In Vitro Evidence of Anti-Inflammatory and Anti-Obesity Effects of Medium-Chain Fatty Acid–Diacylglycerols

Seungmin Yu1, Jong Hun Choi2, Hun Jung Kim2, Soo Hyun Park2, Gwang-woong Go3*, and Wooki Kim1*

1Department of Food Science and Biotechnology, Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea
2R&D Center, Nongshim, Seoul 07057, Republic of Korea
3Department of Foods and Nutrition, Kookmin University, Seoul 02707, Republic of Korea

Introduction

Multiple dietary approaches have been attempted to evaluate the effects of various lipids on obesity and lipid metabolism [1–4]. Among these, medium-chain fatty acids (MCFAs), consisted of 6–12 carbons, mostly saturated, have been reported to exert anti-inflammatory and anti-obesity effects [4, 5]. Compared with conventional long-chain fatty acids (LCFAs), MCFAs do not require carnitine palmitoyl transferase for mitochondrial transport in cellular energy production. Consequently, MCFAs are rapidly oxidized to acetyl-CoA via β-oxidation [6, 7], resulting in increased energy expenditure, reduced fat mass, and downregulated adipogenic gene expression compared with LCFAs [8, 9].

With regard to fatty acid composition on the glycerol backbone of dietary lipids, diacylglycerols (DAGs) exhibited potent suppression in lipid accumulation compared with triacylglycerols (TAGs). Studies have revealed that, during intestinal digestion, TAGs are hydrolyzed to two free fatty acids (FFAs) and 2-monoacylglycerol (2-MG) by pancreatic lipase, followed by absorption in intestinal epithelial cells. TAGs are re-synthesized from absorbed 2-MG and FFAs by monoacylglycerol acyltransferases (MGATs) and diacylglycerol acyltransferases (DGATs) in the epithelial cells. In contrast, dietary 1,3-DAG was shown to be hydrolyzed to 1-MG and FFA by lipase and absorbed into intestinal cells. Absorbed 1-MG and FFA are not synthesized to TAG owing to the lack of 1-MG-specific MGAT in humans [10–12]. Therefore, retarded re-acylation of 1-MG to TAG prevents postprandial hyperlipidemia, in part, through increased availability of lipoprotein lipases and decreased activities of DGATs.
resulting in reduced lipid accumulation [12, 13].

Obese subjects are reported to be in an inflammation-prone condition [14], which in turn worsens obesity, hyperglycemia, and insulin resistance [15, 16]. With respect to the contribution of obesity to inflammation, it was previously reported that infiltration of macrophages into white adipose tissues was increased in obese status [17], which further increased lipolysis. Macrophage-induced lipolysis subsequently promoted hepatic gluconeogenesis and diminished glucose uptake in skeletal muscle, resulting in insulin resistance and ectopic lipid accumulation in the liver. Of interest, recent studies also demonstrated the direct contribution of adipocytes to the development of inflammatory micromilieu, indicating their involvement in endocrine and/or paracrine modulator functions [18]. Specifically, adipocytes were shown to produce proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, which further modulate both adipocytes and macrophages [14].

Macrophages have evolved to engulf foreign cells and recognize microbiome-specific pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides (LPS). The recognition of PAMPs via extracellular and intracellular receptors, such as Toll-like receptors and nucleotide-binding oligomerization domain-like receptors, is further implicated in inflammatory responses for their release of nitric oxide (NO), cytokines, and proinflammatory lipid metabolites such as prostaglandins and leukotrienes. Phagocytic macrophages also trigger adaptive immune responses by expression of antigen-presenting molecules on their surfaces, followed by reciprocal costimulation of T/B-lymphocytes [19, 20].

With advantages of DAGs and MCFAs known, the current study sought to determine the beneficial effects of an edible oil containing MCFA-enriched DAG (MCDG) on adipocytes and macrophages. Conventional canola oil and olive oil served as controls in order to test the substitution by MCDG.

Materials and Methods

MCDG Preparation

MCDG was prepared from canola oil (MSM Milling Company, Australia) and MCFA-triacylglycerols (MCTs) (Ilshinwells, Korea). Canola oil mainly consisted of TAGs (>96.80%), and its fatty acid composition was determined as oleic acid (C18:1, 60%), linoleic acid (C18:2, 22%), linolenic acid (C18:3, 8%), palmitic acid (C16:0, 5%), and stearic acid (C18:0, 2%). MCT was determined to be composed of caprylic acid (C8:0, 55%) and capric acid (C10:0, 45%). For the production of MCDG, the mixture of canola oil and MCT was hydrolyzed by a lipase in order to liberate glycerols and FFAs. The pool of glycerols and FFAs was re-esterified using a reverse reaction of 1,3-specific immobilized lipase (Novozym 40086; Novozyme, Denmark) [21], followed by distillation for the removal of unesterified FFAs and MAGs. MCDG was further decolorized and deodorized using bleaching earth at 100°C for 1 h and steam at 180°C for 1 h, respectively. The consequent MCDG was determined to be enriched in 1,3-DAG (55%) along with 1,2-DAG (25%) and TAG (20%). For application to the cells, MCDG as well as control oils were dissolved in DMSO (Daejung Chemical, Korea) and further diluted in the culture medium at the working concentration. The final concentration of DMSO in the cell culture medium was maintained at less than 0.01% (v/v), for which toxicity was not observed previously [22].

Cell Culture

The murine macrophage RAW264.7 cell line was purchased from the Korea Cell Line Bank (KCLB, Korea). The cells were seeded on 24-well plates at a concentration of 1.0 × 10^4 cells/ml with Dulbecco’s modified Eagle’s medium (DMEM; Welgene, Korea) supplemented with 10% fetal bovine serum (FBS; Welgene) and 1% antibiotic/antimycotic solution (10,000 U/ml penicillin G, 10,000 µg/ml streptomycin, and 25 µg/ml amphotericin B; Welgene) at 37°C in a 5% CO₂ incubator (Model BB15; Thermo Scientific, USA) with or without oil intervention for 24 h. To induce onset of inflammatory responses, macrophages were stimulated with 300 ng/ml of LPS (Sigma-Aldrich Co., USA) for 24 h. The murine preadipocyte 3T3-L1 cell line was purchased form KCLB. Cells were seeded on 24-well plates and maintained in DMEM supplemented with 10% bovine calf serum (Welgene) and 1% antibiotic/antimycotic solution. Cells were fed every 2 days during growth before confluence. Differentiation of confluent preadipocytes (day 0) to adipocytes was induced by 20 mM dexamethasone (Sigma-Aldrich Co.), 0.1 mM 3-isobutyl-1-methlyxanthine (Sigma-Aldrich Co.), and 10 µg/ml insulin (differentiation induction medium; Sigma-Aldrich Co.) in 10% FBS-supplemented DMEM for 2 days. From day 2, the culture medium was replaced every 2 days with DMEM supplemented with 10% FBS and 10 µg/ml insulin (maturation medium) until full differentiation on day 8. Appropriate oil treatments were applied during the whole differentiation period.

Cell Viability Assay

The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded into 96-well plates at the concentration of 5.0 × 10^3 cells/ml and exposed to MCDG (0.01, 0.1, 0.5, 1.0, 10, and 100 µg/ml) for 24 h. Then, 10 µl of MTT solution (5 mg/ml; Amresco, USA) was added to each well, and the cells were incubated for 4 h at 37°C in a 5% CO₂ incubator. After incubation, the supernatant of the cell culture medium was removed, and the MTT formazan crystals were dissolved in DMSO. The absorbance was assessed by a microplate reader (Bio-Rad, USA) at 590 nm to determine the cell viabilities. The results were represented by the percentage of

absorbance relative to the non-oil-treated control group.

Phagocytic Activity Assessment
The phagocytic activity of RAW264.7 cells was assessed by coculture with fluorescent latex beads. Following oil intervention at appropriate concentrations for 24 h, cells were treated with Fluoresbrite plain yellow green latex beads (1.0 μm, 1 x 10^9 particles/ml; Polyscience, USA) for 30 min at 37°C. Following incubation, the cells were washed once in ice-cold PBS and then analyzed with a flow cytometer (Accuri C6; BD Bioscience, USA) to determine the phagocytic activities. The results were presented as phagocytic index, which was calculated by multiplication of the phagocytic cellular population ratio among total phagocytes and the mean fluorescence intensities (MFI) of up-taken beads in the cells, as previously reported [23].

Proinflammatory Cytokine Quantification
Following incubation of RAW264.7 cells with oil samples for 24 h and LPS stimulation for another 24 h, proinflammatory cytokines (IL-6 and TNF-α) in the culture medium were quantified by sandwich ELISA according to the manufacturer’s instructions (BD Bioscience). Briefly, 96-well plates were coated by capture antibodies overnight at 4°C. Then, excess antibodies were discarded, and assay diluent was applied for blocking of nonspecific protein binding. Culture supernatants and purified cytokine standards were applied at designated concentrations and incubated at room temperature for 1 h. Following a series of washing steps, the captured cytokines were incubated with detection antibodies and streptavidin conjugated with horse radish peroxidase (sAv-HRP) for 1 h at room temperature, and substrate solution was treated for reaction after washing. Then, a stop solution (1 M H₃PO₄) was applied, and the absorbance at 450 nm was assessed with a Bio-Rad microplate reader. Alternatively, for the assessment of proinflammatory cytokine production by 3T3-L1 adipocytes, mouse high-sensitivity ELISA kits (eBioscience, USA) were used, following the manufacturer’s instruction, in which additional signal amplification was performed by a provided solution.

RNA Extraction and Quantitative Reverse-Transcription PCR
Following oil treatments and LPS stimulation, RAW264.7 macrophages were harvested by centrifugation at 300 x g for 5 min. Total RNA of cell pellets was isolated using an RNeasy mini kit (Qiagen, Germany) according to the manufacturer’s instructions. Then, the RNA concentration and purity were quantified by a Nanodrop 2000 spectrophotometer (Thermo Scientific). Inflammatory gene transcription of macrophages was assessed by quantitative reverse-transcription PCR (qRT-PCR) with a QuantFast SYBR Green RT-PCR kit (Qiagen) and a CFX Connect Real-Time System (Bio-Rad) under the following conditions: 50°C for 10 min, 97°C for 5 min, followed by 39 cycles at 95°C for 10 sec and 60°C for 30 sec. The sequences of primers were determined by previous studies as follows: mouse glyceraldehyde 3-phosphate dehydrogenase (mGAPDH) Forward 5'-ATCATCCCTGCATCCACT-3', Reverse 5'-ATCCACGACGGA CACATT-3' [24]; mouse cyclooxygenase (mCOX)-2 Forward 5'- TGCACTATGGTACAAAAGCTGG-3', Reverse 5'-TAGGAAAGC TTCATTATCCCT-3' [25], and mouse inducible nitric oxide synthase (mNOS) Forward 5'-CGAAGCGTCTACCTTCA-3', Reverse 5'-TGAACCTATATGGTGTTGCT-3' [26]. Relative mRNA transcription levels of target genes were calculated by normalization to the internal control gene GAPDH by the ΔΔCt method. ΔCt was calculated as Ct_target − Ct_GAPDH, where Ct represents the amplification detection cycle number of an arbitrary threshold. mRNA transcription was analyzed using Bio-Rad CFX manager software. qRT-PCR was performed in quadruplicate for each gene.

Activation Marker Expression Assay
The surface marker expression levels of RAW264.7 cells following oil treatments and LPS stimulation were determined by flow cytometric analysis with fluorescence-conjugated antibodies, as previously described [16]. Briefly, cells were washed with ice-cold PBS and incubated with anti-mouse CD16/CD32 mAbs (eBioscience) for 10 min at 4°C in order to block nonspecific antibody binding to the fragment crystallizable region (FcR). After blocking FcR, cells were stained with fluorescence-conjugated monoclonal antibodies: fluorescein isothiocyanate-conjugated anti-mouse CD80 (CD80-FITC; eBioscience), phycoerythrin-conjugated anti-mouse CD86 (CD86-PE; eBioscience), and allophycocyanin-conjugated anti-mouse MHC-II (I-A/I-E) (MHC-II-APC; eBioscience), for 10 min at 4°C. Following staining with specific antibodies, the cells were washed with ice-cold PBS by centrifugation at 300 x g for 5 min. The cells were analyzed by flow cytometer (BD Accuri C6) following the gating of macrophages by SSC vs. FSC plot. The expression levels of activation markers CD80, CD86, and MHC-II on macrophages were determined by MFI of FL1, FL2, and FL4 channels, respectively, as assessed by BD Accuri C6 software.

Lipid Accumulation Analysis
The lipid accumulation in 3T3-L1 adipocytes was assessed by Oil Red O (ORO) (Sigma-Aldrich) staining. Differentiated 3T3-L1 adipocytes were washed with PBS and fixed with 10% formaldehyde. Fixed cells were incubated for 1 h at room temperature and washed with 60% isopropanol (Daejung Chemical, Korea) to remove residual formalin. The cells were treated with ORO in 60% isopropanol and incubated for 10 min at ambient temperature. Thereafter, the cells were washed four times with distilled water, and stained ORO dye was eluted with 100% isopropanol. Following elution, the dye was transferred to a 96-well microplate, and the absorbance of each well was measured with a microplate reader (Bio-Rad) at 490 nm. The results were calculated as relative absorbance to that of the non-oil-treated control.

Statistical Analysis
Data are representative of repeated experiments and are presented as the mean ± standard error of the mean (SEM). Statistical significance was determined by one-way analysis of variance.
Results and Discussion

Non-Toxicity of MCDG

The cell viability of RAW264.7 murine macrophages following MCDG treatment was determined by the well-established MTT assay. The MTT assay is a rapid colorimetric assay in which the mitochondria-dependent reduction of MTT to formazan by dehydrogenases in viable cells is calculated by measuring the absorbance of residual purple formazan crystals [27]. Briefly, cells were incubated with MCDG at the concentrations of 0.01, 0.1, 0.5, 1.0, 10, and 100 μg/ml for 24 h, followed by MTT (1 mg/ml) for an additional 4 h. As shown in Fig. 1, MCDG-treated cells exhibited 89–112% viability relative to the non-oil-treated control, and the difference was not statistically significant, indicating that MCDG up to 100 μg/ml does not affect cell viability.

Suppressed Phagocytic Activity by MCDG Oil

Macrophages play a key role in both innate and adaptive immunity [28], and phagocytosis is the first step of macrophage-initiated immune responses [29]. Phagocytosis, the recognition and engulfment of foreign substances by macrophages, depends on interaction between surface receptors of macrophages and ligands on the particle surfaces [30, 31]. This interaction initiates particle internalization and leads to activation of endocytic signaling pathways [32]. Intracellular destruction of the foreign substances results in small molecules such as peptides, which are further presented to T cells via major histocompatibility complex (MHC) molecules for adoptive immune reactions [33]. To assess the effects of dietary oils on phagocytosis, fluorescence flow cytometry was used in the current study. The phagocytic activities of the cells on engulfment of FITC-conjugated latex beads were expressed as the phagocytic index (arbitrary unit), as calculated by multiplication of bead-uptake macrophage ratio and the MFI of the uptaken beads. The phagocytic activities of macrophages treated with MCDG were comparable to those of canola- or olive oil-treated cells. Following 10 μg/ml treatment with canola oil, olive oil, or MCDG, the phagocytic indices exhibited no significant difference compared with that of the non-oil-treated control (no oil 19,963 ± 1,841; canola 28,796 ± 1,945; olive 30,378 ± 4,907; and MCDG 16,101 ± 1,375) (Fig. 2A).
However, MCDG exhibited a significant suppression of phagocytosis compared with the widely consumed canola and olive oils. The suppression of phagocytic activity was not dose-dependent in the concentration range of 0.1–10 μg/ml (Fig. 2B). The current observation of enhanced phagocytosis by canola and olive oils is consistent with a previous report in which unsaturated fatty acids increased the phagocytic capacity of murine macrophages [34]. However, MCDG treatment downregulated the macrophage phagocytosis, indicating that MCDG might be an anti-inflammatory substitute for conventional market oils.

**Suppressed Production of Proinflammatory Cytokines by MCDG**

Proinflammatory cytokines have various roles in the immune system, including intercellular communications for immune cell recruitment and/or activation [35]. Among multifunctional cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), implicated in inflammation and immune regulation, are secreted by macrophages stimulated by PAMP, including LPS. The major function of IL-6, identified as a T and B cell differentiation mediator, is the induction of antibody production [36], whereas TNF-α, identified as an endotoxin-induced factor, is involved in proliferation, differentiation, and apoptosis of various cells. However, their secretion is tightly controlled, for overproduction was reported to be associated with inflammation, immune diseases, and cancers [37–39]. In the current study, the production of proinflammatory cytokines (i.e., IL-6 and TNF-α) in macrophages was determined by ELISA kits. Surprisingly, it was observed that IL-6 production was significantly suppressed by MCDG (11.42 ± 0.42 ng/ml) compared with non-oil-treated controls (14.55 ± 0.63 ng/ml) (Fig. 3A). Furthermore, canola oil was shown to increase IL-6 production (15.57 ± 0.08 ng/ml), whereas olive oil moderately modulated IL-6 secretion (13.29 ± 0.12). As shown in Fig. 3B, MCDG was effective at a concentration of 1 μg/ml for suppression of IL-6 production. In accordance, TNF-α secretion of macrophages was also remarkably reduced, as shown in Fig. 3C. The amount of TNF-α secreted by macrophages was the lowest in the supernatant of MCDG-treated cells (12.40 ± 2.38 ng/ml) in comparison with conventional oils (canola: 26.74 ± 0.99 ng/ml; olive: 23.11 ± 3.53 ng/ml). Following the treatment of MCDG at various concentrations (0.1–10 μg/ml), the production of TNF-α was significantly downregulated in a dose-dependent manner.

![Fig. 3](image_url) Reduced production of IL-6 (A, B) and TNF-α (C, D) by MCDG in LPS-stimulated RAW264.7 macrophages. No oil: non-oil-treated control; CAN: canola oil; OLV: olive oil. Different letters indicate significant differences between groups within the panel (p < 0.05).
manner (Fig. 3D).

These observations are in accordance with previous in vivo reports in which MCT downregulated the production of proinflammatory cytokines in rats [40–42]. Malapaka et al. [43] also found that MCFA acted as a modulator of peroxisome proliferator-activated receptor-γ in COS-7 cells, leading to decreased secretion of proinflammatory cytokines, including TNF-α. However, contradictory reports claim that MCT increased the production of IL-6 and IL-12 in vivo [44, 45]. This discordance may come from the different activation materials and dietary doses of MCT used in the studies. Furthermore, it should be highlighted that, in the current study, MCFAs were reconstructed to diacylglycerols. In this regard, the current results indicate that MCDG has suppressive effects on the production of proinflammatory cytokines in macrophages.

Effects of MCDG on Transcription of Proinflammatory Genes

Inflammatory responses are propagated by various molecular mediators. Of these, prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) production from omega-6 polyunsaturated fatty acids by cyclooxygenase-2 (COX-2) and NO production from L-arginine by inducible nitric oxide synthase (iNOS) are known to be key pathways [46]. In this regard, inflammatory stimuli such as bradykinin, LPS, or interferon-γ were reported to induce the synthesis of aforementioned proinflammatory enzymes, through a well-established inflammatory signaling of nuclear factor kappa B (NF-κB)-dependent pathways [47–49]. In the current study, the transcriptional changes of COX-2 and/or iNOS by oil treatments in RAW264.7 macrophages were tested via qRT-PCR. As seen in Fig. 4A, MCDG treatment induced suppressed COX-2 transcription relative to untreated control and olive oil treatments. However, canola oil did not affect the transcriptional products (mRNA) in macrophages. Furthermore, pre-incubation of cells with MCDG oil at 0–10 μg/ml resulted in a significant reduction of COX-2 transcription in a dose-dependent manner (Fig. 4B). A similar inhibitory tendency of MCDG on iNOS transcription was observed. Specifically, MCDG treatment showed downregulation of iNOS transcription compared with conventional olive oil (Fig. 4C). iNOS transcription in macrophages was not significantly different along with the doseincrement of MCDG, although a tendency was observed (Fig. 4D). The current observation of reduced transcription of COX-2 and iNOS is in accordance

![Fig. 4](image-url) Suppressed transcription of COX-2 (A, B) and iNOS (C, D) by MCDG in LPS-stimulated RAW264.7 macrophages. No oil: non-oil-treated control; CAN: canola oil; OLV: olive oil. Different letters indicate significant differences between groups within the panel (p < 0.05). ns: no significant difference.
with previous reports, in which MCT inhibited PGE\(_2\) production in RAW264.7 macrophages [50], decreased activation of NF-\(\kappa\)B, expression of COX-2, and production of TNF-\(\alpha\) in rat liver cells [51], and reciprocally regulated ketogenesis increment and reduction of COX-2 expression in mouse glioma cells [52, 53].

**Comparable Expression of Activation Markers Following Oil Treatments**

Macrophages not only play a key role in innate immunity, but also express various surface proteins to trigger adaptive immune responses via antigen presentation under stimulation [54]. In this cell-to-cell communication, the expression of surface molecules dictates the “education” of lymphocytes (i.e., T/B-cells) against pathogenic challenges [55]. Of these surface proteins, MHC-II presents peptide antigens to receptors of T lymphocytes [56, 57]. The activation and differentiation of antigen-primed T cells are tightly regulated by costimulation via cluster of differentiation (CD)24 with B7 molecules, also termed CD80 (B7.1) and CD86 (B7.2), on the antigen-presenting cells [58–60]. Therefore, in the current study, the expression of those surface markers (i.e., MHC-II, CD80, and CD86) was determined by staining with fluorochrome-conjugated antibodies and flow cytometry. As a result, the expression of the aforementioned surface proteins was not affected by the oil treatment, indicating that MCDG does not downregulate pathogen-specific adoptive immune responses (Fig. 5). However, the expression of CD80 and CD86 in human monocytes/macrophages following treatment of MCT was reported to be downregulated [61]. The apparent discrepancy might be derived from cellular model systems as well as the fatty acid compositions in DAG.

**Reduced Lipid Accumulation in 3T3-L1 Adipocytes by MCDG Treatment**

Adipocytes are critical in metabolic and endocrine systems [62], in part, by secreting various hormones that further affect distal cells, regulating food intake, energy expenditure, and metabolism of carbohydrates and lipids [63]. Insulin induces adipocytes to promote uptake of glucose and lipoprotein-derived fatty acids for TAG synthesis (lipogenesis), while inhibiting the breakdown of TAG for energy expenditure (lipolysis) [64]. In cellular model systems, this phenomenon is easily visualized by cytoplasmic staining of lipid droplets with a well-established dye, ORO (Figs. 6A–6D) [65]. For numeric quantification of fat deposition in 3T3-L1 adipocytes, ORO was eluted, and the absorbance was determined at 490 nm. Surprisingly, the lipid accumulation of adipocytes treated with MCDG was significantly reduced compared with the control oil-treated groups (Fig. 6E). Interestingly, at a lower dose of MCDG at 0.1 \(\mu\)g/ml, lipid accumulation was determined to be increased compared with the non-oil-treated controls (Fig. 6F), indicating that the extracellular pool of lipids is autonomously incorporated into cells to some extent. However, over a dose of 1 \(\mu\)g/ml, MCDG exhibited an inhibitory effect of lipid accumulation. The current observation is consistent with previous in vivo studies showing that MCFA and DAG suppressed body fat accumulation in mice and rats [66, 67], although dissected studies are required for the molecular description of mechanisms at lower vs. higher doses.

**Effect of MCDG on IL-6 Production in 3T3-L1 Adipocytes**

IL-6 is one of the major inflammatory cytokines, and its production by adipocytes has been implicated in the augmentation of low-grade inflammatory conditions in...
obese subjects [68]. In addition, moderate increment of IL-6 in serum is associated with the risk of insulin resistance and, consequently, development of type 2 diabetes [69]. Earlier studies have shown that a high concentration of adipose-derived IL-6 both in vivo and in vitro is correlated with adipocyte hypertrophy and recruitment of macrophages to adipocytes, resulting in development of inflammation [69–71]. In this regard, culture media of fully differentiated 3T3-L1 adipocytes (day 8) following oil treatments were assessed for IL-6 secretion using a commercial high-sensitivity ELISA assay kit. Interestingly, conventional oils (i.e., canola and olive oils) at a concentration of 10 μg/ml exhibited elevated production of IL-6 (23.03 ± 2.22 and 24.55 ± 5.74 pg/ml, respectively) compared with non-oil-treated controls (7.38 ± 0.72 pg/ml) (Fig. 7). However, MCDG (10 μg/ml) showed no significantly different IL-6 production (15.73 ± 0.72 pg/ml) from non-oil-treated controls, indicating that MCDG tends to downregulate IL-6 production in adipocytes. These data, together with the reduction of lipid accumulation, indicate that MCDG could be an alternative to conventional market oils for the purpose of obesity prevention/treatment.

Ample data indicate that both macrophages and adipocytes contribute to and/or communicate for the development of low-grade inflammatory status in obese subjects. Previous studies also suggested that dietary sources, including lipids, affect the inflammatory signaling in macrophages and adipocytes. Therefore, the current study sought to determine the effects of newly introduced MCDG on model systems of inflammation using LPS-stimulated RAW264.7 macrophages, and of obesity using differentiated 3T3-L1 adipocytes.
MCDG exerted potent inhibitory effects on inflammatory responses. Specifically, phagocytic activity, transcription of inflammatory enzymes (COX-2), and secretion of proinflammatory cytokines (IL-6 and TNF-α) were significantly downregulated by MCDG in a dose-dependent manner. As for the regulation of adipocytes by MCDG, lipid accumulation and secretion of proinflammatory cytokine IL-6 were significantly downregulated by MCDG treatments compared with conventional oils, indicating that the newly developed MCDG might be an alternative to the market oils for the prevention/treatment of obesity-relevant inflammatory diseases. The limit of the current study is that the direct effects of MCDG on either cell type (i.e., macrophage and adipocyte) were not sought, although accumulating data indicate that those cells cross-talk for the synergistic augmentation of inflammatory/obesity signals. Therefore, the current results illustrate the need for further studies to elucidate the communication between these two contributors by cross-culture of cells in culture supernatants.

Acknowledgments

This research was supported by the National Research Foundation (NRF-2016R1D1A1B03934359) and by a grant from Kyung Hee University in 2014 (KHU-20140425).

References


53. Stafford P, Abdelwahab MG, Kim DY, Preul MC, Rho JM,


