053	0.225 ± 0.049	0.174 ± 0.028

a, b $p < 0.01$, c, d $p < 0.05$

Organ weights from each group of the mice (Table 2) were divided with 100x body weights of the same group (Fig.3b). Data are expressed as the means ± standard deviation. Statistical comparisons were performed using a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. P -values < 0.05 were considered statistically significant. (a, b) p -values < 0.01 , (c, d) p -values < 0.05 .

fed fructose fed mice did not stain. These results indicate that fructose induces triglyceride storage, while glucose and sugar induces both glycogen and triglyceride storage. The liver of mice fed sugar with added egg yolk had almost normal pattern (Fig. 3d). Both serum ALT and AST levels were markedly increased with the glucose, fructose or sugar diet. Adding egg yolk decreased the ALT and AST levels, whereas adding amino acids did not decrease these enzyme levels (Fig. 3e). The level of serum glucoses increased two-fold in mice fed glucose, fructose and sugar following the addition of egg

yolk (Fig. 3f). Total ketones and 3-HB levels were considerably higher in mice fed fructose. Adding raw egg yolk to sugar increased total ketones and 3-HB levels. As shown in Figure 3g, a similar effect was observed with amino acids. (Fig. 3g).

3-4. One week of egg yolk reversed the fatty liver caused by one week of a sugar only diet.

Because we found that the sugar only diet induced a NAFLD within only one week and considering the possible effect of eggs in suppressing fat accumulation in hepatocytes, we next examined

Fig.4a

Fig.4c

Fig.4b

Fig.4d

whether egg yolk-only diet could reverse the NAFLD caused by a sugar only diet. Five weeks-old female mice were first fed a sugar with vitamins in water for one week, then they were fed raw egg yolk in water for another one weeks. As control, they were fed only egg yolk in water (Fig. 4a). At 2 weeks all group had a reduction in weights and appeared similar (Fig. 4b, 4c) . Liver

histology showed many vacuoles in the hepatocytes of the mice fed sugar. However, the number of vacuoles was greatly reduced in the liver of the mice fed an egg-only diet for 1 week after 1 week of sugar-only diet (Fig. 4d). Total ketones and 3-HB levels were considerably higher in the mice fed an egg-only diet and in the mice fed an egg-only diet for 1 week after 1 week of

Figure 4: One week of egg yolk reversed the fatty liver caused by one week of a sugar only diet.

(a) Five-week-old B6 female mice were fed 1) 30% sugar with vitamins in water for one week then changed to raw egg yolk in water for one week, 2) raw egg yolk in water for one week then changed to 30% sugar with vitamins in water for one week, 3) 30% sugar with vitamins for two weeks, 4) raw egg yolk in water for two weeks. (b) Body weights of each group were measured at 1 and 2 weeks (n=5). (c) Photographs taken at 2 weeks. (d) Liver tissues removed at 2 or 4 weeks were fixed in 4% paraformaldehyde and blocked in paraffin. Paraffin-embedded 4-μm sections were stained with hematoxylin and eosin. They were observed by Olympus microscopy and pictures were taken. (e) Serum ketone bodies (T-KB and 3-HB) were measured at 2 weeks. (f) Serum triglyceride, free fatty acids and cholesterol were measured at 2 weeks. Data are expressed as means ± standard deviations. Statistical comparisons were performed by a one-way analysis of variance (ANOVA) and Tukey-Kramer tests. P-values < 0.05 were considered statistically significant. *p-values < 0.05 **p-values < 0.01.

sugar-only diet than those in the mice fed a sugar-only diet (Fig. 4e). Serum triglyceride, fatty acids and cholesterol levels were higher in the mice fed an egg-only diet for 1 week after 1 week of sugar-only diet than those of the mice fed sugar-only diet (Fig. 4f).

4. Discussion

This study showed that mice fed only sugar, fructose or glucose quickly develop fatty liver (within 1-2 weeks), whereas hen egg that contained high fat did not induce accumulation of fat in the liver. Hen egg contains very small amount of carbohydrates. These carbohydrates including sugar are converted to monosaccharides and used either as an energy source or converted to glycogen or TG in the liver to provide energy during period of starvation. These results indicate that rapid accumulation of fat in the liver mainly caused by intake of carbohydrates rather than intake of TG. There is evidence that 30% fructose or glucose and 60% sugar cause fatty liver¹⁶⁾.

We showed using PAS staining that ingestion of sugar or glucose caused deposition of glycogen in the liver, whereas glycogen deposition in the liver of mice fed fructose was lower than in those fed glucose. Higher amounts of TG were deposited in the liver of mice fed fructose. Fructose induces NAFLD in a

greater extent than glucose¹⁷⁻¹⁹⁾. Studies of patients with NAFLD showed that ~ 59% of intrahepatic triglycerides are derived from circulating FA, whereas only ~ 26% are derived from DNL and ~ 15% from dietary fat²⁰⁾. However, DNL is important in the pathogenesis of NAFLD. For example the ~ 26% of DNL measured in NAFLD patients was reported to be three-fold higher than that observed in control subjects²¹⁾. A recent study also demonstrated that fructose activated hepatic transcription factor Carbohydrate response element-binding protein (ChREBP) and Sterol regulatory element-binding protein 1c (SREBP1c), both of which increased expression of ChREBP target genes and hepatic steatosis^{22,23)}.

Our study that boiled egg or raw egg yolk inhibited the induction of NAFLD by glucose or fructose (Fig. 2e). Egg contains about 60% E of fat, which is similar to the fat content of experimental high fat diet (HFD), that has between 45-75% E of fat. The HFD is therefore similar to the diet that causes NAFLD in humans. Studies have reported fatty liver is associated with obesity, insulin resistance, and increased levels of serum free fatty acids, with these changes observed after approximately 10 weeks²⁴⁻²⁶⁾. Egg has a very low carbohydrates content, whereas the HFD contains various amount of carbohydrates.

Fig.S1

Fig.S2

Fig.S3

Fig.S4

Supplemental Figures Energy intake

Fig.S1 corresponds to the energy intake of the mice used in Figure 1 (Fig.S2 to Fig.2, Fig.S3 to Fig.3 and FigS4 to Fig.4). Energy intakes of each group were calculated. Value indicates mean energy intake of each mice (kcal).

Shuang recently investigated the effects of different carbohydrates in HFD. C57BL/6 mice were fed HFD (58% fat) + 0.1% carbs, HFD (58% fat) + 5% carbs, HFD (58% fat) + 10% carbs, or HFD (58% fat) plus 25.5% carbs for 5 weeks. It was found that although HFD stimulated accumulation of fat in the liver, the addition of dietary carbohydrate did not influence the level of fat accumulation induced by the HFD²⁷⁾. The composition of the hen egg diet used in our experiment was composed of 60% E fat and less than 5% E carbohydrates, similar to the diet used by Shuang's group. However,

the egg-only diet did not induce fatty liver as we found that more than 6 months consumption of this diet did not induce the condition (data not shown). This raises the question: why does the high fat content of the egg-only diet not induce NAFLD and even suppress carbohydrate-induced NAFLD? Hen egg contains several important nutrients including choline, which may prevent the development of fatty liver. Triglycerides in the liver are introduced into very low-density lipoproteins (VLDL) and secreted into circulation. If production and entrance of triglycerides in the liver exceed the secretion of

triglycerides, triglycerides accumulate in the hepatocytes as fat droplets. VLDL is a lipoprotein having a single-layer phospholipid in outer shell, and which contains triglycerides and cholesterol-ester internally. Choline is essential for biosynthesis of phosphatidylcholine (PC), and is needed for the export of TG out of hepatocytes via VLDL²⁸⁾. VLDL made by choline and other nutrients in the egg might work to export TG made by DNL from carbohydrate. Choline-deficiency is now thought to have an impact on diseases such as liver disease²⁹⁾.

5. Conclusions

Taken together, these results showed that mice fed either a sugar-, glucose- or fructose-only diet developed overt NAFLD with a large number of vacuoles observed in hepatocytes and high serum levels of ALT and AST occurring within 2 weeks. Addition of egg to monocarbohydrate diet or changing to egg from sugar diet suppressed the development of NAFLD.

6. Conflict of Interest

The authors declare no competing financial interests.

7. Acknowledgments

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8. References

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