Effect of docosahexaenoic acid monoacylglyceride on systemic hypertension and cardiovascular dysfunction

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1SCF Pharma, Sainte-Luce, Quebec, Canada; 2Department of Physiology and Pharmacology, Université de Sherbrooke, Sherbrooke, Quebec, Canada; 3Department of Obstetric Gynecology, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada, and 4Department of Biology, Université du Québec à Rimouski, Rimouski, Quebec, Canada

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Morin C, Rousseau E, Blier PU, Fortin S. Effect of docosahexaenoic acid monoacylglyceride on systemic hypertension and cardiovascular dysfunction. Am J Physiol Heart Circ Physiol 309: H93–H102, 2015. First published April 24, 2015; doi:10.1152/ajpheart.00823.2014.—ω-3 Fatty acid supplementation has been associated with lower blood pressure. Cardiovascular diseases are also known to be linked directly to an increase in ω-6 and a reduction in ω-3 fatty acid levels in blood circulation and tissues. To determine the effect of docosahexaenoic acid monoglycerides (MAG-DHA) on blood pressure, lipid profiles, and vascular remodeling in rats fed a high-fat/high-carbohydrate (HFHC) diet. Studies were performed in male rats subjected to 8 wk of HFHC diet supplemented or not with 3 g/day MAG-DHA. After 8 wk of daily MAG-DHA treatment, rats in the HFHC + MAG-DHA group had lower arterial blood pressure and heart rate compared with the HFHC group. Moreover, MAG-DHA prevented the increase aortic wall thickness, whereas lipid analysis of aortic tissues revealed an increase in DHA/AA ratio correlated with the production of resolvin D2 and D3 metabolites. Histological analysis revealed that MAG-DHA prevented the development of LVH in the HFHC group. Serum lipid profile analysis further showed a decrease in total cholesterol (TC) and LDL, including very low-density lipoprotein (VLDL) and triglyceride (TG) levels, together with an increase in HDL levels after 8 wk of MAG-DHA treatment compared with the HFHC group. Furthermore, daily MAG-DHA treatment resulted in reduced proinflammatory marker levels such as CRP, IL-6, TNFα, and IL-1β. Altogether, these findings revealed that per os administration of MAG-DHA prevents HFHC-diet induced hypertension and LVH in rats.

docosahexaenoic acid; hypertension; cardiac hypertrophy; inflammation

DESPITE AGGRESSIVE TREATMENT with current therapies, hypertension and heart failure remain major public health problems due to poor quality of life, frequent hospitalizations, and death (5). New therapeutic approaches are needed to prevent the development of heart failure and reverse the progression of established dysfunction. Current evidence from experimental animal studies, epidemiological studies, and clinical trials suggests that ω-3 polyunsaturated fatty acids (n-3 PUFA) of marine origin may be protective against cardiovascular disease (5, 26). For example, McLennan et al. (27) reported that docosahexaenoic acid (DHA) was more effective than eicosapentaenoic acid (EPA) in delaying the development of hypertension in spontaneously hypertensive rats (SHR). Moreover, DHA inhibited ischemia-induced cardiac arrhythmias at low dietary intakes in Hooded Wistar rats (27), whereas in the stroke-prone SHR model, dietary DHA intake was found to prevent the development of hypertension (20). Other studies also found that treatment with a mixture of DHA and EPA in animal models of chronic left ventricular (LV) dysfunction resulted in beneficial effects on pressure overload-induced cardiac disease (7, 11, 12, 27, 41).

Epidemiological studies have demonstrated a significant inverse association between the dietary ω-3 PUFA intake to saturated fatty acids and coronary heart disease mortality in 25 population samples drawn in 16 countries (5). Indeed, intake of fish oils has been associated with a significant reduction in blood pressure (4), heart rate, triglycerides (TG), and very low-density lipoprotein (VLDL) cholesterol (37, 49). In a clinical trial, daily fish meals that provide 3.65 g/day of ω-3 fatty acids were found to significantly reduce blood pressure in overweight treated hypertensive subjects (3). Furthermore, a study conducted on mildly hyperlipidemic men at increased risk of cardiovascular disease revealed that purified DHA but not EPA reduced ambulatory blood pressure and heart rate (37, 38). Finally, a clinical trial involving a 5-wk, double-blind, placebo-controlled dietary supplementation with either DHA or active placebo revealed that 2 g/day DHA supplementation reduced the level of risk factors of coronary heart disease, improving blood pressure, heart rate, and lipid profiles in hypertensive, hypercholesterolemic subjects who did not eat fish on a regular basis (37).

The aim of the present study was thus to assess the impact of long-term treatment with a clinically relevant dose of docosahexaenoic acid monoacylglyceride (MAG-DHA) on blood pressure and left ventricular structure and function in rats fed with a high-fat/high-carbohydrate (HFHC) diet. Specifically, we assessed the effects of MAG-DHA treatment on arterial blood pressure, LV wall thickness, inflammatory marker levels [C-reactive protein (CRP), IL-6, IL-1β, and TNFα], and lipid profiles [total cholesterol (TC), HDL, LDL, and TG]. Studies were performed in male rats subjected to 8 wk of HFHC diet and either untreated or treated with a human equivalent of 3 g/day MAG-DHA. Having shown that this diet results in hypertension and LV hypertrophy, we hypothesized that treatment with MAG DHA would decrease blood pressure and improve LV function and lipid profiles compared with untreated HFHC rats.

MATERIALS AND METHODS

Synthesis of MAG-DHA. MAG-DHA was synthesized as described previously, using highly purified DHA ethyl ester as starting material.
In the resulting molecule, DHA is attached at the sn-1 position of glycerol (16, 17).

**Animal model of hypertension.** Adult (10 wk) male Wistar rats weighing 200–250 g were obtained from Charles River Laboratories (Montreal, QC, Canada). Rats were housed in our animal facilities on a 12:12-h light-dark cycle at 22 ± 2°C ambient temperature and maintained on normal rodent chow and tap water ad libitum. Rats were acclimated 7 days prior to the start of the experiments. All studies involving animals were approved by the Institutional Animal Care Committee of the Université du Québec à Rimouski (protocol no. CPA-53-13-119). Rats were randomly assigned into three groups and fed either normal chow (control) (no. 5002; LabDiet, St. Louis, MO), a HFHC diet, or a HFHC diet + MAG-DHA treatment (HFHC + MAG-DHA); n = 6/group. HFHC diet consisted of 175 g of fructose, 395 g of sweetened condensed milk, 200 g of beef tallow, 155 g of powdered rat food, 25 g of Hubble, Mendel, and Wakeman salt mixture, and 50 g/kg diet of water. In addition, the drinking water for the HFHC groups was supplemented with 25% fructose. MAG-DHA (318 mg/kg) was given orally directly in the back of the mouth with a pipette tip. MAG-DHA treatments were administrated daily (32, 35, 43). The oral dose of 318 mg/kg corresponds to 60% of the maximum daily dose (5 g/day) allowed for human beings by Health Canada Guidelines. Measurements of body weight and food and water intakes were taken daily for 8 wk. Blood pressure measurement was taken every 7 days for 8 wk using noninvasive tail cuff systems (Kent Scientific, Torrington, CT). At the end of the experiment, all three groups of rats were euthanized by lethal dose of pentobarbital sodium, and blood was collected by cardiac puncture.

**Histological analysis.** Cardiac, aortic, and hepatic tissue samples were fixed in 10% buffered formalin and paraffin embedded, after which thin sections (3 μm thick) were stained with hematoxylin-eosin according to standard protocols (34, 35). Images were acquired with a Hamamatsu ORCA-ER digital camera attached to a Nikon Eclipse TE-2000 inverted microscope (Nikon-Canada, Mississauga, ON, Canada). Images were obtained (×40 objective) from aorta, heart, and liver sections derived from control, HFHC, and HFHC + MAG-DHA-treated rats. To reveal vascular remodeling, artery wall thickness and liver fat droplets were determined by image analysis of tissue sections derived from control and HFHC diet-fed rats either untreated or treated with MAG-DHA.

**Plasma biochemistry.** Blood samples were collected in EDTA and SST tubes by cardiac puncture. Plasma and serum were separated by centrifugation at 2,500 g for 15 min and stored at −80°C for later analysis. Serum TG, TC, and HDL cholesterol were measured by enzymatic methods (BioAssay Systems, Hayward, CA). LDL-VLDL cholesterol concentrations were calculated using the Friedewald equation. Plasma CRP, TNFα, IL-6, and IL-1β levels were determined using specific ELISA kits (eBioscience, San Diego, CA).

**Fatty acid composition of plasma and tissues using gas chromatography/flame ionization detector.** Plasma and tissue fatty acid compositions were measured using a modified direct transesterification method in which toluene was used instead of benzene and acetyl chloride was replaced by sulfuric acid (23). Fatty acids were chromatographed as methyl esters on a 60-m fused silica column with an internal diameter of 0.25 mm. The column was wall-coated with 0.15 mm of DB-23. Analysis was performed on a Thermo Scientific TRACE GC Ultra gas chromatograph equipped with a flame ionization detector. Helium was used as a carrier gas and nitrogen as a makeup gas with a split ratio of 20:1. Injection port and detector temperatures were 230 and 280°C, respectively. The oven temperature was programmed to initiate at 50°C for 1 min, after which the temperature was first raised to 140°C at a rate of 25°C/min, then raised to 195°C at a rate of 3°C/min and subsequently maintained for 5 min, and finally increased to 225°C at a rate of 4°C/min and maintained at this final level for 5 min. The gas chromatograph was calibrated using a standard mixture (Supelco 37 Component FAME Mix).

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**Fig. 1. Effect of docosahexaenoic acid monoglycerides (MAG-DHA) on systolic and diastolic blood pressure.** A and B: time course of tail-cuff systolic (A) and diastolic blood pressure (B) measured from control and high-fat/high-carbohydrate (HFHC) diet-fed as well as HFHC + MAG-DHA-treated rats. Results represent means ± SE; 12 measurements were taken for each animal, and 6 animals/group were tested. C: weight (g) as a function of time (day) was taken every week from control, HFHC diet-fed, and HFHC + MAG-DHA-treated rats (n = 6/group). *P < 0.05.
MAG-DHA PREVENTS HIGH-FAT DIET-INDUCED HYPERTENSION

RESULTS

Effect of MAG-DHA on systolic and diastolic blood pressure in HFHC rats. Noninvasive blood pressure (BP) and heart rate (HR) were measured in conscious restrained rats using the tail-cuff method with heating at 35°C. Rats were allowed to acclimate to this procedure for 5 days before experiments were performed. BP and HR values were recorded every 7 days for 8 wk and were averaged from at least 15 consecutive readings obtained from each rat. Figure 1, A and B, illustrates the time course of systolic and diastolic BP measured from control animals fed normal chow and HFHC rats fed a HFHC diet (as described in MATERIALS AND METHODS) as well as MAG-DHA-treated rats fed a HFHC diet. MAG-DHA treatments were administered daily per os (318 mg/kg) directly in the back of the mouth with a pipette tip. Results show that MAG-DHA treatment prevented the increase in systolic and diastolic BP induced by the HFHC diet (Fig. 1, A and B). Moreover, analyses revealed no significant difference in tail-cuff blood pressure measurements between control rats receiving regular chow and MAG-DHA-treated animals fed HFHC diet during the 8 wk of dietary intervention (Fig. 1, A and B). After 8 wk, rats in the HFHC + MAG-DHA group had lower HR measurements (356.3 ± 6.5) compared with the HFHC group (397.4 ± 5.9). Results also demonstrated that following 6 wk of MAG-DHA treatment, a slight but significant reduction in body weight of HFHC rats was quantified (Fig. 1C).

Effect of MAG-DHA treatment on blood lipid profiles following HFHC diet. Cardiovascular diseases are known to be linked directly to an increase in ω-6 and a reduction in ω-3 fatty acids, MUFA, PUFA, AA, EPA, and DHA in plasma, RBC, heart, aorta, and liver derived from control, HFHC-fed, and HFHC + MAG-DHA-treated rats.

Table 1. Gas chromatography data analyses of saturated fatty acids, MUFA, PUFA, AA, EPA, and DHA in plasma, RBC, heart, aorta, and liver derived from control, HFHC-fed, and HFHC + MAG-DHA-treated rats

<table>
<thead>
<tr>
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<th>Control, %</th>
<th>HFHC, %</th>
<th>HFHC + MAG-DHA, %</th>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
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<tr>
<td>Saturated</td>
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<td>52.56 ± 0.82</td>
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<td>MUFA</td>
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<td>PUFA</td>
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<td>AA</td>
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<td>13.68 ± 0.59</td>
<td>8.64 ± 0.28</td>
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<tr>
<td>EPA</td>
<td>0.48 ± 0.11</td>
<td>0.14 ± 0.01</td>
<td>1.47 ± 0.17</td>
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<tr>
<td>DHA</td>
<td>3.49 ± 0.17</td>
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<td><strong>RBC</strong></td>
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<tr>
<td>Saturated</td>
<td>62.39 ± 0.41</td>
<td>61.34 ± 0.57</td>
<td>56.47 ± 2.95</td>
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<td>MUFA</td>
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<td>EPA</td>
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<td>DHA</td>
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<td><strong>Heart</strong></td>
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<tr>
<td>Saturated</td>
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<td>DHA</td>
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<td>0.76 ± 0.12</td>
<td>0.33 ± 0.33</td>
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<td><strong>Liver</strong></td>
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<tr>
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<td>44.16 ± 2.11</td>
<td>45.33 ± 2.23</td>
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<tr>
<td>MUFA</td>
<td>18.60 ± 1.54</td>
<td>34.78 ± 1.71</td>
<td>25.48 ± 1.25</td>
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<td>21.06 ± 1.45</td>
<td>29.19 ± 2.69</td>
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<td>AA</td>
<td>10.56 ± 1.83</td>
<td>5.93 ± 0.88</td>
<td>3.66 ± 0.53</td>
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<tr>
<td>EPA</td>
<td>0.57 ± 0.07</td>
<td>0.21 ± 0.02</td>
<td>1.07 ± 0.15</td>
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<tr>
<td>DHA</td>
<td>6.65 ± 0.06</td>
<td>3.59 ± 0.35</td>
<td>12.86 ± 1.18</td>
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</table>

Values are means ± SE; n = 6/group. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cells; HFHC, high-fat/high-carbohydrate; MAG-DHA, docosahexaenoic acid monoglycerides.

Online solid-phase extraction-liquid chromatography-mass spectrometry/mass spectrometry analysis of DHA metabolites. DHA metabolites were detected in lung homogenates using the method described by Kortz et al. (21). An 1100 series HPLC system coupled with a tripleQuad 6410 mass spectrometer (Agilent) with electrospray ionization in negative ion mode was used. The resolvin RvD2 was quantified by multiple reaction monitoring using the transition (375.2 → 141.2).

Data analysis and statistics. Results are expressed as means ± SE, with n indicating the number of experiments. Statistical analyses were performed using Sigma Plot 11 and SPSS 14.0 (SPSS-Science, Chicago, IL) via one-way ANOVA followed by Dunnett’s post hoc test. Differences were considered statistically significant when P < 0.05.
fatty acid levels in blood circulation and tissues (5, 46). Fatty acid content was thus determined by gas chromatography/flame ionization detector (GC/FID) in plasma and red blood cells derived from control and HFHC as well as HFHC + MAG-DHA-treated rats. Lipid analyses revealed a decrease in arachidonic acid (AA) and an increase in EPA and DHA levels in plasma derived from MAG-DHA-treated rats compared with levels recorded in plasma from HFHC animals (Fig. 2A). As a result, the DHA/AA ratio was significantly increased by fivefold in plasma derived from HFHC + MAG-DHA-treated animals compared with the ratio calculated in HFHC rats (Fig. 2A, inset). Red blood cell (RBC) analyses also revealed a decrease in AA and an increase in the ω-3 fatty acids EPA and DHA in RBC derived from HFHC + MAG-DHA-treated rats compared with RBC from the HFHC group (Fig. 2B). After 8 wk of dietary intervention, the DHA/AA ratio was increased twofold in RBC derived from HFHC + MAG-DHA-treated rats compared with that quantified in HFHC-fed animals (Fig. 2B, inset). Table 1 illustrates lipid analyses of saturated fatty acids, monounsaturated fatty acids, PUFA, AA, EPA, and DHA in plasma, RBC, heart, aorta, and liver derived from control, HFHC-fed, and HFHC + MAG-DHA-treated rats.

Effect of MAG-DHA on proinflammatory mediator expression. Inflammation plays a key role in the pathogenesis and progression of cardiovascular disease and hypertension (45). Inflammatory markers such as CRP, TNFα, IL-6, and IL-1β are associated with vascular lesions in humans and are predictive of cardiovascular outcome (45). In our experiments, CRP levels were determined by specific ELISA in serum derived from control rats fed regular chow and rats fed HFHC diet in the absence and presence of MAG-DHA treatment. As seen in Fig. 3A, serum CRP levels were significantly higher in the HFHC group (47.6 ± 2.4 μg/ml) compared with the control group (21.5 ± 1.6 μg/ml), whereas MAG-DHA treatment reduced serum CRP levels approximately twofold (28.6 ± 1.0 μg/ml) compared with the levels found in serum of HFHC animals.

The primary regulators of CRP are IL-6, IL-1β, and TNFα, which are secreted by neutrophil granulocytes and macrophages at sites of injury. Thus, IL-6, TNFα, and IL-1β levels were measured by ELISA in plasma derived from the three animal groups. As shown in Fig. 3, B–D, daily MAG-DHA treatment significantly decreased the levels of IL-6, TNFα, and IL-1β in sera by 64, 75, and 65%, respectively, compared with the levels detected in the sera of HFHC animals.

Effect of MAG-DHA on aortic remodeling in rats fed HFHC diet. Increased wall thickness is a common structural feature of hypertensive-resistant vessels (15) and conduit arteries such as the aorta (6). Hypertensive structural alterations of the aortic wall may in turn affect arterial mechanics. To define the level...
of vascular remodeling, morphometric analysis were performed on aortic thin sections derived from all three experimental groups and stained with hematoxylin-eosin. Histological analyses revealed that aortic wall thickness was significantly lower in the HFHC + MAG-DHA-treated rats than in HFHC animals (Fig. 4, B and C). The thickness of the smooth muscle tunica media and the number of elastin bands were markedly reduced in HFHC + MAG-DHA-treated rats compared with the HFHC group (Fig. 4, B and C) such that media histology was comparable with that of control samples (Fig. 4A). Quantitative morphometric analyses confirmed that aortic wall thickness was increased by 94% in HFHC rats (73.3 ± 3.5 μm) compared with control animals (37.8 ± 0.9 μm; Fig. 4D).

In contrast, 8-wk MAG-DHA treatment in HFHC-fed rats significantly reduced aortic wall thickness by 43% (42.3 ± 1.0 μm; Fig. 4D) compared with HFHC animals. Lipid analyses were also performed by GC/FID on aortic homogenates derived from the three animal groups, as illustrated in Fig. 4E. The DHA/AA ratio was significantly increased threefold in aorta derived from HFHC + MAG-DHA-treated animals compared with the ratio obtained in the HFHC group (Fig. 4E).

DHA metabolites were also determined by solid-phase extraction-liquid chromatography-mass spectrometry/mass spectrometry (SPE-LC-MS/MS) in aortic tissue derived from control, HFHC, and HFHC + MAG-DHA-treated rats (24 h after the last dose of MAG-DHA). SPE-LC-MS/MS analyses revealed the presence of resolvin D2 (RvD2), resolvin D3 (RvD3), protectin D1, and 4-hydroxy-DHA, 7-hydroxy-DHA, 10-hydroxy-DHA, 13-hydroxy-DHA, 14-hydroxy-DHA, and 17-hydroxy-DHA in aorta derived from the three animal groups. The results revealed a concentration of 62.10 ± 24.15 pg/mg RvD2 in aortic tissues derived from HFHC + MAG-DHA-treated rats compared with no detectable level in control and HFHC animals (Fig. 4F).
Effect of MAG-DHA on LV hypertrophy in HFHC rats. LV hypertrophy (LVH) is one of the pathological hallmarks of hypertension and is the most common and important adaptation of the heart to repeated increases in afterload (40, 48). LV hypertrophy develops as a compensatory mechanism designed to maintain a normal cardiac output in the presence of an increased arterial pressure. Histological analysis of LV tissues showed an increased myocyte thickness in HFHC rats compared with tissues derived from control animals (Fig. 5, A and B). However, MAG-DHA treatment resulted in lower myocyte thickness compared with the HFHC group (Fig. 5, B and C). Further morphometric analysis revealed a 2.7-fold increase in HFHC rats (21.3 ± 2.5 μm) compared with control animals (7.8 ± 0.9 μm; Fig. 5D), whereas MAG-DHA-treated HFHC rats displayed a twofold decrease in myocyte thickness (10.4 ± 1.0 μm) compared with HFHC animals (Fig. 5D). LVH index was determined as the ratio of LV weight to total body weight. Analyses revealed an increased LVH index in HFHC animals (1.83 ± 0.04 mg/g) compared with control rats (1.47 ± 0.06 mg/g), whereas in the presence of MAG-DHA treatment, the LVH index was markedly reduced (1.53 ± 0.03 mg/g; Fig. 5E). Lipid analyses performed by GC/FID on heart tissues derived from the three animal groups revealed a larger reduction in AA and an increase in DHA levels in hearts derived from HFHC + MAG-DHA-treated rats compared with corresponding fatty acid levels quantified in HFHC heart tissues (Fig. 5F). Moreover, the cardiac DHA/AA ratio was increased significantly, by 3.2-fold, in HFHC + MAG-DHA-treated animals compared with the ratio calculated in the HFHC group (Fig. 5G).
A, C, and D), and no significant change in HDL (Fig. 6B) in the HFHC group compared with the control group. However, there was a significant reduction in TC, LDL/VLDL, and TG levels along with an increase in HDL levels after 8 wk of MAG-DHA treatment in the HFHC + MAG-DHA group compared with the HFHC group (Fig. 6, A–D).

**Effect of MAG-DHA supplementation on liver physiology.** Histological analysis showed increased hepatic lipid deposition in liver sections derived from HFHC rats compared with control animals (Fig. 7, A and B). Following 8 wk of MAG-DHA treatment, a lower hepatic lipid deposition was observed when compared with tissue sections from nonsupplemented HFHC diet-fed rats (Fig. 7, B and C). The liver weight-to-body weight ratio was significantly reduced in the HFHC + MAG-DHA group (25.3 ± 0.6 mg/g) compared with the ratio observed in the HFHC group (30.7 ± 0.9 mg/g; Fig. 7D). Moreover, no significant difference was found between HFHC + MAG-DHA-treated rats and control rats (26.4 ± 0.7 mg/g; Fig. 7C). Lipid analyses of liver tissues derived from the three different groups revealed a 5.6-fold increase in DHA/AA ratio in tissues derived from the HFHC + MAG-DHA group compared with the ratio measured in the HFHC group (Fig. 7D).

**DISCUSSION**

In the present study, we investigated the ability of MAG-DHA to prevent systemic hypertension and LVH induced by a fat- and carbohydrate-rich diet. Long-term administration of MAG-DHA (human equivalent of 3 g/day) for 8 wk was found to decrease arterial blood pressure, LV wall thickness, and proinflammatory marker levels (CRP, IL-6, TNFα, and IL-1β) as well as improve lipid profiles (TC, HDL, LDL, and TG). Hence, we propose that MAG-DHA is able to prevent hypertension and vascular remodeling in a rat model fed a HFHC diet.

Most studies that have assessed the potential cardiovascular benefits of ω-3 fatty acids have focused largely on the importance of EPA, with little attention given to the relative effect of DHA. However, studies suggest that DHA and EPA may have differential effects on BP and HR (30, 46). In this study, we assessed the systemic effects of MAG-DHA treatment on various tissues, including blood, aorta, heart, and liver in rats fed a HFHC diet for 2 mo. Fatty acids in monoacylglyceride form confer an enhanced bioavailability to ω-3 and are generally recognized as safe as well as widely used as emulsifying agents in the food industry. In the current literature, animal studies have demonstrated DHA to be more effective than EPA in decreasing or preventing hypertension in rat models. McLennan et al. (27) reported that DHA was more effective than EPA in delaying the development of hypertension in SHR, but not in adult SHR with already established hypertension. In addition, DHA but not EPA inhibited ischemia-induced cardiac arrhythmias at low dietary intakes in Hooded Wistar rats (27). At moderate- to high-dietary intakes, DHA was also more effective than EPA in inhibiting thromboxane-like vasoconstrictor responses in aortas from SHR (27). Kimura et al. (20) also showed that DHA, compared with a DHA-free diet, prevented the development of hypertension in stroke-prone SHRs by inhibiting the increase in SBP in a dose-dependent manner.

Considering that 31% of Americans are hypertensive and another 30% are prehypertensive, whereas only 47% of those...
with hypertension are adequately controlled, hence, it is of key clinical interest to find a stable, well-absorbed $\text{H}_3$ fatty acid that would exert beneficial effects on lowering blood pressure and thereby potentially reduce the risk of cardiovascular disease (46). A clinical study by Liu et al. (24) comprised of generally healthy adults taking neither antihypertensive medications nor fish oil supplements revealed a positive correlation between greater DHA content in serum phospholipids and lower BP in both clinic and ambulatory settings. Sagara et al. (44) further demonstrated that a 5-wk clinical trial with 2 g/day of DHA supplementation reduced coronary heart disease risk factor levels, improving blood pressure, heart rate, and lipid profiles (decreased TC/HDL cholesterol and non-HDL cholesterol/HDL cholesterol and increased HDL cholesterol ratios) in middle-aged hypertensive, hypercholesterolemic Scottish men who did not eat fish on a regular basis. Another clinical trial by Mori (31) in mildly hyperlipidemic men supplemented with 4 g/day purified DHA in their usual diets for 6 wk resulted in increased plasma phospholipid DHA levels while reducing both ambulatory BP and HR. On the other hand, 4 g/day purified EPA had no significant effect on either ambulatory BP or HR (31). These interesting results suggest that DHA is the principal $\omega$-3 fatty acid in fish and fish oils and is likely responsible for their BP- and HR-lowering effects in humans. Existing literature suggests a range of mechanisms through which $\omega$-3 fatty acids may affect BP, including but not limited to 1) interacting with the nitric oxide pathway and endothelial function, 2) lowering vascular tone via blockade of the angiotensin pathway, 3) inhibition of thromboxane production and thromboxane-induced vasoconstriction, and 4) modulating autonomic tone (8, 31). Current evidence regarding the effects on these pathways tends to be greater for DHA than EPA (30). It is well documented that membrane fatty acid composition is modified by diet (19). In the present study, MAG-DHA treatment increased the level of DHA in plasma and red blood cells as well as in aorta, heart, and liver tissues, suggesting a high DHA bioavailability and thereby likely contributing to reducing vascular remodeling and inflammation. This endogenous rise in DHA also lowered the level of $\omega$-6 AA found in blood circulation and tissue samples, thus resulting in an increased DHA/AA ratio. This increased DHA level may also contribute to the production of specific metabolites and to the reduction of excess inflammation markers or potentially that of inflammatory signaling molecules.

Although the fact that MAG-DHA administration was able to increase DHA/AA ratio, we have not provided evidence that lowering $\omega$-6/$\omega$-3 was directly associated with an improvement in BP management and reduced cardiovascular disease risks. Moreover, Mozaffarian et al. (36) demonstrated that dietary $\omega$-3 PUFA from both seafood and plant sources may reduce coronary heart disease risk, with no apparent influence from $\omega$-6 PUFA intake (36). In animal study, Slee et al. (47) demonstrated that the dietary ratio of $\omega$-6/$\omega$-3 PUFA has no influence on long-chain $\omega$-3 PUFA cellular incorporation from fish oil. The OPTILIP clinical study, performed to determine the optimal $\omega$-6/$\omega$-3 ratio in the UK diet, provided important

Fig. 7. Physiological role of MAG-DHA on liver functions. A–C: hematoxylin-eosin staining of liver ($\times$20) showing fat deposition in control (A), HFHC (B), and HFHC + MAG-DHA-treated rats (C). $fd$, Fat droplets. Scale bar, 50 $\mu$m. Three tissue sections were analyzed for each animal; $n = 6$ /group. D: liver weight (mg) as a function of body weight (g) was measured in control, HFHC, and HFHC + MAG-DHA-treated rats. Results represent means ± SE ($n = 6$ /group). E: bar histogram displaying mean DHA/AA ratios in liver homogenates derived from control, HFHC, and HFHC + MAG-DHA-treated rats ($n = 6$). *$P \leq 0.05$. 

H100 MAG-DHA PREVENTS HIGH-FAT DIET-INDUCED HYPERTENSION

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evidence to show that the ratio of ω-6/ω-3 PUFA is of no practical value in predicting changes in cardiovascular disease risk factors (18).

Animal studies have moreover demonstrated that ω-3 fatty acids are incorporated into myocardial cells and have potent antiarrhythmic effects (22). DHA has also been shown to be the major ω-3 fatty acid incorporated into myocardial membranes, even when animals were fed fish oils in which EPA was the predominant fatty acid (42). Hence, these findings suggest a key role for DHA metabolites in cardiac functions. The antiarrhythmic effects of ω-3 fatty acids are thought to be related to their ability to inhibit myocardial Ca²⁺ overload, thromboxane production, ischemic acidosis, and ischemic K⁺ loss (1, 22).

Hypertensive structural alterations of the aortic wall may affect arterial mechanics. Aortic wall thickness has been found to be negatively associated with ω-3 fatty acids of all tissues but positively associated with TC and TG concentrations (29). Analysis of the present data showed that the aortic wall was significantly thinner, with TC, TG, and LDL-VLDL levels being significantly lower in the MAG-DHA-treated group relative to the untreated HFHC group. Moreover, our data clearly demonstrate that MAG-DHA treatment increased HDL levels. Engler et al. (13) also showed previously that DHA supplementation decreased vascular wall thickness in SHR. Moreover, fish intake has been shown to reduce coronary artery atherosclerosis progression (14), whereas trans fat accelerated its progression (28). Concomitantly, previous studies consistently found that fish oil consumption decreased TG, TC, and LDL levels and increased HDL cholesterol levels (25, 29, 39).

Hypertension and cardiovascular disease are low-grade systemic inflammatory conditions in which there is an increase in proinflammatory cytokines, reactive oxygen species, and proinflammatory eicosanoids coupled with a decrease in cellular antioxidants, anti-inflammatory cytokines, and certain polyunsaturated fatty acids. These latter decreases also include a reported reduction in their anti-inflammatory products such as lipoxins, resolvins, protectins, maresins, and nitrolipids (10). A mild but significant chronic imbalance between pro- and anti-inflammatory signaling molecules in these diseases suggests that therapeutic strategies directed at suppressing inappropriate inflammation may facilitate recovery from such diseases. In the present study, we demonstrate that MAG-DHA treatment was able to reduce CRP level and proinflammatory cytokines such as TNFα, IL-6, and IL-1β. n-3 PUFA may directly or indirectly trigger anti-inflammatory effects on the pattern of inflammatory cytokines (i.e., TNFα, IL-1β, IL-6, IL-8, and interferon-γ) produced by various cell types at sites of inflammation (10). Moreover, anti-inflammatory effects of ω-3 fatty acids have been studied in humans, with CRP levels believed to reflect a chronic, low-grade inflammatory process, and associated with increased cardiovascular disease (2, 9, 38, 50). Animal studies have shown that fish oil consistently reduced CRP in rat model of hypertension. Our current data further reveal that MAG-DHA administration resulted in the production of RvD1, as witnessed by the elevated levels of this metabolite found in aortic tissue following MAG-DHA treatment. Overall, these data suggest that the anti hypertensive and anti-inflammatory effects of MAG-DHA are likely related to the production of resolving metabolites. Indeed, in a previous study, we also demonstrated that RvD1, protectin D1, and 19,20-EpDPE, three active lipid mediators of MAG-DHA derived from 15-lipoxygenase and CYP450 epoxygenase pathways, displayed anti-inflammatory and vasorelaxant effects in human pulmonary arteries (33, 34). As a result, MAG-DHA was proposed to not only improve the plasma and cell/tissue content of DHA but also augment the production of beneficial metabolites such as resolvins and thus prevent and/or improve hypertension, metabolic syndrome, and cardiovascular disease.

In conclusion, the present findings indicate that MAG-DHA given in prevention is able to modify the ω-3/ω-6 ratio, thus conferring antihypertensive and anti-inflammatory properties to DHA metabolites. Furthermore, when administrated per os, MAG-DHA represents a stable compound deprived of toxicity that could serve as a precursor to generate a variety of PUFA-derived mediators such as resolvins, which are known to directly mediate anti-inflammatory, antiproliferative, and pro-resolving effects through specific receptors. Consequently, MAG-DHA could provide a safe and interesting new approach for the prevention of hypertension and cardiovascular diseases.

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DISCLOSURES

Only S. Fortin declares a potential conflict of interest since he is the owner of SCF Pharma, including a worldwide exclusive license on patented uses of MAG-DHA and composition containing MAG-DHA.

AUTHOR CONTRIBUTIONS

C.M., E.R., and S.F. conception and design of research; C.M. performed experiments; C.M. analyzed data; C.M. and S.F. interpreted results of experiments; C.M. prepared figures; C.M. drafted manuscript; C.M., E.R., P.U.B., and S.F. edited and revised manuscript; C.M., E.R., P.U.B., and S.F. approved final version of manuscript.

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