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Effects of increasing ratios of dietary omega-6/omega-3 fatty acids on human monocyte immunomodulation linked with atherosclerosis

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ABSTRACT

The aim of this study was to identify a reliable ratio of omega-6/omega-3 fatty acids for regulating monocyte immunomodulation linked with atherosclerosis. We evaluated monocyte behavior on treatment with various dietary fatty oils with increasing fatty acid ratios (ω6:ω3): canola oil (ω6,5.6:ω3,1), olive oil (ω6,13.4:ω3,1), corn oil (ω6,52:ω3,1), coconut oil (saturated), and fish oil (ω6,1:ω3,2). Oxidized LDL-treated monocytes were treated with 0.5% and 2% oil emulsions (v/v) for 0, 2, 4, and 8 h. Oil red 'O' and Nile red staining demonstrated 70% and 30% lipid accumulation only in coconut and corn oil. The levels of IL-1β, IL-12β, IL-6, macrophage colony stimulation factors (IFNγ, IKBKγ_1, IKK), and atherosclerosis-related immunomodulatory genes (TLR-4, TGFBR$_2$, and IL$_{33}$) were significantly increased after 8 h only in corn and coconut oil. Canola (5.6:1) and fish (2:1) oils are beneficial for immunoregulation and suppress macrophage-induced foam cell formation and atherosclerosis development.

1. Introduction

Dietary fat is an important component of the human diet; however, an imbalance in the saturated/unsaturated fats ratio or a higher intake of fat can have negative effects on the immune system and consequently health (Jump, 2011). Consumption of excessive saturated fat (SFA) promotes lipid storage and inflammation linked with risk of inflammatory cardiovascular disease (Jump, 2008). In contrast dietary polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acid (MUFA) play a protective role by controlling synthesis and oxidation of SFA, lower hepatic fat content and hypercholesterolemia. The PUFAs omega-3 fatty acids (α-linolenic acid, ALA) have anti-inflammatory properties, and thus might be useful in the management of inflammatory and autoimmune diseases. Omega-6 fatty acid (linoleic acid, LA) is a precursor for the proinflammatory cytokines interleukin (IL)-1 and leukotriene-B4 (LTB4), and elevation of these cytokines is linked to insulin resistance, coronary heart disease, psoriasis, depression, aging, and cancer (Simopoulos, 2008).

A balanced ratio of omega-6 to omega-3 PUFAs plays an essential role in multiple biological processes and maintains metabolic homeostasis (Wang, Chen, Hao, & Yang, 2016). In the Western diet, the omega-6/omega 3 fatty acid ratio has been determined to range from 15/1 to 16.7/1 (Simopoulos, 2002). A deficiency in omega-3 and excessive omega-6 promote the pathogenesis of many diseases such as an influence on arterial smooth muscle cells that leads to cardiovascular diseases (Simopoulos, 2008). In particular, intake of foods with an imbalanced omega-6/omega-3 ratio plays a major role in the development of hyperlipidemia that often leads to atherosclerosis, which is characterized by endothelial dysfunction, arterial macrophage accumulation, inflammation, and vascular smooth cell proliferation (Tiniakos, Vos, & Brunt, 2010). LA increases low-density lipoprotein (LDL)-oxidation, and oxidative modification increases the atherogenicity of LDL-cholesterol. Arterial oxidized LDL (oxLDL) is then taken up by scavenger receptors and is internalized by vessel macrophages, leading to foam cell formation and arterial smooth muscle cell inflammation (Reaven et al., 1991).

ALA or omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, other autoimmune disorders, and cancer (Simopoulos, 1991). LA, an 18-carbon fatty acid with two double bonds (18:2, omega-6), is the primary dietary omega-6 PUFA. LA cannot be synthesized by humans, and higher dietary intake of LA increases the symptoms of coronary disease since omega-6 fatty acid further breaks down to form arachidonic acid (AA), which is the substrate for the synthesis of a variety of proinflammatory molecules. Thus, reducing LA intake should help to reduce the tissue AA content, which might in turn reduce the inflammatory potential and therefore, lower the risk of coronary disease (Ross, 1999).

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Table 1
Composition analysis using GC–MS for canola oil, olive oil, corn oil, coconut oil and fish oils purchased from local Saudi market shown 99–95% similarity in the database.

S. No	Compound Name	Peak area (%)				
		Canola oil	Olive oil	Corn oil	Coconut oil	Fish oil
1	o-Ethylhydroxylamine 1- propanamine	0.20	–	0.11	–	
2	9-Hexadecenoic acid	0.21	0.79	0.31	–	0.06
3	Hexadecenoic acid	4.38	8.93	11.13		0.09
4	9,12-Octadecadienoic acid (ω-6)	18.84	9.83	49.25	1.8	0.43
5	9-Octadecenoic acid (Oleic acid)	64.20	52.23	21.93	5.5	0.08
6	9,12,15-Octadecatrienoic acid (ω-3)	1.75	–	–	1.1	0.37
7	Ethyl oleate	–	–	–	–	7.36
8	Octadecanoic acid	–	–	–	–	3.76
9	Cis-5,8,11,14,17-Eicosapentaenoic acid (ω-3)	–	–	–	–	0.28
10	Cis-5,8,11,14,17-Eicosapentaenoic acid (ω-3)	–	–	–	–	0.10
11	Cis-5,8,11,14,17-Eicosapentaenoic acid (ω-3)	–	–	–	–	0.83
12	Cis-11-Eicosenoic acid	1.03	–	0.17	–	3.62
13	Ethyl-5,8,11,14,17-Eicosapentaenoate (ω-3)	–	–	–	–	20.28
14	Methyl-8,11,14,17-Eicosatetraenoat	–	–	–	–	1.55
16	Eicosanoic acid	–	–	–	–	1.56
17	4,7,10,13,16,19-Decosahexaenoic acid (ω-3)	–	–	–	–	0.30
18	Methyl-6,9,12,15,18-henicosapentaenoate (ω-3)	–	–	–	–	0.39
19	4,7,10,13,16,19-Decosahexaenoic acid (ω-3)	–	–	–	–	0.25
20	Butyl 4,7,10,13,16,19-Decosahexaenoate (ω-3)	–	–	–	–	16.57
21	Ethyl-5,8,11,14,17-Eicosapentaenoate (ω-3)	–	–	–	–	3.99
22	Ethyl-13-decosenoate	–	–	–	–	5.87
23	Squalene	–	15.70	–	–	–
24	Capric acid	–	–	–	7.0	–
25	Lauric acid	–	–	–	44.6	–
26	Myristic acid	–	–	–	20.4	–
27	Palmitic acid	–	–	–	11.2	–
28	Stearic acid	–	–	–	2.6	–
29	Arachidic acid	–	–	–	1.4	–

*Peak area = The percentage of particular compound present in overall sample (100%); Percentages have not rounded up to 100%, due to other constituents not listed.

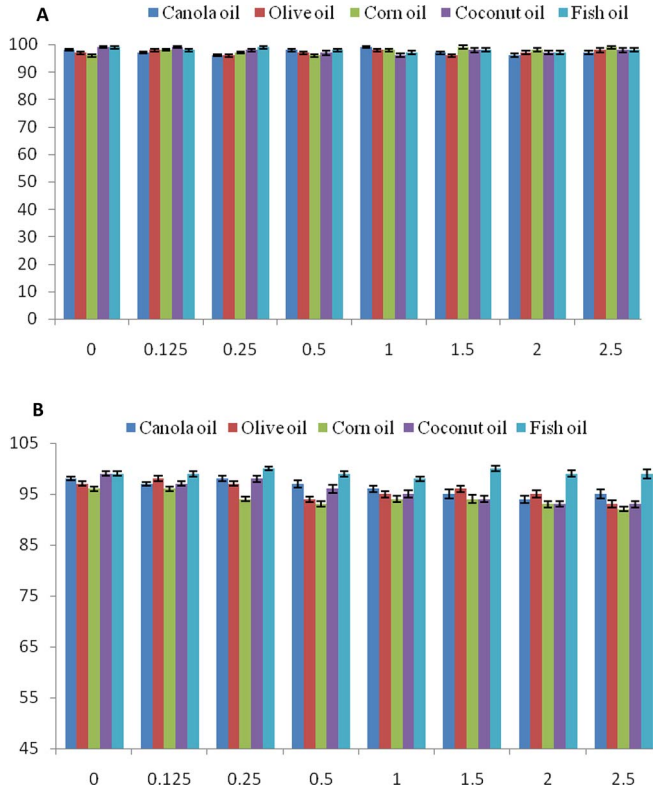

Fig. 1. (A). *In vitro* cytotoxic effect of vegetable oil (Canola oil, Olive oil, Corn oil & Coconut oil) and Fish oil on human monocytes after 6 h. (B). *In vitro* cytotoxic effect of vegetable oil (Canola oil, Olive oil, Corn oil & Coconut oil) and Fish oil on human monocytes after 12 h. Results are presented as the mean ± standard deviation (SD) (n = 6 in all the groups).

AA is the substrate for the production of a wide variety of eicosanoids (20-carbon AA metabolites), such as prostaglandin E2, thromboxane A2, and leukotriene B4. These are described as proinflammatory, vasoconstrictive, or proaggregatory factors that lead to coronary heart disease, psoriasis, depression, aging, and cancer (Simopoulos, 2008).

In the present study, we aimed to identify the appropriate healthy ratio of omega-6/omega-3 essential fatty acids from the major types of cooking oils used in Saudi Arabia that could prevent macrophage-stimulated proinflammatory cytokine development and foam cell formation. We chose oils with an increasing ratio of omega-6/omega-3 fatty acids, including canola oil (5.6/1), olive oil (13.4/1), corn oil (52/1), coconut oil (saturated), and fish oil (1/2). Oxidized-LDL-induced peripheral blood monocytes were treated with 0.5% and 2% doses (selected based on the results of the cell proliferation assay) of different oil emulsions (v/v) for 0 h, 2 h, 4 h, and 8 h, respectively. We then analyzed the influence of increasing ratios of omega-6/omega-3 on lipid accumulation, aggregation, and foam cell formation in macrophages. Further, alterations in the mRNA expression levels of proinflammatory cytokines [IL-12β, tumor necrosis factor-alpha (TNFα), IL-1β, IL-4, IL-6, and interferon-gamma (IFN-γ)] and macrophage-induced inflammatory cardiovascular disease-related genes (CCL_2, $IKBK\gamma_1$, KKB_2, IKK, TLR-4, $TGFBR_2$, $VCAM_1$, $ICAM_1$, and IL_{33}) were analyzed after 8 h of treatment. The results of this study could help to determine the optimal ratio of dietary omega-6/omega-3 fatty acids for the prevention of proinflammatory cytokine development and enhancement of anti-inflammatory agents that would be beneficial for a healthier life.

2. Material and methods

2.1. Chemicals and reagents

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), oil Red 'O', Nile red, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's