

Title: Increased corn oil intake during an allergy sensitization period aggravates food allergy severity after oral allergen exposure

Running Title:

Corn-oil intake & food allergy in allergen exposure

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Word count: 2060 words

Abstract

Egg white (EW) allergy model mice underwent 15-day oral immunotherapy (OIT) using diets with different corn-oil content and 1% EW. After OIT, mortality following an oral food challenge in OIT groups were approximately 90%. However, in previous studies, mortality was 0%. This phenomenon is to be associated with increased corn-oil intake during allergy sensitization and allergen exposure for 2 weeks.

Keywords: corn oil, egg white, fat, food allergy, oral immunotherapy

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Oral immunotherapy (OIT) aims to induce allergen desensitization through continuous intake of food allergens and is a new approach to food allergy treatment (Ebisawa *et al.* 2017). Previous studies have reported a relationship between allergic disorders and fatty acid and fat intake (Hogenkamp *et al.* 2011; Kunisawa *et al.* 2015; Li *et al.* 2013; Hussain *et al.* 2019). Linoleic acid is metabolized to arachidonic acid. However, no studies to date have examined the relationship between OIT outcomes and fatty acid intake. The present study aimed to examine the relationship between OIT efficacy and corn oil intake level in a mouse model of food allergy.

In a preliminary experiment, we performed a 2-week OIT in a mouse egg white (EW) allergy model (Supplementary Fig 1A and Supplementary material). Following an oral food challenge (OFC), administered on day 16, half of the mice that were fed a 13% corn oil diet supplemented with 1% EW died (Supplementary Fig 1B). In contrast, the mice that were fed a diet without EW remained alive. In our previous studies (Maeta *et al.* 2018; Katahira *et al.* 2019), EW-allergic mouse models treated with OIT using a 5% corn oil diet supplemented with 1% EW for 2 weeks were alive after the OFC. We confirmed the reproducibility of this finding and compared the present results with those of our previous studies (Maeta *et al.* 2018; Katahira *et al.* 2019) to investigate factors associated with the death of mice after an OFC.

The care and treatment provided to the experimental animals were in accordance with the Mukogawa Women's University (Hyogo, Japan) guidelines for the ethical treatment of laboratory animals (No. FSN-01-2018-01-A). Six-week-old BALB/c female mice (n=27) were purchased from CLEA Japan (Tokyo, Japan) and were maintained under specific-pathogen-free conditions. The mice were individually housed at 22 °C with 60% humidity and under a 12-h light (08:00–20:00)/dark (20:00–08:00) cycle. The EW allergy mouse model was prepared as previously described (Maeta *et al.* 2018; Katahira *et al.* 2019) (Supplementary material). The corn oil concentrations in the diets were 5% (low-fat [LF]), 13% (medium-fat [MF]), and 34% (high-fat [HF]) (Supplementary Table 1). EW was diluted 3-fold in water, mixed for 1

h, filtered through a gauze, and freeze-dried. All diets were prepared in-house. The concentration of linoleic acid in corn oil is approximately 55% (Ministry of Education, Culture, Sports, Science and Technology 2020). Moreover, the concentrations of saturated fatty acids, oleic acid, and α -linolenic acid in corn oil are approximately 13%, 30%, and 1%, respectively (Ministry of Education, Culture, Sports, Science and Technology 2020). The diet used during the sensitization period was the MF diet (Table 1).

The main experimental protocol is shown in Fig. 1A. Mice allergic to EW (n=27) were divided into the following four groups according to the diets they were fed for 15 days: MF, LF-EW, MF-EW, and HF-EW (Table 1). On day 15, plasma samples were obtained from blood samples collected from the submandibular vein under isoflurane anesthesia. All mice underwent an OFC on day 16 using the following method (Maeta *et al.* 2018; Katahira *et al.* 2019): OFC with 40 mg EW in 200 μ L saline was initiated in all mice following an overnight fast. Mice rectal temperature was measured using a thermometer (KN-91-AD-1687-M; Natsume Seisakusyo Co. Ltd., Tokyo, Japan) before and 15 and 90 min after the OFC. Mice that were unlikely to recover body temperature (<30 °C) after the OFC were euthanized by cervical dislocation.

We measured plasma allergen-specific immunoglobulin (Ig) levels by enzyme-linked immunosorbent assay (ELISA). Ovalbumin (OVA)- and ovomucoid (OM)-specific IgE and IgA plasma levels were determined by capture ELISA (Maeta *et al.* 2018; Katahira *et al.* 2019) (Supplementary material). In addition, OVA- and OM-specific IgG1 and IgG2a levels were determined by indirect ELISA (Maeta *et al.* 2018; Katahira *et al.* 2019) (Supplementary material). Plasma triglyceride levels were measured using a triglyceride E-test kit (Wako Pure Chemical Industries, Osaka, Japan). Comprehensive lipid metabolite measurements were outsourced to Human Metabolome Technologies (HMT) Inc. (Yamagata, Japan) (Supplementary material). Hierarchical cluster analysis was performed by HMT's proprietary MATLAB.

Except for mortality rates and comprehensive lipid metabolite assessment values,

variables are presented as means $\pm$ standard error. Data of comprehensive lipid metabolites was shown the ratio calculated by relative levels of EW groups/relative levels of MF groups. Statistical differences except for comprehensive lipid metabolites were analyzed by one-way ANOVA followed by Tukey's multiple-comparison test. Differences with $p < 0.05$ were considered significant. The GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA) was used for all analyses.

On day -1, temperature change 15 min after the OFC was -1.5 ± 0.1 °C ($n=27$) in the mice, confirming the induction of EW allergic reaction. On day 16, in the LF-EW, MF-EW, and HF-EW groups, rectal temperature 90 min after the OFC was significantly lower than that in the MF group (Fig 1B). Moreover, mortality rates after the OFC in the MF, LF-EW, MF-EW, and HF-EW groups were 0% (0/7), 100% (6/6), 85.7% (6/7), and 85.7% (6/7), respectively (Fig 1C and Table 1). The preliminary and main experiment findings suggest this phenomenon to be reproducible. Moreover, these findings indicate that the deaths of mice in the LF-EW, MF-EW, and HF-EW groups following the OFC were caused by anaphylactic shock. However, in our previous experiments (Maeta *et al.* 2018; Katahira *et al.* 2019), mice deaths were not observed after an OFC during OIT (Table 1). The difference between the previous (Maeta *et al.* 2018; Katahira *et al.* 2019) and present experiments is in the use of LF and MF diets (Table 1), respectively, in the sensitization period. These findings suggest that in a mouse model of EW allergy, the amount of corn oil intake during the sensitization period exacerbates allergy during the OIT period.

Plasma OVA- and OM-specific IgE levels on day 15 were comparable across the groups (Supplementary Table 2). OVA-specific IgA level was higher in the LF-EW, MF-EW, and HF-EW groups than in the MF group (Supplementary Table 2). In previous experiments (Maeta *et al.* 2018; Katahira *et al.* 2019), OVA-specific IgA levels were 43.9 ± 6.2 and 197 ± 39 $\mu\text{g/mL}$ in the EW allergy mice that were fed the reference LF (R-LF) and LF-EW (R-LF-EW) diets during the 2-week OIT, respectively. The mice in the R-LF-EW groups did not die after the OFC (Table 1) (Maeta *et al.*

2018; Katahira *et al.* 2019). Increased OVA-specific IgA levels may be associated with antigen intake and may not affect mortality risk.

Plasma triglyceride levels in the LF-EW, MF-EW, and HF-EW groups were lower than those in the MF groups (Fig 2A). In comprehensive lipid metabolite analysis, 278 lipid metabolites were detected; hierarchical cluster analysis findings are presented in Fig 2B. The levels of 52 lipid metabolites in the LF-EW, MF-EW, and HF-EW groups were lower than those in the MF group (Supplementary Table 3 and Supplementary Fig. 2). Plasma triglyceride levels of EW-allergic mice fed R-LF diet after a 2-week OIT in our previous study were 81.1 ± 6.2 mg/dL (Katahira *et al.* 2019), and these levels in the MF group were higher than those in the R-LF group. Corn oil concentrations in the LF and MF diets were 5% and 13%, respectively. The MF diet during the sensitization and OIT periods were associated with an increase in the plasma triglyceride levels in a mouse model with EW allergy. In the main experiment, the plasma triglyceride and lipid metabolite levels in the LF-EW, MF-EW, and HF-EW groups were lower than those in the MF group. In our previous study, the plasma triglyceride levels in the R-LF group (81.1 ± 6.2 mg/dL) were comparable to those in the R-LF-EW group (90.7 ± 10.2 mg/dL) (Katahira *et al.* 2019). These findings indicate that the MF diet may increase lipid levels during the sensitization period and that allergen exposure over a 2-week period after the sensitization period may promote lipid metabolism. A previous study has shown that blood fatty acid composition was associated with allergy in children aged 2-9 years (Mikkelsen *et al.* 2017); meanwhile, other studies reported a relationship between ω -3 fatty acid metabolites and allergy alleviation (Kunisawa *et al.* 2015; Saika A *et al.* 2021). Overall, changes to lipid metabolism associated with fat intake and allergen exposure may aggravate food allergy.

Diets high in fat are associated with obesity, which may aggravate food allergy due to dysregulated intestinal effector mast cell responses, increased intestinal permeability, and gut dysbiosis (Hussain *et al.* 2019). The body weights of mice in the present and previous study (Maeta *et al.* 2018) before OIT were 23.6 ± 0.2 and 23.8 ± 0.2 g/mouse,

respectively. The MF diet that included 13% of corn oil did not induce obesity in the sensitization period. Hussain *et al.* reported that a high-fat diet may affect gut microbiota regardless of the obesity status, which may be sufficient to confer enhanced susceptibility to food allergy (Hussain *et al.* 2019). Intestinal bacteria are critical for regulating allergic responses (Feehley *et al.* 2019). Miyamoto *et al.* reported that 10-hydroxy-cis-12-octadecenoic acid, which is a gut microbial metabolite of linoleic acid, ameliorated intestinal epithelial barrier impairment (Miyamoto *et al.* 2015). However, in our experiments, the change of microbiota by MF diet and OIT were not determined. Future studies are required to elucidate the relationship between gut microbiota change associated with corn oil intake, allergen exposure, and OIT outcomes.

In conclusion, deaths among mice allergic to EW after a 2-week OIT period were associated with anaphylactic shock following the OFC. This phenomenon may be associated with increased corn oil intake in the sensitization period and allergen exposure for 2 weeks after sensitization. In the future, it is necessary to clarify, in detail, the mechanisms underlying the aforementioned phenomenon. Moreover, it is need to examine how the severity of allergies changes when using a low corn oil sensitized diet and a high corn oil OIT diet. The present findings indicate that nutrient intake during and before OIT may affect treatment outcomes.

Acknowledgements

We thank Editage for English language editing.

Author contributions

AM and KT designed the study. AM and RK analyzed the samples. AM drafted the manuscript. All authors approved the final manuscript.

Disclosure statement

The authors have no conflicts of interest to declare.

Funding

None

Data Availability

The data underlying this article are available in the article and from the corresponding author upon request.

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Table 1. Diets in the sensitization and oral immunotherapy (OIT) periods, and mortality rates after oral food challenge (OFC)

	Diets ¹		Day 16
	Sensitization period (day -1)	OIT period (days 1–15)	Mortality after OFC
<i>Preliminary experiment</i>			
MF	MF (13% corn oil)	MF (13% corn oil)	0/10 (0%)
MF-EW	MF (13% corn oil)	MF-EW (13% corn oil, 1% EW)	5/10 (50%)
<i>Main experiment</i>			
MF	MF (13% corn oil)	MF (13% corn oil)	0/7 (0%)
LF-EW	MF (13% corn oil)	LF-EW (5% corn oil, 1% EW)	6/6 (100%)
MF-EW	MF (13% corn oil)	MF-EW (13% corn oil, 1% EW)	6/7 (86%)
HF-EW	MF (13% corn oil)	HF-EW (34% corn oil, 1% EW)	6/7 (86%)
<i>Previous experiments (Maeta et al. 2018; Katahira et al. 2019)</i>			
R-LF	LF (5% corn oil)	LF (5% corn oil)	0/17 (0%)
R-LF-EW	LF (5% corn oil)	LF-EW (5% corn oil, 1% EW)	0/17 (0%)

¹ Diet compositions are presented in Supplementary Table 1.

EW, egg white; LF, low-fat; HF, high-fat; MF, medium-fat; R-LF, reference low fat

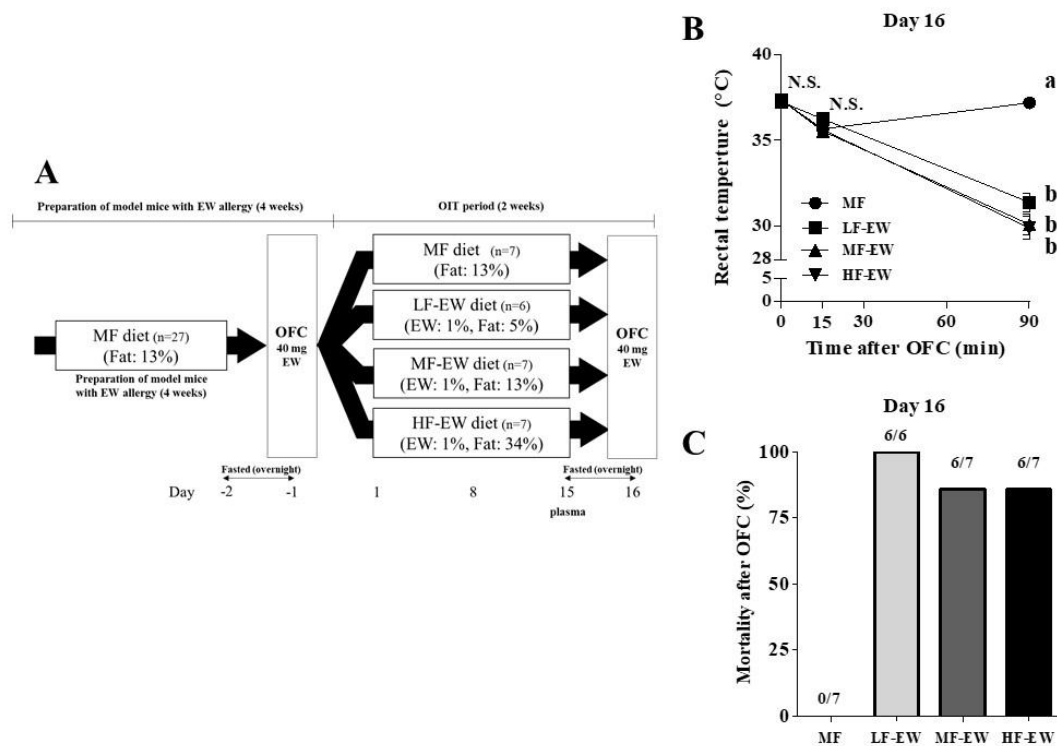


Fig 1. Study design schematics for the main experiments (A) and the rectal temperature (B) and the mortality (C) following oral food challenge (OFC) after oral immunotherapy.

Rectal temperature values are shown as mean $\pm$ standard error. P-values of <0.05 were considered significant and are indicated by lower-case letters (a, b). EW, egg white; LF, low-fat; HF, high-fat; MF, medium-fat; N.S., not significant

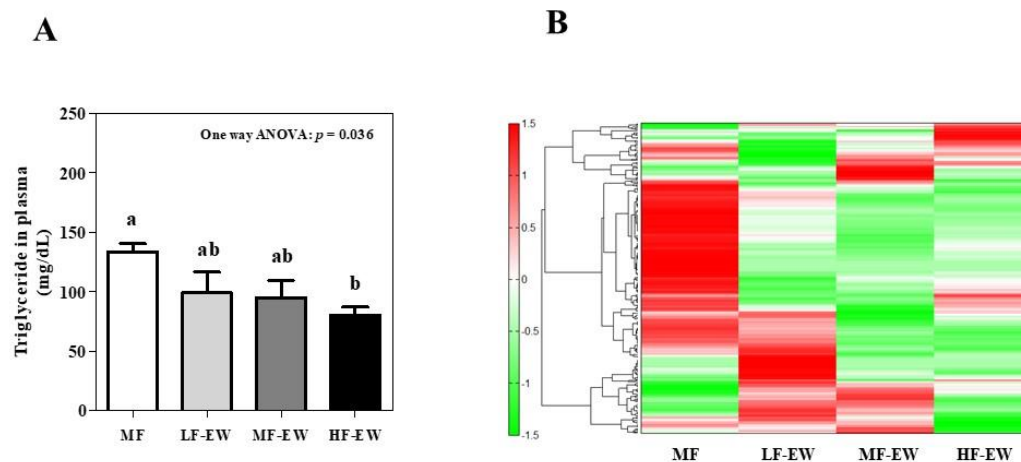


Fig 2. Plasma triglyceride levels (A) and hierarchical cluster analysis of comprehensive lipid metabolites (B).

Triglyceride values are presented as mean $\pm$ standard error. P-values of <0.05 were considered significant and are indicated by lower-case letters (a, b). Heat map of the relative levels of comprehensive lipid metabolites in pooled plasma volume of each group is presented. The darker the green, the smaller the mean of all groups, and the darker the red, the larger the mean of all groups. EW, egg white; LF, low-fat; HF, high-fat; MF, medium-fat.