Boffin Access
FOSTERING KNOWLEDGE

Open Access Journal | Volume: 3.4

Biomedical Research and Reviews

ISSN 2631-3944

Are Mice Born Normally With an Egg-only Diet?

Ken-ichi Isobe[1,3]
Naomi Nishio[2]

[*1]Department of Food Science and Nutrition, Nagoya Women's Uuniversity, 3-40 Shioji-cho, Mizuho-ku, Nagoya, Aichi, 467-8610 Japan

[2]Faculty of Education, Saitama University, 255 Shimo Okubo, Sakura-ku, Saitama 338-8570, Japan

[3]Faculty of Medical Sciences, Shubun University, 6 Nikko-cho, Ichinomiya, Aichi 491-0938, Japan

Abstract

Chicken eggs (egg) 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 mated 8-week-old mice consuming an egg-only diet, and examined whether mice got pregnant and mice gave birth. Then we continued to raise newborn mice only consuming an egg-only diet compared to normal chew diet. We found that the rate of pregnancy of the mice consuming an egg-only diet was not different from that of the mice consuming normal chew diet. Further, we found that the number of pups born from the mother consuming only eggs was not different from that of the mother consuming normal chew diet. However, the newborn mice from the mother fed only eggs did not grow healthy when they consume egg-only diet. Only few mice were alive at one month after birth. Then we change the diet just before or just after birth from egg only diet to normal chew diet and we found that both group grew healthy more than one month. These results indicate that mice are born healthy only with egg only diet.

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]. Chick develops independently from mother. Fetus of mammal receives nutrients from the mother via umbilical vein, whereas fetus of birds get nutrients only from albumin and yolk independent to mother.

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 [1].

Recently, low carbohydrate diets (LCD) have been extensively investigated in several clinical states [2,3]. However, a carbohydrate-restricted diet was reported to increase mortality [4,5].

To understand the deep biological meaning of low carbohydrate nutrition, we designed the experiments to feed mice only hen egg. Here, we asked whether murine pups could develop in the mother fed egg-only diet. Here we set up the experiments to examine the rate of pregnancy between the mice fed only eggs and the rate of healthy new born.

Materials and Methods

Mice

Five to eight -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.

Article Information

DOI:	10.31021/brr.20203132
Article Type:	Research
Journal Type:	Open Access
Volume:	3 Issue: 4
Manuscript ID:	BRR-3-132
Publisher:	Boffin Access Limited
Received Date:	28 October 2020
Accepted Date:	04 November 2020
Published Date:	06 November 2020

***Corresponding author:**

Ken-ichi Isobe
Faculty of Medical Sciences
Shubun University
6 Nikko-cho, Ichinomiya
Aichi 491-0938, Japan
Tel: +81-586-45-2101
Fax: +81-45-4410
E-mail: isobe.k@shubun.ac.jp

Citation: Isobe K, Nishio N. Are Mice Born Normally With an Egg-only Diet?. Biomed Res Rev. 2020 Nov;3(4):132.

Copyright: © 2020 Isobe K, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 international License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

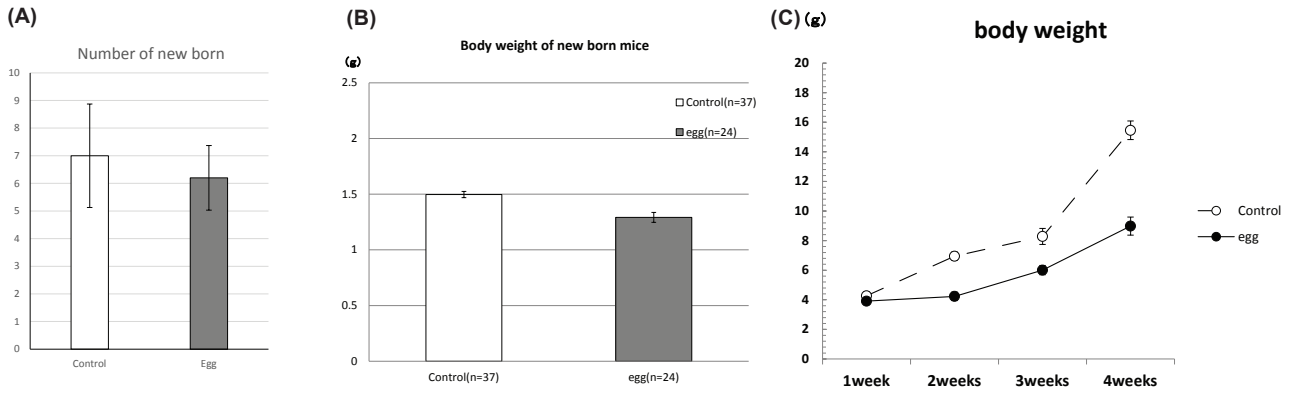

Figure 1: The number and body weight of the newborn mice from the mother fed only eggs

Eight-week-old one male mice and five or six female mice were housed together in a cage. They were fed 1) only CE-2 (control) or 2) only eggs, ad libitum. Pregnant mouse was moved to a new cage and fed the same diet.

a) Number of mice born from the mother fed normal diet or egg-only diets.

b) Body weights of the newborn mice from the mother fed CE-2 (control) or egg-only diet were measured at birth. Data shown are the mean ratios ± standard deviations (control n=37, egg n=24).

Body weights of newborn mice nursed by the mother fed CE-2 (control) or egg were measured at 1, 2, 3, 4 weeks.

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 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.

Measurement of body weight organ weight

Body weights were measured regularly and organ weights were measured at the time of sacrifice.

Biochemical analysis

Serum samples were obtained from the mice at the time of sacrifice. Serum glucose, ketone bodies were measured by enzymatic colorimetric assays. 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). Wavelengths were measured with an xMark™ Microplate Absorbance Spectrophotometer (Bio Rad).

Statistical analyses

Data are expressed as means ± standard deviations (SD).

Results

1. **The number and body weight of the new born mice from the mother fed only eggs were almost same as those of the mother fed chew diet.**

In order to examine whether the mice fed only eggs give birth, we set up the experiments of mating the mice in a cage with one male and five or six female mice consuming only eggs or consuming control diet (CE-2). Among 11 female mice 9 mice became pregnant in egg-only diet in one month (82%). Among 10 mice 8 mice became pregnant in control diet (80%). These indicate that the rate of pregnancy was not different between egg-only diet and control diet. Further, average number of newborn mice from the mother fed only eggs was 6.2, whereas average number of newborn mice from the mother fed control diet was 7.0 (Figure 1a). We found that the appearance of the newborn mice from the mother fed only eggs was healthy. The average body weights of newborn mice from the mother fed only eggs was 1.29g, whereas the average body weights of newborn mice from the mother fed control diet was 1.50g (Figure 1b). This means that newborn mice born from the mother fed egg-only diet were slightly lower than the mice born from the mice fed control diet.

2. **The newborn mice from the mother fed only eggs did not grow healthy when they consume egg-only diet.**

We continue to breed newborn mice by same diet. At one week they were healthy and body weights were almost the same as the newborn mice from the mother fed control diet. However, at two weeks they were smaller than those born from the mother of normal diet. The weights of liver of 2, 3, 4 weeks of the mice born from the mother egg-only diet were lower than those born from the mother of normal diet (Figure 1c).

3. **The mice bred by ND just before or after birth from egg-only diet grow healthy.**

In order to know whether the mice born from egg-only mother can not survive by milk feeding, we changed the diet just before or just after birth from egg only diet to control diet (Figure 2a). Almost all mice of second group (egg to control diet before birth) and the mice of third group (egg to control diet after birth) survived more than four weeks, whereas the mice of third group died after 1 week and many mice were dead at 4 weeks (Figure 2b). At birth the numbers of mice of all groups were almost same (Figure 2c) and looked healthy (Figure 2d). At birth body weight of all groups were not different (Figure 2e). Body weights of second and third group (egg to control diet before birth and after birth) were not different from fourth group (control diet) until four weeks, although the body weights of first group (egg-only diet) were much lower than those of other groups (Figure 2e). By autopsy we measured liver and heart weight from 1 week to 4 weeks. We found that liver weight of all groups were almost same

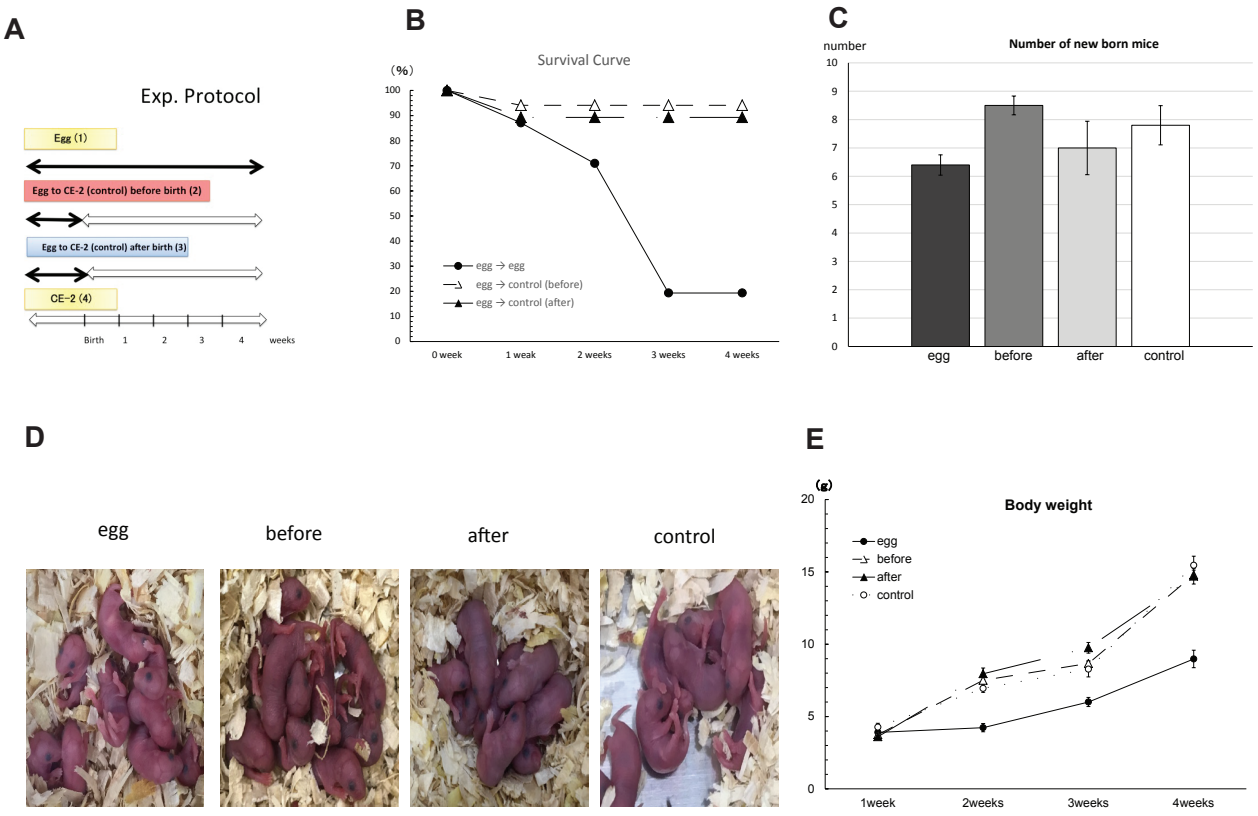

Figure 2: The mice bred by control diet just before or after delivery from egg-only diet

Pregnant mice fed only eggs were bred by 1) egg-only diet, 2) CE-2 (control) diet just before delivery or 3) control diet just after delivery. 4) Pregnant mice fed control diet were continued to be bred by control diet after delivery.

(a) Experimental Protocol

(b) Survival curve. Number of mice alive at 0,1,2,3 and 4 weeks

(c) Average number of newborn mice from one female of each group

(d) Photographs of newborn mice of each group

(e) Average body weights of mice of each group at 1, 2, 3, and 4 weeks

Data are expressed as means ± standard deviations.

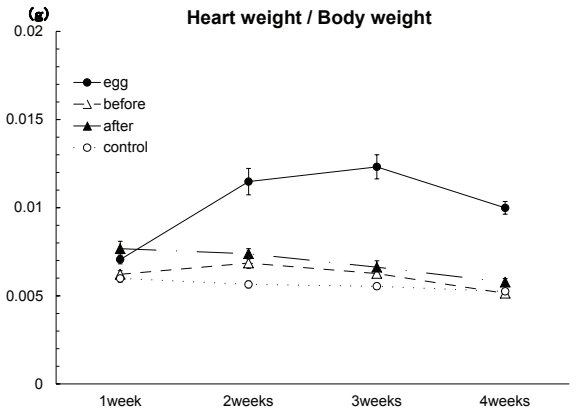

Figure 3: Organ weights of the mice bred by control diet just before or after delivery from egg-only diet

(a) Liver tissues of each group were removed from the mice used in Figure 2 and liver weights were measured.

(b) Heart tissues of each group were removed from the mice used in Figure 2 and heart weights were measured.

(c) Heart tissue weights were divided with 100x body weights of the same group.

Data are expressed as means ± standard deviations.

at 1 week. Liver weights were much lower in only first group (egg-only mother) from 2 weeks to 4 weeks than those of other groups (Figure 3a). On the contrary heart weights of the mice bred by the mother of first group (egg-only diet) were higher than those of other group (Figure 3b). Especially heart weight/ body ratio of the mice bred by mother of egg-only diet was much higher than those of other group (Figure 3c). This means that changing the diet from egg to control diet at birth rescue the mice from some problems of the milk of the mother bred egg-only diet.

4. Keep the glucose level and high ketone bodies in egg-only pregnant and nursing mice.

In order to know the gluconeogenesis of the pregnant and nursing mice fed only eggs, we measured the blood glucose around 13th to 16th of pregnant mother. We found that pregnant mice fed only eggs had almost the same level of blood glucose as those of pregnant mice fed control diet (Figure 4a). In nursing mice, the blood glucose levels of the mother fed only eggs were almost the same as those of the mice fed control diet. However, the blood glucose levels of the mother fed control changed from eggs after birth were slightly higher than those of the mice fed only eggs (Figure 4b).

The ketone bodies in the serum of the egg-only pregnant and nursing mice were examined and compared with those of the control diet mice. Both T-KB and 3-HB were much higher in

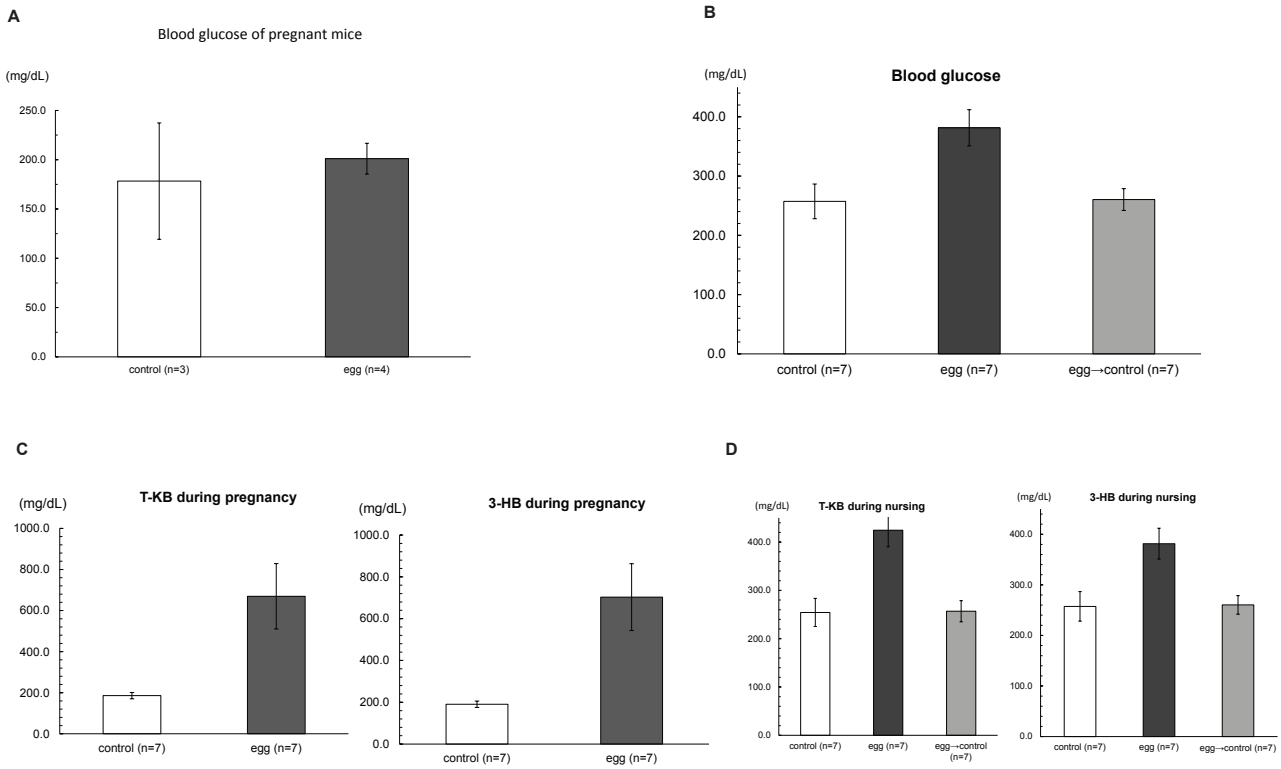

Figure 4: Keep the glucose level and high ketone bodies in egg-only pregnant and nursing mice

(a,c) Serum from the around 13th to 16th of pregnant mice bred only eggs or normal diet were taken in the morning and levels of the serum glucose (a) or levels of the serum T-KB and 3-HB were measured.

(b,d) Serum from each group of the mice in Figure 2 were taken in the morning, and levels of the serum glucose (c) or levels of the serum T-KB and 3-HB (d) were measured.

Data are expressed as means ± standard deviations.

the serum of the egg-only pregnant mice than in the serum of the control pregnant mice (Figure 4c). Both T-KB and 3-HB were higher in the serum of the egg-only nursing mother mice than either serum of normal diets of nursing mother mice or serum of egg to normal diet of nursing mother mice (Figure 4d).

Discussion

1. **The mice were born healthy from the mother fed only eggs.**

In this paper we have shown that female mice fed only eggs can successfully deliver term babies. The new born mice are healthy, because almost all new born mice from egg-only mother are healthy and gain same weight at 1 month after birth as those of the mice born from the mother fed ND when they were nursed with the mother fed ND. Because, chick and mammal use liver as a major organ of gluconeogenesis, they need glucose at the first stage of development until functional liver is developed. Glucose is used as an energy and a source of nucleic acids (through pentose phosphate pathway) for quickly dividing tissue cells [6]. The albumin was made up of 82% water, 17% protein, and 0.5% carbohydrates, whereas the yolk was made up of 53% water, 15.6% protein, 27.4% fat, and less than 0.5% carbohydrates [7]. In the studies of nutritional need of chick development, it has been demonstrated that carbohydrates are used for the initial phase of chick development as a source of nucleic acids for quickly dividing cells. Then at middle phase of chick development proteins are mainly used. The last phase of chick development (after 14th days of incubation) fats are used for energy source as β-oxidation [8,9]. Chicken embryos grow exponentially during the final week of incubation, with about 90% of the total energy for growth derived from β-oxidation of yolk fatty acids [10].

Embryonic development of mice is different from that of chicken. Mice get energy from umbilical vein, which contain high amount of glucose for energy. Human fetuses receive nutrients from the mother, but after birth, humans receive nutrients in different ways depending on their regional habits or religion.

2. High keton bodies in egg-only pregnant

We have shown that the levels of T-KB and 3-HB were more than 3 times higher in the egg-only pregnant mice than in normal diet mice. In nursing those levels were also higher in the egg-only nursing mice than in normal diet mice. In mammals, ketone bodies are produced predominantly in the liver from FAO-derived acetyl-coenzyme A (CoA), and they are transported to extra hepatic tissues for terminal oxidation. This physiology provides an alternative fuel that is augmented by relatively brief periods of fasting, which increases fatty acid availability and diminishes carbohydrate availability [11]. Multiple studies suggest diverse signaling roles for ketones, even in carbohydrate replete states, a subset of which could be independent of their metabolism [12].

3. Controversial clinical studies of low-carbohydrate and high fat diet in gestational diabetes Mellitus.

Gestational diabetes Mellitus (GDM) is one of the urgent medical problems in the world. The management of GDM still poses many unanswered questions [13]. The restriction of dietary carbohydrate (CHO) has been started for the treatment of GDM at a century ago [14]. Meta-analysis of low- CHO for treatment of GDM has been done recently [15,16]. These analysis

indicated that many studies of low- CHO for treatment of GDM indicated a larger decrease in fasting and postprandial glucose associated with lower infant birth weight. However, they critically evaluated the many low- CHO studies as low to very low quality [16]. Further low- CHO with high lipids increased fasting total cholesterol and FFA, which increased inflammation and insulin resistance [17]. WHO guideline recommended a low-fat diet (<30% of energy) and limiting saturated fatty acids to less than 10% of energy intake by replacing them with unsaturated fatty acids [18]. WHO guideline was based on the studies of North America and Europe, where people consume an excess energy with a low carbohydrate [19]. However, outside these regions especially in Asia most people consume very high carbohydrate diets such as refined rice. Recently several epimediological studies showed controversial results. Increased dietary fat intake reduced mortality and was not linked to cardiovascular disease [20]. Increased dietary fat intake reduced type 2 diabetes and cancer [21]. Intake of dietary saturated fatty acids (SFAs), which has long been perceived as unhealthy, was not associated with increased risk of insulin resistance or cardiovascular disease [22,23]. More recent study showed that people having diets comprised 64E% fat, 16E% protein, and 20E% carbohydrate for 6 weeks with stable body weight, insulin/glucose homeostasis in the fasting state was changed in a non-diabetic direction, as fasting plasma insulin concentration decreased after high-fat diets. Same results were obtained by saturated and unsaturated fats [24].

4. A model of the study of low carbohydrate diet for the treatment of gestational diabetes.

There exist several studies of the animal model of GDM. Exposure to high–saturated-fat diet (HFD) before 1 month and during gestation has been shown to induce GDM. Glucose metabolism and HFD-associated placental oxidative stress have been shown to the cause of GDM [25,26]. In our model of egg-only diets C57BL/6 mice are not obese during pregnancy, whereas mice become obese during pregnancy in HFD.

We presented the results here that pups born from the mother consuming only eggs looked healthy and they grow normally when they were bred by normal diet in nursing. Our results indicate that hen eggs having low-CHO and high fat are safe for murine mother.

Acknowledgments

This research was supported by 16H05280 and 19K11687 of the Ministry of Education, Science, Technology, Sports and Culture, Japan.

References

1. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med 2006 May;23(5):469-480.
2. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, et al. Dietary Intervention Randomized Controlled Trial (DIRECT) Group. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. N Engl J Med 2008 Jul;359(3):229-241.
3. Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, et al. Low-carbohydrate nutrition and metabolism. Am J Clin Nutr. 2007 Aug;86(2):276-84.
4. Lagiou P, Sandin S, Lof M, Trichopoulos D, Adami HO, et al. Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. BMJ. 2012 Jun;344:e4026.
5. Sjögren P, Becker W, Warensjö E, Olsson E, Byberg L, et al. Mediterranean and carbohydrate-restricted diets and mortality among elderly men: a cohort study in Sweden. Am J Clin Nutr. 2010 Oct;92(4):967-974.
6. Hamburgar V, Hamilton HL. A series of normal stages in the development of the chick embryo. 1951. Dev Dyn. 1992 Dec;195(4):231-272.
7. Z.Uni, L.Yadgary, R.Yair. Nutritional limitations during poultry embryonic development. J Applied Poultry Res. 2012 Mar;21(1)175-184.
8. Romanoff AL, Romanoff AJ. Biochemistry of the Avian Embryo. The Quarterly Review of Biology .1967;43(3).
9. Speake BK, Murray AM, Noble RC. Development of the Avian Embryo, Chapman and Hall, London, UK. (1974).
10. Speake BK, Murray AM, Noble RC. Transport and transformations of yolk lipids during development of the avian embryo. Prog Lipid Res. 1998 May;37(1):1-32.
11. Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr. 2006;26:1-22.
12. Puchalska P, Crawford PA. Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. Cell Metab. 2017 Feb;25(2):262-284.
13. Simmons D. GDM and Nutrition—Answered and Unanswered Questions—There's More Work to Do! Nutrients. 2019 Aug;11(8):1940.
14. Westman EC, Yancy WS Jr, Humphreys M. Dietary treatment of diabetes mellitus in the pre-insulin era (1914-1922). Perspect Biol Med. 2006 Winter; 49(1):77-83.
15. Jovanovic-Peterson L, Peterson CM. Dietary manipulation as a primary treatment strategy for pregnancies complicated by diabetes. J Am Coll Nutr. 1990 Aug;9(4):320-325.
16. Yamamoto JM, Kellett JE, Balsells M, García-Patterson A, Hadar E, et al. Gestational Diabetes Mellitus and Diet: A Systematic Review and Meta-analysis of Randomized Controlled Trials Examining the Impact of Modified Dietary Interventions on Maternal Glucose Control and Neonatal Birth Weight. Diabetes Care. 2018 Jul;41(7):1346-1361.
17. Farabi SS, Hernandez TL. Low-Carbohydrate Diets for Gestational Diabetes. Nutrients. 2019 Aug;11(8):1737.
18. WHO. World Health Organization healthy diet fact sheet number 394. (accessed Aug 19, 2017).
19. Puska P. Fat and heart disease: yes we can make a change—the case of North Karelia (Finland). Ann Nutr Metab. 2009;54:33-38.
20. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. Lancet. 2017 Nov;390(10107):2050-2062.
21. Schwab U, Lauritzen L, Tholstrup T, Haldorssoni T, Riserus U, et al. Effect of the amount and type of dietary fat on cardiometabolic risk factors and risk of developing type 2 diabetes, cardiovascular diseases, and cancer: a systematic review. Food Nutr Res. 2014 Jul;10;58.
22. Astrup A. A changing view on saturated fatty acids and dairy: from enemy to friend. Am J Clin Nutr. 2014 Dec;100(6):1407-1408.
23. Morio B, Fardet A, Legrand P, Lecerf JM. Involvement of dietary saturated fats, from all sources or of dairy origin only, in insulin resistance and type 2 diabetes. Nutr Rev. 2016 Jan;74(1):33-47.
24. Lundsgaard AM, Holm JB, Sjoberg KA, Myrmel LS, Fjaere E, et al. Mechanisms Preserving Insulin Action during High Dietary Fat Intake. Cell Metab. 2019 Jan;29(1):229.
25. Liang C, DeCourcy K, Prater MR. High–saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. Metabolism.2010;59(7):943-950.
26. Kretschmer T, Schulze-Edinghausen M, Turnwald EM, Janoschek R, Bae-Gartz I, et al. Effect of Maternal Obesity in Mice on IL-6 Levels and Placental Endothelial Cell Homeostasis. Nutrients. 2020 Jan;12(2):296.