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Human Crystalline Lens Phospholipid Analysis With Age

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Paired human crystalline lenses (n = 21, patient ages 20–79 years) were extracted for lipids with chloroform–methanol 2:1, using the Folch method. The extracted crude lipids were analyzed at 202.4 MHz by phosphorus-31 magnetic resonance spectroscopy (^{31}P NMR). Fourteen membrane phospholipids were detected including phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylcholine plasmalogen (PC plas), phosphatidylethanolamine (PE), phosphatidylethanolamine plasmalogen, lysophosphatidylethanolamine (PA), phosphatidylglycerol (PG), lysophosphatidylglycerol, phosphatidylserine (PS), phosphatidic acid, phosphatidylinositol, sphingomyelin (SPH), and two uncharacterized phospholipids. The uncharacterized phospholipid at 0.13 was the predominant phospholipid, comprising 43.20% of the lens phospholipid profile. A decrease in mole percent of phosphorus concentrations of PE, PC plas, and PC and an increase in SPH correlated with age. The following computed indices decreased with age: PC/PG and PE/PS; PC + PE; (PC + PC plas); and PC/PS. The following computed indices increased with age: (PC + SPH)/(PE + PS), SPH/PG, (PC + SPH)/(PE + PS), LPC/PC, LPE/PE, SPH/PE, and SPH/PC. Changes in membrane phospholipids of the crystalline lens with age as detected by ^{31}P NMR can be used to fingerprint lens maturation. Invest Ophthalmol Vis Sci 32:549–555, 1991

The phospholipids are one of the principal lipid components of biologic membranes. Since membrane phospholipid composition is most likely related to membrane function and phospholipids are continually turned over, modified, synthesized, and degraded accordingly, it is reasonable to suspect that tissue age and pathology will affect tissue phospholipid profiles. Alterations in phospholipids have been implicated in lenticular aging. Lens phospholipid profiles have been reported in lenses of various ages.[1-11] Although the more recent studies were done on phospholipid fractional spots obtained by various thin-layer chromatographic techniques on lens extracts, the establishment of a method for analysis of tissue extracts using high-resolution phosphorus-31 nuclear magnetic resonance (^{31}P NMR)[12-13] permits increased resolution of phospholipid signals, detection of increased numbers of phospholipids, and a high degree of confidence level for quantitative precision. Moreover, differences in age of the crystalline lens using high-resolution ^{31}P NMR were illustrated in a preliminary study.[14] A comprehensive and methodologic review of human ocular lipids has been published.[15] The current study establishes the nature of crystalline lens phospholipid ^{31}P NMR profiles with aging and determines whether there are differences between human lenses over a wide spectrum of ages.

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Materials and Methods

Surgical

Paired human eyebank globes (n = 21; patient ages 20, 22, 27, 30, 35, 41, 42, 47, 53, 54, 55, 56, 60, 61, 62, 66, 68, 73, and 79 years) were enucleated from cadavers within 2 hr post mortem and placed in a standard moist chamber environment. Before corneal excision, slit-lamp biomicroscopic examination of each lens revealed a clear transparent tissue, except in the more aged tissues where mild nuclear sclerotic changes were detected. From each globe the cornea with scleral rim was then excised. The iris with ciliary body was then separately dissected, revealing the crystalline lens with zonular attachments. The zonules were then cut and the intact lenses in toto dissected free and immediately frozen in liquid nitrogen.

Chemical Procedures

A simple Folch extraction of the lens phospholipids was done.[15,16] Lens-pair tissue specimens frozen in

liquid nitrogen were pulverized to a fine powder with a stainless steel mortar and pestle chilled with liquid nitrogen. The homogenized tissue (average, 0.6 g/lens pair) was then added to 20 ml of chloroform–methanol 2:1 (v/v). The homogenate, with only one liquid phase was filtered into a separatory funnel and washed with 4 ml volume of 0.1 M KCl and allowed to separate thoroughly (approximately 24 hr). The chloroform phase was recovered and evaporated using a rotary evaporator at 37°C. The analytic medium for the ^{31}P NMR phospholipid analysis was identical to that previously described for NMR phospholipid analysis.[12,13] The medium consists of reagent-grade chloroform and methanol containing benzene-d_6 and dissolved cesium ethylenediamine tetraacetate (Cs-EDTA), pH 6.0. The prepared lipid was dissolved in 2.0 ml 2:1 chloroform–methanol containing benzene-d_6 and placed in a 10-mm NMR sample tube. A single phase was obtained. To this solution, 1.0 ml of the methanol-Cs-EDTA salt was added and the mixture stirred. At this time two phases were obtained, a major chloroform phase and a minor water phase. The Teflon plug (Wilmad Glass Co., Inc., Buena, NJ) of the NMR sample tube was advanced, expulsing the minor water phase from the solvent side of the NMR tube which was then removed. The sample turbine was adjusted so that the chloroform phase was detected by the NMR spectrometer's receiver coil.

^{31}P Magnetic Resonance Spectroscopy

The NMR spectrometer used in this investigation was a heteronuclear General Electric (Freemont, CA) 500NB NMR spectrometer system operating at 202.4 MHz for ^{31}P. The spectrometer was equipped with an Oxford Instruments (Oxford, England) 500/52 magnet and cryostat with an operating magnetic field of 11.75 Tesla, deuterium field-frequency stabilization, and an automatic field-homogeneity adjustment capability that adjusted the spectrometer room temperature shims to improve field homogeneity during sample acquisition (essential). Analytic samples were placed in standard 10-mm NMR sample tubes and spun at 8 Hz during the data acquisition period. Samples were analyzed with proton broad-band decoupling to eliminate ^{1}H–^{31}P NMR multiplets. Under these conditions each spectral resonance corresponds to a single phosphorus functional group, representing a single generic phospholipid species. Chemical-shift data are reported relative to the standard of 85% inorganic orthophosphoric acid;[17] however, the primary internal reference standard was a naturally occurring phospholipid derivative phosphatidylcholine (PC; chemical shift, −0.84 δ). Spectrometer conditions used for analytic extract analyses were as follows: pulse sequence, one pulse; pulse width, 18 μsec (45° spin-flip angle); acquisition delay, 83 μsec; cycling delay, 500 μsec; number of acquisitions, 12,000; number of points per free-induction decay, 4096; acquisition time, 1.36 sec; and sweep width, ± 1000 Hz. The average time per analysis was 6 hr. In addition, a computer-generated filter time constant, introducing 0.6-Hz line broadening, was applied. Data reductions, including peak area, chemical-shift measurements, and spectral curve analysis, were calculated using the spectrometer's computer. To compensate for relative saturation effects among various phosphorus signals detected in a single ^{31}P NMR spectroscopic profile, the NMR spectrum must be standardized against measured amounts of tissue-profile metabolites when these are known. The procedure for this calibration—so that an accurate quantitative measurement is obtained from the ^{31}P NMR spectral profile—has been described.[18,19] Chemical shifts follow the convention of the International Union of Pure and Applied Chemistry and are reported in field independent units of δ.

Data Analyses

Phospholipid concentrations in mean relative mole percentages of phosphorus were computed for all detected resonances in the analyzed lens pairs. From these data, 27 indices were calculated: PC plas + PE plas; PC + PE; (PC plas + PE plas)/(PC + PE); PC plas/PC; PE plas/PE; LECITHIN (PC + PC plas); CHOLINE (PC + PC plas + sphingomyelin [SPH]); NONCHOLINE (phosphatidylinositol [PI] + PE + phosphatidylserine [PS] + PE plas + phosphatidic acid [PA] + PG); LECITHIN/CEPHALIN, (PC + PC plas/PE + PE plas); OUTSIDE (PC + SPH); INSIDE (PE + PS); LEAFLET (PC + SPH)/(PE + PS); ANIONIC/NEUTRAL (PI + PS + PA + PG)/(PC + PC plas + SPH + PE + PE plas); SPH/PC; SPH/PE; SPH/PS; SPH/PG; PC/PE; PC/PS; PC/PG; PE/PS; PE/PG; PS/PG; LYSO (lysophosphatidylcholine [LPC] + lysophosphatidylethanolamine plasmalogen [LPE plas]) + lysophosphatidylglycerol [LPG]); NONLYSO (PC + PE + PG); LPC/PC; and lysophosphatidylethanolamine (LPE)/PE. These theoretic parameters, given as ratios of individual or grouped phospholipids, were generated to compare phospholipids or groups of phospholipids and provide more pathway-specific metabolic interrelations for discussion.

Individual metabolite concentrations and indices were correlated with age by computing Pearson's correlation coefficients (r). The significance for each

of the coefficients was based on a two-tailed test with the probability of rejection of a significant correlation $P < 0.05$. Data were also evaluated by discussing coefficients of determination (R^2), the square of the correlation coefficients. The R^2, a computer statistical parameter, identifies the fraction of the linear change in the dependent variable, in this case metabolite concentration or metabolic index, attributable to the correlated independent variable, in this case, age. In our study, the remaining fraction represented by ($1 - R^2$) is the fraction of the linear change not attributable to age, but to other factors such as hereditary, metabolic, environmental, and traumatic factors. An increase or decrease in the linear changes modeled by the Pearson's procedure is given by the sign of the correlation coefficient. When significance was determined, regression lines were plotted.

Results

A ^{31}P MR phospholipid profile of a 35-year-old human lens is given in Figure 1. Fourteen phospholipids detected in the spectral profiles include PC, LPC, PC plas, PE, PE plas, LPE, PG, LPG, PS, PA, PI, SPH, and two uncharacterized resonances. The relative mean mole percentage of phosphorus concentrations are given for the individual phospholipids in Table 1.

Correlation coefficients (r), correlating the relative mole percentage of phosphorus concentrations of the 20 lens pairs with age, are also given in Table 1. Of the 14 detected phospholipids, a significant negative correlation with age was noted in the mole percent of phosphorus concentrations for PC ($r = -0.678$, $P < 0.001$), PC plas ($r = -0.640$, $P < 0.005$), and PE ($r = -0.475$, $P < 0.05$). Likewise, a positive correlation with age was noted for SPH ($r = 0.637$, $P < 0.005$). For these significantly changing phospholipids, R^2, the coefficient of determination, varied from 23% for PE to 46% for PC.

Of 27 computed indices (Table 2), 12 were found to change significantly with age. The following indices decreased significantly with age: PC/PG ($r = -0.553$) and PE/PS ($r = -0.543$) ($P < 0.01$), PC + PE ($r = -0.591$) ($P < 0.005$), LECITHIN ($r = -0.790$), and PC/PS ($r = -0.644$) ($P < 0.001$). The following indices increased significantly with age: LEAFLET ($r = -0.441$), SPH/PG ($r = 0.513$), LYSO ($r = 0.465$), LPC/PC ($r = 0.464$), and LPE/PE ($r = 0.529$) ($P < 0.05$); SHP/PE ($r = 0.627$) ($P < 0.005$); SPH/PC ($r = 0.821$) ($P < 0.001$).

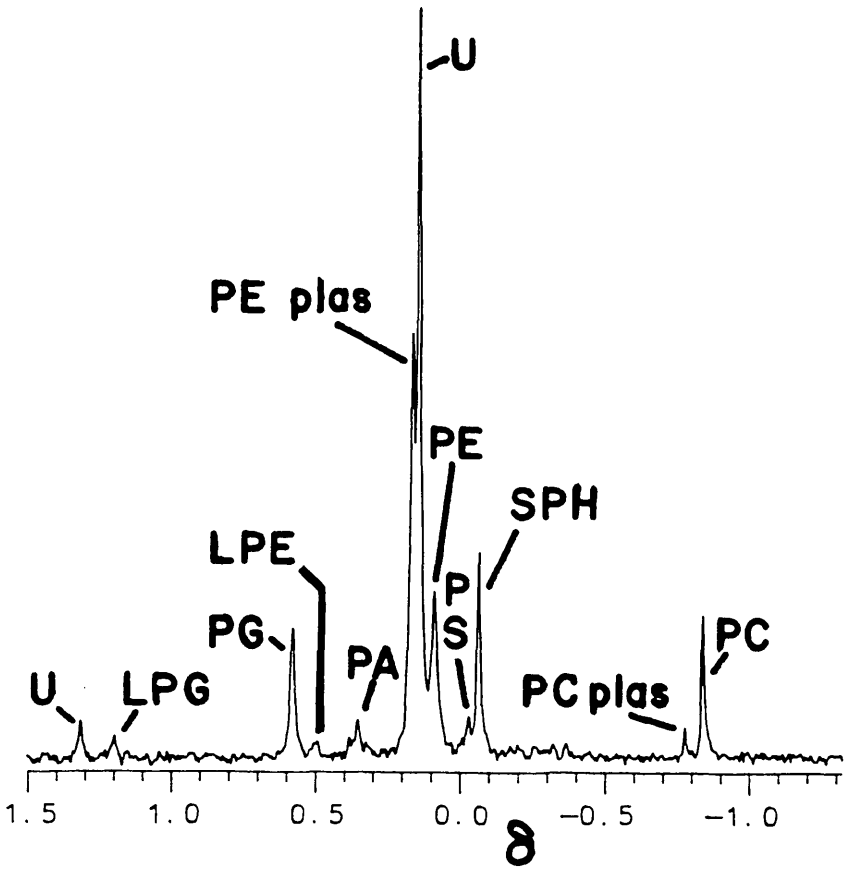

Fig. 1. ^{31}P magnetic resonance phospholipid profile of 35-year-old human crystalline lenses. The resonance signals from downfield (left) to upfield (right) are as follows: uncharacterized resonance signal (U) at 1.31 δ, lysophosphatidylglycerol (LPG) at 1.19 δ, phosphatidylglycerol (PG) at 0.57 δ, lysophosphatidylethanolamine (LPE) at 0.43 δ, phosphatidic acid (PA) at 0.34 δ, phosphatidylethanolamine plasmalogen (PE plas) at 0.15 δ, uncharacterized resonance signal (U) at 0.13 δ, phosphatidylethanolamine (PE) at 0.08δ, phosphatidylserine (PS) at −0.05 δ, sphingomyelin (SPH) at −0.08 δ, lysophosphatidylcholine at −0.31 δ, phosphatidylcholine plasmalogen (PC plas) at −0.78 δ, and phosphatidylcholine (PC) at −0.84 δ.

Table 1. ^{31}P magnetic resonance phospholipid profiles of the human crystalline lens correlated with age

*Phospholipid**	*Chemical shift (δ)*	*Mole percentage of phosphorus (±SD)*		*Pearson's r†*
PC	−0.84	2.81	0.94	−.6778‡
LPC	−0.31	1.47	0.89	.3126
PC plas	−0.78	1.14	0.49	−.6401§
PE	0.08	6.14	1.52	−.4745‖
PE plas	0.15	14.51	5.90	−.3120
LPE	0.43	0.99	0.52	.4238
PG	0.57	9.89	1.05	.1123
LPG	1.19	1.80	0.82	.2826
PS	−0.05	1.89	0.63	.3248
PA	0.34	2.64	0.78	−.2280
PI	−0.35	0.78	0.41	.1233
SPH	−0.08	9.65	1.93	.6365§
U	0.13	43.69	6.82	.2515
U	1.31	2.60	1.31	.2741

* PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PC plas, phosphatidylcholine plasmalogen; PE, phosphatidylethanolamine; PE plas, phosphatidylethanolamine plasmalogen; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; LPG, lysophosphatidylglycerol; PS, phosphatidylserine; PA, phosphatidic acid; PI, phosphatidylinositol; SPH, sphingomyelin; U, uncharacterized.
† Pearson's correlation coefficient—r.
‡ $P < 0.001$.
§ $P < 0.005$.
‖ $P < 0.05$.

For the significantly changing indices R^2, varied from 19% for leaflet to 67% for SPH/PC. Regression lines were computed for the significantly changing phospholipids. These regression lines are presented to show the linear changes attributable to age in PC, PC plas, PE, and SPH (Fig. 2). The regression line of leaflet was also computed and is presented in Figure 3.

Discussion

The ^{31}P NMR technique allows the detection and quantitation of a large number of phospholipids in a single assay. A comparison of phospholipids reported in previous studies is difficult since the most sophisticated technique used to detect and quantitate human lens phospholipids was thin-layer chromatography (TLC).[10] With increased resolution to detect and quantitate phospholipids, several problems have been encountered when attempting to compare the data with previous data on the human lens and that of other species, not the least of which is the detection of uncharacterized phospholipids in significant quantity rendering difficult, a comparison of TLC and ^{31}P NMR data. In our phospholipid profile, the majority of the resonance signals detected were assigned with certainty to known phospholipids (Fig. 1). The lens phospholipid profiles exhibit two signals that are uncharacterized, one of which is the largest phospholipid component of the lens (Table 1). The uncharacterized phospholipids do not correspond to any phospholipid commonly associated with mammalian membrane systems. The dominant uncharacterized phospholipid of the human lens at −0.13 δ was previously identified by TLC techniques as SPH.[2,3,9] By ^{31}P NMR this phospholipid is not SPH, since standard test SPH comes into resonance at −0.08 δ. Furthermore, it cannot be saponified to yield phosphodiester polar head-group fragments suggesting that it is either a phosphorylsphingosine derivative or a plasmalogen. The dominant uncharacterized phospholipid is not ethanolamine plasmalogen; however, it is ninhydrin negative and has a chemical shift (Table 1) that is distinct from that of PE plas. Full

Table 2. ^{31}P magnetic resonance phospholipid indices of the human crystalline lens correlated with age

Phospholipid index*	*Pearson's† r*
PC plas + PE plas	−.3562
PC + PE	−.5908‡
(PC plas + PE plas)/(PC + PE)	.0084
PC plas/PC	−.0245
PE plas/PE	−.0665
LECITHIN	−.7896§
CHOLINE	−.1720
NONCHOLINE	−.3525
LECITHIN/CEPHALIN	−.0835
OUTSIDE	.3279
INSIDE	−.3535
LEAFLET	.4409‖
ANIONIC/NEUTRAL	.3584
SPH/PC	.8212§
SPH/PE	.6274‡
SPH/PS	−.0464
SPH/PG	.5125‖
PC/PE	−.3948
PC/PS	−.6443§
PC/PG	−.5527¶
PE/PS	−.5429¶
PE/PG	−.3918
PS/PG	.1858
LYSO	.4646‖
NONLYSO	−.3858
LPC/PC	.4642‖
LPE/PE	.5294‖

* PC plas + PE plas, phosphatidylcholine plasmalogen + phosphatidylethanolamine plasmalogen; PC + PE, phosphatidylcholine + phosphatidylethanolamine; LECITHIN, PC + PC plas; CHOLINE PC + PC plas + SPH (sphingomyelin); NONCHOLINE, PI + PS + PE + PE plas + PA + PG (phosphatidylinositol, phosphatidylserine, phosphatidic acid, phosphatidylglycerol); LECITHIN/CEPHALIN, (PC + PC plas)/(PE + PE plas); OUTSIDE, PC + SPH; INSIDE, PE + PS; LEAFLET, (PC + SPH)/(PE + PS); ANIONIC/NEUTRAL, (PI + PS + PA + PG)/(PC + PC plas + SPH + PE + PE plas); LYSO, LPC + LPE + LPG (lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol); NONLYSO, PC + PE + PG.
† Pearson's correlation coefficient—r.
‡ $P < 0.005$.
§ $P < 0.001$.
‖ $P < 0.05$.
¶ $P < 0.01$.

characterization awaits its isolation and functional group analysis.

The earlier phospholipid human lens aging study by Broekhuyse[2] may be compared with our present study. Both studies agree with regard to the correlation with age of PC; however the studies differ with regard to the relative amount of PC detected. The earlier study[2] indicating about threefold more PC than shown in Table 1. The data of both studies agree with respect to LPC. In terms of relative amounts of PC plas detected, both studies agree. Broekhuyse,[2] however, reports an increase with age; in this study, the correlation with age was negative. Regarding the summed quantity of PE and PE plas, both studies agree. However, with respect to the relative quantity of each, there is disagreement. The data in Table 1 indicate that the greater ethanolamine phospholipid is the plasmalogen; Broekhuyse[2] assigned the greater component to PE. The correlation with age of the summed ethanolamine component is in agreement. Considering only the plasmalogen component, however, a negative correlation with age is shown in Table 1. In the earlier work,[2] a positive correlation was indicated. These differences may be attributable to inaccurate assignment of the phospholipid signals. The quantity of lyso ethanolamine detected by ^{31}P NMR is only 10% of that detected in the earlier work. In both studies PG is indicated as a minor component. By ^{31}P NMR, this phospholipid is readily resolved and quantitated. By two-dimensional TLC, it is detected as a trace contaminant of the PE spot. Lysophosphatidylglycerol was not detected by Broekhuyse. Both studies agree for both the concentration and the change in the concentration of PS with lens age. As in the case of PG, both studies indicate the presence of PA as a minor component, with the concentration of PA at about the 1% level. Again, by ^{31}P NMR, PA is readily resolved and quantitated. By two-dimensional TLC, it is detected as a trace contaminant of the unidentified component spot. Neither study detected the elevated levels reported by Feldman et al.[20] Both studies are in accord regarding PI. Again, neither study detected the elevated levels reported by Feldman et al.[20]

In the identification of SPH, the major phospholipid of the lens, the current study and the earlier work[2] are at variance. Broekhuyse identified the major component as SPH.[2] By ^{31}P NMR, the major phospholipid of the lens is not SPH, although it may

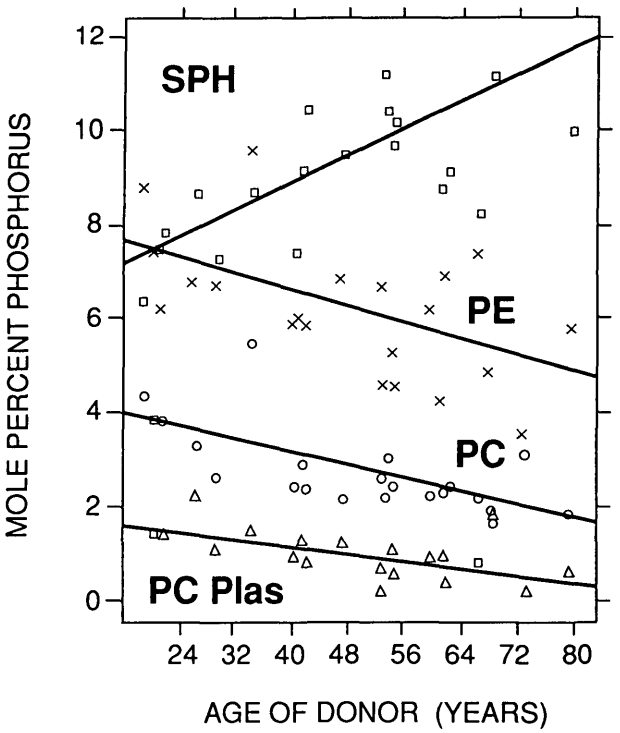

Fig. 2. Regression lines of human crystalline lens phospholipids found to change significantly with age by ^{31}P magnetic resonance spectroscopy. Significant age-dependent changes in the human crystalline lens sphingomyelin (SPH, $P < 0.005$), phosphatidylethanolamine (PE, $P < 0.001$), phosphatidylcholine (PC, $P < 0.001$), and phosphatidylcholine plasmalogen (PC Plas, $P < 0.005$) concentrations in mole percentages of phosphorus as determined by ^{31}P magnetic resonance spectroscopy. Regression line equations are given in slope (A) − intercept (B) form (y = Ax + B) as: [SPH] = 0.072 (Age) + 6.063, [PE] = −0.042 (Age) + 8.241, [PC] = −0.037 (Age) + 4.665, and [PC Plas] = −0.018 (Age) + 2.05.

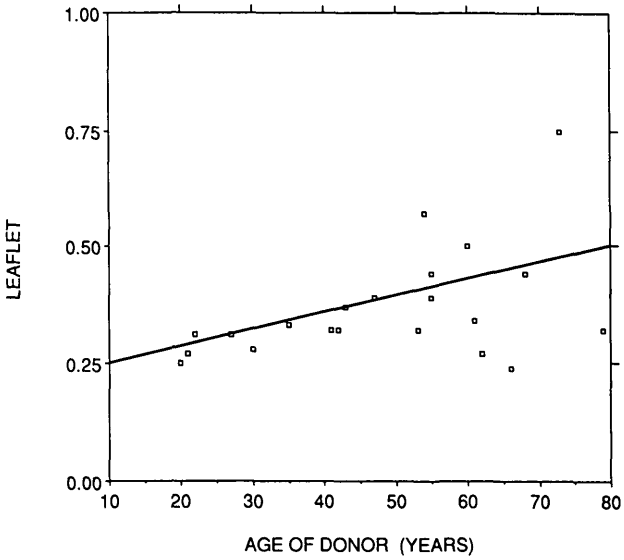

Fig. 3. Regression line of the human crystalline lens phospholipid index LEAFLET found to increase significantly with age by ^{31}P magnetic resonance spectroscopy. Significant age-dependent, linear changes in the human crystallins lens phospholipid index LEAFLET [(phosphatidylcholine + sphingomyelin)/(phosphatidylethanolamine + phosphatidylserine), $P < 0.05$] as determined by ^{31}P magnetic resonance spectroscopy. The regression line equation, in the slope (A) − intercept (B) form (y = Ax + B) is given as: LEAFLET = 0.003 (Age) + 0.217.

be an SPH derivative; SPH was detected at the 10% level.

Broekhuyse[2] detected the presence of a significant unidentified phospholipid in about the same concentration range as indicated in Table 1 for SPH. By TLC, however, this unidentified component could not be identified as a sphingosine phospholipid. Perhaps, Broekhuyse's unidentified phospholipid corresponds to the uncharacterized phospholipid of Table 1, detected at 1.31 δ. A second unidentified phospholipid detected in the earlier work[2] also may be a candidate for the uncharacterized ^{31}P NMR component. With regard to the variation in the concentration of the major human lens phospholipid with age, however, both studies agree.

Broekhuyse[2] identified cardiolipin, phosphatidylmonomethylethanolamine, and phosphatidyldimethylethanolamine as components of a two-dimensional TLC streak that also was reported to contain triglycerides. These phospholipids "were identified without the use of standards,"[2] by comparing R_f values with those reported in the literature. These phospholipids were not detected by ^{31}P NMR, although had they been present in the quantities indicated in Table II of Broekhuyse's report,[2] the cardiolipin and phosphatidyldimethylethanolamine would have been detected.

Both PC and PE concentrations decrease significantly with age. This finding is consistent with previous reports.[2,9] These changes coincide with parallel reductions in their ether analogues: PC plas and PE plas; however, only the reduction in PC plas was found to be significant. The changes in these four phospholipids are further represented by the first group of indices (Table 2, PC plas + plas + PE plas through PE plas/PE), where the decrease in the plasmalogens and their ester derivatives, while only significant for the grouped ester derivatives, is such that the balance between the enol-ether content and corresponding ester content is maintained with age.

The use of indices in this analysis and in the general interpretation of NMR spectral data is helpful because indices, representing sums and ratios of individual or grouped phospholipids, can be used to describe known biochemical processes and pathways or provide insight into unknown process or overall cellular phospholipid composition. The fact that an index cannot be interpreted in terms of a particular process should not detract from the values of the index since it may represent a process unique to the human lens or aging. Even though these indices have not been established in studies involving the human lens, they are nonetheless relevant.

It has been suggested that the more common phospholipids found in mammalian cell membranes are asymmetrically distributed between the inner and outer leaflets of the membrane.[21] In healthy tissues,[20] this asymmetry has shown PC and SPH to be the major components of the outer membrane leaflet and PE and PS, the major components of the inner membrane leaflet. Evaluation of the rate of change of the four significantly changing phospholipids (Fig. 2) shows that the rate of change of PC, −0.037 relative mean mole percent of phosphorus per year (RMPP/YR) is decreasing at nearly the same rate that PE is decreasing (−0.042 RMPP/YR). Combined, the rates of these decreasing relative phospholipid concentrations are theoretically possible. When evaluating the significant phospholipids in such a nonrandom fashion, these arguments imply that the choline in the outer leaflet is being incorporated into SPH and that the PE of the inner leaflet is also being replaced by SPH. The remaining phospholipid found to change significantly with age, PC plas, changes with a rate of (−0.018 RMPP/YR) but is found in concentrations of approximately 1% or less in the analyzed tissues. The evaluation of changes in the phospholipid composition of the human lens with age, in terms of their rates of change, suggests that changes in phospholipids with age may be compensatory. Even though the greatest rate of change in relative mole percent of phosphorus concentration with time was seen in SPH with a rate of 0.072 RMPP/YR, it was PC that underwent a change most attributable to age, $r = -0.6778$, with a coefficient of determination (R^2) of 46%.

Support for the hypothesis that changes in phospholipid composition with age in the human lens are compensatory is found by examining indices that address the asymmetry and polarity of the membrane (Table 2, LECITHIN through ANIONICS/NEUTRALS and SPH/PC through PS/PG). The most significant changes attributable to age are seen in the indices containing PC, SPH, or both. The significant decrease in PC and the significant increase in SPH, or a combination of the two, account for the significant decrease in the index that measures the total lecithin, LECITHIN, and the stability of the choline-containing phospholipid indices CHOLINE and OUTSIDE. Since PC and PE combined and SPH change at approximately the same rate (Fig. 2), the net result is that LEAFLET, the index that combines the effects of all three significantly changing phospholipids, changes significantly with age (Fig. 3). This is due primarily to the denominator that decreased with age. It should be noted that the leaflet index, INSIDE, does not significantly decrease with age.

All possible combinations of ratios of the five phospholipids: PC, SPH, PS, PE, and PG were computed to represent relationships among the principle known

membrane lipids and their replacement products. Four of these indices did not change significantly with age. Three of these indices—SPH/PS, PE/PG, and PS/PG—contain the phospholipids PS and PG that do not change with age, and the fourth—PC/PE—has a numerator and denominator with near equal rates of change.

Of the indices that represent phospholipase activity (LYSO, NONLYSO, LPC/PC, and LPE/PE), all changed significantly with age. The lysophospholipid derivatives increase with a simultaneous decrease in the parent phospholipids from which they are derived, indicating a shift in the phospholipase equilibrium toward the direction of phospholipid breakdown. It is generally assumed that with age the phospholipase equilibrium shifts so as to increase the content of human lens lysophospholipids, and this agrees with the findings of Broekhuyse[2] and with the interpretations we presented.

Key words: lens, magnetic resonance, P-31, aging, phospholipids

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