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Original Article

# The interaction between *ChREBP* rs3812316 gene variant and FiberCreme-IMO cookies on triglyceride levels of hyperlipidemic subjects

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## Abstract

A high-fiber diet can reduce the triglyceride levels of hyperlipidemic subjects. FiberCreme-IMO is a non-dairy creamer product containing fiber that potentially improves the triglyceride profile. Genetic variants of *ChREBP* rs3812316 may affect triglyceride response on the consumption of FiberCreme-IMO cookies. This study investigated the effect of FiberCreme-IMO on triglyceride levels and the interaction between FiberCreme-IMO and genotypes of *ChREBP* rs3812316 on triglyceride levels of hyperlipidemic subjects. Fifty adults with hyperlipidemia were assigned into two groups: one group consumed cookies made from FiberCreme-IMO (n=25), and another consumed control cookies containing coconut milk powder (n=25). Each subject consumed 80 grams of cookies per day for four weeks. Serum triglycerides were measured at baseline and four weeks after treatment. Genotypes of *ChREBP* rs3812316 were analyzed at the end of the study using PCR-RFLP. FiberCreme-IMO cookies reduced the triglyceride levels compared to control cookies, but the reduction was not statistically significant ($p>0.05$). There was no significant interaction between FiberCreme-IMO cookies and *ChREBP* rs3812316 on the triglyceride levels. This study suggests that FiberCreme-IMO ameliorated the triglyceride levels of hyperlipidemic subjects after four weeks of consumption.

**Keywords:** dietary fiber, FiberCreme-IMO, hyperlipidemia, nutrigenetic.

## Introduction

Hyperlipidemia is a high blood lipid level characterized by high total cholesterol and/or triglyceride levels [1]. Based on Indonesian Baseline Health Research (Riskesdas) 2013 and 2018, hypertriglyceridemia increased within five years [2]. An imbalanced diet is one of many factors that cause hypertriglyceridemia [3]. Improving dietary patterns, such as enhancing dietary fiber intake, can ameliorate the triglyceride profile of hyperlipidemic subjects [4].

A widely marketed creamer product containing fiber is FiberCreme-IMO. FiberCreme-IMO uses isomaltooligosaccharide (IMO) to replace glucose components in conventional creamer. IMO is a complex carbohydrate resistant to enzymatic digestion [5]. FiberCreme-IMO is also made with fully hydrogenated coconut oil, making this creamer free of trans fat; thus, it can be used as a replacement for coconut milk. Moreover, a previous study by Marsono *et al.* (2020) reported the hypolipidemic effect of FiberCreme-IMO in diabetic rats [6].

Genetic variation among individuals may contribute to the difference in triglyceride response to diet [7]. Genome-wide association studies (GWAS) have identified genetic variants associated with triglyceride metabolism; one of them is *ChREBP* rs3812316 [8]. Ortega-Azorin *et al.* (2014) reported that the mutation of *ChREBP* rs3812316 c.771C>G affected triglyceride response in the Mediterranean diet, in which the mutant allele with high adherence to the Mediterranean diet

showed a lower hypertriglyceridemia proportion diet compared to wild-type [9]. Therefore, this study investigated the effect of FiberCreme-IMO cookies on triglyceride levels in hyperlipidemic adults and analyzed the probability of a genetic variant of *ChREBP* rs3812316 affecting individual triglyceride responses.

## Material and methods

### Subjects

Adults aged 20–60 years were recruited, and the screening was based on the ICD-10 standard in which subjects with fasting triglyceride levels ≥150 mg/dL and/or fasting total cholesterol ≥200 mg/dL are categorized as hyperlipidemia [1]. Participants with a history of complications, pregnant and breastfeeding women, are excluded from this study. This study was approved by the Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/0404/EC/2021). The patients signed the informed consent forms before participating in this study.

### Intervention

Fifty eligible subjects were divided into two treatment groups: 1) FiberCreme-IMO cookies (n=25) and 2) control cookies containing coconut milk (n=25). The composition of treatment cookies is presented in Table 1. Subjects consumed 80 grams of cookies each day for four weeks.

### Data collection

Data were obtained twice, before and four weeks after cookie treatment. Blood specimens were collected in the morning after 8 hours of fasting. In order to estimate their dietary intake, subjects were assigned to complete the semi-quantitative food frequency questionnaire (SQ-FFQ). The usage of lipid-lowering drugs and anthropometric measurements were also assessed.

### Biochemical measurement

Serum total cholesterol and triglyceride were analyzed using a commercial kit (DiaSys, Germany). Genomic DNA was extracted from peripheral blood using standard protocols (Promega Wizard™ Genomic DNA Purification Kits). Genotypes of *ChREBP* rs3812316 were analyzed using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). 545 bp fragment was amplified using primers 5'-CACCTGAGATGTCCATGAAGTC-3' (forward) and 5'-CAGAGGTGTGAACAGACAGACC-3' (reverse). PCR conditions were the following: pre-denaturation at 95°C for 7 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute, then final extension at 72°C for 7 minutes. PCR products were then digested using *the Cail* restriction enzyme.

### Statistical analysis

Data were analyzed using SPSS version 25 (IBM Corp, USA). Chi-square and Fisher's exact tests were used to compare the proportions of the two groups. Paired t-test was used to analyze the difference in triglyceride levels before and after treatment. Two-way ANOVA was used to compare the mean differences in triglyceride changes according to genotypes and treatment groups. The *General Linear Model* (GLM) was used to determine the interaction between cookies and genetic variation on changes in triglyceride levels, with adjustment for the potential confounders such as dietary intake and medication (lipid-lowering drugs). Statistical significance was set at $p<0.05$.

Table 1: Composition of treatment cookies.

| Components | FiberCreme-IMO cookies (100 grams) | Coconut milk cookies (100 grams) |
|---|---|---|
| **Carbohydrate** | 71.89 | 69.82 |
| **Fat** | 11.45 | 11.98 |
| **Protein** | 5.04 | 4.83 |
| **Water** | 4.73 | 7.31 |
| **Fiber** | 5.78 | 4.09 |
| **Resistant starch** | 2.21 | 1.74 |

## Results

### Baseline characteristics

Table 2 shows the baseline characteristics of the 50 subjects by cookies groups. Triglyceride and total cholesterol levels between the two groups were not statistically different.

### The effect of treatment cookies on triglyceride levels

After receiving 4 weeks of treatment, subjects who consumed FiberCreme-IMO cookies experienced decreased triglyceride levels. In contrast, those who consumed control cookies showed increased triglyceride levels (Table 3), though these changes were not statistically significant.

This study did not observe the wild-type genotype of *ChREBP* rs3812316. The proportion of CG and GG genotypes was 92% and 8%, respectively. As presented in Table 4, the reductions of triglyceride levels between *ChREBP* rs3812316 genotypes were not significantly different (p=0.432). There was no significant interaction between genetic variants of *ChREBP* rs3812316 and FiberCreme-IMO cookies on the triglyceride levels.

## Discussion

A reduction of triglyceride levels in the FiberCreme-IMO group was probably due to the higher fiber and resistant starch contained in FiberCreme-IMO cookies compared to coconut cookies. Dietary fiber, notably resistant starch, cannot be hydrolyzed easily by small intestine digestive enzymes [10]. Resistant starch could persist in the small intestine for 120 minutes without being digested before entering the colon [11]. Undigested fiber will undergo an enzymatic fermentation by colonic bacteria and produce short-chain fatty acids (SCFA), including propionate, acetate, and butyrate [12]. Several studies reported the ability of propionate to reduce the production of free fatty acids in the liver [11]. According to Sunarti *et al.* (2020), SCFA suppressed the SREBP-1c expression, a transcriptional regulator of genes involved in fatty acids and triglyceride synthesis [13]. Isomaltooligosaccharide in FiberCreme-IMO is a complex carbohydrate with similar

Table 2: The baseline characteristics of study subjects.

| Parameters | FiberCreme-IMO cookies (n=25) | Control cookies (n=25) | P-value |
|---|---|---|---|
| **Age** | 45.00±2.25 | 49.84±1.74 | 0.130* |
| **Body mass index** | 27.25±0.91 | 25.24±0.62 | 0.044* |
| **Sex (M/F)** | 7/18 | 14/11 | 0.086[a] |
| **Triglyceride (mg/dL)** | 169.53±15.71 | 150.42±15.94 | 0.345* |
| **Total cholesterol (mg/dL)** | 225.59 (165.26–318.53) | 227.23 (133.33–288.96) | 0.915[#] |
| **% Whole body fat** | 35.12±1.15 | 29.76±0.80 | <0.001* |
| **% Visceral fat** | 11.02±0.97 | 9.64±0.93 | 0.266* |
| **Energy intake (kcal)** | 2020.20±126.52 | 1880.60±122.88 | 0.425* |
| **Carbohydrate (% energy)** | 44.54±1.80 | 46.12±1.49 | 0.502* |
| **Total fat (% energy)** | 45.97±1.66 | 45.30±1.51 | 0.764* |
| **Protein (% energy)** | 13.53±0.70 | 13.81±0.59 | 0.759* |
| **PUFA (% energy)** | 11.73±0.81 | 11.14±0.86 | 0.595* |
| **Dietary fiber (g)** | 19.01±1.80 | 19.43±1.65 | 0.867* |
| **Lipid-lowering drugs (%)** | 4 (16) | 5 (20) | 1.000[b] |

Note: Data are presented as mean±SE or median (min-max) for continuous variables and number (%) for categorical variables. P-value was determined by independent t-test*, except total cholesterol, sex, and use of lipid-lowering drugs, which were determined by [#] Mann-Whitney test, [a] Chi-square and Fischer's exact test respectively; PUFA, polyunsaturated fatty acid.


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Table 3: Triglyceride levels between cookies groups (mg/dL).

| | FiberCreme-IMO cookies | Control Cookies |
|---|---|---|
| **Before treatment** | 169.53±15.71 | 150.42±15.94 |
| **After treatment** | 155.63±16.23 | 163.15±18.02 |
| **ΔTG** | -13.90±11.70 | 12.73±14.22 |
| **P value** | 0.083 | 0.170 |

Note: Data are presented as mean±SE; ΔTG – changes in triglyceride levels before and after treatment; p-value compared the mean of triglyceride levels before and after treatment.

Table 4: Triglyceride levels between *ChREBP* rs3812316 genotypes before and after treatment (mg/dL).

| | FiberCreme-IMO cookies | | Control cookies | | P interaction |
|---|---|---|---|---|---|
| | CG (n=2) | GG (n=23) | CG (n=2) | GG (n=23) | |
| **Before** | 97.51±5.40 | 175.79±16.44 | 114.10±45.90 | 153.58±16.95 | 0.379 |
| **After** | 90.63±3.07 | 161.28±17.15 | 193.79±77.08 | 160.48±18.91 | |
| **ΔTG** | -6.88±2.33 | -14.51±12.74 | 79.69±31.18 | 6.91±14.72 | |
| **P-value** | 0.098 | 0.095 | 0.062 | 0.301 | |

Note: Data are presented as mean±SE; ΔTG – changes in triglyceride levels before and after treatment; p-value compared the mean of triglyceride levels before and after treatment.

effects to dietary fiber [5]. Additionally, Grubic *et al.* (2019) reported the effect of IMO supplementation on lipid profile improvement [14].

Although FiberCreme-IMO contains less saturated fat than coconut cookies, it might account for the insignificant reduction in triglyceride levels. Excessive intake of saturated fat increases triglyceride levels [15], resulting in ineffective triglyceride reduction. The negligible interaction effect between FiberCreme-IMO cookies and *ChREBP* rs3812316 on the triglyceride levels may be due to the small sample size; thus, it was not strong enough to detect significant changes [16]. Although the reduction of serum triglyceride in this study was not statistically significant, reducing triglyceride levels could impede the risk of cardiovascular diseases. Various intervention studies showed that every one mmol/L (18 mg/dL) decrease in triglyceride levels could reduce the probability of cardiovascular diseases by 0.8 times (95% CI; 0.76–0.85; p<0.0001) [17]. Further study using various doses of FiberCreme-MO cookies is needed to determine the effective amount of FiberCreme-IMO cookies in reducing triglyceride levels.

This study was not without limitations: firstly, there was a possible recall bias when completing the SQ-FFQ, as this method was influenced by memory, individual perception, and knowledge [18]. To maintain standardized assessment and reduce potential biases, we conduct a briefing with the enumerators before data collection. Secondly, we did not assess the physical activity and the duration of medication with lipid-lowering drugs.

## Conclusion

FiberCreme-IMO can improve the triglyceride profile in hyperlipidemic subjects, suggesting its suitability as an alternative to replace coconut milk. However, no significant interaction was observed between the genotype of *ChREBP* rs3812316 and FiberCreme-IMO cookies in relation to triglyceride levels, possibly due to the limited sample size. Further studies with a larger sample size are necessary to confirm these preliminary findings.

## Conflict of interest

The authors declare no conflict of interest.

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