Appl Biochem Biotechnol
DOI 10.1007/s12010-012-9963-6

Lipid Biomarkers of Lens Aging

Bimal Prasanna Mohanty · Soma Bhattacharjee · Prasenjit Paria · Arabinda Mahanty · Anil Prakash Sharma

Received: 28 July 2012 / Accepted: 6 November 2012

Abstract Lipids are important structural components of cell membranes and have profound effect on membrane fluidity. Lipid profiling and lipidomics have captured increased attention due to the well-recognized roles of lipids in numerous human diseases. Investigating lipid profiles not only provides insights into the specific roles of lipid molecular species in health and diseases, but can also help in identifying potential preventive or therapeutic biomarkers. Cataract, the loss of transparency of eye lens, is a disease of protein aggregation. There are several factors contributing to the stability in protein conformation. Age-related changes in lipid composition could be a contributing factor for altered protein–lipid interaction leading to protein aggregation and cataract. Keeping this in view, in the present study, fatty acid profiling from different age groups of lenses was carried out, using a freshwater catfish as the model. Total lipids were extracted from lenses of three different age groups of fishes (young, adult, and aged) and fatty acid methyl esters (FAME) were prepared and FAME analysis was carried out using gas chromatography–mass spectrometry. The results showed that three fatty acids viz. heneicosylic acid (C21), docosahexaenoic acid (C22:6), nervonic acid (C24:1) which were not present in the adult lens, appeared in the aged lens. On the other hand, eicosenoic acid (C20:1) present in the adult lens was found to be absent in the aged lens. The appearance or disappearance of these fatty acids can possibly serve as biomarkers of aging lens which is the most vulnerable stage for cataract development.

Keywords Lipid biomarkers · Eye lens · Aging · GC–MS · Lipid profiling · Lipidomics

Introduction

Biomarkers are key molecular or cellular events that link a specific pathophysiology, environmental exposure, or disease to a health outcome. Clinically useful biomarkers are required to inform regulatory and therapeutic decision making regarding candidate drugs

B. P. Mohanty (✉) · S. Bhattacharjee · P. Paria · A. Mahanty · A. P. Sharma
Biochemistry Laboratory, ICAR- Central Inland Fisheries Research Institute, Barrackpore, Kolkata 700120, India
e-mail: bimalmohanty12@rediffmail.com

Published online: 21 November 2012

and their indications in order to help bring new medicine to the right patients faster than they are today [1]. Much progress has been made in identifying and validating new biomarkers that can be used in early detection of various diseases. Biomarkers can be proteins, lipids, metabolic enzymes, or other such biomolecules. The omics technologies (genomics, proteomics, and metabolomics) have provided the right platform for biomarkers discovery research. Recently, developed and emerging proteomics technologies have significant potential implications for human and ecological risk assessment issues. The use of proteomics is gaining importance in industry for biomarkers and drug discovery and as an application in clinical diagnosis by monitoring pattern of protein changes or protein expression signature. Numerous genomic, proteomic, and phosphoproteomic studies have focused on discovering gene expression signatures and protein signaling pathways associated with exposure diseases. Disease development, in turn, is a complex process involving many factors including intrinsic physiological changes and harmful environmental exposure and the causes can be related to genetic abnormalities, infections, aging, exposure to toxicants, etc. [2]. The advancement in modern molecular techniques have shed lights on the mechanism of disease development and some of the key genes, proteins, pathways have been identified and these molecules are serving as the targets or biomarkers for modern drug development.

Like the "protein expression signature", signature lipid biomarker analysis has been a newer useful tool for lipid biomarker discovery [3]. Lipids are a diverse group of molecules which are hydrophobic or amphiphilic in nature; the diverse nature of lipid structure and their properties enable these compounds to fulfill many biological functions from energy storage through membrane structure to signaling intermediates [4]. Lipidomics technology, the systematic study of the lipidome, is a lipid-targeted metabolomics approach aiming at comprehensive analysis of lipids in biological systems [5] and often focuses on either the identification or validation of lipid biomarkers or the characterization of lipid metabolism with a view to understanding the system/disease under investigation. Lipids are important structural components of cell membrane; most of the lipids have a profound effect on membrane fluidity. Lipid profiling has captured increased attention due to the well-recognized roles of lipids in numerous human diseases to which lipid-associated disorders contribute, such as diabetes, obesity, and Alzheimer's disease [5]. There are several classes of lipids and each has specific biological functions. As we move into the lipidomics era, the potential to accurately and rapidly measure hundreds of individual lipid species provides the opportunity to use more complex lipid profiles as biomarkers of chronic disease.

Among the different groups of lipid molecules, fatty acids have great value and useful properties to be biomarkers. Fatty acids, the long chain organic acids having from four to 24 carbon atoms, are building-block components of most of the lipids. They play the leading role in the construction and maintenance of all healthy cells, more importantly the cell membrane. They serve as the cell's gatekeeper, operating the sodium–potassium pump that regulates the opening and closing of the metabolic pathways. Through a chemical transformation, they become prostaglandins that protect the body against unhealthy outside agents. The fatty acids are grouped into saturated and unsaturated fatty acids, based on number of double bonds in them. The group consists of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). PUFAs include the essential fatty acids, viz. α-linolenic acid (18:3, *n*-3; ALA) and linoleic acid (*n*-6 fatty acid) which are important for human health. They form the starting point for the creation of long-chain polyunsaturated fatty acids like the eicosapentaenoic acid (20:5, *n*-3; EPA) and the docosahexaenoic acid (22:6, *n*-3; DHA). EPA and DHA are known to reduce the risk of coronary heart diseases, protect against diabetes mellitus and exhibit anti-inflammatory action, and also associated with brain development, vision, and reproduction. They are important in treatment of atherosclerosis,

cancer, rheumatoid arthritis, psoriasis, and diseases of old age such as Alzheimer's and age-related macular degeneration.

Cataract, the opacification of the eye lens, is a leading cause of human blindness. About 17 million cases of worldwide per year and 28,000 new cases are reported daily worldwide [6]. Presently, surgical intervention is the only approach to manage cataract. Understanding the molecular mechanism for cataract formation could be useful for designing alternative treatment or for designing drugs that may delay the onset of the process. Most investigations concerning the causes of cataracts focus on the changes in lens proteins (crystallins) [7, 8] since cataract is a disease of protein aggregation [9]; however, since protein stability is maintained by protein–protein, protein–lipid, and other such interactions, alterations in lipid composition of lens could also be a contributing or predisposing factor for development of cataract.

In this back drop, in the present study, we have analyzed the age-related changes in fatty acid composition of eye lens with the objective of identifying biomarkers that are associated with lens aging. *Rita rita*, a freshwater catfish [10–12], has been used as the model fish.

Materials and Methods

Collection of Lens

Live fishes *Rita* were collected from their natural riverine habitat (river Ganga) and were divided into three groups on basis of body weight, viz: group I, 50–100 g; group II, 400–500 g; and group III, 900–1,200 g (N=30, for each group). Lenses were dissected out from euthanized (in 0.2 mM tricaine solution) fishes and were cleaned using ice cold phosphate buffer saline. All lenses appeared to be transparent.

Lipid Extraction and Preparation of Fatty Acid Methyl Esters

Lipid extraction was carried out as per Folch et al. [13]. In brief, frozen lenses were homogenized (using a motor pestle) in the organic solvent mixture (chloroform–methanol, 2:1), keeping the solvent/tissue ratio 20:1, and washed by centrifugation. Washing was repeated five times with fresh solvent mixture. The chloroform fractions, enriched with lipids, were collected, pooled, and dried in a rotary evaporator. The dried lipids were weighed, dissolved in chloroform, and stored in small amber glass laboratory bottles at −20 °C. Fatty acid methyl esters (FAME) were prepared from the extracted fat as per Matecalfe, Schmitz, and Petha [14].

Fatty Acid Analysis by GC–MS

The gas chromatography–mass spectrometry (GC–MS) analyses of the fatty acids were carried out using a GC (Trace GC Ultra, Thermo Scientific) equipped with a capillary column (TR-FAME, 30 m × 0.25 mm, 0.25 μm film thickness) and a MS (ITQ 900, Thermo Scientific) attached to it. For separation of saturated fatty acids, the oven temperature program was set as follows: 1 min initial hold at 50 °C, temperature rise from 50 to 220 °C at the heating rate of 20 °C/min followed by a hold of 5 min at 220 °C, temperature rise from 220 to 250 °C at the heating rate of 10 °C/min, and a final hold of 10 min at 250 °C. For separation of unsaturated fatty acids, the oven temperature program was as follows; 1 min initial hold at 80 °C, temperature rise from 80 to 150 °C at the heating rate of 20 °C/min followed by a hold of 15 min at 150 °C, 150–240 °C at the heating rate of 10 °C/min, and a final hold of 2 min

at 240 °C. Helium was used as a carrier gas with column flow of 1.0 ml/min. The MS conditions were as follows; ionization voltage 70 eV, mass range of 45–600 and the scan time equal to the GC run time. The fatty acids were identified by comparison of their retention times with those of standards (Supelco ME14-1KT unsaturated and ME19-1KT saturated) and by using the NIST Library (version 2.0, 2008).

Statistical Analysis

Statistical analysis was carried out using SPSS (16.0) software. Results were expressed as mean value±standard deviation. The differences between the mean values of three size groups were calculated using one-way analysis of variance and statistically significant differences were reported at $p<0.05$.

Results

Lipids and lipid-soluble compounds are essential constituents of the cells and tissues that comprise the eye, and defects in their synthesis, intracellular and extracellular transport, and turnover underlie variety of significant, common, and often severely debilitating eye disease [15].

The fatty acid composition analysis of *Rita* lens showed the presence of saturated (6:0. 7:0, 8:0, 9:0, 10:0, 11:0, 12:0,13:0, 14:0, 15:0, 16:0, 18:0, 20:0, 21:0, and 23:0), monounsaturated (14:1,16:1, 18:1, 20:1, and 24:1) and polyunsaturated (18:2 ω6, 20:5 ω3, 22:6 ω3) fatty acids. All fatty acids detected by GC–MS in lenses from the different age groups of *R. rita* were identified and quantified and the full data set is presented (Table 1). The most abundant fatty acids identified in all three groups were EPA (C20:5), followed by palmitic acid (C16) and stearic acid (C18).

Changes in Fatty Acid Composition of Eye Lenses of Different Age Groups of *R. rita*

The relative contents of saturated fatty acid (SFA) remained constant with increase in age. The major SFA in the *Rita* lens were palmitic acid (C16) and stearic acid (C18). Interestingly, it was observed that one SFA, pelargonic acid (C9) with the molecular weight 158 mass unit was present only in lower age group and another SFA species heneicosanoic acid (C21), the molecular weight 326 mass unit was present only in upper age group of eye lens. Thus, these fatty acyl groups were unique to the specific age group of Rita lens. In the present study, PUFA contributed to the majority of unsaturated fatty acid content in rita lens. The relative abundance of PUFAs in lower, middle and upper age groups was 44.50, 48.32, and 37.13 % of the total fatty acid contents, respectively. The major PUFA in the three age groups of Rita lens was EPA (20:5). The amounts of EPA in the three age groups of lenses were 44.14±0.12, 46.19±0.17, 30.71±0.14, respectively ,in terms of total area percentage of the chromatogram. Docosahexanoic acid (22:6) with the molecular weight 328 mass unit was present only in the upper age group lens.

The relative content of MUFA in the upper age group of fish lens was significantly high as compared to lower and middle age group fish lens (Fig. 1). The MUFA content in lower, middle, and upper age group were 4.08, 4.07, and 12.48 % of the total fatty acid contents, respectively. The major MUFAs in the *R. rita* lens was myristoleic acid (14:1) which was present in higher concentration in the young and aged fish lenses. Eicosenoic acid (20:1) with the molecular weight 326 mass unit which belongs to MUFA group was present only in

Table 1 Fatty acids detected in the lens extracts of *R. rita* (expressed as total area percentage)

Fatty acids	Relative percentages of the fatty acids detected in petroleum-ether extracts of *R. rita* lens			
	Middle age group, MAG (adult)	Upper age group, UAG (aged)	Lower age group, LAG (young)	Remarks
Saturated fatty acids (SFAs)				
C6 (Caproic acid)	0.15±0.02 a	0.09±0.008 b	0.14±0.007 c	
C7 (Enathicacid)	0.10±0.02 a	0.13±0.008 b	0.07±0.01 c	
C8 (Caprylic acid)	0.81±0.03 a	0.48±0.008 b	1.27±0.04 c	
C9 (Pelargonic acid)	nd	nd	0.08±0.02	
C10 (Capric acid)	0.12±0.03 a	0.21±0.01 b	0.29±0.03 b	
C11 (Undecylic acid)	nd	0.09±0.008 a	0.19±0.03 b	
C12 (Lauric acid)	0.77±0.12 a	0.63±0.008 b	0.65±0.04 b	
C13 (Tridecylic acid)	1.31±0.13 a	1.19±0.01 a	1.29±0.07 a	
C14 (Myristic acid)	3.24±0.274 a	5.68±0.14 b	2.37±0.05 c	Significantly high in UAG
C15 (Pentadecylic acid)	nd	2.17±0.03	1.28±0.03	
C16 (Palmitic acid)	22.53±0.44 a	15.37±0.45 b	15.74±0.23 b	
C18 (Stearic acid)	15.29±0.180 a	19.27±0.19 b	20.37±0.07 c	
C20 (Arachidic acid)	1.67±0.14 a	1.31±0.05 b	1.34±0.045 b	
C21 (Heneicosylic acid)	nd	1.32±0.03	nd	Present in UAG only
C22 (Behenic acid)	1.06±0.219 a	0.32±0.24 b	1.57±0.03 c	
C23 (Tricosylic acid)	4.8±0.14 a	1.47±0.01 b	3.21±0.05 c	
Monounsaturated fatty acids (MUFAs)				
Myristoleic acid 14:1	0.69±0.09 a	9.27±0.10 b	2.67±0.03 c	Significantly high in UAG
Palmitoleic acid 16:1	0.66±0.01 a	0.47±0.02 b	0.32±0.01 c	
Oleic acid 18:1	nd	0.33±0.03 a	0.16±0.02 b	
Vaccenic acid 18:1	1.32±0.165 a	1.19±0.04 b	0.46±0.03 c	
Elaidic acid 18:1	1.40±0.08 a	1.22±0.04 b	0.47±0.008 c	
Eicosenoic acid 20:1	1.29±0.065	nd	nd	Present in MAG only
Nervonic acid 24:1	nd	2.31±0.14	nd	Present in UAG only
Polyunsaturated fatty acids (PUFAs)				
Linoleic acid 18:2	0.41±0.049 a	0.17±0.01 b	0.28±0.02 c	
Linolelaidic acid 18:2	0.22±0.02 a	0.07±0.01 b	0.081±0.007 b	
EPA 20:5	46.19±0.17 a	30.71±0.14 b	44.14±0.12 c	
DHA 22:6	nd	3.87±0.06	nd	Present in UAG only

Values are presented as mean±standard deviation

Lowercase letters (a, b, c) indicate the groups in which the values are significantly ($p<0.05$) different

nd not detected, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

middle age group lens; another MUFA species, nervonic acid (24:1) was present only in upper age group lens.

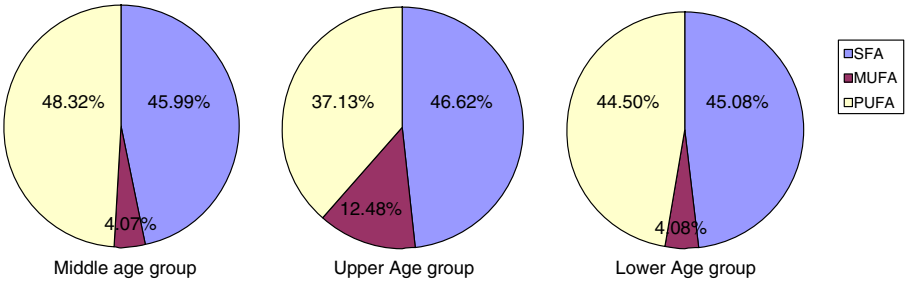

Fig. 1 Relative abundance of SFA, MUFA, and PUFA in eye lens of three different age groups of *R. rita*

Biomarkers of Aging Lens

In the present study, we observed appearance of three fatty acids viz. docosahexanoic acid DHA (C22:6), nervonic acid (24:1), and heneicosylic acid (C21) and absence of one fatty acid, i.e., eicosenoic acid (20:1) in the upper age group lens which can be used as biomarkers of lens aging (Fig. 2, Table 2).

Discussion

In the present study, it was observed that PUFAs contributed to the majority of unsaturated fatty acid content. The major PUFA detected was EPA that acts as a precursor for prostaglandin 3 (which inhibits platelet aggregation), thromboxane 3, and leukotriene 5 groups (all eicosanoids). It has been reported that in the Antarctic tooth fish (*Dissostichus mawsoni*) lens PUFAs

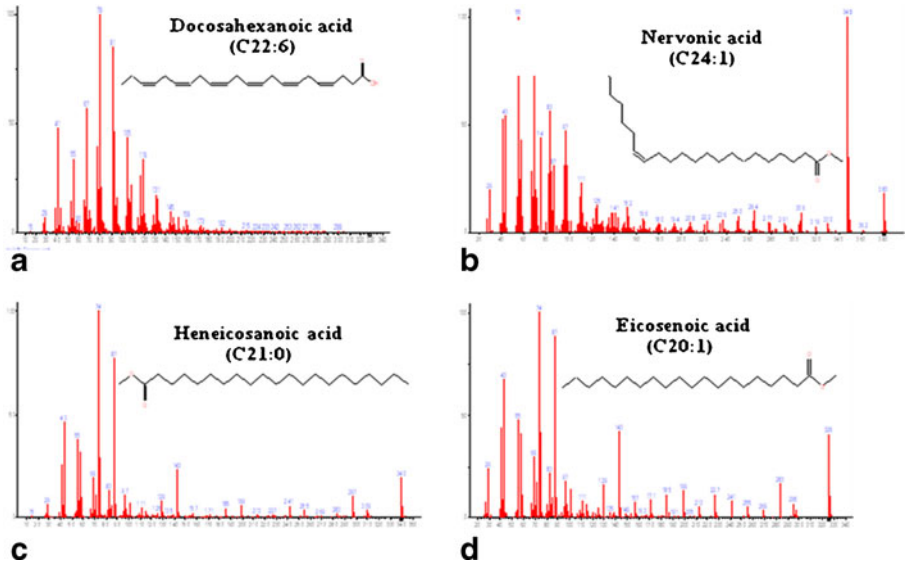

Fig. 2 GC–MS spectra of lipid biomarkers of aging lens. Three fatty acids viz. docosahexanoic acid (**a**), methyl nervonate (nervonic acid) (**b**), and heneicosanoic acid (**c**) were detected exclusively in the lens of fish of upper age group. Eicosenoic acid methyl ester (**d**), was present in lens of middle age group (adult) lens only and was absent in the aging lens (upper age group)

Table 2 Status of lipid biomarkers

Lipid biomarkers	Status (presence/absence in aging lens)	Formula/MW	References
Heneicosylic acid (C21:0)	+	$C_{21}H_{42}O_2$/326	http://www.chemicalbook.com/ChemicalProductProperty_EN_CB8686602.htm
Docosahexaenoic acid (C22:6)	+	$C_{22}H_{32}O_2$/328	NIST Library 2.0
Nervonic acid (C24:1)	+	$C_{24}H_{46}O_2$/366	http://www.chemicalbook.com/ChemicalProductProperty_EN_CB5304545.htm
Eicosenoic acid (C20:1)	−	$C_{20}H_{38}O_2$/310	NIST Library 2.0

+ present, − absent

also contributed to the majority of unsaturated fatty acid [16]. Consistent with adaptation to a low temperature environment, the fish lens fractions possessed higher degrees of unsaturation or short chain fatty acid compared with the mammalian species. In concert with the current findings, the most abundant SFA in *Rita* lens were palmitic acid and stearic acid. It has been reported that human lens also contain largely palmitic acid and a monounsaturated 24-carbon chain fatty acid, while in bovine and rabbit lenses a monounsaturated 18-carbon chain fatty acid was predominated [17]. Saturated fats are needed for energy, hormone production, cellular membranes, and for organ padding. Certain saturated fatty acids are also needed for important signaling and stabilization processes in the body. The saturated fatty acids that play important roles in these processes are the 16-carbon palmitic acid, the 14-carbon myristic acid, and the 12-carbon lauric acid. When these important saturated fatty acids are not readily available, certain growth factors in the cells and organs are not be properly aligned. This is because the various receptors, such as G-protein receptors, need to be coupled with lipids in order to provide localization of function. As a lens fiber cell matures, it loses all its organelles and becomes a large cell packed full of crystallins. Therefore, fiber cell plasma membrane is the sole source of lens lipids and they represent the fatty acids analyzed herein. Stearic acid is one of the abundant saturated fatty acid in fish lens with an 18 carbon chain. Stearic acid is produced from carbohydrates via the fatty acid synthesis machinery via acetyl-CoA.

Lipid and fatty acid analyses are important from the biomedical perspectives, as lipid oxidation has been used as marker to infer lens damage, crystallin aggregate formation, and cataract in both fish and man [16]. It has been reported that the lens growth is accompanied by the increase of protein and lipid content. The lipid mass increases predominantly at the expense of phospholipids and cholesterol and the age-related increase of cholesterol/phospholipid coefficient suggest increase membrane rigidity [18]. Human lenses are characterized by the predominance of saturated and monoenic acids, while the proportion of polyenic acids amounts to 2 % only [19]. Changes in lipid composition might affect the properties of lens fiber membranes crucial for lens homeostasis [20].

Biomarker is in general a substance used as an indicator of a biological state which indicates a change in expression or state of a biologically active molecule that correlates with the risk or progression of a disease. In the present study, we identified presence (appearance) of three fatty acids viz. docosahexanoic acid DHA (C22:6), nervonic acid (24:1), and heneicosanoic acid (C21) and absence of one fatty acid, i.e., eicosenoic acid (20:1) in the upper age group lens which can be used as biomarkers of lens aging (Fig. 2). Specific fatty

acids, especially phospholipids, have useful properties to be biomarkers. DHA is an omega-3 fatty acid. The role of DHA may be related to its biophysical effects on the cell membrane. DHA influences the biophysical properties of membranes via its high polyunsaturation and may help to create a membrane that accommodates the dynamic behavior of rhodopsin during the photoreceptive process [21]. In addition, DHA is known to modulate the activity of membrane-bound enzymes and receptors, and the kinetics of membrane transport systems, as well as being a precursor for the synthesis of other biologically active molecules. DHA is good for the eyes and is helpful in recovery from certain visual dysfunctions. DHA has a positive effect on diseases such as hypertension, arthritis, atherosclerosis, depression, diabetes mellitus, myocardial infarction, thrombosis, heart disease, and some cancers. It is also important for the brain during aging [22]. A series of studies over the past several years, have shown that the retinal pigment epithelium (RPE) synthesizes NPDI from DHA, the most prevalent fatty acid present in photoreceptor outer segment membranes. This fatty acid may not only protect RPE cells from oxidative stress but also is thought to bolster survival of the photoreceptor cells.

Nervonic acid is a monounsaturated omega-9 fatty acid which helps to maintain brain health by assisting in the biosynthesis and maintenance of nerve cell. It serves as an electrical insulator that speeds up the conduction of nerve impulses. It is found in the sphingolipids of white matter in human brain. Nervonic acid has been identified as important in the biosynthesis of nerve cell myelin. Therapeutically, nervonic acid is suggested to improve brain functions (e.g., memory) defer caducity, put off tiredness, prevent demyelination, and keep low fat in the blood. It has been reported that the eicosenoic acid and its salt have potential medical use for treating diabetes and improving lipid metabolism. The role of heneicosanoic acid in the eye lens is not well understood.

As we move into the lipidomics era, the potential to accurately and rapidly measure hundreds of individual lipid species provides the opportunity to use more complex lipid profiles as biomarkers of chronic disease. Importantly, many of the risk factors contributing to disease are likely to also influence lipid metabolism and consequently will be reflected in the lipid profiles of an individual [4].

In the present study, three fatty acids, namely heneicosanoic acid, DHA, methyl nervonate (nervonic acid) appeared exclusively in the lens of upper age group. The function of heneicosanoic acid is not well understood. Therefore, it is difficult to predict how the presence or absence of this fatty acid impacts the structure and function of the lens. These three fatty acids have some beneficial health effects like DHA protects retinal pigment epithelium cells from oxidative stress. DHA is good for the eyes and is helpful in recovery from certain visual dysfunctions. Nervonic acid is an essential fatty acid which helps to maintain brain health by assisting in the biosynthesis and maintenance of nerve cell. Eicosenoic acid and its salts have potential medical use for treating diabetes and improving lipid metabolism. Therefore appearance (or presence) of these three fatty acids may give protection to the aged lens and make them less susceptible to any lens pathology. On the other hand, disappearance of eicosenoic acid methyl ester in upper age group can be harmful to the lens. On basis of the observations, it can be suggested that these fatty acids collectively or individually can act as biomarkers of aging lens. Since these fatty acids are present in the aging lens and three of them are known to have beneficial role, perhaps the ophthalmic preparations containing such substances could be good for keeping the lens healthy; however, it is necessary to check whether these fatty acids are also present in aging human lens since there are species variations in lens lipid composition [23].

Acknowledgments This work was supported by the Indian Council of Agricultural Research under CIFRI Core Project No. FHE/ER/07/06/005 to BPM. SB is thankful to the Department of Biotechnology, Govt. of India for financial assistance under the DBT Post Doctoral Fellowship. PP is a NFBSFARA-ICAR Senior Research Fellow. AM is a Outreach Activity#3, Fisheries Division - ICAR Research Fellow.

References

1. Frank, R., & Richard, H. (2003). Clinical biomarkers in drug discovery and development. *Nature Reviews, 2*, 566–580.
2. Yu, L.-R. (2011). Pharmacoproteomics and toxicoproteomics: the field of dreams. *Journal of Proteomics, 74*, 2549–2553.
3. Piotrowska-Seget, Z., & Mrozik, A. (2003). Signature lipid biomarker (SLB) analysis in determining changes in community structure of soil microorganisms. *Polish Journal of Environmental Studies, 12*, 669–675.
4. Meikle, P., Barlow, C., & Weir, J. (2009). Lipidomics and lipid biomarker discovery. *Australian Biochemistry, 40*, 12–16.
5. Hu, C., van der Heijden, R., Wang, M., van der Greef, J., Hankemeier, T., & Xu, G. (2009). Analytical strategies in lipidomics and applications in disease biomarker discovery. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences, 877*, 2836–2846.
6. Kupfer, C., Underwood, B., & Gillen, T. (1994). Leading causes of visual impairment worldwide. In D. M. Albert & F. A. Jakobiec (Eds.), *Principles and practice of ophthalmology* (pp. 1249–1255). Philadelphia: Saunders.
7. Bloemendal, H., De Jong, W., Jaenicke, R., Lubsen, N. H., Slingsby, C., & Tardieu, A. (2004). Ageing and vision: structure, stability and function of lens crystallins. *Progress in Biophysics and Molecular Biology, 86*, 407–485.
8. Sharma, K. K., & Santhoshkumar, P. (2009). Lens aging: effects of crystallins. *Biochimica et Biophysica Acta, 1790*, 1095–1108.
9. Wang, S. S. S., Wu, J. W., Yamamoto, S., & Liu, H. S. (2008). Diseases of protein aggregation and the hunt for potential pharmacological agents. *Journal of Biotechnology, 3*, 165–192.
10. Mohanty, B. P., Bhattacharjee, S., Mondal, K., & Das, M. K. (2010). HSP70 expression profiles in white muscles of riverine catfish *Rita rita* show promise as biomarker for pollution monitoring in tropical rivers. *National Academy Science Letters, 33*, 177–182.
11. Mohanty, B. P., Bhattacharjee, S., & Das, M. K. (2011). Lens proteome map and α-crystallin profile of the catfish *Rita rita*. *Indian Journal of Biochemistry & Biophysics, 48*, 35–41.
12. Bhattacharjee, S., Mohanty, S., Sharma, A. P., & Mohanty, B. P. (2011). Effect of storage temperature as a preanalytical variable on the lens crystallins protein quality for proteomic studies. *Proteomics Clinical Applications, 5*, 504–512.
13. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.
14. Matcalfe, L. D., Schmitz, A. A., & Petha, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry, 38*, 514–515.
15. Fliesler, S. J. (2010). Lipids and lipid metabolism in the eye. *Journal of Lipid Research, 51*, 1–3.
16. Kiss, A. J., Devries, A. L., & Kiss, M. M. (2010). Comparative analysis of crystallins and lipids from the lens of Antarctic tooth fish and cow. *Journal of Comparative Physiology. B, 180*, 1019–1032.
17. Rosenfield, L., & Spector, A. (1981). Changes in lipid distribution in the human lenses with the development cataract. *Experimental Eye Research, 33*, 641–650.
18. Toivonen, L. V., Sidorov, V. S., Nefedova, Z. A., & Yurovitskii, Y. G. (2003). Age related features of cataractogenesis in salmon fry. I. Lipid composition of lens during normal development. *Russian Journal of Developmental Biology, 35*, 49–56.
19. Rosenfeld, L., & Spector, A. (1982). Comparison of polyunsaturated fatty acid levels in normal and mature cataractous human lenses. *Experimental Eye Research, 35*, 69–75.
20. Zelenka, P. S. (1984). Lens lipids. *Current Eye Research, 3*, 1337–1359.
21. Gibson, N. J., & Brown, M. F. (1993). Lipid head group and acyl chain composition modulate the MI-MII equilibrium of rhodopsin in recombinant membranes. *Biochemistry, 32*, 2438–2454.
22. Horrocks, L. A., & Younk, K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research, 40*, 205–206.
23. Panz, T., Lepiarczyk, M., & Żuber, A. (2011). Comparing the contents of lipids derived from the eye lenses of various species. *Folia Histochemica et Cytobiologica, 49*, 425–430.