THE INFLUENCE OF LONG TERM FOOD DEPRIVATION ON THE PHYSICOCHEMICAL CHARACTERISTICS OF LENTICULAR TISSUE

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Abstract. Food and water deprivation-frequent torture methods occur for a variety of reasons. It may be used as a political tool or a method of self-harm. To the best of our knowledge, no solid data are available relating the long-term food deprivation and the eye lens, therefore this study aims to clue this relation if any. Rats were food deprived for one, three and six days. The lenses were enucleated where both lipids and proteins were extracted for the analysis. The data showed no specific changes in cholesterol, phospholipids and soluble lens protein contents; this is concomitant with changes in the thermal behavior of lens lipids. It may be concluded that the phase transition behavior and the different lipid microdomains play an essential role in the response of the lens to different food deprivation periods.

Key words: Lipids, proteins, DSC, DC conductivity, food-deprivation, UV-spectroscopy.

INTRODUCTION

Intense research in organisms ranging from fungi to vertebrates has uncovered beneficial properties of dietary restriction in delaying aging and increasing cellular stress resistance [4]. Food restriction can change energy balance by decreasing energy expenditure as a mechanism of energy conservation, which is very important for survival and for species preservation [15]. However, reducing nutrient intake may be due to economic reasons such as poverty or may be used as a political tool (hunger strike) or a method of self harm, i.e. commits suicide. Kasdallah *et al*. (2005) [8] demonstrated that water deprivation, food deprivation and their combination stimulated the adrenal cortex, thereby suggesting a stress state. On the other hand, it seems that nutritional stress modifies the pituitary-thyroid axis through mechanisms different from those of osmotic stress. Surgeons and anesthesiologists are currently re-thinking the advisability of fasting patients the night before surgery. Overnight fasts are not completely innocuous because

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even brief starvation may cause loss of body and liver weights, depletion of hepatic glycogen, decrease of blood glucose and increase of gluconeogenesis from amino acids [9, 16]. Li *et al*. [10] found that calorically restricted mice have superior resistance to reactive oxygen radicals (H_2O_2) in the lens epithelium. To extend our knowledge about dietary restriction we evaluate its impact on the lens constituents.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Nitrogen gas was used to de-oxygenate the "Analar" grade organic solvents used in this study. All materials were used without further purification.

Rats – *Rattus norvegicus* – weighing 200–300 g were randomly divided into four groups each composed of 15 rats (30 eyes). One of them was served as control (normal) group, while animals of the other three groups were subjected to food-deprivation for one, three and six days respectively. At the end of each feed-deprivation period the eyes were removed from the globes and opened so that the lenses can be removed. The lenses were weighed to determine their wet weight and then were homogenized separately in a known volume of phosphate-buffered saline (pH 7.2) using cell homogenizer type Tübingen 7400 (Germany). After centrifugation in a cooling centrifuge, the supernatant was used to determine the total soluble proteins, calcium and glucose contents according to Lowry *et al*., [12], Ginder and King [7] and Trinder [21] respectively. The remaining debris was used for lipid extraction and purification according to Folch *et al*., [6]. The resultant pure lipids were weighed and dissolved in a known volume of chloroform in dark-glass bottles and kept stored under nitrogen atmosphere at –20ºC for further analysis. Total cholesterol as well as total phospholipids were determined in aliquots of the pure lipids after removing the solvent according to the procedures previously described by Zigman *et al*. [23] and Broekhyse [5]. The typical UV-spectra of pure lipids were recorded on Shimadzu UV-vis spectrometer (model UV-240a, Japan).

As previously mentioned by Sherif *et al*. [18], the DSC experiments were run and analyzed using a Perkin-Elmer (Wellesley, MA) Sapphire different scanning calorimeter. Ten μL of lipids in heptane: methanol (5 mg lipids) were hermetically sealed in aluminum pans and data normally acquired during three cycles of heating and cooling from –5 to 50 ºC at a rate of 5 ºC/min.

The estimation of malondialdehyde (MDA, the end product of lipid peroxidation) in the lens homogenates was done by the method of Niehans and Samuelson [14]. The color produced by the reaction of thiobarbituric acid with MDA was measured at 530 nm with the help of the spectrometer.

The electrical conductivity of the supernatant previously obtained after lens homogenization was measured at 25 ºC using YSI Field/Laboratory conductance meter model 32 FL (Yellow Springs, Ohio, USA), which was calibrated using standard liquid at 1413 μS. Five minutes were allowed for reading to equilibrate.

All values are expressed as mean ±SD. The statistical analysis was performed using ANOVA procedure where the significance level was set at $p < 0.05$. On the other hand, all the spectral analysis was performed with OriginPro 7.5 software (Origin Lab Corporation, MA).

RESULTS AND DISCUSSION

Figure 1 shows the typical absorption spectra of lens lipids as a function of the food-deprivation period. Regarding our results, the normal lipid pattern prepared from rat lenses is totally different from the pattern described by Babizhayev [1]. The mean absorption peak which corresponds to the native lipid (isolated double bonds of hydrocarbon chains) was detected at 273 nm. The additional two peaks appeared at 270 nm and 286 nm are due to the triene conjugates and an unsaturated component(s) respectively. It is known that double bonds can absorb UV-light in the range 200–289 nm, therefore as the degree of lipid unsaturation increased, the absorbed wavelength will increase until the lipid becomes colored and absorbs light in the visible region [3]. Fourier transformation was used to analyze the obtained lipid spectra for all studied groups, in order to reveal any overlapped peaks, where all the UV-spectra indicate the presence of three peaks. As a result of food deprivation, no specific changes could be detected in the lens lipid UV-spectra. All the spectra have the same characteristic three peaks previously mentioned in the normal pattern at the same corresponding wavelengths. The mean difference in these peaks is the area. The area under the peak in the UV-absorption spectroscopy is indicative for the concentration of that component.

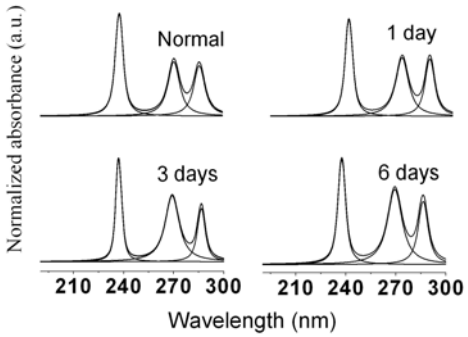

Fig. 1. Typical UV-spectra of rat lens lipids as a function of the food deprivation period.

As shown in Table 1, there are significant fluctuations in the concentrations of the different lipid constituents. The decrease in the native lipid content was directly proportional with the food restriction period till three days, then it returns

normal after six days of food deprivation. This change was concomitant with the same behavior for the unsaturated component(s).

The lipid peroxidation products (A_{270} nm) were found to be in the normal range till three days and significantly increased in the day six of food deprivation. The increase in the value of the absorption peak A_{270} was confirmed by the increase in the level of the malondialdehyde as shown in Table 2; such increase may be due to the fact that food deprivation blocks the antioxidant mechanisms. The other lens constituents shown in Table 2, soluble lens proteins, total cholesterol, total phospholipids and glucose concentrations, were found to oscillate around the mean normal values. The lens fiber membrane – as all other membranes – is a lipid bilayer associated with proteins (crystallins). Therefore, the chronic food deprivation for six days does not influence these lenticular constituents.

Table 1

The area under the UV-absorption peaks estimated by Lorentz model

Food-deprivation period	Peak (cm^2) at 237 nm	Peak (cm^2) at 270 nm	Peak (cm^2) at 286 nm
Normal	0.7 ± 0.02	0.6 ± 0.003	0.57 ± 0.04
1 day	0.66 ± 0.01	0.6 ± 0.001	0.44 ± 0.03†
3 days	0.4 ± 0.004†	0.6 ± 0.01	0.25 ± 0.03†
6 days	0.71 ± 0.04	0.98 ± 0.07†	0.6 ± 0.05

† Statistically significant

Although the concentration of the cholesterol and phospholipids was not changed due to chronic food deprivation, the area under the native lipid peak (237 nm) was decreased from the first day to the third day of food restriction. This contradictory behavior is due to the change in the content of the unsaturated component.

As also noted from Table 2, the calcium content of the lens fibers as a function of the food-deprivation period significantly decreased from day one to day six of feed restriction. Calcium is the most abundant mineral in the human body, and has several important functions [2, 19, 22]. A constant level of calcium is maintained in body fluid and tissues so that these vital body processes function efficiently. Cataract is defined as the transformation of soluble lens protein (low molecular weight proteins, LMW) to non-soluble aggregates (high molecular weight aggregates, HMW). Spector *et al*. [20] demonstrated that calcium is usually associated with these high molecular weight aggregates suggesting that the increased level of calcium is required to modify the protein structure so that it aggregates to the non-soluble form. By reducing the calcium content associated with these aggregates, the HMW protein was partially converted to LMW proteins. Human lens cells normally have very low Ca^{2+} permeability [12]. Rat lenses as a result of the chronic food restriction decrease its calcium content so that reduce the sensitivity of the protein to denaturate (i.e. aggregate).

The DC conductivity of the lens homogenate (Table 2) was linearly decreased than the normal till day three of food restriction, then increased at the day six. The measurement of conductivity is an important tool in the quality control of food industry; it used to measure the protein content in the milk where its decreased value indicates low protein content, as well as of the lipid content, since increased lipid content reduces the value of the conductivity. According to our results, the protein and the main lipid constituents (cholesterol and phospholipids) content did not alter. Severinsen and Munch [17] found that there was a reduction in the deep body temperature of rats fed with a reduced food amount and, their thermal conductance was reduced by 30%. This mechanism is important for the semi-starved rats to keep their core body temperature close to the normal. Our results showed that it is not only the thermal conductance, but also the electrical conductance, used as a thermoregulatory "set-point".

Table 2

Different lens constituents at different food-deprivation periods

	Normal	1 day	3 days	6 days
Soluble protein**(mg/g lens*)	379± 8	374 ± 11	382± 9	370± 15
Cholesterol** (mg/g lens*)	1.11± 0.2	1.17± 0.1	1.12±0.2	0.97±0.2
Malondialdehyde (nmol/g*)	3.2± 0.4	2.9±0.8**	3.5±0.6**	5.0±0.6†
Phospholipids** (mg/g lens*)	2.8± 0.3	2.9± 0.1	2.8± 0.3	2.9±0.2
Calcium (μg/g lens*)	69.4± 3	57.8± 5†	49.2± 5†	44.9±8†
Glucose** (μg/g lens*)	91.7± 7	89.6 ± 6	100± 16	95.8±4
Lens homogenate DC Conductivity (μS/cm)	277± 9	245± 12†	178± 6†	330±14†

** Statistically non-significant * Wet weight
† Statistically significant

Lipids are characterized by a unique physical phenomenon that is the phase transition behavior. Lipids may exist in two physical states, the gel phase (hydrocarbon chains are ordered and closely packed) and the liquid crystalline phase (hydrocarbon chains are loosely packed and mobile) with specific interconversion temperature known as the phase transition temperature. DSC measurements were employed to investigate the influence of different food-deprivation periods on the phase transition behavior of lenticular lipids. Figure (2a) shows the characteristic phase transition behavior of the extracted lens lipids studied at the temperature range 5–50ºC. The endothermic (hydrocarbon chain melting during heat given) phase transition of the normal lipids indicates the presence of seven endothermic peaks appeared in the temperature range 22–49ºC,

with the major one at 33ºC. After the first day of food deprivation, the endothermic peaks were reduced to five peaks and appeared in the temperature range 28–49ºC, where the main phase transition was found at 36ºC. Increasing the food deprivation period to three days resulted in increasing the endothermic peaks to ten peaks that covered the temperature range 14–49ºC. There are two mean phase transition temperatures detected at 33 and 38ºC. After six days of food deprivation, the thermogram indicates the presence of seven endothermic peaks in the temperature range 10–49ºC with a main phase transition at 30ºC and another one in the pre-transition region at 17ºC.

The phase transition of a single homogeneous lipid component is sharp and much easier to interpret than a mixer of lipids. Due to the heterogeneity of the lipid mixture, the phase transition behavior is complicated due to the cooperative interaction of these different hydrocarbon chains; this results in the multiple endothermic peaks shown in figure (2a), which reflect the existence of different lipid microdomains (i.e. lipid rafts). It is obvious from this figure that food restriction influences the thermal behavior of lens lipids.

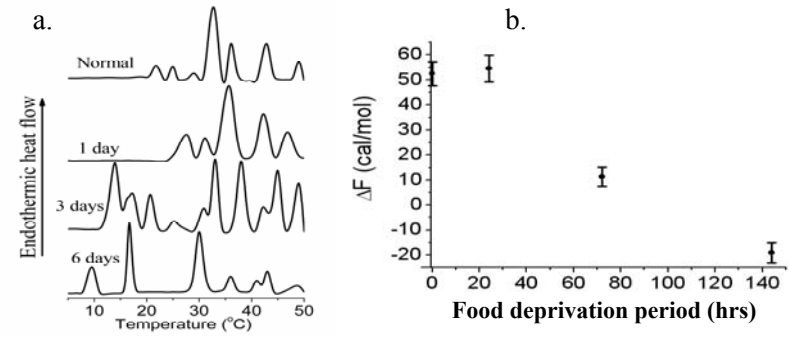

Fig. 2a. Thermograms of different lipid samples studied at the temperature range 5–50 ºC and b. The maximum free energy of different lipid samples.

In fact, these multiple endothermic peaks reflect the polymorphic changes of the lens lipids. The pronounced polymorphic changes are noticed after three days of food restriction. This lateral phase separation is required by the membrane to function since membrane function depends on the correct orientation. For example, there are many different membrane-bound proteins that carry out vital biological functions that may require different lipid environment to function. It is possible to accommodate such requirements in the phase transition region of a multiple-component mixture of lipids, where there are different phases with different lipid characteristics. It is also interesting noting that the increase in lipid polymorphism after three days of food restriction was associated with the decrease in the lipid unsaturated component that appeared at 287 nm (A_{287}) in the UV-spectrum as well

as the decreased calcium content. In the study done by Mitton *et al*., [13] on cold cataract of lenses from selenite-treated animal, there was an altered polyion content in these lenses before nuclear cataract formation, that contributes to the greater thermal stability of transparency in these lenses leading to lowering the temperature at which "cold cataract" forms. Thus reducing calcium concentration does not only reduce the sensitivity of protein to aggregate, but it also influences the thermal behavior and the formation of these different lipid microdomains.

Figure (2b) shows the change in the maximum free energy of the extracted lipids calculated using the equation:

$$\Delta F = -RT \ln K \tag{1}$$

where R is the universal gas constant, T is the absolute temperature and K is the effective concentration. On the first day of food restriction, ΔF is mimicking the normal value. As the feed deprivation period increased to three days, ΔF is significantly reduced and finally its value was dramatically changed on day six. According to Gibbs equation, ΔF of the system is the change in its internal energy during reaction, i.e., it measures the maximum energy that is available from the total internal energy of the system.

$$\Delta F = \Delta H - T\Delta S \text{ (Gibbs equation)} \tag{2}$$

where H is the enthalpy (heat content) and S is the entropy (disorder of the system). Therefore, the value of ΔF may have three different meanings: (1) zero value; the system is at equilibrium [isoergonic], (2) positive value; endergonic [non spontaneous] reaction and (3) negative value; exergonic [spontaneous] reaction. Since the energy of the molecule is stored in its chemical bonds, the observed variation in ΔF is also affected either by the decreased value in the lipid unsaturated component(s) or by the pronounced increase in the lipid polymorphism till day three of food restriction, i.e., the formation of different lipid microdomains (polymorphism) does not require energy and it formed by itself, and this is in contrast to the noticed change after day six of food restriction, where the value of ΔF indicates that the polymorphic changes require energy. The variation in ΔF was found to be in the same direction as the conductivity measured.

The present study provides data showing that long term dietary restriction has significant effects on the degree of lipid unsaturation of the lens. The major change due to this chronic food restriction was found in the thermal characteristics of the lens lipids where the formation of different lipid microdomains was different according to the period of food restriction. This implies that lens membranes change their function to buffer the stress resulted from chronic food deprivation and that the physical behavior of membranes is important.

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