Biological Response in Various Compartments of the Rat Lens after In Vivo Exposure to UVR-B Analyzed by HR-MAS ^{1}H NMR Spectroscopy

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Purpose. The purpose of the present study was to investigate metabolic changes in different compartments of the rat lens (anterior, nuclear, posterior, and equatorial) after exposure to an acute double threshold dose of ultraviolet-B radiation (UVR-B) by using high-resolution magic angle spinning (HR-MAS) ^{1}H nuclear magnetic resonance (NMR) spectroscopy and pattern recognition (PR) methods.

Methods. One eye in each of 28 6-week-old female albino Sprague-Dawley rats was exposed to in vivo 7.5 kJ/m^2 UVR-B for 15 minutes. The contralateral eye was left unexposed. One week after irradiation, all rats were killed, and both lenses were isolated. Each lens was cored by a trephine, and the cylinder was sliced into three portions (anterior, nuclear, and posterior). The lens material that remained after the coring process was analyzed as the equatorial region. Analysis of lens metabolism was performed by HR-MAS ^{1}H NMR spectroscopy (14.1 T; Avance DRX600; Bruker BioSpin GmbH, Rheinstetten, Germany), and the metabolic profiles were statistically analyzed by the PR method of principal component analysis (PCA).

Results. Metabolic differences were detected among the compartments in the lens, both in samples from the contralateral nonexposed lenses and in samples from lenses exposed to in vivo UVR-B. In the rat lens, exposure to UVR-B caused changes in GSH, phosphocholine, *myo*-inositol, succinate, formate, and adenosine triphosphate (ATP)/adenosine diphosphate (ADP) and in levels of the amino acids phenylalanine, taurine, hypotaurine, tyrosine, alanine, valine, isoleucine, and glutamate, that varied among lens compartments.

Conclusions. HR-MAS ^{1}H NMR spectroscopy, combined with PR methods (PCA), is effective for analysis of separate parts of the intact rat lens. To understand the biochemistry of the lens, it is important to divide the lens into sections, representing functionally and anatomically distinct compartments. (*Invest Ophthalmol Vis Sci.* 2006;47:5404–5411) DOI:10.1167/iovs.05-1633

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Exposure to UVR-B (280–315 nm) is thought to be a major cause of cataract, and because of the thinning of the ozone layer, an increase in solar UVR-B is of current concern. The ozone layer prevents normally 70% to 90% of the UVR-B to reach the earth's surface, and the incoming radiation varies considerably with geographic, physical, and meteorological factors.[1] When UVR-B at 300-nm reaches the eye, the cornea, and the aqueous humor attenuates 97% of the incident radiation.[2] The remaining 3% is completely attenuated in the lens.[2] Evidence for cataractogenesis caused by the high-energy wavelengths of UVR-B is found both in animal experiments and in human studies.[3]

The effects of UVR-B on the lens differ between acute high-dose and chronic low-dose exposure.[4] Acute high-dose exposure to UVR-B causes cortical opacities[5] and induces several changes in the lens, such as alterations in enzyme activity caused by an increased amount of free radicals and inactivation of free radical scavenging systems.[6] Electrolyte transport problems[7] and DNA damage[8,9] are associated with osmotic changes in the lens, leading to cortical opacification. Chronic low-dose exposure to UVR-B affects tryptophan and may form crystalline links, which cause sclerosis and opacities in the nucleus of the lens.[4]

The pathogenic effects of UVR-B vary among lens compartments. Over the course of a lifetime, lens fiber cells differentiate to form different parts of the lens. The cortical region is histologically different from the nuclear area. The epithelial cells at the equator of the lens proliferate and transform into new fiber cells. These cells further develop as the cortical area and thereafter as the nuclear part of the lens. The nucleus consists of the oldest and deepest lens fiber cells, which have lost all organelles. Exposure to previous UVR-B, is suggested to be a reason for nuclear opacity.[5] In contrast, opacities observed in the cortical area, consisting of relatively newly developed fiber cells, might be a result of recent UVR-B exposure.[5]

In the investigation of photochemical damage of the lens, studies of metabolism are helpful in understanding the underlying processes. In previous studies of metabolism, substantial differences have been observed between different compartments of the lens.[10] In studies of the biochemical properties of the whole lens, only the average of the ingredient substances have been analyzed.[10,11] Data on the topography of biochemical alterations in the lens are limited. Separate analyses of different lenticular compartments will increase the understanding of pathophysiology in the lens.

High-resolution magic angle spinning (HR-MAS) ^{1}H nuclear magnetic resonance (NMR) spectroscopy of metabolic changes in different compartments of the intact rat lens can be investigated with quality as good as from extracts.[11] NMR-based metabonomics is a modern but well-known statistical method of handling the large amount of data from NMR spectra.[12] These methods have only recently been applied to the eye.[13–16] HR-MAS ^{1}H NMR spectroscopy has revealed considerable changes in the rat lens biochemistry after exposure to UVR-B.[11] A significant decrease was found in several amino

acids such as valine, phenylalanine, tyrosine, taurine, hypotaurine, glycine, and glutamate.[11] However, these results presented just the average of the ingredient substances in the whole lens.

In the present study, we investigated the biochemical response alterations in the various compartments of the albino rat lens after in vivo exposure to UVR-B. This is the first study to present such detailed biochemical data on intact tissue from different sections of the lens. Because of histologic differences in the lens, we hypothesized that the metabolic response of UVR-B exposure would be different between the lens compartments. A detailed biochemical analysis using ^{1}H HR-MAS NMR spectroscopy may contribute to a better understanding of the mechanisms by which UVR-B induces cataractogenesis in various parts of the lens.

METHODS

Animal Experiments and Sample Preparation

Six-week-old female outbred albino Sprague-Dawley rats (n = 28, ~150 g) were anesthetized with 11 mg/kg xylazine and 80 mg/kg ketamine, intraperitoneally. Before irradiation, 1 drop of 0.5% tropicamide was instilled in both eyes to dilate the pupils. One eye of each animal was exposed to UVR-B and the contralateral eye served as a nonexposed control. The UVR-B dose was 7.5 kJ/m^2, measured in the corneal plane, and the exposure time was 15 minutes. The UVR-B source was a 350-W high-pressure Hg lamp (Oriel Instruments, Stratford, CT) equipped with water filter, dual monochromator set to λ_{MAX} at 300 nm and full bandwidth at half maximum (FWHM) 10 nm, and collimating optics. The irradiance was measured with a thermopile calibrated by the Swedish National Bureau of Standards. The maximum intensity was at 300.1 nm, and true FWHM was 9.5 nm. All animals were kept and treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

One week after exposure, the rats were killed by CO_2 asphyxiation, the eyes were enucleated, and the lenses were placed in physiologic saline (BSS; Alcon, Stockholm, Sweden) and dissected from the remnants of ciliary body and zonular fibers. The lenses were stored at −20°C for 30 to 60 minutes before compartmentalization of the frozen lenses. A Bonn Sectioning Device (Department of Experimental Ophthalmology, University of Bonn, Germany)[17] was cooled by circulating methanol from a water bath kept at −5°C. All accessories used for the compartmentalization was cooled by submersion in liquid nitrogen. Each rat lens was cored with a trephine (2.5 mm bore) and sliced into three regions. The lens core was divided into a 1-mm anterior portion and a 1-mm nuclear portion, and the remaining posterior portion had a thickness varying from 0.7 to 1.5 mm. The lens material left after the coring process was analyzed as the equatorial region. Samples for NMR analysis were made from 28 exposed lenses and 28 control lenses, where each NMR sample consisted of pooled equivalent parts from 4 different lenses. The average weight (± SD) of the pooled samples were 22.0 ± 2.9 (anterior), 22.6 ± 1.7 (nucleus), 19.3 ± 2.8 (posterior), and 19.6 ± 3.5 (equatorial) mg. Two NMR samples were discarded because of destruction in the laboratory. Immediately after sectioning, the pooled lens fractions were stored at −80°C.

HR-MAS ^{1}H NMR

HR-MAS ^{1}H NMR spectroscopy was performed (Avance DRX600 14.1-T spectrometer; Bruker BioSpin GmbH, Rheinstetten, Germany) operating at 600.132 MHz for protons. The spectra were recorded at 4°C, with a 4-mm ^{1}H/^{13}C MAS probe. The samples were immersed in deuterium oxide (D_2O) containing 1 mM TSP (sodium-3′-trimethylsilylpropionate-2,2,3,3-d_4) in a zirconium MAS rotor (50 μL, 4 mm diameter). The samples were spun at a rate of 5000 Hz. To suppress signals from lipids and macromolecules, the ^{1}H spectra were obtained by using a T_2 filter and the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence: $[90° - (\tau\text{-}180° - \tau)_n - \text{acquisition}]$.[18] The T_2 filter contained delays of 500 μs (τ) and 72 loops (n), giving an effective echo time of 72 ms ($2n\tau$ = 72 ms). The acquisition time was 2.28 seconds, and 512 transients were collected, using a 7.2-kHz spectral region of 32K data points. An exponential line-broadening of 0.30 Hz was applied to the raw data before Fourier transformation. Water suppression was achieved with a selective presaturation pulse. Homonuclear correlated spectra (^{1}H ^{1}H-COSY) and J-resolved spectra were recorded, to assign complicated coupling patterns. Peak assignments were performed according to previous reports.[11,16,19–22]

Multivariate Analysis

NMR spectra were statistically analyzed by principal component analysis (PCA; The Unscrambler ver. 7.01; CAMO, Trondheim, Norway). PCA is a common statistical technique for finding patterns in data with multiple response variables. It reduces dimensionality and produces new uncorrelated variables (principal components), describing the amount of variation in the data set. A selected region (0.65–4.7 ppm) from the HR-MAS ^{1}H NMR spectra (54 samples), was used as input for PCA. This area included the most abundant metabolites in a single spectrum of the rat lens. The dominating resonances of phosphocholine (3.16−3.21, 3.47−3.51 and 4.02− 4.07 ppm) were removed from the selected regions. In this way, potential differences between the compounds of lower intensities were easier to reveal. The low-field region (above 5 ppm) had a lower signal-to-noise ratio and was not included in the analysis. The results are based on a matrix consisting of 54 samples × 25,380 variables. PCA was performed with full cross-validation, indicating that the same samples were used for both calibration and validation. The number of principal components (PCs) included in the cross-validation analysis was determined by the explained variance. The number of PCs that gave the minimal total residual variance was considered to be the required number of PCs. To find group divisions and reveal the relationship between samples, the score plot from the various PCs was interpreted.

Additional Data Analysis

Individual compounds from NMR spectra of exposed tissue were compared with normal tissue. Metabolite content is given in *institutional units*, calculated from the NMR peak integral (from raw output data) divided by sample wet weight. Statistical analysis was performed by t-test; the level of significance was set to $P < 0.05$ and the confidence level to 95%.

RESULTS

Lens Macroscopic Appearance

Cortical opacification was observed in all the excised rat lenses from UVR-B exposed eyes. All the contralateral nonexposed lenses had a clear appearance.

HR-MAS ^{1}H NMR Spectroscopy

A characteristic HR-MAS ^{1}H NMR spectrum of the anterior portion of a nonexposed rat lens is shown in Figure 1. Twenty-six different metabolites from different groups of compounds were assigned in the spectra. Of these, 16 different metabolites were judged to provide a sufficient signal to be analyzed quantitatively.

Principal Component Analysis

PCA demonstrated variation among the different compartments in nonexposed and in exposed rat lenses, as seen from the scores of the first principal component (PC1; Fig. 2). The second principal component (PC2) showed a clear separation between UVR-B−exposed samples (filled symbols) and the unexposed samples (open symbols). PC1 and PC2 explained 79% of the total variation in the NMR spectra. The samples from the nucleus had the highest score for PC1, and the equatorial parts showed the lowest score for PC1 (Fig. 2). The samples from the nucleus and the equator were therefore

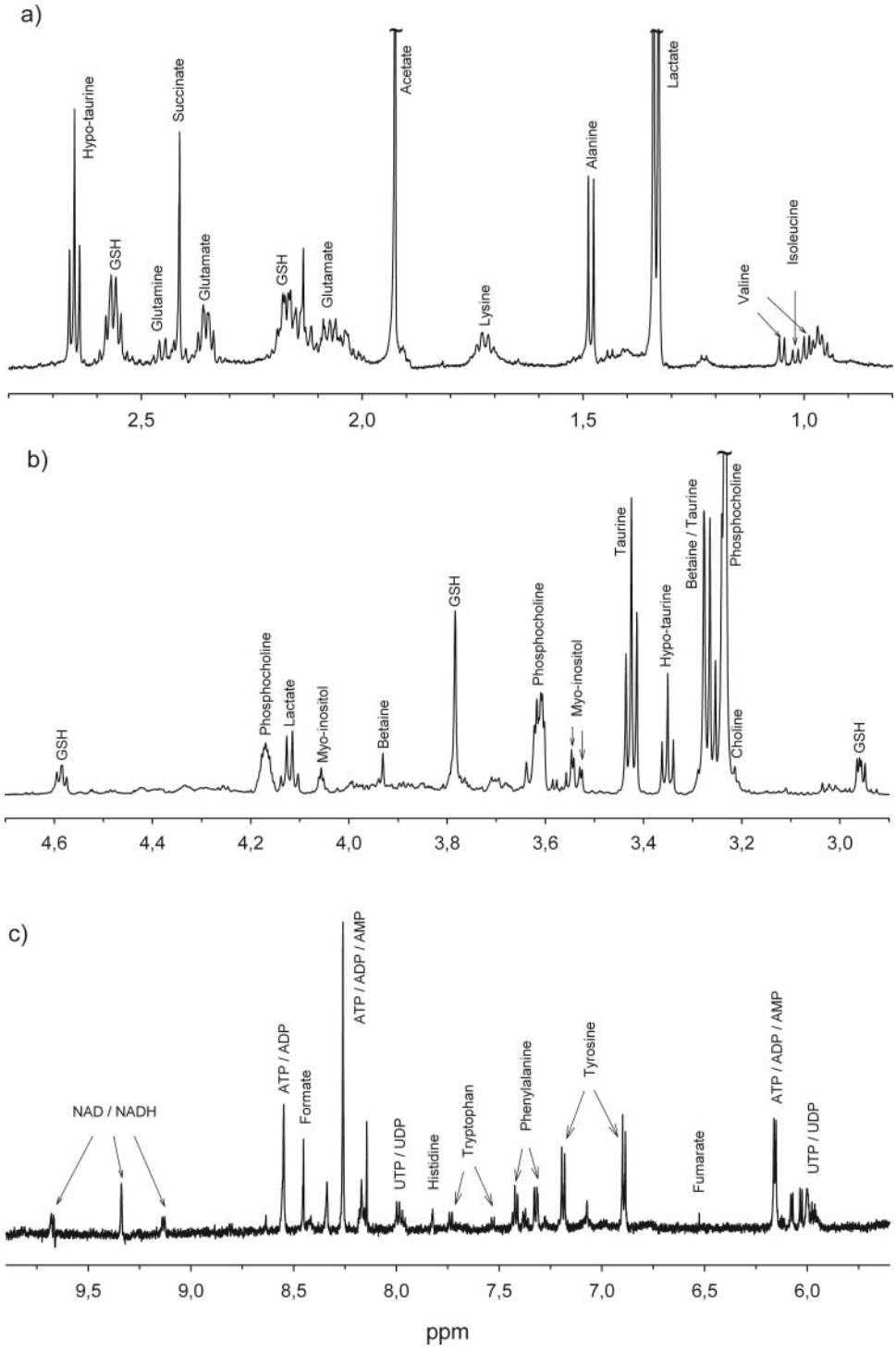

FIGURE 1. A characteristic HR-MAS ^{1}H NMR spectrum of a sample of the anterior part of the rat lens excised from control eyes: (**a**) 0.8–2.8 ppm, (**b**) 2.9 to 4.7 ppm, and (**c**) 5.6–9.9 ppm.

clearly separated (Fig. 2, marked by surrounding circles). This high score was true both for exposed and for nonexposed contralateral lenses. The samples from the anterior and the posterior cortex had intermediate scores for PC1 and did not show separation within UVR-B–exposed or contralateral nonexposed lenses.

Regional Alterations of Specific Metabolites

The concentrations of metabolites in the different compartments of the rat lens within UVR-B–exposed and contralateral nonexposed lenses are presented in Figure 3. The concentrations are presented as the mean of peak integrals, as obtained from the HR-MAS ^{1}H NMR spectra, divided by lens wet weight (institutional units). The metabolic effect of in vivo UVR-B exposure is presented in Figure 4. Metabolite levels in exposed tissue are given as a percentage of that in normal tissue. Among the quantified compounds, the most substantial changes after UVR-B exposure were found in the anterior part of the rat lens, whereas the nucleus showed the smallest metabolic changes of all the examined lens parts.

Amino Acids. The essential amino acid isoleucine occurred relatively evenly throughout the lens, in both exposed and nonexposed lenses (Fig. 3). The UVR-B exposure caused a decrease in isoleucine in all the compartments in the lens (Fig.

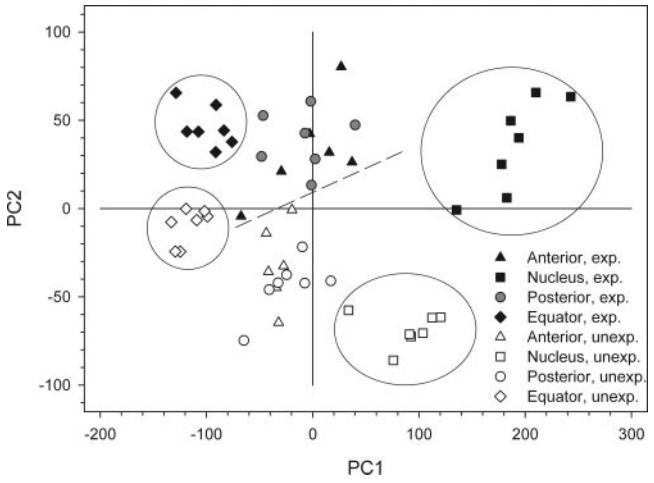

FIGURE 2. Scores for the first and the second principal components from the PCA (representing 79% of total variation). The nucleus and the equatorial region in UVR-B–exposed and in nonexposed samples were clearly separated by the first principle component (*circles*), but the anterior and the posterior samples were distributed without any clear pattern. The UVR-B–exposed samples were separated from the contralateral nonexposed samples by the second principal component. exp., exposed; unexp., unexposed.

4a: anterior [A], −60%; nucleus [N], −46%; posterior [P], −57%; and equator [E], −53%). Valine occurred in a slightly lower concentration in the nucleus of nonexposed lenses (Fig. 3b), and after exposure to UVR-B the concentration decreased in all regions (Fig. 4a: A, −60%; N,−48%; P, −59%; and E, −66%). Evenly distributed decrease was shown in the concentration of these amino acids after exposure to UVR-B. Phenylalanine (A, −68%; N, −53%; P, −58%; and E, −69%) decreased substantially in all compartments after UVR-B exposure (Fig. 4) and was evenly distributed between the regions both in nonexposed and exposed samples (Figs. 3b, 3d). Possible alterations in levels of the detected essential amino acids lysine, tryptophan, and histidine were not analyzed.

The nonessential amino acids tyrosine and alanine were relatively evenly present throughout the nonexposed contralateral lens (Figs. 3b, 3d). Glutamate showed a lower concentration in the nucleus of the nonexposed lenses. After the exposure to UVR-B, the concentration of tyrosine (A, −71%; N, −56%; P, −56%; and E, −70%) decreased in all regions, whereas that of alanine increased in the nuclear region (Fig. 4a; N, +23%). Glutamate decreased in the anterior compartment of the lens (Fig. 4a; A, −32%). Glutamine was not analyzed.

The metabolized amino acids taurine and hypo-taurine occurred in a lower concentration in the nucleus than in the other compartments of contralateral nonexposed lenses (Fig. 3d). After the exposure to UVR-B, the concentration in all the lens compartments of both taurine (A, −58%; N, −35%; P, −36%; and E, −41%) and hypo-taurine (A, −61%; N, −39%; P, −45%; and E, −52%) were significantly decreased (Fig. 4a). The decline in concentration of taurine and hypo-taurine was most expressed in the anterior cortex (Fig. 4a).

Antioxidants. The concentration of the antioxidant glutathione was much lower in the nucleus than in other parts of the nonexposed lens (Fig. 4b). Exposure to UVR-B reduced the glutathione concentration in the anterior cortex (Fig. 4b; A, −40%). In the other regions there was no clear change in the relative concentration of glutathione.

Metabolism. Succinate, an intermediate compound in aerobic metabolism, had a lower concentration in the nucleus than in the rest of the lens (Fig. 3d). After exposure to UVR-B, the relative concentration of succinate was decreased in the anterior and in the equator regions (−38%, −36%) of the lens (Fig. 4b). The concentration in the nucleus and in the posterior compartments was maintained. Acetate and fumarate were not analyzed. The end metabolite of anaerobic metabolism, lactate, was relatively evenly distributed in contralateral nonexposed lenses, except with a lower concentration in the nucleus (Fig. 3d). After exposure to UVR-B, there was an increase of lactate in the nucleus (Fig. 4b; N, +21%). Another end metabolite, formate, was lower in the nucleus than in other parts of the nonexposed lenses (Fig. 3b). The exposure to UVR-B reduced formate concentration in the anterior cortex, but increased the concentration in the posterior cortex (Fig. 4b: A, −40%; P, +20%).

Energy Transfer. The concentration of the energy transfer molecules ATP/ADP was lower in the nucleus than in the other parts of the nonexposed contralateral lens (Fig 3b). After exposure to UVR-B, the concentration in the anterior cortical region was lower (Fig. 4b; A, −49%).

Methyl Group Donors and Membrane Building Blocks. The level of the methyl group donor betaine was lower in the nucleus than in other parts of the nonexposed lens (Fig. 3b). There was no clear change in betaine concentration after UVR-B exposure. The membrane building block phosphocholine was lower in the nucleus than in the other parts of the contralateral nonexposed lens (Fig. 3d). Exposure to UVR-B reduced the concentration of phosphocholine in all compartments of the lens (Fig. 4b; A, −43%; N, −16%; P, −20%; and E, −31%).

Cell Signaling and Osmoregulation. The concentration of the cell signaling substance and osmolyte *myo*-inositol was lower in the nucleus than in other parts of the nonexposed contralateral lens (Fig. 3b). The exposure to UVR-B reduced the concentration in all the compartments (Fig. 4b; A, −67%; N, −53%; P, −54%; and E, −58%).

DISCUSSION

HR-MAS ^{1}H NMR spectroscopy provided metabolic profiles of the anterior, posterior, nuclear, and equatorial compartments of the UVR-B–exposed and the contralateral nonexposed rat lenses. NMR-based metabonomics showed that metabolic profiles from different compartments of the UVR-B–exposed lenses were different from the contralateral nonexposed lenses (Fig. 2). Differences were observed both between the lens compartments within unexposed samples and within UVR-B exposed samples. This was confirmed by the regional investigations of the individual metabolites (Figs. 3, 4). The level of individual metabolites in the contralateral nonexposed lenses showed a more profound difference between the lens compartments than the metabolites in the UVR-B–exposed lenses (Fig. 3).

The dose−response relationship between UVR-B exposure and lens opacities in rats has previously been quantified.[23,24] Intensity of forward light-scattering in the lens of albino rats increased exponentially with increasing UVR-B dose between 0.1 and 14 kJ/m^2.[24] The UVR-B dose (7.5 kJ/m^2) used in this study is above threshold for permanent lenticular damage, and forward light-scattering with this dose increases to 140% of that in unexposed lenses.[11,24] The observation of anterior cortical lens opacities in all the UVR-B–exposed rat eyes in the present study is in agreement with previous reports. Therefore, the biochemical changes observed in the lens reflect UVR-B–induced cataractogenesis. Another important factor when studying UVR exposure to the eye is the time of measurement after irradiation. In the present study, rats were killed 1 week after exposure to UVR-B, because previous data demonstrated that the intensity of light-scattering, after exposure to similar doses, has reached its maximum after 1 week.[24]

The control eye was not exposed at all in this study. One option would have been exposure to an equivalent amount of

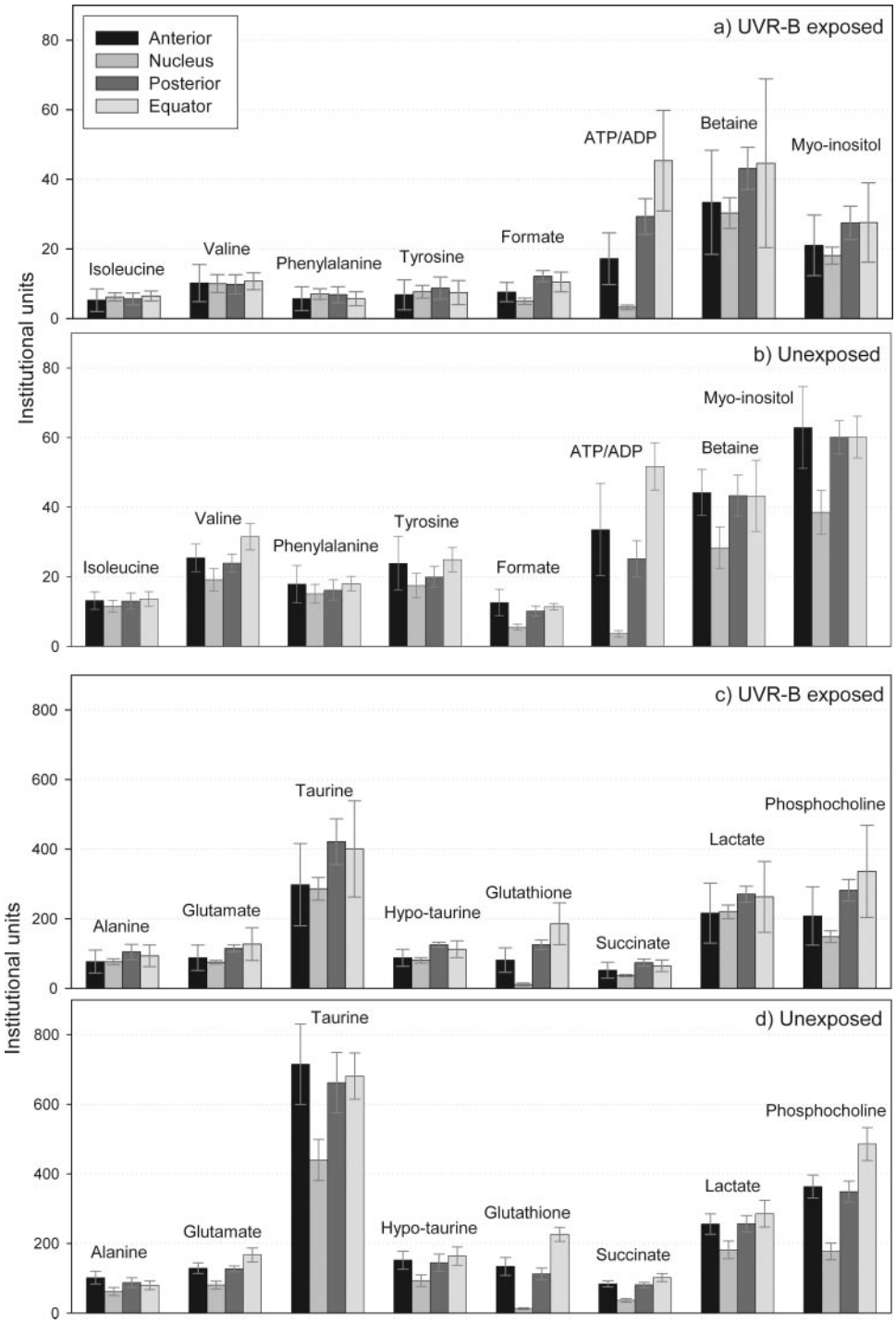

FIGURE 3. Comparison of the metabolic composition of the anterior, nuclear, posterior, and equatorial portions of the rat lens. Low concentrations: (**a**) UVR-B–exposed lenses; (**b**) unexposed lenses. High concentrations: (**c**) UVR-B–exposed lenses; and (**d**) unexposed lenses. The error bars represent the 95% CI for the mean difference ($n = 6$ for the anterior region and $n = 7$ for the other compartments).

energy from visible light. Although interesting, such a control may be of little value, since there are several studies showing an effect of visible light only in pro-oxidative environments, such as a high oxygen level[25,26] or with addition of a photosensitizer.[27,28]

Hockwin et al.[10] reviewed the cataract research in the 20th century and discussed the errors and misunderstandings in several studies. Topography of lens metabolism is one of the main critical points discussed in their review. Usually, biochemical studies start with homogenization of the whole lens. The data recorded are therefore averages of the compounds from the homogenized tissue.

In the present study, concentrations of metabolites varied among compartments, especially between the inner and outer layers (Fig. 3). In the contralateral nonexposed lenses, 12 of 16 compounds were present at lower concentration in the nucleus than in the anterior cortex (Figs. 3b, 3d). For most of the compounds in the nonexposed lenses, the concentration was lower in the nucleus than in the posterior cortex and the equator. This difference between the nuclear region and its surroundings may be a reflection of lower metabolic activity in the nucleus. In the anterior and the posterior cortex in the nonexposed lenses (Figs. 3b, 3d), the concentrations were similar, indicating that the metabolic activity in the two compartments is similar.

After exposure to in vivo UVR-B in the present study, a reduction in amino acid concentration (Fig. 4), with the exception of alanine, is in agreement with Risa et al.,[11] who used

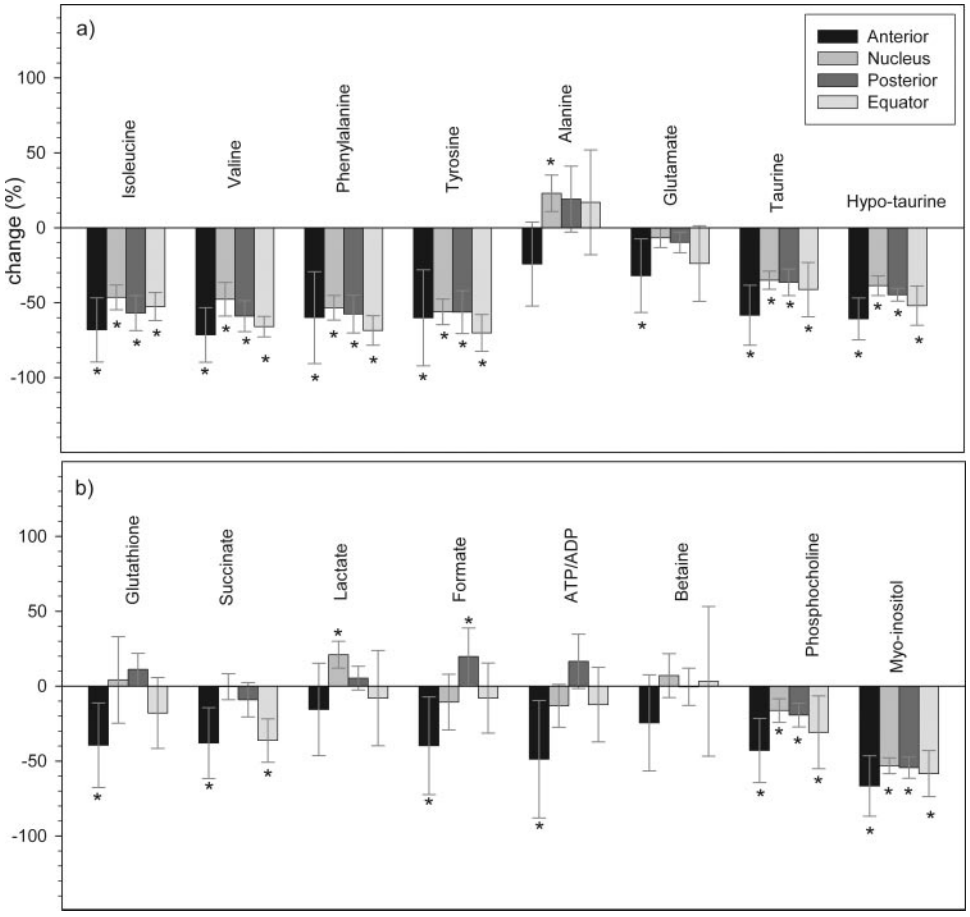

FIGURE 4. The change in concentration (%) of compounds between exposed and contralateral nonexposed samples from the anterior, nuclear, posterior, and equatorial portions of the rat lens. (**a**) Amino acids; (**b**) other assigned compounds. The error bars represent 95% CI for the mean difference ($n = 6$ for the anterior region and $n = 7$ for the other compartments). *Significant difference, $P < 0.05$.

the same dose and the same HR-MAS ^{1}H NMR method. However, their results were obtained from analysis of whole-lens samples, representing the average of the concentration of all the compartments. The present study shows that the reduction in amino acids were strongest in the anterior part of the lens, whereas the changes observed in the nucleus were not as distinctive (Fig. 4a). The current general decrease in amino acids after a suprathreshold dose of UVR-B may be related to impaired protein synthesis or the antioxidant function of amino acids.[29] However, the individual investigated amino acids have several reported functions.

The concentrations of the essential amino acids isoleucine and valine were observed to decrease substantially (isoleucine 46%–60%; valine, 48%–66%) in all lens compartments after UVR-B exposure (Fig. 4a). In a previous work in our laboratory, these substances were found to increase in the aqueous humor of rabbits after exposure to UVR-B.[15] Together, these findings may indicate that UVR-B causes impairment of transport or permeability of isoleucine and valine from the aqueous humor to the lens or that UVR-B increases permeability of these amino acids from the lens to the aqueous humor. However, other mechanisms such as antioxidant effects[29] and osmolytic functions[30] may, in addition, cause the observed decrease.

Tyrosine is an amino acid suggested to be a hydroxyl radical scavenger, singlet oxygen quencher, and weak photosensitizer.[6] However, little is known regarding the function of tyrosine in the eye.[6] The significant decreases of 71% in the anterior part of the rat lens and 56% in the nucleus, observed after in vivo UVR-B exposure (Fig. 4a) may be a result of these potential functions. Furthermore, the first step in the metabolic degradation pathway of phenylalanine is a hydroxylation to tyrosine. A decrease of 53% to 69% of phenylalanine observed in the various lens compartments after UVR-B exposure (Fig. 4a) may therefore affect the concentration level of tyrosine. UVR exposure of phenylalanine in the presence of hydrogen peroxide has been reported to convert phenylalanine into alanine.[31] The observed 23% increase of alanine in the nucleus (Fig. 4a) may be a result of UVR-induced transformation. This increased level of alanine after in vivo UVR-B exposure is in agreement with a previous whole-lens study from our laboratory,[11] and a study demonstrating a substantial increase in alanine (107%) in lenses exposed to UVR-B in combination with dexamethasone.[14]

Glutamate is a free amino acid found in the lens and is largely formed by transformation of glutamine.[32] Both glutamate and glutamine were detected in this study, but glutamine was not quantified, because of overlap between the resonances. Glutamate penetrates tissue poorly when compared with other amino acids. It is used for production of CO_2, proteins, and other metabolites.[32] After in vivo UVR-B exposure, a 32% decrease was detected in the anterior region (32%), but in the other compartments, the concentration remained unchanged (Fig. 4a). Previous findings have shown that the epithelial cells have the highest rate of glutamate synthesis, whereas the fiber cells have low permeability to glutamate.[32] Thus, most of the glutamate metabolism takes place in the outer region of the lens. In previous studies performed in our laboratories, glutamate concentration in whole-lens tissue was not changed after in vivo exposure to the same dose of UVR-B.[11] Therefore, the present study illustrates the importance of analyzing concentration of compounds in lenses fractioned into different compartments.

Taurine is known as the most abundant free amino acid in the lens,[33] which was confirmed by this study (Fig. 3d). It is known as an antioxidant,[34] a membrane stabilizer,[34] and an osmolyte.[35] The 41% to 58% decrease observed in the rat lens compartments after exposure to UVR-B (Fig. 4a) may be in

association with these reported functions. The present results are consistent with those of Risa et al.,[11] who showed a significant decrease in taurine (~50%) in whole lenses after exposure to UVR-B of the same dose. Agreement was further found between our study and that of Risa et al. in the case of hypo-taurine. Hypo-taurine is the precursor to taurine and showed in this study a stronger decrease (52%–61%, Fig. 4a) than taurine. This depletion of hypo-taurine may affect taurine production. Hypo-taurine may also have antioxidant effects or the UVR-B may disturb the synthesis of hypo-taurine, as discussed before by Tessem et al.[16] Hypo-taurine has been discussed as a compound with a tendency to decrease the most in the lens after exposure to UVR-B.[11]

The finding of a high anterior and equatorial concentration of reduced glutathione (GSH) in the unexposed lenses (Fig. 3d) is consistent with previous information stating that GSH is a highly concentrated antioxidant in the lens, vital for maintenance of lens transparency.[36] GSH detoxifies potentially damaging oxidants such as H_2O_2 and has a hydroxyl radical–scavenging function in lens epithelial cells.[36] The high concentration in the anterior cortex and the equatorial region of nonexposed lenses (Fig. 3d) may reflect protection of cell proliferation and lens fiber elongation against oxidation. The current finding of a very low concentration of nuclear GSH (Fig. 3d) is consistent with previous studies of normal lenses.[36,37] After exposure to UVR-B, the concentration of GSH decreased significantly (−40%) in the anterior part of the rat lens, but the other parts remained statistically unchanged (Fig. 4b). This is in agreement with previous results obtained in cultured rabbit lenses after exposure to UVR-B.[7] The decrease in GSH was possibly due to oxidation by oxidizing compounds produced by the UVR-B. However, no oxidized glutathione (GSSG) was observed in the NMR spectra. The oxidation process produces mainly protein-linked glutathione that may be difficult to observe with the pulse sequence (CPMG) used in this study.[11] Another explanation may be that the exposure to UVR-B in some way impaired the synthesis of GSH.

In nonexposed contralateral lenses, succinate and ATP/ADP were higher in the equatorial compartments than in the other parts of the lens (Figs. 3b, 3d), probably reflecting the high demand of energy for replication in the germinative zone and lens fiber elongation in the equatorial zone. The similar findings for phosphocholine and glutamate probably mirror the need for membrane and protein synthesis.

The decrease of succinate in the anterior (38%) and the equatorial (36%) part of the lens (Fig. 4b) may be explained by enzyme inhibition in the Krebs cycle caused by UVR-B exposure. This idea is supported by the fact that the concentration of succinate remained unchanged in the nuclear part of the lens (Fig. 4b) and metabolism in the lens is primarily confined to the epithelial cells. The fact that lactate concentration increased 21% in the nucleus of the lens (Fig. 4b) is probably due to increased anaerobic glycolysis, catalyzed by lactate dehydrogenase (LDH). The anterior cortex of the lens showed a slightly decreased level of lactate concentration which agrees with observations by Löfgren and Söderberg[38] at shorter postexposure intervals.

In a previous report from our laboratory, formate concentration increased in the aqueous humor after exposure to UVR-B.[15] In the current study, formate concentration decreased 40% in the anterior cortex, but increased 20% in the posterior cortex of the rat lens after UVR-B exposure (Fig. 4b). The observed decrease in the anterior cortex may be due to increased leakage from the lens. However, the connection between UVR-B exposure to the eye and changes of formate concentration in various lens compartments is still unknown.

Choline is an essential phospholipid precursor and is phosphorylated to form phosphocholine.[39] The accumulation of choline has been used to study effects of osmotic or oxidative cataractogenic stress.[40] In our HR-MAS ^{1}H NMR spectra, choline was detected, but in very small amounts, and the peak was not quantified. However, oxidative stress from photo-oxidation is found initially to affect choline transport into the lens and later to affect phosphocholine synthesis.[39] Phosphocholine is among the organic phosphate compounds that decrease in concentration in some cataracts and in lenses that have been exposed to osmotic agents or oxidative stress.[41] The current 16% to 43% decrease in phosphocholine after exposure to UVR-B (Fig. 4b) may be a result of disturbed choline transport and subsequent synthesis of phosphocholine caused by oxidative stress.[41] In a previous study, the decrease in synthesis of phosphocholine was found to parallel a decrease in ATP concentration.[41] After the current in vivo UVR-B exposure, ATP/ADP concentration decreased 41% in the anterior part of the rat lens, but no significant differences were found in the other parts (Fig. 4b). Risa et al.[11] showed a similar decrease in these high-energy phosphates and in phosphocholine after UVR-B exposure of the same dose.

The 53% to 67% decrease of *myo*-inositol found in the various lens compartments after UVR-B exposure (Fig. 4b) confirms previous results obtained in our laboratory on whole-lens analysis after UVR-B exposure.[11] Myo-inositol is known to have an osmolytic function[42] in lens epithelial cells, to be a cellular signal transducer and to play a role in growth and differentiation.[43] UVR-B exposure in the present study may have disturbed these mechanisms of myo-inositol in the lens compartments. In a previous study on aged cataractous rat lenses, a similar rapid decrease in *myo*-inositol has been reported.[44]

The most substantial decrease in compound concentrations after UVR-B in the present study was observed in the anterior cortex (Fig. 4). This finding is in agreement with the observed cataract in the anterior cortical region. The nuclear region of the lens generally had the lowest concentrations of most of the compounds (Fig. 3) and showed the lowest decrease or no decrease after exposure to UVR-B (Fig. 4). The compound concentrations in the posterior and equatorial regions varied, but were generally higher than in the concentration in the nucleus (Fig. 4). All these observations are in agreement with the fact that the lens is composed of functionally distinct anatomical compartments that have different metabolic activity.

An interesting observation of the present study is that the variation in compound concentration among the various lens compartments became smaller after exposure to UVR-B (Figs. 3a, 3c). GSH and ATP/ADP were the only compounds of 16 of which there was a substantially larger concentration in the anterior cortex than in the nucleus of the exposed lenses. In the contralateral nonexposed lenses, this was true of 12 of 16 compounds. The similarity between the anterior and the posterior cortex both for UVR-B exposed and for contralateral nonexposed lenses is probably because both compartments are made up of the same elongated fiber cells. Although the UVR-B is exposed into the anterior part of the lens, the cellular damage seems to be spread throughout the whole lens, involving also the posterior region. Generally, the exposure to UVR-B seems to reduce metabolic activity in the active anterior area of the lens. The alterations in compound concentration observed after exposure to UVR-B may be a result of biochemical mechanisms such as oxidation (tyrosine, GSH, taurine, valine, isoleucine), osmotic changes (*myo*-inositol, taurine), membrane dysfunction (choline, p-choline), and/or problems with cell transportation (choline, formate).

This study demonstrates the importance of performing biochemical analysis of functionally distinct compartments of the lens. Exposure to UVR-B induced a reduction of several compounds in the anterior cortex and in the other compartments of the albino rat lens.

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