RESEARCH ARTICLE

Effect of intake of virgin coconut oil (*Cocos nucifera* L.) on the spleen and small intestinal immune cells and liver lipid of mice

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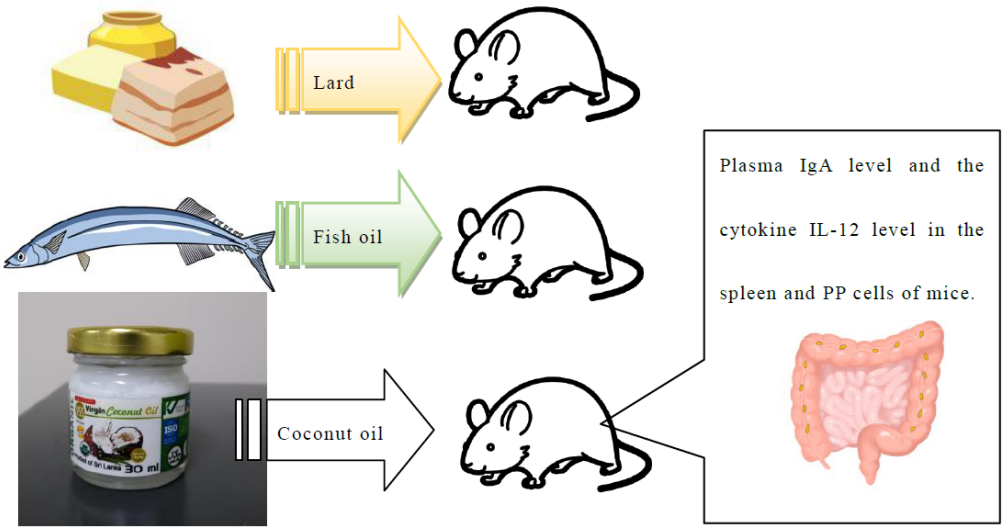

Highlights

- Feed lard diet, fish oil diet and coconut oil diet in mice for 6 weeks.
- Virgin coconut oil (*Cocos nucifera* L.) has a high proportion of medium-chain fatty acids (MCFAs).
- The cytokine IL-12 level in the spleen cells was highest in the C group, followed in order by the F and L groups.

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RESEARCH ARTICLE

Effect of intake of virgin coconut oil (*Cocos nucifera* L.) on the spleen and small intestinal immune cells and liver lipid of mice

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Abstract: Virgin coconut oil (*Cocos nucifera* L.) (VCO) is consumed worldwide and is rich in medium-chain fatty acids (MCFAs) and lauric acid (LA). The aim of this study was to examine the immuno-stimulation effect of VCO produced in Sri Lanka. Three groups of mice were fed either a lard diet (L), a fish-oil diet (F) or a coconut oil diet (C) for 6 weeks. During the experimental period, no significant differences in total food intakes or body weights of mice were observed among the three groups. Perirenal fat tissue weight, plasma Triacylglycerol (TG) level and total liver lipids were lower in the F group mice than those in the L and C group mice. The total cholesterol levels in livers of mice in the C group showed higher than that of the other groups. The cytokine IL-12 levels in spleen cells were highest in the C group mice, followed in order by the mice in F and L groups. Our studies clearly show that the plasma IgA and the cytokine IL-12 levels in the spleen and PP cells of mice increased in spite of the accumulation of liver lipids in mice by the intake of VCO produced in Sri Lanka.

Keywords: Coconut oil; medium-chain fatty acids; IL-12; lard; fish oil.

INTRODUCTION

Virgin coconut oil (*Cocos nucifera* L.), extracted from fresh coconut pulp is known to have a high proportion of medium-chain fatty acids (MCFAs), lauric acid (LA) and polyphenols with antioxidant activity (Cardoso *et al.*, 2015). Coconut oil is easily absorbed and can be an excellent energy source for physical performance (Alves *et al.*, 2015). The scientific literature has demonstrated benefits of extra virgin coconut oil to the reduction of body fat (Lipoeto *et al.*, 2004). In a study by Voon *et al.* (2011) diets rich in saturated fatty acids prepared with either palm oil or coconut oil and high in oleic acid did not alter postprandial or fasting plasma concentrations. Serum cholesterol, triglyceride and glucose levels were also found to be lower in virgin coconut oil (VCO) treated mice (Yeap *et al.*, 2015).

We focused on the immune-stimulation effect of VCO from Sri Lanka, because this effect has been little studied to date. We examined the body and fat tissue weight, plasma lipid concentration and plasma immunoglobulin A (IgA) in the spleen and the small intestinal immune cells of mice. Peyer's patch (PP) cells from the small intestine and spleen cells were studied, and interleukin (IL)-12 level was investigated. We also compared the effects of coconut oil with those of lard and fish oil.

MATERIALS AND METHODS

Animals and diets

Fifty-four three-week-old male ICR mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). All animals were housed individually in stainless steel cages under controlled conditions at a temperature of 22 ± 1 °C and 50% relative humidity with a 12 h dark/light cycle (19:00-7:00). Animals were randomly divided into three dietary treatment groups with equal mean body weight: the lard diet (L) group (n=18), the fish oil diet (F) group (n=18), and the coconut oil diet (C) group (n=18). The experimental diet was based on the AIN-93G diet (Reeves *et al.,* 1993). The composition of the fatty acid composition of the lard, fish oil and coconut oil diets are shown in Tables 1. It was added soybean oil (3 g/100 g diet) avoid an n-6 fatty acid deficiency. Casein, lard, soybean oil and dietary components were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Fish oil was received from Nihon-Suisan (Tokyo, Japan), and VCO was purchased from VSS Products (PVT) Ltd. (Dankotuwa, Sri Lanka). The mice were fed the L, F or C diet for 6 weeks. Feed intake was recorded daily, and body weight was measured on alternate days. After feeding periods of two, four and six weeks, five mice were fasted for 16 h and sacrificed without affliction under ether anesthesia, and liver tissues and perirenal fat tissues were collected. Blood was collected by heart puncture with a heparinized syringe. The blood was maintained at 4 °C and centrifuged at 1,000 g for 15 min. Plasma and liver samples were stored at -80 °C until they were analysed.

All procedures were performed in accordance with the Animal Experimentation Guidelines of the Laboratory Animal Care Committee of Seitoku University (No.192).

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Table 1: Fatty acid composition of the diets (% of total fatty acids)

Fatty acid	Lard diet	Fish oil diet	Coconut oil diet
Saturated	33.1	25.4	69.9
Monounsaturated	41.6	33.3	11.8
Polyunsaturated	25.2	37.8	18.2
Total n-6	22.8	17.9	16.3
Total n-3	2.3	20.3	1.9
n-6/n-3	9.9	0.9	8.6

Table 2: Body, liver and perirenal fat tissue weights

Week	Group	Final body weight (g)	Liver weight (g/100 g B.W.)	Perirenal fat tissue weight (g/100 g B.W.)
2	L	30.6 ± 3.38	6.02 ± 0.61	0.43 ± 0.31
	F	33.0 ± 4.11	5.58 ± 0.74	0.37 ± 0.18
	C	33.5 ± 2.11	5.51 ± 0.49	0.50 ± 0.23
4	L	41.5 ± 6.13	4.73 ± 0.05	0.67 ± 0.38
	F	39.0 ± 5.04	4.53 ± 0.28	0.70 ± 0.21
	C	38.3 ± 8.01	4.46 ± 1.33	0.69 ± 0.28
6	L	46.1 ± 5.40	3.73 ± 0.41	1.06 ± 0.10^{a}
	F	40.6 ± 3.30	3.87 ± 0.43	0.72 ± 0.30^{b}
	C	43.6 ± 4.20	3.71 ± 0.24	1.11 ± 0.23^{a}

Values represent mean ± SD (n=5). Within a grouping, values not sharing a common superscript letter are significantly different at $p < 0.05$.

Biochemical assays of plasma and liver

Liver lipids were extracted following the method described by Folch *et al.* (1996). Triacylglycerol (TG), total cholesterol (T-cho) concentrations in plasma, and liver extracts were measured using test kits (Triglyceride E-tests Wako and Cholesterol E-tests were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan). The IgA level of plasma in mice was measured using test kits (Mouse IgA ELISA Kit, ICL, Inc., Portland, OR, USA).

Cytokine analysis

Cells were prepared following the procedure described by Aoki-Yoshida *et al*. (2016). Cells were cultured in RPMI1640 (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal calf serum (FCS; GIBCO, Grand Island, NY, USA), 2 g/L $NaHCO_3$, 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μM 2-mercaptoethanol, and 300 mg/L L-glutamine at 37 °C in 5% CO_2 in the air.

PP cells from ICR mice fed for 6 weeks were isolated from the small intestines as follows, PP cells were removed from the small intestines and washed with Roswell Park Memorial Institute (RPMI) (+). The PP cells were crushed in RPMI (+) treated with 1 mg/mL collagenase IV (Sigma Aldrich, St Louis, MO, USA) in a 50 mL tube with gentle stirring at 37 °C in the air for 60 min. After collagenase treatment, the preparation was filtered with gauze and the cells were washed with Phosphate-buffered saline (PBS) followed by centrifugation at 4 °C and, 1300 rpm for 5 min. The supernatant was suspended with 1 mL of RPMI (+).

Spleen cells were crushed in 5 mL of RPMI (+) and filtered with gauze. The cell suspension was then centrifuged at 4 °C and, 1300 rpm for 5 min. The supernatant was suspended with 1 mL of RPMI (+).

One hundred μL of isolated PP cells (1×10^5 cells/well) and spleen cells (1×10^5 cells/well) were added in a 96-well flat-bottomed plate. Determination of the IL-12 level in the supernatants was performed by a sandwich enzyme-linked immunosorbent assay (ELISA). The mouse IL-12 ELISA Ready-SET-Go! (eBioscience, Inc., San Diego, CA, USA) was used according to the manufacturer's instructions.

Statistical analysis

Values were expressed as means ± SD. Repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of groups. Differences in mean values between groups were tested by Scheffe's multiple range test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Body, liver and perirenal fat tissue weights

No significant differences in final body weight or liver weight were observed among the three groups (Table 2). There were no significant differences in perirenal fat tissue weights were observed among the three groups up to 4 weeks. However, weights in the F group at 6 weeks were lower than those of the L and C groups ($p < 0.05$).

Liver, plasma lipids and plasma IgA profiles

During the experimental period, there were no significant differences in liver TG concentration among mice in the three groups (Figure 1b). No differences were observed in total lipids or in liver T-cho concentrations among three groups until 4 weeks. The total lipids or the liver T-cholesterol concentrations in the C group at 6 weeks showed higher levels than those in the L and F groups (Figure 1a, c) ($p < 0.05$).

On the other hand, the plasma TG concentration in the F group was lower than those in the L and C groups at 6 weeks (Figure 2a) ($p<0.05$). Additionally, the plasma T-cho concentrations in the F group were lower than those in the L and C groups at both 2 and 6 weeks (Figure 2b) ($p<0.05$). No differences were observed in plasma IgA levels among the three groups up to 2 weeks. However, plasma IgA levels of the F and C groups showed higher levels at 4 weeks than that of the L group (Figure 2 c) ($p<0.05$).

IL-12 levels of spleen and PP cells

The cytokine IL-12 level in the spleen cells was highest in the C group, followed in order by the F and L groups (Figure 3a) ($p<0.05$). Level of the cytokine IL-12 in the PP cells of the C group was higher than that in the L and F groups (Figure 3b) ($p < 0.05$).

DISCUSSION

Fish oil is known to reduce plasma TG levels in patients with hyperlipidemia and is useful for improving CVD (Nestel *et al.*, 1990; Guichardant *et al.*, 2015). The lipid-lowering action of n-3 PUFAs results primarily from the inhibition of lipogenesis and the stimulation of FA oxidation in the liver (Halminski *et al.*, 1991; Komatsuzaki *et al.*, 2010). Nakashima *et al.* (2009) report that plasma lipid concentrations in mice fed a fish oil diet for 6 weeks were lower than those in mice fed a lard diet. The present experimental results also showed that perirenal fat tissue weight, plasma TG, and T-cho concentrations in the F group were lower than those in the L and C groups at 6 weeks (Table 3, Figure 2a, b) ($p<0.05$).

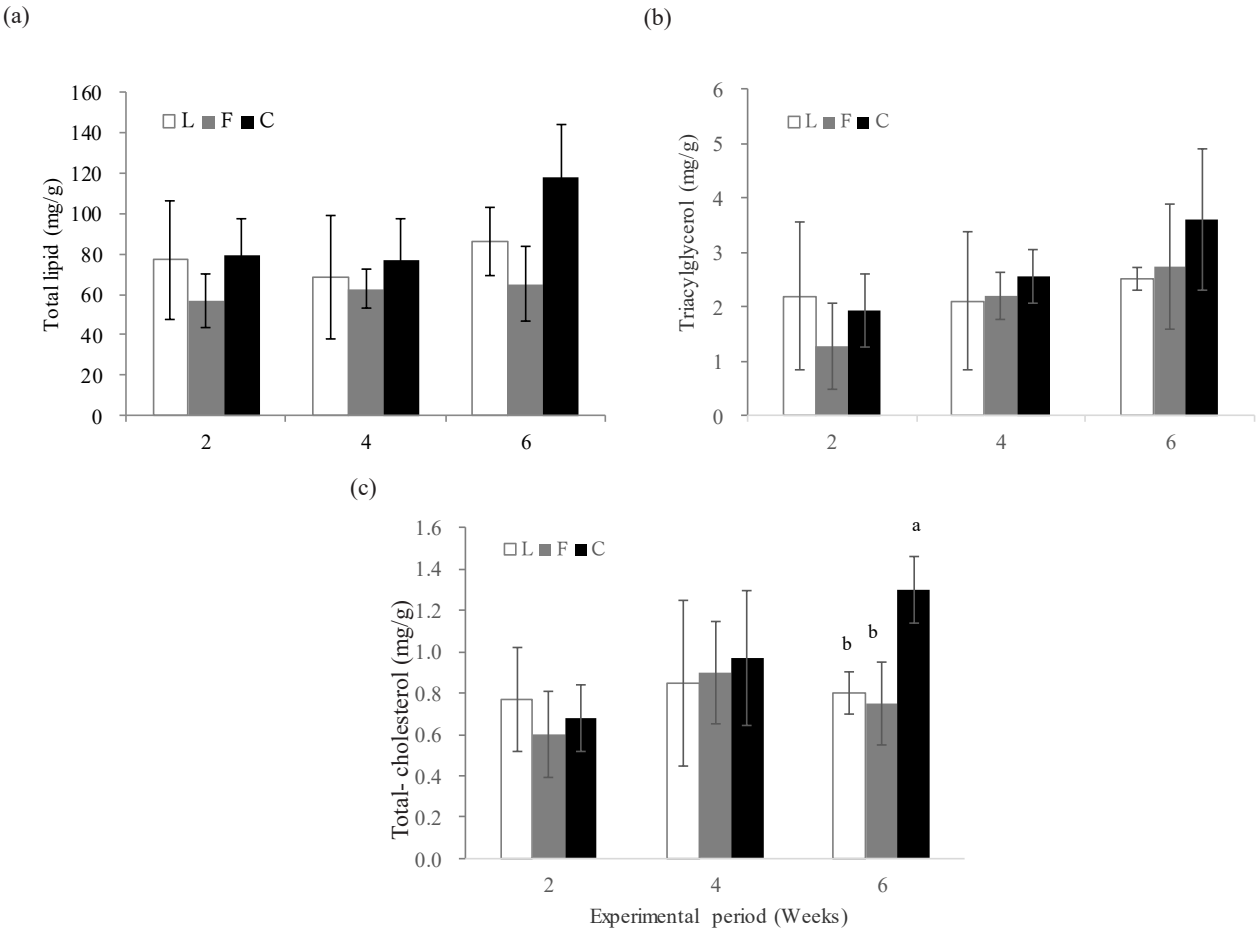

Figure 1: Liver lipids in mice fed experimental diets; L: lard diet; F: fish oil diet; C: coconut oil diet. Values represent mean ± SD (n=5). Within a grouping, values not sharing a common superscript letter are significantly different at $p < 0.05$.

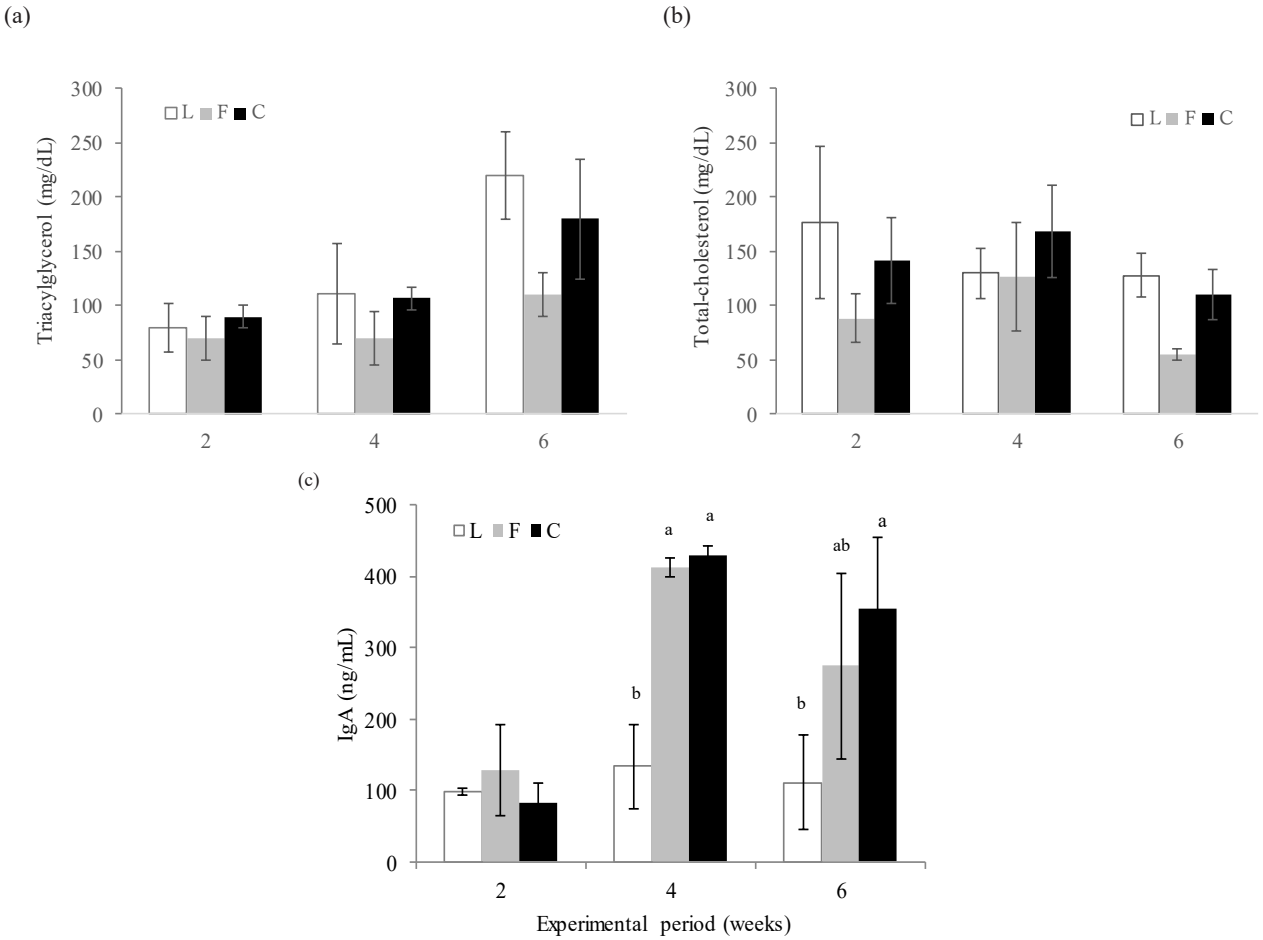

Figure 2: Plasma lipids and IgA levels in mice fed experimental diets. L: lard diet; F: fish oil diet; C: coconut oil diet.Values represent the mean ± SD (n=5). Within a grouping, values not sharing a common superscript letter are significantly different at $p < 0.05$.

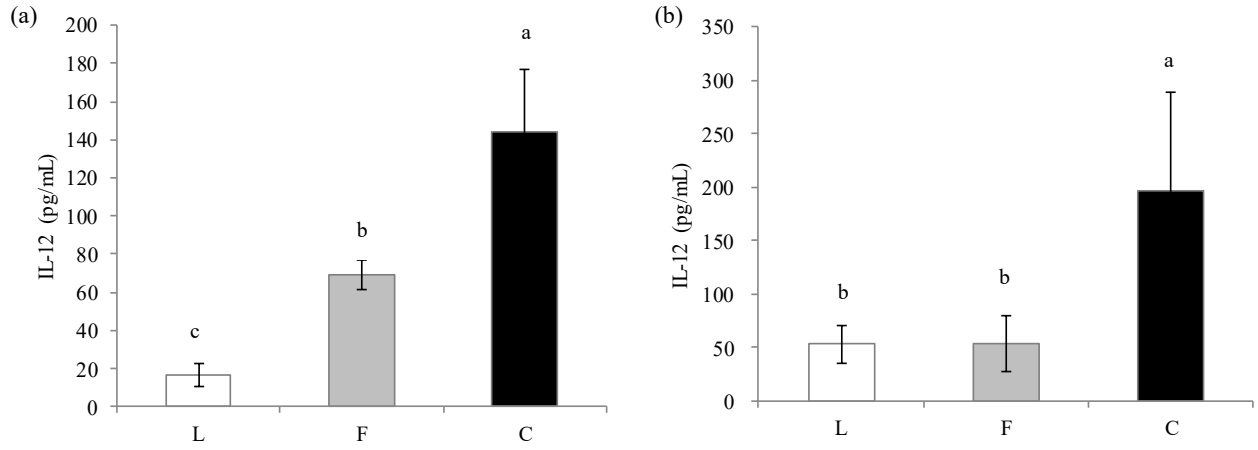

Figure 3: Production of the cytokine IL-12 in the spleen cells and Peyer's patch (PP) cells in mice fed experimental diets after 6 weeks; (a) spleen cells. (b) PP cells. L: lard diet; F: fish oil diet; C: coconut oil diet. Values represent the mean ± SD (n=5). Within each panel, values not sharing a common superscript letter are significantly different at $p<0.05$.

On the other hand, the effect of the hypolipidemic action of coconut oil was not observed as well as the effect of lard (Figure 2a, b). The total lipids and liver T-cho concentrations in the C group at 6 weeks were 118 ± 26 mg/g and 1.3 ± 0.2 mg/g, respectively (Figure 1a, c) (p<0.05), and liver T-cho concentrations in the C group was twice as high as those in the L group at 6 weeks. MCFAs are absorbed intact from the small intestine, and do not undergo degradation or re-esterification processes (Pehowich *et al.*, 2000). They are transported to the liver through the portal vein like glucose and amino acids, which are degradation products of sugar and proteins respectively (McCarty *et al.*, 2016). LA concentrations in mice fed the lard diet and the coconut oil diet in the present experiment were 0.1% and 34.3%, respectively. This demonstrates clearly that fats can easily accumulate in the liver of mice fed coconut oil for a long time.

Plasma IgA levels in the F and C groups were found to have increased at 4 weeks after the start of feeding (Figure 2c). IgA is present in the blood, intestinal tract and mother's milk, and plays an important role in immunity. It has been reported that fish oil enhances intestinal IgA and B cell functions *in vivo* (Gurzell *et al.*, 2013). It is important to investigate whether the coconut oil has an immune-stimulating effect, as DHA does.

Recent experimental studies on VCO suggest antioxidant, anti-inflammatory, and immunostimulatory effects of VCO (Voon *et al.*, 2011; Nair *et al.*, 2016). The cytokine IL-12 produced by immune cells works to activate important cell-mediated immunity factors in the early period of infection (Komatsuzaki *et al.*, 2017). In order to clear the immune-stimulation effect of coconut oil, the IL-12 production of the spleen cells and intestinal PP cells of mice was investigated. IL-12 levels in the spleen and the PP cells of the C group were 144 ± 33 pg/mL and 197 ± 91 pg/mL, respectively (Figure 3) and notably, the IL-12 level of spleen cells in the C group was approximately twice that in the F group (Figure 3 a). According to Voon *et al.* (2011), no significant difference was observed in the effects of a coconut oil diet containing LA (8.5%) on serum inflammatory markers such as tumor necrosis factor (TNF)-α IL-6 and interferon (IFN)-γ. In the present study, the LA composition of the diets of the C group was 34.3% (Table 2). When adding 6-10C other than LA, the total MCFA in the coconut oil diet was 46.9%. There are many immunologically active substances in the spleen and PP cells. IL-12 production was detected in both spleen and PP cells in the C group in this experiment, and we speculate that the LA contained in the coconut oil induces cytokine production.

Ye *et al.* (2020) reported that plasma IL-12 levels were increased in many types of atherosclerosis and atherosclerotic cardiovascular diseases. It was speculated that the accumulation of perirenal fat tissues and the increased plasma lipids in the C group stimulated the PP cells and then the plasma IL-12 levels. Therefore, it was suggested that VCO was one of the factors causing cardiovascular diseases although it activated immune cells in the C group.

In a recent report, the polyphenols in VCO were found to be contributed to the prevention of Alzheimer's disease (Chatterjee *et al.*, 2020). Thus, further research is needed on the health effects of VCO. LA is known to exhibit antimicrobial activity against some bacteria, but it is unclear whether the antimicrobial activity of VCO is comparable to that of LA (Nagase *et al.*, 2017). In future research, we would like to examine the antimicrobial activity of VCO in greater details.

CONCLUSION

The present study clearly shows that the plasma IgA and the cytokine IL-12 levels in the spleen and PP cells of mice increased in spite of accumulation of liver lipids in mice by the intake of VCO produced in Sri Lanka.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no financial conflicts of interest to report.

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