ω-6 (18:2) and ω-3 (18:3) fatty acids in reconstituted high-density lipoproteins show different functionality of anti-atherosclerotic properties and embryo toxicity

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Abstract

Among unsaturated fatty acids, epidemiologic studies have demonstrated that ω-6 (linoleic acid) and ω-3 (linolenic acid) fatty acids show different associations with risk of cardiovascular disease (CVD), although its molecular mechanisms remain unclear. To determine why consumption of ω-3 fatty acid is associated with lower risk of CVD, we investigated the biological functions of ω-6 (linoleic acid) and ω-3 (linolenic acid) in reconstituted HDL (rHDL) after encapsulation using human cells and zebrafish embryo. Apolipoprotein A-I (apoA-I) is the principal protein behind the beneficial functions of HDL, which include potent anti-oxidant, anti-inflammatory, and anti-atherosclerotic activities in blood. Several rHDLS were synthesized with apoA-I and different molar ratio of ω-6 or ω-3 fatty acid. Both fatty acids showed similar solubility in rHDL up to a molar ratio of 95:5:1:10 (palmitoyloleoyl phosphatidylcholine:cholesterol:apoA-I:fatty acid). Although both rHDL showed similar structural properties and α-helical contents, ω-6-rHDL showed loss of anti-oxidant ability against LDL oxidation. Uptake of acetylated LDL into macrophages was inhibited by ω-3-rHDL but not ω-6-rHDL, suggesting that ω-6-rHDL has higher pro-atherosclerotic activity. ω-3-rHDL showed more enhanced cholesterol efflux activity with less accumulation of triglyceride in the macrophage. ω-6-rHDL caused more senescence in human dermal fibroblast cells with cytotoxicity, while ω-3-rHDL treatment inhibited the senescence. In zebrafish embryo survivability, ω-3-rHDL-injected embryos showed 86±3% survival, whereas ω-6-rHDL-injected ones showed 72±2% survival as well as an elevated inflammatory response in zebrafish embryos. In conclusion, ω-6-rHDL and ω-3-rHDL show different physiological activities in atherosclerosis, inflammation, and cellular senescence.

Keywords: Linoleic acid (ω-6); Linolenic acid (ω-3); Reconstituted HDL; Zebrafish; Inflammation; Atherosclerosis

1. Introduction

It is well known that ω-6 fatty acid has inflammatory [1] and atherogenic properties [2], whereas ω-3 polyunsaturated fatty acid (PUFA) displays protective effects against atherosclerosis [3]. Major ω-3 fatty acid reportedly inhibits carcinogenesis through anti-inflammatory activity [4]. On the other hand, ω-6 fatty acid or linoleic acid is known to induce carcinogenesis through oxidative damage and pro-inflammatory mechanisms as well as endoplasmic reticulum stress and apoptosis in human hepatoma cells [5,6]. α-Linolenic acid (ALA, ω-3) is an abundant and natural precursor of dietary ω-3 PUFA, which exerts anti-inflammatory effects, and has been shown to inhibit pro-inflammatory cytokine production [7]. ALA-enriched oil also reduces oxidative stress and CD40 ligand expression [8]. Prospective studies have suggested that there is a weak association between increased dietary ALA intake and reduced risk of prostate cancer [9]. Further, ALA plays a protective role against cancer development. To investigate the differential roles of ω-3 and ω-6 fatty acids in lipoprotein metabolism, we synthesized reconstituted HDL (rHDL) with apolipoprotein A-I (apoA-I) and ω-3 or ω-6 fatty acid.

HDL is a protein–lipid complex in plasma and exerts potent cardioprotective, anti-oxidant, anti-inflammatory, and anti-atherosclerotic activities [11]. Further, HDL-cholesterol is inversely correlated with incidence of coronary heart disease [10]. ApoA-I, the major protein of HDL, possesses several beneficial effects. Many researchers, including our group, have reported that the quality of HDL is highly dependent on structural and functional correlations with apoA-I during aging [12–14]. In support of this, modification of apoA-I is directly related with production of dysfunctional HDL, which is known to exacerbate cellular senescence through atherogenic and inflammatory effects [15,16]. Taken together, these results strongly suggest that the functionality of HDL is highly affected by its composition.

Although it is well known that ω-6 fatty acid has unhealthy effects, its role in lipoprotein metabolism remains unclear. Since the dietary should be transported through the bloodstream, interaction of trans fatty acid with lipoprotein should be investigated. Especially, the functionality and
structure of HDL can be modified by foreign molecules such as trans fat [17] and smoking [18].

In this study, we compared the physiological functions of two types of unsaturated 18-carbon fatty acids, linoleic acid (18:2, Δ^9,12) and linolenic acid (18:3, Δ^9,12,15). As fatty acids are insoluble in water, we synthesized rHDL containing either fatty acid at different molar ratios. Although they share the same carbon number and very similar structures, it is possible that their physiological roles in lipoprotein metabolism and embryonic development are different.

2. Materials and methods

2.1. Materials

Linoleic acid (18:2, ω-6; Cat# L1376) and linolenic acid (18:3, ω-3; Cat# L2376) were purchased from Sigma Chemical (St. Louis, MO, USA). Human plasma was obtained from the Blood Bank of Yeungnam University Medical Center (Daegu, Korea).

2.2. Purification of plasma apoA-I

ApoA-I was purified from human plasma by ultracentrifugation and column chromatography following a previously described method [19].

2.3. Synthesis of rHDL

Discoidal HDL containing ω-3 or ω-6 fatty acid was prepared by the sodium cholate dialysis method [20] using initial molar ratios of palmityloleyl phosphatidylcholine (POPC):cholesterol:apoA-I:fatty acid:sodium cholate of 95:5:1:x:150, where x represents 1, 5, or 10. For example, A-1:ω-6 (1:10)-rHDL indicates a molar ratio of 95:5:1:10:150 for POPC:cholesterol:apoA-I:linoleic acid (ω-6):sodium cholate. The rHDL particles were washed without further purification due to their high homogeneity, and their sizes were determined by 8–25% native polyacrylamide gradient gel electrophoresis (PAGE, Pharmacia PhastSystem, GE Healthcare, Uppsala, Sweden) and compared with those of standard globular proteins (Cat# 17-0445-01; Amersham Pharmacia, Uppsala, Sweden). Relative migrations were compared via densitometric scanning analysis using a Gel Doc XR (Bio-Rad, Hercules, CA, USA) with Quantity One software, version 4.5.2. The number of apoA-I molecules per rHDL particle as well as self-association properties of lipid-free proteins were determined by cross-linking with BS3 using the method described by Staros [21]. Reaction products were analyzed by sodium dodecyl sulfate (SDS)-PAGE on precast 8–25% gradient gels (Amersham Pharmacia).

2.4. Inhibition of LDL oxidation

Fresh LDL was incubated with lipid-free or rHDL-associated apoA-I at a concentration of 2 μM in the presence of 10 μM CuSO4 for up to 3 h. During incubation, the quantity of conjugated dienes formed was determined by measuring the absorbance at 234 nm (A234) and 37°C [22,23] using a Beckman DU 800 spectrophotometer (Fullerton, CA, USA) equipped with a MultiTemp III thermocirculator (Amersham Pharmacia, Uppsala, Sweden).

To verify spectroscopic data, oxidized samples were subjected to electrophoresis on 0.5% agarose gels to compare their electromobilities [24]; migration of each lipoprotein is known to depend on its intact charge and size. Gels were then dried and the bands stained with 0.125% Coomassie Brilliant Blue.

2.5. Acetylation of LDL

Acetylation of LDL (acetylated LDL (acLDL)) was performed using saturated sodium acetate and acetic anhydride according to a previously described method [25]. After acetylation and subsequent dialysis, acLDL protein content was determined and filtered through a 0.22-μm filter (Millex; Millipore, Bedford, MA, USA). To visualize phagocytosis of acLDL, a fluorescent cholesterol derivative (22-(N-7-nitrobenzoxo-2-oxa-1,3-diazol-4-yl)amino-21,24-bisnor-5-cholen-3-yl) and 0.1 mg of sodium per milligram of apoA-I was added to acLDL particles.

2.6. LDL phagocytosis assay

THP-1 cells, a human monocytic cell line, were obtained from the American Type Culture Collection (HTIB-202; Manassas, VA, USA) and maintained in RPMI-1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) until needed for experimentation. Cells that had undergone no more than 20 passages were incubated in medium containing phorbol 12-myristate 13-acetate (PMA; final concentration 150 nM) in 24-well plates for 24 h at 37°C in a humidified incubator (5% CO2 and 95% air) to induce differentiation into macrophages. Differentiated and adherent macrophages were then rinsed with warm phosphate-buffered saline (PBS) and incubated with 400 μl of fresh RPMI-1640 medium containing 15% FBS, 50 μl of acLDL (1 mg of protein per milliliter in PBS), and 50 μl of PBS or each protein (final PBS concentration: 2 μM) for 48 h at 37°C in a humidified incubator. After incubation, cells were washed with PBS three times and then fixed in 4% paraformaldehyde for 10 min. Next, fixed cells were stained with oil-red O staining solution (0.67%) and then washed with distilled water. THP-1 macrophage-derived foam cells were then observed and photographed using a Nikon Eclipse TE2000 microscope (Tokyo, Japan) at a magnification of ×600.

2.7. Lipid extraction and analysis

Cells in 6-well culture plate were washed three times with cold PBS and incubated with 2 ml of hexane/isopropanol (3:2, v/v) to extract total lipid as described previously [26]. The extracts were evaporated under nitrogen and re-dissolved in isopropanol (0.3 ml). Cellular lipid concentrations were determined by enzymatic assays using commercially available kits (total cholesterol, T-CHO and triglyceride, Cleantech TS-S; Wako Pure Chemical, Osaka, Japan). Protein concentrations of cell lysates were determined via the Lowry protein assay, as modified by Markwell et al. [27].

2.8. Circular dichroism

The average α-helical contents of proteins in lipid-free and lipid-bound states were measured by circular dichroism (CD) spectroscopy using a J-715 spectropolarimeter (Jasco, Tokyo, Japan). Spectra were obtained from 250 to 190 nm at 25°C in a 0.1-cm pathlength quartz cuvette, using a bandwidth of 1.0 nm, a speed of 50 nm/min, and a response time of 4 s. The protein samples, which were dialyzed against TBS, were diluted to 0.07 mg/ml for lipid-free proteins to avoid apolipoprotein self-association, whereas lipid-bound proteins were diluted to 0.1 mg/ml. Four scans were accumulated and averaged. The α-helical content was calculated from the molar ellipticity at 222 nm [28].

2.9. Characterization of Trp fluorescence and isothermal denaturation

The wavelengths of maximum fluorescence (WMF) of tryptophan (Trp) residues in apoA-I protein were determined from uncorrected spectra obtained using an LS55 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA) in conjunction with the WinLab software package 4.00 (Perkin-Elmer) and a 1-cm pathlength Suprasil quartz cuvette (Fisher Scientific, Pittsburgh, PA, USA). Briefly, samples were excited at 295 nm to avoid tryosine fluorescence, and emission spectra were then scanned from 305 to 400 nm at room temperature. Effects of urea addition on the secondary structure of apoA-I in a lipid-bound state were determined by measuring WMF.

2.10. Assays of conformational adaptability

To examine the conformational adaptability of POPC-rHDL particles, we examined changes in particle size upon incubation of rHDL with LDL as well as loss of phospholipids, as described previously [29]. Each POPC-rHDL preparation (100 μg of protein in 50 μl) was incubated with human LDL (120 μg of protein in 50 μl) at 37°C for up to 24 h. Structural changes were stopped at designated time intervals by addition of native gel sample buffer and storage at 4°C prior to analysis by native 8–25% PAGE.

[Fig. 1. Electrophoretic patterns of apoA-I-rHDL containing different fatty acids at different molar ratios of apoa-I-fatty acid (1:1, 1:5, and 1:10) in a lipid-bound state. Each POPC-rHDL migrated on an 8–25% native gel gradient without denaturation of proteins. Lane 1, apoA-I-rHDL containing linoleic acid (1:1); lane 2, apoA-I-rHDL containing linolenic acid (1:5); lane 3, apoA-I-rHDL containing linoleic acid (1:10); lane 4, native apoA-I; lane M, molecular weight standard, high-molecular-weight markers (Amersham Pharmacia).]
To compare anti-oxidant and anti-inflammatory activities of HDL between smokers and control, HDL from each group was injected into zebrafish embryos, as described previously [30]. Embryos at 1 day post-fertilization were individually microinjected into a pneumatically pipetted device (PC-10; Narishige, Tokyo, Japan). To minimize bias, injections were performed at the same position on the yolk. Filter-sterilized solution of each sterilizer ([final concentrations of 10 mM PGH and 0.3% PHMG in (50 nl]) was injected into flask's embryos. Following injection, live embryos were observed under a stereomicroscope (Motic SMZ 168; Hong Kong) and photographed using a Motic cam2300 CCD camera.

2.14. Microinjection of zebrafish embryos

After injection with rHDL, changes in reactive oxygen species (ROS) levels in larvae were imaged by dihydroethidium (DHE; Cat# 37291, BioChemika) staining, as previously described [32]. Images were obtained by fluorescence microscopy (Ex=588 nm and Em=605 nm) on a Nikon Eclipse TE2000 instrument (Tokyo, Japan). To avoid bias, red fluorescence was measured in the trunk area away from the injection site. Quantification of stained area was carried out via computer-assisted morphometry using Image Proplus software (version 4.5.1.22; Media Cybernetics, Bethesda, MD, USA).

2.15. Imaging of reactive oxygen species

To compare anti-oxidant and anti-inflammatory activities of HDL between smokers and control, HDL from each group was injected into zebrafish embryos, as described previously [30]. Embryos at 1 day post-fertilization were individually microinjected into a pneumatically pipetted device (PC-10; Narishige, Tokyo, Japan). To minimize bias, injections were performed at the same position on the yolk. Filter-sterilized solution of each sterilizer ([final concentrations of 10 mM PGH and 0.3% PHMG in (50 nl]) was injected into flask's embryos. Following injection, live embryos were observed under a stereomicroscope (Motic SMZ 168; Hong Kong) and photographed using a Motic cam2300 CCD camera.

2.16. Statistical analysis

All data are expressed as the mean±S.D. of at least three independent experiments with duplicate samples. Comparisons between results were made by Student’s t test using the SPSS program (version 12.0; SPSS, Inc., Chicago, IL, USA). Statistical significance was defined as P<0.05.
3. Results

3.1. Synthesis of rHDL

All rHDLs containing fatty acids were well synthesized, and both \( \omega-3 \) and \( \omega-6 \) fatty acids were solubilized in isotonic buffer (TBS) via rHDL formulation as shown in Fig. 1. The rHDL containing fatty acids showed a slightly smaller particle size than control rHDL (94 Å) without fatty acid. As fatty acid ratio increased, particle size decreased from 91 Å (1:1, A-I: \( \omega-6 \)) to 89 Å (1:10, A-I: \( \omega-3 \)) and 90 Å (1:1, A-I: \( \omega-3 \)) to 87 Å for both \( \omega-6 \) and \( \omega-3 \) fatty acids, respectively. Band intensity decreased compared to control at the highest fatty acid content (1:10), especially for \( \omega-3 \) fatty acid. However, there was no displacement of apoA-I in all rHDL particles, indicating that elevated content of linolenic acid affected staining ability of rHDL via putative interactions with apoA-I.

3.2. Secondary structure

Elevation of both fatty acids reduced \( \alpha \)-helicity (less than 70%), whereas \( \alpha \)-helical contents of native rHDL and apoA-I were 73% and 47%, respectively. Elevation of \( \omega-6 \) fatty acid content in rHDL did not alter \( \alpha \)-helicity (around 65–66%), whereas an increase in \( \omega-3 \) fatty acid content in rHDL caused a remarkable decrease in \( \alpha \)-helicity to 56% (1:10, A-I: \( \omega-3 \)). Trp residues in lipid-bound apoA-I without fatty acids moved to the interior of the tertiary structure, resulting in a 3-nm blue shift in WMF (around 347 nm) compared to lipid-free apoA-I. The WMF of \( \omega-6 \)-rHDL was similar to that of control (around 343–344 nm) regardless of molar ratio. However, \( \omega-3 \)-rHDL showed a stronger blue shift in WMF (around 340 nm) at a molar ratio of 1:10, suggesting that Trp residues moved to hydrophobic phase via interactions with \( \omega-3 \) fatty acid. Generally, \( \omega-3 \)-rHDL showed slightly decreased \( \alpha \)-helicity and particle size with increasing fatty acid content in rHDL, as shown in Table 1 and Fig. 1.

3.3. Inhibition of LDL oxidation

In conjugated diene assay, all rHDLs containing fatty acids caused less oxidation of LDL compared to PBS treatment. After 100 min of incubation with LDL in the presence of cupric ion, native rHDL showed 29% less production of conjugated dienes than LDL alone, whereas \( \omega-6 \)-rHDL showed 20% less production of conjugated dienes (Fig. 2A). However, \( \omega-3 \)-rHDL showed the strongest anti-oxidant ability, resulting in 2-fold

![Cellular uptake of acLDL](image_url)
stronger anti-oxidant ability than ω-6-rHDL. In electromobility comparison, ω-6-rHDL-treated LDL showed the fastest mobility as compared to native HDL, whereas ω-3-rHDL-treated LDL showed slower mobility (Fig. 2B). These results suggest that ω-3 fatty acid in rHDL exerts greater anti-oxidant ability against LDL oxidation.

3.4. Anti-atherogenic activity

As shown in Fig. 3, compared to acLDL alone (photo b), phagocytosis of acLDL by macrophages was inhibited by native rHDL (photo c), whereas ω-6-rHDL treatment caused increased uptake of LDL (photos d–f) in a dose-dependent manner. However, ω-3-rHDL treatment inhibited uptake of acLDL in a dose-dependent manner (Fig. 3g–i).

In the image analysis, native rHDL treatment caused 32% reduction of acLDL uptake, whereas ω-6-rHDL (1:1, 1:5) treatments inhibited acLDL uptake by 30% and 26%, respectively. Interestingly, ω-6-rHDL (1:10) showed no inhibitory activity and even facilitated uptake of acLDL, suggesting that there was a discrepancy between the dosage levels. On the other hand, ω-3-rHDL treatment caused up to 47% inhibition of acLDL uptake in a dose-dependent manner, which
matches that of ω-6-rHDL (1:10). These results suggest that linoleic acid is pro-atherogenic and pro-inflammatory, whereas linolenic acid has more anti-atherogenic activities.

Lipid extraction analysis revealed that apoA-I-rHDL treatment caused 48% and 38% removal of triglyceride (TG) and cholesterol from the cell lysate of macrophage, which had been incubated with acLDL to induce phagocytosis. More interestingly, ω-3-rHDL treatment caused 56% and 52% removal of TG and TC, respectively, indicating the strongest cholesterol efflux activity among all kind of rHDL. However, ω-6-rHDL caused 29% and 18% of TG and cholesterol, respectively, indicating the weakest cholesterol activity. These results suggest that functionality of rHDL can be enhanced by encapsulation of ω-3 fatty acid in terms of anti-oxidant ability and cholesterol efflux.

3.5. Anti-senescent effect

As the molar ratio of fatty acids in rHDL increased, ω-6-rHDL treatment resulted in a greater number of SA-β-gal-positive cells, indicating that ω-6 caused more senescence in HDF cells at passage 15 (Fig. 4). However, ω-3-rHDL treatment inhibited cellular senescence in a dose-dependent manner. Especially, ω-6-rHDL (1:10) resulted in 1.4-fold more SA-β-gal-positive cells, whereas ω-3-rHDL (1:10) caused only 30% SA-β-gal-positive cells, indicating that ω-6-fatty acid had a pro-senescence effect while ω-3 fatty acid showed an anti-senescence effect. Indeed, at the same molar ratio of A-I:fatty acid (1:10), ω-6-rHDL treatment caused 4.3-fold greater senescence than ω-3-rHDL. Cell number populations were slightly elevated by treatment with both ω-3-rHDL and ω-6-rHDL fatty acid at a 1:1 molar ratio. However, increased fatty acid content in rHDL at molar ratios of 1:5 and 1:10 resulted in similar cell numbers as rHDL alone.

3.6. Embryo survival

After 48 h post-injection in the presence of oxidized LDL (oxLDL), PBS-injected embryos showed 55% survivability, whereas native rHDL-injected embryos showed 88% survival. This result suggests that native rHDL can protect against inflammatory embryonic death caused by oxLDL, as in our previous report [31]. ω-3-rHDL-injected embryos showed 86±3% survival, whereas ω-6-rHDL-injected ones showed 72±2% survival. Although ω-6-rHDL also showed some protective activity, ω-3-rHDL showed stronger protective effects against oxidative stress caused by oxLDL. In DHE staining for ROS visualization, PBS-injected embryos showed the strongest red intensity (2.5-fold stronger than that of native rHDL-injected embryos). Further, both ω-6-rHDL- and ω-3-rHDL-injected embryos showed 2.0- and 1.5-fold stronger red intensity compared to native rHDL-injected embryos, respectively. This result shows that ω-3 fatty acid caused less production of ROS than ω-6 fatty acid.

4. Discussion

It is well known that ALA (ω-3, 18:3) has protective effects against cardiovascular and inflammatory diseases via down-regulation of pro-inflammatory genes, including adhesion molecules and cytokines [33,34]. However, besides its absorption in the gut and delivery in blood, the physiological effect of ALA in serum lipoprotein metabolism via putative interactions has not been fully understood. This study was designed to investigate interactions of ω-3 and ω-6 fatty acids with apoA-I and HDL during blood circulation.

As fatty acid content in HDL increased, ω-3-rHDL showed relatively lower α-helicity as well as smaller particle size compared to ω-6-rHDL (Table 1 and Fig. 1). However, Trp residues in apoA-I of ω-3-rHDL moved to a more hydrophobic region (blue shift in WME), and there was no displacement of apoA-I, suggesting that synthesis of rHDL occurred up to a molar ratio of 1:10 (A-I:fatty acid), ω-3-rHDL exerted stronger inhibitory activity against LDL oxidation (Fig. 2) and uptake of LDL into macrophages (Fig. 3). Senescence of dermal cells was more accelerated by ω-6-rHDL and attenuated by ω-3-rHDL (Fig. 4). This result is in good agreement with a recent review suggesting that diet supplementation with evening primrose oil and ω-3 fatty acid may be appropriate for treating certain inflammatory skin diseases such as atopic dermatitis [35]. Further, consumption of ω-3-fatty acid is inversely associated with melanoma and squamous cell carcinoma [36]. Our results are also supported by a previous report showing that anti-oxidants exert inhibitory effects against melanogenesis [37,38] as well as our own study demonstrating the facial skin whitening effect of vitamin C [39].

The protective effects of ω-3-rHDL from embryonic death (Fig. 5) are supported by other reports demonstrating that ALA-treated oocytes produce more blastocysts [40] while ALA treatment improves oocyte maturation and development with a lower number of apoptotic cells [41].

It is well known that ω-3 fatty acid is involved in anti-inflammatory signaling to increase cholesterol efflux and decrease TG synthesis [42]. ALA has been shown to inhibit NF-κB activation as well as production of TNF-α and interleukin-6 following TLR4 activation in response to LPS binding and signaling. Similarly, HDL inhibits NF-κB through the same anti-inflammatory pathway, as we reported previously. However, glycated HDL and HDL containing transfat show loss of anti-inflammatory signaling [43,17]. HDL containing oleic acid displays anti-inflammatory activity, whereas HDL containing elaidic acid is pro-inflammatory in macrophages and zebrafish embryos [17]. Furthermore, we recently reported that incorporation of growth hormone-2 into HDL significantly enhances anti-oxidant activity [44]. These reports collectively suggest that the beneficial functions of HDL can be modified by altering lipid and protein composition to produce dysfunctional HDL. Since absorbed dietary fatty acids are released into the bloodstream as free fatty acids, fatty acids can be incorporated into lipoproteins via putative interactions in serum. Therefore, there is a possibility of HDL with different compositions containing either ω-3 or ω-6 fatty acid and exhibiting different functions.

The current results show that incorporation of ω-6 fatty acid induced more dysfunctional HDL versus that of ω-3 fatty acid. Moreover, microinjection of oxLDL into live embryos showed that ω-3-rHDL exerted more protective effects from oxidative stress caused by oxLDL than ω-6-rHDL (Fig. 5), suggesting that ω-3 fatty acid (ALA) has anti-inflammatory and anti-oxidant activities. This result demonstrates that ALA has protective effects against cardiovascular and neurodegenerative diseases [42]. Although unsaturated fatty acids are beneficial for suppression of aging-related diseases regardless of double-bond location, there exist distinct functional differences between ω-3 and ω-6.

It is well known that ω-6 fatty acid can be converted into arachidonic acid, which is a substrate for cyclooxygenase to produce pro-inflammatory eicosanoids such as thromboxane, leukotriene, and prostaglandin. On the other hand, ω-3 fatty acid (ALA) is involved in anti-inflammatory and anti-atherosclerotic signaling via suppression of liver X receptor-dependent sterol regulatory element binding protein [45]. Recently, we reported functional and structural impairment of HDL in young smokers, suggesting that the functional status of HDL can be a good biomarker for extent of inflammation signaling and aging. Similarly, another group reported an association between higher ω-3 index and increased insulin sensitivity in middle-aged overweight men [46]. Specifically, overweight middle-aged men showed 2-fold higher ALA content in their highest ω-3 index tertile as well as significantly less inflammatory markers such as C-reactive protein and HOME-IR. Therefore, C18:3n-3, ω-3, ALA contributes more to higher insulin sensitivity and more favorable metabolic profiles than C18:3n-6, ω-6, γ-linolenic acid.

In conclusion, HDL functionality and metabolic advantage is strongly enhanced by increased ω-3 content via anti-oxidant and
Fig. 4. Comparison of cellular senescence in HDFs by rHDL containing fatty acids. (A) Senescence was compared by senescence-associated β-galactosidase (SA-β-gal) staining at the designated passage in the presence of equal amounts of rHDL (final apoA-I concentration: 0.8 μM). Cell image was captured using a Nikon Eclipse TE2000 microscope (Tokyo, Japan) at a magnification of ×400. (B) Graph shows number of HDF cells per 1 ml of cell culture media compared by trypan blue staining and SA-β-gal-positive cells per 7.4 mm² of cell culture area during treatment with each rHDL. Data are shown as the mean±S.D. from three independent experiments performed in duplicate. *P<.05; **P<.01; §P<.01 versus A-I-rHDL.
Incubation time (hours)

Survivability (%)

oxLDL + PBS (n=50)

oxLDL + A-I-rHDL (n=48)

oxLDL + (A-I:ω-6)-rHDL (1:10) (n=46)

oxLDL + (A-I:ω-3)-rHDL (1:10) (n=51)

No-injection (n=53)

Fluorescence area (AU)

52 hpf

DHE stained area (AU, pixel)
anti-senescent activities. α–3–HDL showed atheroprotective and embryoprotective activities in human cells and zebrafish.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2015.08.008.

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