REVIEW

Fatty Acids and Lipid Derivatives Protecting Photooxidative Attack in Age-related Macular Degeneration

Diana Matías-Pérez and Iván Antonio García-Montalvo*

Division of Graduate Studies and Research, National Technology of Mexico/Technological Institute of Oaxaca, Avenida Ing. Víctor Bravo Ahuja No. 125 Esquina Calzada Tecnológico, C.P. 68030, Oaxaca, MEXICO

Abstract: The objective is the systematic review of studies published in Scielo, Redalyc, Dialnet, Web of Science, Scopus and Pubmed, related to the inclusion of fatty acids and lipid derivatives in the daily diet to prevent or delay the appearance or progression of Age-Related Macular Degeneration (AMD). The analysis of the research results consulted shows that AMD is one of the most frequent causes of blindness in subjects over 55 years of age. AMD is characterized by decreased vision, metamorphopsia, macropsies, micropsies, and central scotoma. Disease that must be diagnosed early as it can lead to irreversible blindness. Among the components of the diet that in numerous epidemiological studies have shown an association in the treatment of AMD and that are reviewed in this work are fatty acids, vitamins and carotenoids. There is ample evidence that fatty acids and lipid derivatives can be included in the diet plans of subjects with AMD.

Key words: AMD, photooxidative attack, ROS, fatty acids, lipid derivatives

1 Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in industrialized countries, among the clinical symptoms are: a gradual loss of central vision, distortion of images and straight lines, the presence of blurred and dark areas of central vision, among others. These visual disturbances affect the lifestyle of patients, compromising their daily activities¹⁻³⁾. AMD affects approximately 8.7% of the elderly population worldwide (> 55 years affecting; 10% of people older than 65 years and more than 25% of people older than 75 years)¹⁾, the number of AMD cases is expected to increase to 288 million by 2040⁴⁾.

2 Age-related Macular Degeneration (AMD)

This condition occurs in the small central part of the retina, known as the macula lutea, which is essential for the visualization of fine details and image resolution. In the macula lutea, the most common characteristics of this disease can be recognized in the form of drusen (aggre-

gates of extracellular material) and the growth of the chorioidal vessels called chorioidal neovascularization^{5, 6)}. The appearance of drusen and neovascularization is the result of chronic changes of the macula and in particular of the retinal pigment epithelium (RPE), of the choriocapillaris (CC), as well as photoreceptors (rods and cones) and Bruch's membrane (BRM)⁷⁻¹⁰⁾. Two types of drusen are known, hard drusen and soft drusen. Hard drusen are typically smaller than 63 μm and have a flattened shape on stereoscopic photographs, while those larger than 125 μm are often classified as soft drusen (125 μm is the approximate diameter of the retinal veins at the edge of the optic disc)⁷⁾. Hard drusen are nodular accumulations of hyaline material on the outer part of the basement membrane of the retinal pigmented epithelium. Whereas soft drusen are considered groups of hard drusen, in addition, there are indistinct soft drusen which are localized detachments of the retinal epithelium and which can group together to form a retinal drusenoid detachment¹¹⁾. The molecular pathway that underlies geographic atrophy (GA) and vision loss. This pathway implies that RPE death consequently leads to photoreceptor loss, which progressively results in visual

*Correspondence to: Iván Antonio García Montalvo, Division of Graduate Studies and Research, National Technology of Mexico/ Technological Institute of Oaxaca, Avenida Ing. Víctor Bravo Ahuja No. 125 Esquina Calzada Tecnológico, C.P. 68030, Oaxaca, MEXICO

E-mail: ivan.garcia@itoaxaca.edu.mx

Accepted December 16, 2020 (received for review November 6, 2020)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

<http://www.jstage.jst.go.jp/browse/jos/> <http://mc.manuscriptcentral.com/jjocs>

loss over time. Low levels of the ribonucleic acid (RNA)-cleaving enzyme, DICER1, in RPE cells were observed in patients with dry AMD. It was reported that decreased levels of DICER1 lead to a decreased rate in the breakdown of RNA-Alu molecules, which are noncoding sequences of RNA. The overabundance of cytoplasmic RNA-Alu activates inflammatory proteins such as NLRP3 in the inflammasome, which results in the activation of a cascade of molecular reactions that result in RPE cell loss. Furthermore, mitochondrial dysfunction has been associated with the development of dry AMD. The mitochondrial dysmorphology observed in the RPE in individuals with AMD was consistent with severe dysfunction^{12–17}.

According to the fundoscopic abnormalities observed, two forms of AMD can be distinguished: the dry or non-exudative atrophic form and the neovascular or exudative form also called the wet form¹⁸. The dry or non-exudative atrophic form, known as geographic atrophy, it is characterized by the progressive accumulation of drusen between the RPE and the CC. Excessive drusen between the RPE and the CC hinder the transport of oxygen and nutrients, which degenerates the RPE system and photoreceptors, it can also progress to the wet form, which is the most aggressive form of AMD characterized by choroidal neovascularization. The angiogenesis process leads to the formation of very fragile blood vessels, which are responsible for bleeding and destruction of RPE cells (see Fig. 1)^{19, 20}.

AMD is considered a multifactorial disease that involves an interaction between genetic and environmental factors²¹. Environmental factors include: aging and smoking contribute significantly to the increased risk of AMD^{22, 23}. The disease increases with age and with it the loss of photoreceptors, mainly the rods, thus acting as a common cause of the development of AMD²⁴. Cigarette smoke contains a high amount of toxic substances, which contribute to atherosclerosis, endothelial dysregulation,

and angiogenesis. The presence of oxidative compounds in tobacco is associated with an increase in the formation of reactive oxygen species (ROS) and therefore with oxidative damage at the cellular level of RPE^{25–29}, in addition, eating habits contribute significantly to the progressive development of the disease³⁰.

3 Fatty Acids and Lipid Derivatives in Age-Related Macular Degeneration

Age-related macular degeneration and maculopathies are the result of a photooxidative attack, supplementation of the diet with vitamins C, E, lutein, zeaxanthin, carotenoids and fatty acids have been shown to slow the progression of atrophic macular degeneration, avoiding progress towards its most severe form and / or neovascular forms^{31–37}. They have been proposed to limit damage to photoreceptors at the macular level, by protecting against the cumulative effects of oxidative stress, which would be a mechanism of cellular injury that is caused by reactive oxygen intermediaries³¹. The retina is considered susceptible to oxidative stress due to its high oxygen consumption and the high natural exposure of irradiation that accumulates^{38–41}. In the case of omega-3 and omega-6 fatty acids, eicosapentaenoic acid (EPA) and arachidonic acid (AA) were used respectively for their effects against oxidative stress and inflammatory processes. AA acts as a precursor for pro-inflammatory processes known as series 2 prostanoids, while EPA provides precursors for the production of anti-inflammatory mediators of series 3 prostanoids. Inflammation is a process in the innate immune system, the excessive production of pro-inflammatory products during chronic inflammation can have detrimental effects, increasing susceptibility to disease, due to the increase in reactive oxygen species, the induction of a state of cellular stress,

Fig. 1 Types of age-related macular degeneration. a) Wet AMD b) Dry AMD.

the alteration in inactive molecules such as: growth factors, remodeling of matrix proteins and tissue structure⁴²⁾.

PUFAs are important components of the outer segments of retina photoreceptors and have been shown to interact with rhodopsin, suggesting their role in phototransduction. DHA is the major polyunsaturated fatty acid in the retina and brain. Its presence in the disk membrane of retinal photoreceptor outer segment is indispensable for retinal function and protects against damage from bright light and oxidative stress. DHA accounts for 20–30% of the fatty acids in phosphatidylcholine(PC) or phosphatidylethanolamine(PE) of outer segment disk membranes. It has been also suggested that particularly VLC-PUFAs (>C26) are suited to build highly curved membranes in photoreceptor outer segment disks. During the process of daily renewal, photoreceptors are constantly shedding their outer segment membranes, which are phagocytosed by the RPE where PUFAs are recycled and further processed. The membrane-bound (n-3) and (n-6) PUFAs are cleaved by phospholipases A1 and A2, leading to metabolism by three major pathways, the cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 oxidases, resulting in metabolites with diverse functions, n-3 metabolites such as prostaglandins are anti-inflammatory and n-6 metabolites such as PGE2 and TXA2 are inflammatory. DHA serves as a precursor to neuroprotective docosanoids, while the release of longer PUFAs (>C30) can lead to the formation of elovonoids, two more classes of pro-homeostatic lipid mediators^{43–50)}.

Vitamin C, vitamin E, lutein and zeaxanthin act in the central area of the macula, the macular pigment can protect the underlying photoreceptor cell layer from light damage by filtering blue light⁵¹⁾, the Blue Mountain Eye study reported that the increase in lutein and zeaxanthin in the diet, showed a reduction in the risk of early incidence and neovascularization of AMD with a period of

between 5 and 10 years⁵²⁾. The Age-Related Eye Disease Study (AREDS)⁴²⁾ and the Age-Related Eye Disease Study 2 (AREDS2)^{51–53)} have improved the scientific understanding of the effect of nutritional supplementation mainly based on antioxidants in reducing the development of AMD. In the AREDS2 study, supplementation with lutein, minerals, omega-3 polyunsaturated fatty acids and fatty acid derivatives were used (Table 1)^{48–50, 54)}.

4 Conclusions

The high consumption of saturated fatty acids, snuff, alcohol, sedentary lifestyle has led to an increase in diseases that are now considered public health problems, development of AMD is multifactorial, however, nutritional support is a challenge partly because to the regulatory environment and difficulties in designing clinical trials to answer these questions, environmental factors such as the quitting smoking and eating a healthy diet serve to prevent or slow the progression of AMD. In subjects who already present some degree of the pathology, they are recommended to take AREDS-type supplements, based on available scientific evidence. These findings enhance the understandings of dietary impacts on neovascular AMD and provide a context for future nutritional intervention studies.

Authors' Contributions

IAGM, DMP participated in the study concept, design, writing and critical review of the manuscript.

Table 1 Participation of lipid derivatives and fatty acids in Age-Related Macular Degeneration.

Nutritional component	Relationship with AMD
LUTEIN AND ZEAXANTHINE	Natural yellow pigments serve as antioxidants, in AMD it acts as filters to blue light, also reduces the oxidative effect, this because in the macula it is susceptible to oxidative damage, which would cause a progressive deterioration of the pigment epithelium, which can be counteracted by the various existing defense mechanisms, an example of which is enzymes with antioxidant activity. In the specific case of zeaxanthin, it participates in the inhibition of cell neoplastic transformation.
VITAMIN E	The specific mechanism of its antioxidant action is not fully elucidated. The most probable hypothesis is based on the fact that tocopherol reacts with peroxy radicals (ROO-), interrupting the oxidation chain reaction, most likely in this way it acts to protect the macula from oxidative stress.
OMEGA-3	Omega-3 involved in the formation of EPA and DHA acids, which in turn participate in brain development and retina, omega-3 are related to the prevention of cardiovascular diseases, dermal processes and inflammatory processes, which is an important part of AMD.

References

- 1) Ke, K. The direct, indirect and intangible costs of visual impairment caused by neovascular age-related macular degeneration. *Eur. J. Health. Econ.* **11**, 525-531 (2010).
- 2) Lim, L.; Mitchell, P.; Seddon, J.; Holz, F.; Wong, T. Age-related macular degeneration. *Lancet* **379**, 1728-1738 (2012).
- 3) Gopinath, B.; Liew, G.; Burlutsky, G.; Mitchell, P. Age-related macular degeneration and 5-year incidence of impaired activities of daily living. *Maturitas* **77**, 263-266 (2014).
- 4) Wong, W.; Su, X.; Lietai, X. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: A systematic review and meta-analysis. *Lancet. Glob. Health* **2**, e106-116 (2014).
- 5) Mullins, R.; Russell, S.; Anderson, D.; Hageman, G. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *FASEB J.* **14**, 835-846 (2000).
- 6) Van Leeuwen, R.; Klaver, C.; Vingerling, J.; Hofman, A.; De Jong, P. Epidemiology of age-related maculopathy: a review. *Eur. J. Epidemiol.* **18**, 845-854 (2003).
- 7) Hageman, G.; Luthert, P.; Chong, N.; Johnson, L.; Anderson, D.; Mullins, R. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog. Retin. Eye. Res.* **20**, 705-732 (2001).
- 8) Anderson, D.; Mullins, R.; Hageman, G.; Johnson, L. A role for local inflammation in the formation of drusen in the aging eye. *Am. J. Ophthalmol.* **134**, 411-431 (2002).
- 9) Bok, D. Evidence for an inflammatory process in age-related macular degeneration gains new support. *Proc. Nat. Acad. Sci. USA* **102**, 7053-7054 (2005).
- 10) Spraul, C.; Lang, G.; Grossniklaus, H. Morphometric analysis of the choroid, Bruch's membrane, and retinal pigment epithelium in eyes with age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **37**, 2724-2735 (1996).
- 11) Raffai, R.; Dong, L.; Farese, R.; Weisgraber, K. Introduction of human apolipoprotein E4 «domain interaction» into mouse apolipoprotein E. *Proc. Natl. Acad. Sci. USA* **98**, 11587-11591 (2001).
- 12) Yang, Z.; Stratton, C.; Francis, P.; Kleinman, M.; Tan, P.; Gibbs, D.; Tong, Z.; Chen, H.; Constantine, R.; Yang, X.; Chen, Y.; Zeng, J.; Davey, L.; Ma, X.; Hau, V.; Wang, C.; Harmon, J.; Buehler, J.; Pearson, E.; Patel, S.; Kaminoh, Y.; Watkins, S.; Luo, L.; Zabriskie, N.; Bernstein, P.; Cho, W.; Schwager, A.; Hinton, D.; Klein, M.; Hamon, S.; Simmons, E.; Yu, B.; Campochiaro, B.; Sunness, J.; Campochiaro, P.; Jorde, L.; Parmigiani, G.; Zack, D.; Katsanis, N.; Ambati, J.; Zhang, K. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. *N. Engl. J. Med.* **359**, 1456-1463 (2008).
- 13) Shaw, P.; Stiles, T.; Douglas, C.; Ho, D.; Fan, W.; Du, H.; Xiao, X. Oxidative stress, innate immunity, and age-related macular degeneration. *AIMS. Mol. Sci.* **3**, 196-221 (2016).
- 14) Ambati, J.; Ambati, B.; Yoo, S.; Ianchulev, S.; Adamis, A. Age-related macular degeneration: Etiology, pathogenesis, and therapeutic strategies. *Surv. Ophthalmol.* **48**, 257-293 (2003).
- 15) Tarallo, V.; Hirano, Y.; Gelfand, B.; Dridi, S.; Kerur, N.; Kim, Y.; Cho, W.; Kaneko, H.; Fowler, B.; Bogdanovich, S.; Albuquerque, R.; Hauswirth, W.; Chiodo, V.; Kugel, J.; Goodrich, J.; Ponicsan, S.; Chaudhuri, G.; Murphy, M.; Dunaief, J.; Ambati, B.; Ogura, Y.; Yoo, J.; Lee, D.; Provost, P.; Hinton, D.; Núñez, G.; Baffi, J.; Kleinman, M.; Ambati, J. DICER1 loss and Alu RNA induce age-related macular degeneration via the NLRP3 inflammasome and MyD88. *Cell* **149**, 847-859 (2012).
- 16) Nordgaard, C.; Karunadharma, P.; Feng, X.; Olsen, T.; Ferrington D. Mitochondrial proteomics of the retinal pigment epithelium at progressive stages of age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* **49**, 2848-2855 (2008).
- 17) Al-Zamil, W.; Yassin, S. Recent developments in age-related macular degeneration: A review. *Clin. Interv. Aging* **12**, 1313-1330 (2017).
- 18) Green, W. Histopathology of age-related macular degeneration. *Mol. Vis.* **5**, 27 (1999).
- 19) Algvere, P.; Seregard, S. Drusen maculopathy: a risk factor for AMD. Can we prevent visual loss? *Acta. Ophthalmol. Scand.* **81**, 427-429 (2003).
- 20) Friberg, T.; Bilonick, R.; Brennen, P. Risk factors for conversion to neovascular age-related macular degeneration based on longitudinal morphologic and visual acuity data. *Ophthalmology* **119**, 1432-1437 (2012).
- 21) Smith, W.; Assink, J.; Klein, R. *et al.* Risk factors for age-related macular degeneration: pooled findings from three continents. *Ophthalmology* **108**, 697-704 (2001).
- 22) National Eye Institute, *Age-Related Macular Degeneration (AMD)*, National Eye Institute (2014).
- 23) Velilla, S.; García-Medina, J.; García-Layana, A. *et al.* Smoking and age related macular degeneration: Review and update. *J. Ophthalmol.* **2013**, 1-11 (2013).
- 24) Curcio, C.; Owsley, C.; Jackson, G. Sparetherods, save the cones in aging and age-related maculopathy. *Invest. Ophthalmol. Vis. Sci.* **41**, 2015-2018 (2000).
- 25) Fowles, J.; Dybing, E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tobacco Control.* **12**, 424-430 (2003).

- 26) Roth, F.; Bindewald, A.; Holz, F. Key pathophysiologic pathways in age-related macular disease. *GRAEF. Arch. Clin. Exp.* **242**, 710-716 (2004).
- 27) Benowitz, N. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. *Prog. Cardiovasc. Dis.* **46**, 91-111 (2003).
- 28) Deisinger, P.; Hill, T.; English, J. Human exposure to naturally occurring hydroquinone. *J. Toxicol. Environ. Health. Sci. Part A* **47**, 31-46 (1996).
- 29) DeCaprio, A. The toxicology of hydroquinone—relevance to occupational and environmental exposure. *Crit. Rev. Toxicol.* **29**, 283-330 (1999).
- 30) Zampatti, S.; Ricci, F.; Cusumano, A.; Marsella, L.; Novelli, G.; Giardina, E. Review of nutrient actions on age-related macular degeneration. *Nut. Res.* **34**, 95-105 (2014).
- 31) Klein, B.; Knudtson, M.; Lee, K. *et al.* Supplements and age-related eye conditions the beaver dam eye study. *Ophthalmology* **115**, 1203-1208 (2008).
- 32) San Giovanni, J.; Chew, E.; Clemons, T. *et al.* The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS report No. 22. *Arch. Ophthalmol.* **125**, 1225-1232 (2007).
- 33) Evans, J.; Lawrenson, J. Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. *Cochrane. Database. Syst. Rev.* **6**, CD000253 (2012).
- 34) Chong, E.W.; Wong, T.; Kreis, A.; Simpson, J.; Guymer, R. Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *Brit. Med. J.* **335**, 755-759 (2007).
- 35) Millen, A.; Volland, R.; Sondel, S. *et al.* Vitamin D status and early age-related macular degeneration in postmenopausal women. *Arch. Ophthalmol.* **129**, 481-489 (2011).
- 36) Christen, W.; Glynn, R.; Chew, E.; Albert, C.; Manson, J. Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the women's antioxidant and folic acid cardiovascular study. *Arch. Intern. Med.* **169**, 335-341 (2009).
- 37) Kuzniarz, M.; Mitchell, P.; Flood, V.; Wang, J. Use of vitamin and zinc supplements and age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmic Epidemiol.* **9**, 283-295 (2002).
- 38) Beatty, S.; Koh, H.; Phil, M.; Henson, D.; Boulton, M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv. Ophthalmol.* **45**, 115-134 (2000).
- 39) Downie, L.; Keller, P. Nutrition and age-related macular degeneration: Research evidence in practice. *Optom. Vis. Sci.* **91**, 821-831 (2014).
- 40) Buring, J.; Hennekens, C. Antioxidant vitamins in cancer: the Physicians' Health Study and Women's Health Study. in *Nutrients in cancer prevention and treatment* (Prasad, K.N.; Santamaria, L.; Williams, R.M. eds.). Experimental Biology and Medicine: Book 27. Totowa, NJ. Humana pp. 223-234 (1995).
- 41) Stampfer, M.J.; Hennekens, C.; Manson, J.; Colditz, G.; Rosner, B.; Willett W. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* **328**, 1444-1449 (1993).
- 42) Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.* **119**, 1417-1436 (2001).
- 43) Bazan, N. Docosanoids and elovanoids from omega-3 fatty acids are pro-homeostatic modulators of inflammatory responses, cell damage and neuroprotection. *Mol. Asp. Med.* **64**, 18-33 (2018).
- 44) Gong, Y.; Fu, Z.; Liegl, R. *et al.* Omega-3 and omega-6 long-chain PUFAs and their enzymatic metabolites in neovascular eye diseases. *Am. J. Clin. Nutr.* **106**, 16-26 (2017).
- 45) Skowronska-Krawczyk, D.; Chao, D. Long-chain polyunsaturated fatty acids and age-related macular degeneration. *Adv. Exp. Med. Biol.* **1185**, 39-43 (2019).
- 46) Kaarniranta, K.; Koskela, A.; Felszeghy, S.; Kivinen, N.; Salminen, A.; Kauppinen, A. Fatty acids and oxidized lipoproteins contribute to autophagy and innate immunity responses upon the degeneration of retinal pigment epithelium and development of age-related macular degeneration. *Biochimie* **159**, 49-54 (2019).
- 47) Souied, E.; Aslam, T.; García-Layana, A.; Holz, F.; Leys, A.; Silva, R.; Delcourt, C. Omega-3 fatty acids and age-related macular degeneration. *Ophthalmic. Res.* **55**, 62-69 (2016).
- 48) Mukhtar, S.; Ambati, B. The value of nutritional supplements in treating Age-Related Macular Degeneration: A review of the literatura. *Int. Ophthalmol.* **39**, 2975-2983 (2019).
- 49) Chapman, N.; Jacobs, R.; Braakhuis, A. Role of diet and food intake in age-related macular degeneration: a systematic review. *Clin. Exp. Ophthalmol.* **47**, 106-27 (2019).
- 50) Lister, T. Nutritional, alternative, and complementary therapies for age-related macular degeneration. *Integr. Med. (Encinitas)*. **18**(6), 30-36 (2019).
- 51) Age-Related Eye Disease Study 2 Research Group. Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: The Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *JAMA* **309**, 2005-2013 (2013).
- 52) Bazán-Salinas, I.; Matías-Pérez, D.; De Gyves-Escobar, E.; Rivera-Santiago, J.; Pérez-Campos, E.; García-Mon-

- talvo, I. El consumo de aceites vegetales y la prevención de enfermedades cardiovasculares. *Avan. C. Salud. Med.* **2**, 22-30 (2014).
- 53) Sin, H.; Liu, T.; Lam, D. Lifestyle modification, nutritional and vitamins supplements for age-related macular degeneration. *Acta Ophthalmol.* **91**, 6-11 (2013).
- 54) Tan, J.; Wang, J.; Flood, V.; Rochtchina, E.; Smith, W.; Mitchell, P. Dietary antioxidants and the long-term incidence of age-related macular degeneration: The Blue Mountains Eye Study. *Ophthalmology* **115**, 334-341 (2008).
-