Do mice grow normally with an egg-only diet?

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Chicken egg (egg) is a conventional food that contains every nutrient required for the growth of the chicken embryo. Eggs contain high amounts of important proteins and fat with a very low amount of carbohydrates, and also contain all vitamins and minerals needed for the chick to develop. We took 5-week-old mice and raised them for 6 months consuming an egg-only diet, and examined their health conditions. We found that mice raised on this diet grew to the same weight as mice fed a normal chow diet (ND), and remained healthy until they were 8 months old. As expected, the levels of total ketone bodies and 3-hydroxybutyrate were approximately 3 times higher in the egg-only mice than in the ND mice. Total cholesterol, HDL cholesterol, triglycerides, and free fatty acids in the serum of the egg-only mice were higher than those in the ND mice. Surprisingly, serum glucose levels in the egg-only mice were nearly the same as those of the ND mice at all points. Because of the high amount of fat in the diet (approximately 60% of total calories), we expected that the egg-only mice would develop fatty liver or other metabolic diseases. However, over the 6-month examination period, we observed no pathological changes in the livers of the egg-only mice serologically and histologically. Serum alanine transaminase and aspartate transaminase levels were normal and no fat droplets were observed in the livers of the egg-only mice.
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Abstract

Chicken egg (egg) is a conventional food that contains every nutrient required for the growth of the chicken embryo. Eggs contain high amounts of important proteins and fat with a very low amount of carbohydrates, and also contain all vitamins and minerals needed for the chick to develop. We took 5-week-old mice and raised them for 6 months consuming an egg-only diet, and examined their health conditions. We found that mice raised on this diet grew to the same weight as mice fed a normal chow diet (ND), and remained healthy until they were 8 months old. As expected, the levels of total ketone bodies and 3-hydroxybutyrate were approximately 3 times higher in the egg-only mice than in the ND mice. Total cholesterol, HDL cholesterol, triglycerides, and free fatty acids in the serum of the egg-only mice were higher than those in the ND mice. Surprisingly, serum glucose levels in the egg-only mice were nearly the same as those of the ND mice at all points. Because of the high amount of fat in the diet (approximately 60% of total
calories), we expected that the egg-only mice would develop fatty liver or other metabolic diseases. However, over the 6-month examination period, we observed no pathological changes in the livers of the egg-only mice serologically and histologically. Serum alanine transaminase and aspartate transaminase levels were normal and no fat droplets were observed in the livers of the egg-only mice.

Key words: egg-only diet; mice; low carbohydrates
Introduction

Chicken egg (egg) is a conventional food that contains every nutrient needed for the normal growth of the chicken embryo. Eggs contain high amounts of important proteins and fat, with a very low amount of carbohydrates. Eggs contain all vitamins and minerals needed for chick development [1]. Human fetuses receive nutrients from the mother, but after birth, humans receive nutrients in different ways depending on their regional habits or religion.

Obesity and metabolic syndrome are currently serious problems worldwide. Type 2 diabetes, nonalcoholic fatty liver disease (NAFLD), atherosclerosis, stroke, and ischemic heart disease occur in people with those conditions [2]. To overcome obesity, people have been trying various diets. Long-term caloric restriction with adequate intake of nutrients reduces the risk of developing type 2 diabetes, hypertension, and cardiovascular diseases [3]. The Ramadan diet, characterized by fasting during daylight hours with food consumption predominantly consisting of a
high-calorie, high-carbohydrate evening meal, has been associated with beneficial
effects [4]. A Mediterranean dietary pattern can reduce body weight and body mass
index [5]. The Dietary Approaches to Stop Hypertension (DASH) diet emphasizes
the consumption of vegetables, fruits, whole grains, legumes, nuts, fish, and
poultry, and limits that of sugar-sweetened foods and beverages, added fats, and
red meat. DASH has favorable effects on blood pressure and cardiovascular
disease [6]. Recently, low carbohydrate diets (LCD) have been extensively
investigated in several clinical states [7,8]. LCD are followed by many people in
the world to prevent obesity and Type 2 diabetes. On the other hand, LCD,
especially the ketosis diet, have been shown to have adverse effects. Low
carbohydrate-high protein diets were demonstrated to be associated with an
increased risk of cardiovascular diseases. Moreover, a carbohydrate-restricted diet
was reported to increase mortality [9,10].

In order to answer the question whether egg consumption is good for health, we
set up experiments in which young mice were fed only eggs. Such mice grew to
the same weight as the mice fed a normal diet. Surprisingly, the weight of the mice
and the size of the livers of the mice fed only eggs were almost the same as those of the mice fed a normal diet (ND mice). Because of the low amount of carbohydrate, it is likely that energy was derived from lipid oxidation and ketone bodies. However, the blood glucose level of the mice fed only eggs was almost the same as that of the mice fed a normal diet.

Materials and Methods

Mice

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

Mouse diets and feeding
The CLEA Rodent Diet CE-2 was the ND; it contains 8.84% water, 25.48% protein, 4.61% fat, and 61.07% carbohydrate, along with vitamins and minerals. The energy of 100g of CE-2 is 339.1 kcal (Chubu Kagaku, Japan). For the egg-only diet, we used boiled eggs. Eggs were obtained from firms in Aichi, Japan. The nutritional analysis of the boiled eggs was performed at Japan Food Research Laboratories (Nagoya, Japan). Boiled egg has a high water content (75.6g/100g), and 12.9g/100g protein, 9.6g/100g fat and 1.1g/100g carbohydrate and vitamins and minerals, which corresponds to 36.1% of calories (E) from protein, 60.8% E from lipid and 3.1% E from carbohydrates.

**Histological analysis**

Liver tissue was fixed in 4% paraformaldehyde buffered with PBS (pH 7.4) and blocked in paraffin. Paraffin-embedded 4-μm sections were stained with hematoxylin for 5 minutes and with eosin for 3 minutes.

**Biochemical analysis**

Serum samples were obtained from the mice at the time of sacrifice. 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 (555nm) using WAKO assay kits (Wako Pure Chemicals, Osaka, Japan). Blood glucose was measured by using the LaboAssayTM Glucose kit (Wako Pure Chemicals, Osaka, Japan). Ketone bodies, including total ketone bodies (T-KB) and 3-hydroxybutyrate (3-HB) were measured by AUTO WAKO Total Ketone Bodies and 3-HB (Wako Pure Chemicals, Osaka, Japan). Total cholesterol, HDL cholesterol and triglycerides (TG) were measured using the Wako E test, and free fatty acids (FFA) (non-esterified fatty acids; NEFA) were measured using the NEFA C test (Wako Pure Chemicals, Osaka, Japan). Wavelengths were measured with an xMark™ Microplate Absorbance Spectrophotometer (Bio Rad).

Statistical analyses

Data are expressed as means ± standard deviations (SD). Statistical comparisons were performed by Test. P-values < 0.05 were considered statistically significant.

Results
1. Mice fed only eggs attained the same weight as the mice fed a normal chow diet (ND), and remained healthy for the duration of the experiment (until 8 months old).

Five-week-old female mice were raised on an ND or egg-only diet. The body weights of the egg-only mice were the same as those of the ND mice until 27 weeks of feeding (Fig. 1a). Mice were sacrificed at 1 week, 3 weeks and 27 weeks for examination of organ appearance, organ histology, and metabolic analysis. At 27 weeks (aged 8 months), both the egg-only–fed mice and the ND mice appeared healthy (Fig.1b). At 27 weeks, the fat accumulation in the abdominal cavities of the egg-only mice was slightly higher than that of the ND mice. The organs inside the abdominal cavity had no abnormal characteristics, although the intestines of the egg-only mice contained egg yolk and looked yellowish (Fig. 1c).

2. Serum glucose levels of the egg-only mice were almost the same as those of the ND mice.

Because eggs contain a very small amount of carbohydrate (3.1% E), we thought that the serum glucose levels of the egg-only mice would be lower than those of
the ND mice. Surprisingly, the serum glucose levels of the egg-only mice were almost the same as those of the ND mice (Fig.2).

3. Ketone bodies of the egg-only mice were higher than those of the ND mice.

Eggs contain a high amount of fat. The TG in fats is digested into monoglycerides and FFAs. They are absorbed and stored in mainly fat tissues. TG is hydrolyzed to monoglycerides and FFA. FFA is oxidized in tissues for energy production. β-oxidation is the main pathway and produces acetyl-coA, which enters the tricarboxylic acid (TCA) cycle or produces ketone bodies. The ketone bodies in the serum of the egg-only mice were examined and compared with those of the ND mice. T-KB and 3-HB were measured at 1, 2, 3, 4 and 27 weeks. As early as 1 week, both T-KB and 3-HB were higher in the serum of the egg-only mice than in the serum of the ND mice. Similar results were observed at each time point (Fig. 3a,b).

4. Livers of the egg-only mice were healthy histologically and serologically.

Because eggs contain large amounts of TG and proteins and a very small amount of carbohydrates, we expected that the livers of the egg-only mice would be
different from those of the ND mice, and that the egg-only diet would induce pathological changes. However, the liver weights were similar between the groups at 1, 2, 3, 4 and 27 weeks. (Fig.4a). There were also no significant differences in the levels of serum ALT and AST between the groups. (Fig.4b). We also observed no pathological changes in the livers of the egg-only mice for 1, 2, 3, 4 and 27 weeks (Fig.4c).

5. Total cholesterol, HDL cholesterol, TG, and FA in the serum of the egg-only mice were high.

As expected, the total cholesterol, HDL cholesterol, TG, and FFA serum levels in the egg-only mice were higher than those in ND mice (Fig.5).

Discussion

1. The mice fed only eggs were healthy.

We have shown that the egg-only mice were healthy from birth to nearly 6 months after the initiation of the diet. There is a murine model of LCD. The ketogenic diet (KD) is extremely low in carbohydrate [11-14]. The KD has been
suggested to be more effective in promoting weight loss than conventional caloric restriction [15]. The composition of the KD differs from that of the egg-only diet. The KD contains 95.1% E fat, 0.4% E carbohydrate, and 4.5% E protein [1,2], whereas the chicken egg diet used in our study contains 60.8% E fat, 3.1% E carbohydrate, and 36.1% E protein. The egg-only diet is similar to the KD in that both contain very little carbohydrate. However, the KD diet has a lower amount of protein. We have shown that the egg-only mice showed the same weight gain as the ND mice, which is different from mice fed a KD. KD-fed mice develop a distinct metabolic state characterized by weight loss and increased energy expenditure [16]. The appearance and activity of the egg-only mice for 6 months was similar to those of the ND mice (Fig.1c). However, because this was the first preliminary study, we did not examine the physical performance parameters.

2. The low carbohydrate level of the egg-only diet may have induced gluconeogenesis in the mice.

Almost all carbohydrates, which are digested to monosaccharides in the small intestine, are used for ATP production via glycolysis. We thought at first that lipids
would be the main energy source in the egg-only diet, because ATP is produced from fatty acids by β-oxidation. Thus, we thought that the serum glucose level would be low in the egg-only mice. However, we found that the serum glucose levels were only slightly lower in the egg-only mice than in the ND mice. This could be because serum glucose is derived from gluconeogenesis, mainly in the liver. Proteins in the egg are digested to amino acids, which are used for cellular protein synthesis, but are also used for gluconeogenesis. Glycerol, which is derived from the digestion of TG, also might have been used for gluconeogenesis.

3. High serum ketone bodies in mice fed only eggs.

We have shown that the levels of T-KB and 3-HB were more than 3 times higher in the egg-only mice than in ND mice (Fig.3). Because the egg-only diet has a very low amount of carbohydrates, β-oxidation of fatty acids may have been enhanced, which would have induced the production of ketone bodies. This phenomenon is the same as with the KD. Acetyl-CoA generated from β-oxidation is diverted away from the TCA cycle and converted into acetoacetate, 3-hydroxybutyrate, and acetone (ketone bodies) through ketogenesis in the
mitochondria [17]. 3-hydroxy-3-methylglutaryl-CoA synthase 2 catalyzes the rate-limiting conversion of acetoacetyl-CoA and acetyl-CoA into 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), which is further converted into acetoacetate by mitochondrial HMG-CoA lyase [18]. Then, D-beta-hydroxybutyrate dehydrogenase generates 3-hydroxybutyrate [19], and acetoacetate spontaneously undergoes decarboxylation to form acetone. Both acetoacetate and 3-hydroxybutyrate are used as energy sources in extrahepatic tissues [20].

4. Normal liver function and histology with the egg-only diet.

Because the egg-only diet is high in fat (60.8% E), we expected that the mice fed only eggs for 6 months would develop NAFLD. Unexpectedly, we found that the livers of those mice were healthy in terms of liver enzymes and histology. Hepatic lipid accumulation occurs as a result of excess FFA from high fat diets or an increase in hepatic fatty acid synthesis [21]. It has been shown that impairment of TG secretion by VLDL can also contribute to hepatic steatosis under certain conditions [22]. The amount of fat in eggs is almost the same as that in an experimental high-fat diet [23]. It is possible that some nutrients in eggs may have
reduced the accumulation of fats in the livers of the egg-only mice.

Taken together, we have shown that young mice fed only eggs were healthy for 6 months after the initiation of the diet. They were not obese, and had healthy livers. These mice had similar levels of blood glucose and high levels of ketone bodies and cholesterol compared with ND-fed mice.

Contributions

K.I. conceptualized and designed the study. N.N. set up, performed, and analyzed the experiments.

Competing financial interests

The authors declare no competing financial interests.

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References


Figure legends

Figure 1. Healthy appearance of the egg-only mice.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad libitum for 27 weeks.

(a) Body weights of ND (control) or egg-fed female mice were measured at 1, 2, 3, 4 and 27 weeks of feeding. Data shown are the mean ratios ± standard deviations (0w: control n=6, egg n=6; 1w: control n=5, egg n=6; 2w: control n=7, egg n=6; 3w: control n=5, egg n=7; 4w: control n=9, egg n=7; 27w: control n=4, egg n=3.)

(b) Mice from both groups appeared healthy after 27 weeks of feeding. Mice raised on a ND (control) or eggs for 27 weeks were sacrificed, and photographs were taken after opening the abdomen.

Figure 2. Serum glucose levels of the egg-only mice.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad
libitum for 27 weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum samples were taken. Serum glucose levels were measured at each time point. Data are expressed as means ± standard deviations. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. * p-values < 0.05 ** p-values < 0.01.

Figure 3. Comparison of the levels of ketone bodies.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad libitum for 27 weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum samples were taken. Total ketone bodies (Fig.3a) and 3-hydroxybutyrate (Fig.3b) were measured at each time point. Data are expressed as means ± standard deviations. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. ** p-values < 0.01.

Figure 4. Liver histology and function of the egg-only mice.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad libitum for 27 weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum samples were taken.
(a) Liver weights of the ND and the mice fed only eggs were measured at each time point. ** p-values < 0.01.

(b) The levels of liver marker enzymes alanine transaminase and aspartate transaminase were measured at each time point.

Data are expressed as means ± standard deviations. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant.

(c) Liver tissues removed at each time point 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.

Figure 5. Total cholesterol, HDL cholesterol, triglycerides and free fatty acids in the serum of the egg-only mice.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad libitum for 27 weeks. They were sacrificed at 27 weeks and serum samples were taken. Total cholesterol, HDL cholesterol, triglycerides and free fatty acids were measured. Data are expressed as means ± standard deviations. Statistical
comparisons were performed by Test. p-values < 0.05 were considered statistically significant. ** p-values < 0.01.
Healthy appearance of egg only fed mice.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs ad lib for 27-weeks. *(a)* Body weights of ND (control) or egg-fed female mice were measured at 1, 2, 3, 4 and 27 weeks of feeding. Data shown are the mean ratio ± SD (0w; control n=6, egg n=6, 1w; control n=5, egg n=6, 2w; control n=7, egg n=6, 3w; control n=5, egg n=7, 4w; control n=9, egg n=7, 27w; control n=4, egg n=3.). *(b)* Pictures show healthy appearance of the mice of 27 weeks of feeding. Mice bred by ND (control) or eggs for 27 weeks of feeding were killed and photographs were taken after opening the abdomen.
Figure 1a.

Body weight

- Control
- Egg

(g)
Figure 1b.

Control

Egg
Figure 1c.
Control

Egg
Figure 2 (on next page)

Serum glucose levels of the mice fed only eggs.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs ad lib for 27-weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum were took. Serum glucoses were measured at each time point. Data are expressed as means ± SD. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. * p-values < 0.05 ** p-values < 0.01.
Figure 2.

Serum Glucose

(mg/dL)

1W  2W  3W  4W  27W

Control  Egg

**  *
Higher Ketone bodies of the mice fed only eggs.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs ad lib for 27-weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum were took. Total ketone bodies (T-KB) (Fig.3a) and 3 hydroxy butyrate (3-) HB (Fig.3b) were measured at each time point. Data are expressed as means ± SD. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. ** p-values < 0.01.
Figure 3a.

(μmol/L)

- **Control**
- **Egg**

T-KB

- **1W**
- **2W**
- **3W**
- **4W**
- **27W**
Figure 3b.

3-HB (µmol/L)

- Control
- Egg

Significance levels:
- **: p < 0.01
Liver histology and function of the egg-only mice.

Cellular Senescence as the Causal Nexus of Aging Five-week-old female mice were fed 1) only ND (control) or 2) only eggs, ad libitum for 27 weeks. They were sacrificed at 1, 2, 3, 4 and 27 weeks and serum samples were taken. (a) Liver weights of the ND and the mice fed only eggs were measured at each time point. ** p-values < 0.01. (b) The levels of liver marker enzymes alanine transaminase and aspartate transaminase were measured at each time point. Data are expressed as means ± standard deviations. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. (c) Liver tissues removed at each time point 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.
Figure 4a.

Liver weight

Control
Egg

(g/gB.W.)

1W  2W  3W  4W  27W
Figure 4b.
Figure 4c.

Control

1w  2w  3w  4w  27w

Egg

1w  2w  3w  4w  27w
Figure 5 (on next page)

Total cholesterol, HDL cholesterol, TG and FA in the serum of the mice fed only eggs.

Five-week-old female mice were fed 1) only ND (control) or 2) only eggs ad lib for 27-weeks. They were sacrificed at 27 weeks and serum were took. Total cholesterol, HDL cholesterol, TG and FA were measured. Data are expressed as means ± SD. Statistical comparisons were performed by Test. p-values < 0.05 were considered statistically significant. ** p-values < 0.01.
Figure 5.

**HDL-cho**

- Control: [Graph showing HDL-cho levels for control and egg groups.]
- Egg: [Graph showing HDL-cho levels for control and egg groups.]

**Total-cho**

- Control: [Graph showing total-cho levels for control and egg groups.]
- Egg: [Graph showing total-cho levels for control and egg groups.]

*Statistical significance indicated by **.***
Figure 5.

The bar chart shows the comparison of TG (Triglycerides) and FAA (Fatty Acid Acyltransferase) levels in control and egg groups. The TG levels are higher in the egg group compared to the control group, indicated by the ** symbol. Similarly, the FAA levels are also higher in the egg group, with a significant difference as indicated by the ** symbol.