# LOW POWER RED LASER IRRADIATION EFFECTS, AS SEEN IN METABOLICALLY INTACT AND IMPAIRED HUMAN BLOOD CELLS

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*Abstract*. The aim of the present work was to contribute to the understanding of the cellular and molecular mechanisms involved in low power long wavelength laser irradiation effects. Investigating the 680 nm laser light influence on the anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-[(4-trimethyl-amino)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) fluorescence, we report low power red laser induced changes in the lipid order parameter in various regions of the plasma membrane of human platelets and peripheral blood lymphocytes. Using irradiation doses of therapeutic significance, the dependence of the observed membrane effects on the actual metabolic state of cells is pointed out.

*Key words*: AlGaInP/GaAs, steady state fluorescence anisotropy, fluorescent lipid probes, plasma membrane fluidity, serum starvation, metabolic impairment, human peripheral lymphocytes, human platelets.

## INTRODUCTION

After some 30 years of debate, at present, low power laser therapy (LPLT), also named low level laser therapy (LLLT), became part of physiotherapy in most countries. It is successfully used whenever the goal is promotion of wound healing, reduction of inflammation, and/or pain relief [1, 5, 18, 24, 26–27, 30]. There is no doubt nowadays, that low-intensity monochromatic light from lasers acts directly on the organism at the molecular level [8–14], nonetheless – in spite of the growing number of well designed scientific investigations [2, 12–14, 20–22, 28–29] – the detailed molecular and cellular mechanisms involved remain elusive [13]. There is widely accepted that there exists a universal photobiological mechanism of light action on respiratory chain in both eukaryotic and prokaryotic cells, terminal

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enzymes of the respiratory chain being the photoacceptors [8–10, 12–13], and specificity of cellular responses only appearing during secondary reactions [9, 12–14], the cellular membrane being part of the photosignal transduction and amplification chain [13–14]. Irradiation supposedly causes the cellular redox balance to shift toward a more oxidized state (slight oxidative stress), it also optimizes the energy status of a cell [13].

However, there are many still unknown ways how metabolic pathways in a cell are regulated. Therefore, it is not surprising that understanding of light regulation mechanisms of cell metabolism is yet fragmentary [13–14]. Consequently, many short term and long term effects of irradiation should be thoroughly investigated before low power laser therapy will really become a mainstream medical tool.

The aim of the present work was to contribute to the enrichment of the scientific knowledge concerning characteristics and mechanisms involved in soft laser irradiation effects at subcellular level.

Dynamic rearrangement of membrane components at the cell surface and the changes induced in the membrane physico-chemical properties play an essential role in cellular signaling [16, 23]. Multiple cell membrane alterations have been described in humans and animals with various pathologies as well as under the influence of various physico-chemical factors [17, 23]. Using human platelets and peripheral blood lymphocytes, and the lipid probes DPH and TMA-DPH, selected to monitor fluidity/lipid packing density in the core and in the polar headgroup regions of the plasma membrane lipid bilayer respectively, we report steady-state fluorescence anisotropy data revealing membrane effects of the 680 nm laser light. Data analysis is focused on revealing the modulation of the observed lipid order / membrane fluidity changes by the cells actual metabolic state.

## MATERIALS AND METHODS

### MATERIALS

The fluorescent lipid probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1[4-(trimethylammonium)phenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH) was from Molecular Probes, Hepes buffer substance from Sigma Chemical Co., while all other chemicals were the best research grade available.

### CELLS

Peripheral lymphocytes and platelets were prepared from freshly drawn human blood obtained from healthy volunteers who had not received any medical treatment within two weeks before experiment. The blood was collected in a citrate

buffer (100 mM sodium citrate, 7 mM citric acid, 140 mM glucose, pH 6.5) containing 0.1 mM aspirin. Lymphocytes were separated by Ficoll-Hypaque density gradient centrifugation, washed twice by centrifugation / resuspension / centrifugation at 100g for 5 min, and finally resuspended in Hepes buffer HPMI (buffer no. 1, constituted of 100 mM NaCl, 5.4 mM KCl, 0.4 mM $MgCl_2$, 0.04 $CaCl_2$, 10 mM Hepes, 20 mM glucose, 24 mM $NaHCO_3$ and 5 mM $Na_2HPO_4$) at pH 7.4. Cell population obtained by this technique contained ~70% T cells [4]. The platelet-rich plasma was removed with a plastic pipette and transferred into plastic tubes for further utilization. Washed platelets were prepared from platelet-rich plasma by centrifugation for 10 min at 500 g. The platelet pellet was resuspended in HEPES buffer (buffer no. 2, constituted of 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, pH 7.4) [6]. Cell suspensions with viability level assessed by Trypan blue exclusion, higher than 95%, were only used.

## METABOLIC IMPAIRMENT

Metabolic impairment of cells was obtained by serum starvation at temperatures below 37 $^oC$. Human platelets and peripheral lymphocytes were kept various time periods (1 – 48 hours) at room temperature (~ 25 $^oC$) or at 4 $^oC$, without any nutritional supplement (serum, aminoacids or vitamins) in glucose-containing Hepes buffers 2 and 1 respectively. We name “fresh” samples constituted of suspensions of lymphocytes freshly separated within 90 min after blood drawing or of platelets freshly transferred in serum-free buffer from platelet rich plasma within 1 – 4 h after blood drawing.

## SAMPLES

Samples were constituted by continuously stirred, thermostated human platelet and peripheral lymphocyte suspensions of cell densities of $2 \cdot 10^7$ cells/ml and $2 \cdot 10^6$ cells/ml, prepared in Hepes buffers 2 and 1, respectively.

## LABELING OF CELLS WITH FLUORESCENT LIPID PROBES

$4 \cdot 10^{-3}$ M stock solution of DPH and $5 \cdot 10^{-4}$ M stock solution of TMA-DPH prepared in tetrahydrofuran and dimethylformamide, respectively, were kept at –20 $^oC$ until use within a month. Human platelets and lymphocytes were labeled with TMA-DPH by incubating them in the measuring cuvette with 1.25 μM dye at 37 $^oC$ for 3 min at a cell density of $2 \cdot 10^7$ cells/ml and $2 \cdot 10^6$ cells/ml, respectively. For DPH labeling stock solution was dispersed in PBS at a dilution 1:2000 in complete darkness, followed by 1 h continuous stirring of a fluorophore dispersion at 37 $^oC$, also in darkness. Cells were labeled by mixing of a suspension of $5 \cdot 10^6$

lymphocytes/ml or of $2 \cdot 10^7$ platelets/ml respectively, with an equal volume of fluorophore dispersion (2 μM DPH in PBS), and incubating for 30 min at 37 °C in darkness. After washing by centrifugation/resuspension in Hepes or HPMI buffer at a concentration of $2 \cdot 10^7$ cells/ml (platelets) and $2 \cdot 10^6$ cells/ml (lymphocytes), respectively, labeled cell suspensions were used immediately.

## FLUORESCENCE ANISOTROPY MEASUREMENTS

Steady state fluorescence and anisotropy measurements were carried out in a Perkin-Elmer MPF 44B spectrofluorimeter or in a Jobin-Yvon SpectroFluo JY3 spectrofluorimeter, equipped with thermostatted cell holders, stirring devices, Polaroid HN polarizers and connected to IBM PC computers using appropriate data acquisition software: SCOPE and EASYEST, respectively. The excitation and emission wavelengths were selected 340 nm / 425 nm and 355 nm / 430 nm for TMA-DPH and DPH, respectively. A BG12 filter placed in front of the exit slit of the sample compartment protected the detector from dispersed laser light. Steady state fluorescence anisotropy values ($r$) were obtained by quasi-simultaneous (within 12 s) measurements of the intensity components $I_{VV}$ and $I_{VH}$., where VV and VH stand for vertical/vertical (parallel) and vertical/horizontal (perpendicular) positions of the excitation and emission polarizers, respectively. A correction factor $G = \frac{I_{HV}}{I_{HH}}$ for unequal transmission by the optical elements of the vertically and horizontally polarized intensity components was also determined and thus the **steady-state fluorescence anisotropy** values were calculated every $20 - 25$ s according to the formula:

$$r = \frac{I_{VV} - G \cdot I_{VH}}{I_{VV} + 2 \cdot G \cdot I_{VH}} \tag{1}$$

The small autofluorescence of cells and the unavoidable scattered light contribution, determined by measuring unlabeled controls under the same experimental conditions as samples, were subtracted from each intensity component.

The **lipid order parameters** in the core and in the polar headgroup region of the plasma membrane bilayer, $S^{DPH}$ and $S^{TMA\text{-}DPH}$, were computed as functions of limiting initial $r_0$ and long-time $r_\infty$ values of the fluorescence anisotropy:

$$S^{DPH} = \sqrt{\frac{r_\infty^{DPH}}{r_0}} \quad \text{and} \quad S^{TMA-DPH} = \sqrt{\frac{r_\infty^{TMA-DPH}}{r_0}} \tag{2}$$

where $r_0 = r_0^{DPH} = r_0^{TMA\text{-}DPH} = 0.362$, while the limiting long-time fluorescence anisotropy values $r_\infty$ for DPH and for DPH analogs were approximated using the empirical curve obtained by van Blitterswijk *et al*. [25] as

$$r_\infty = 1.270 \cdot r - 0.076 \quad \text{for} \quad 0.13 < r < 0.28 \tag{3}$$

$$r_\infty = 1.100 \cdot r - 0.032 \quad \text{for} \quad 0.28 < r < 0.34 \tag{4}$$

where $r$ represents the DPH and TMA-DPH fluorescence anisotropies respectively, calculated from measured fluorescence intensities according to the formula (1).

**Membrane fluidities**, $f^{DPH}$ and $f^{TMA\text{-}DPH}$, were expressed as reciprocals of the lipid order parameters.

## LASER SOURCE

The radiation source was an AlGaInP/GaAs based semiconductor laser used in the medical practice, Philips CQL806D, with continuous wave output, having emission wavelength of 680 nm, nominal power of 30 mW, elliptical beam size 2.5 mm × 7 mm with speckle area of 17.5 mm$^2$, divergence 3.5$^o$ and polarization ratio 100:1.

## SAMPLE IRRADIATION

Irradiation of the samples was performed using a dedicated experimental set-up and/or directly in a fluorimeter, both equipped with thermostatting and stirring facilities. In the fluorimeter a BG12 filter protected the detector from any scattered laser light. Due to beam divergence of the used laser source, the incident power density values were source-sample distance dependent. At a 2 cm distance from the source the measured incident power was 0.026 W. This resulted in an estimated average incident power density on the 1 cm$^2$ upper surface of the continuously stirred cell suspension of ~260 W/m$^2$, which lies in the upper limit region of intensities typically used in low power long wavelength laser therapy. Moving the source 6 cm away from the sample, beam divergence caused an increase in speckle size and in consequence a ~10% loss in the magnitude of power incident on the sample. In these conditions the estimated average incident power density on the 1 cm$^2$ upper surface of the continuously stirred cell suspension became ~234 W/m$^2$.

The minimum irradiation time of the continuously stirred cell suspension was 12 s, equal to the time needed to measure all four fluorescence intensities in order to calculate one fluorescence anisotropy value, according to formula (1). With the laser source placed at the 6 cm distance from the sample, the estimated average incident energy density per experimental point, gathered in the presence of laser radiation, was ~2.8 kJ/m$^2$.

Duration of irradiation – realized previous to or during steady state fluorescence anisotropy measurements – varied between 12 – 600 s, resulting, at a given power density, in energy densities in the range of 2.8 – 140 kJ/m$^2$.

## STATISTICAL ANALYSIS

Anisotropy, lipid order parameter and membrane fluidity values and changes occurred in these parameters under the influence of laser irradiation are presented as *mean*±*S.D.* calculated from at least 3 independent measurements. Unpaired analysis of data series obtained by measurements made on cells various time periods after their transfer in serum-free buffer, before, during and after laser irradiation, was performed by Student's t-test. Significance was accepted at $p < 0.05$.

## RESULTS

At 37 $^o$C the fluorescence polarization of the hydrophobic lipid probe, DPH, was relatively weak in both human platelets and peripheral lymphocytes. The reduced fluorescence anisotropy values of $0.207 \pm 0.012$ and $0.191 \pm 0.022$ respectively, indicated relatively reduced order (order parameters: $0.734 \pm 0.024$ and $0.700 \pm 0.047$) and corresponding relatively high fluidity ($1.363 \pm 0.043$ and $1.437 \pm 0.100$) in the core of plasma membrane lipid bilayer in these cells. In same conditions the positively charged lipid probe, TMA-DPH, reported in the headgroup region of the plasma membrane lipid bilayer of human platelets and peripheral lymphocytes average fluorescence anisotropy values of $0.289 \pm 0.005$, and $0.270 \pm 0.009$. The corresponding values of the second rank order parameter and fluidity of membrane lipids of $0.896 \pm 0.010$ and $1.116 \pm 0.012$, and of $0.855 \pm 0.016$ and $1.170 \pm 0.022$, in platelets and lymphocytes respectively, indicate higher order in the headgroup region as compared to the core of the plasma membrane in these cells.

Under the influence of the 680 nm laser irradiation apparently significant changes occur in most of these parameters (Figs. 1 – 5). Metabolically impaired cells with intact plasma membrane (more than 88% Trypan blue excluding, data not shown) appear to be more sensitive to laser irradiation (Figs. 6 – 9), as compared to metabolically intact (less than 20% altered membrane potential, higher than 92% viability, data not shown) controls.

Changes induced are less substantial in the core of the plasma membrane in both cells (Figs. 1–2). The statistical significance of the differences observed is also lower.

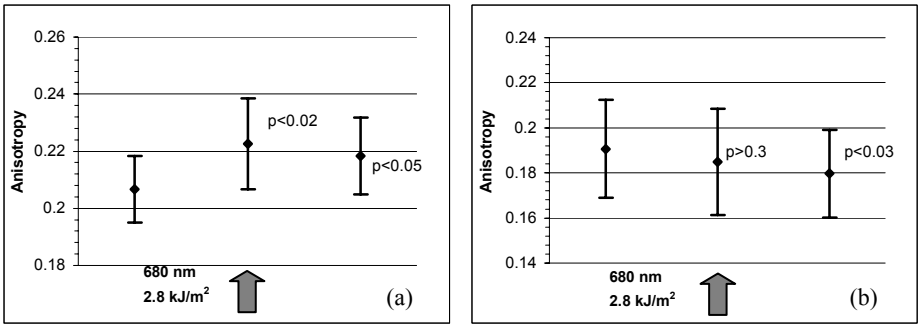


Fig. 1. – DPH fluorescence anisotropy (*r*) data, demonstrating apparently significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) exposed to various periods (0–4h) of previous serum starvation.

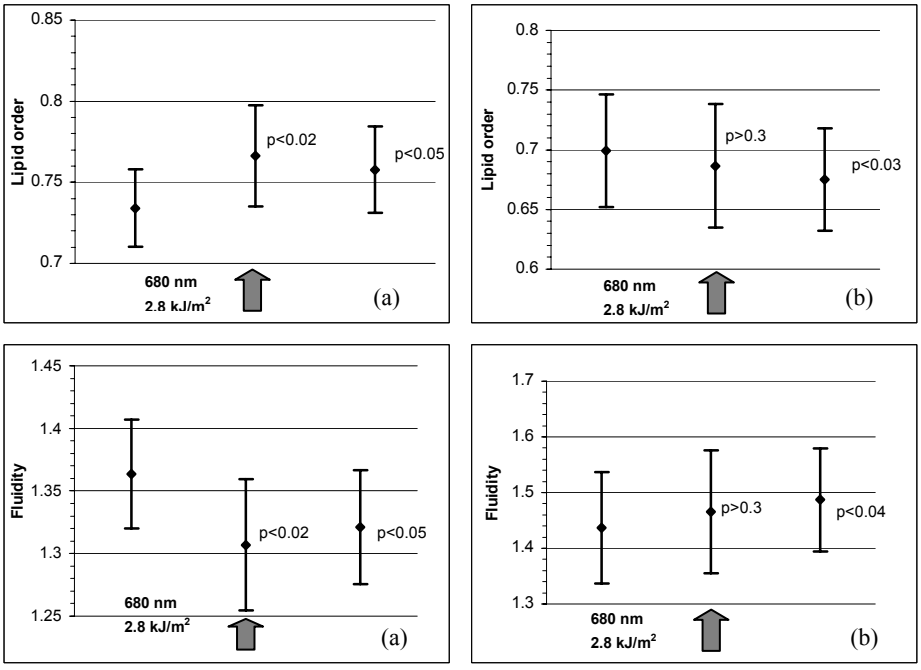


Fig. 2. – Data concerning membrane lipid order (*S*) and fluidity (*f*) in the core of plasma membrane lipid bilayer, demonstrating apparently significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) exposed to various periods (0–4h) of previous serum starvation.

Careful experiment design, using cell populations more homogeneous as concerns the treatment parameters, can even cause the loss of statistical significance of irradiation effects (Fig. 3). However – as it can be seen on Fig. 3B –

in case of the lymphocyte group exposed to 4 h serum starvation, statistically different from the "fresh" sample (measured immediately after its transfer in serum-free buffer), late effects of laser irradiation are statistically significant.

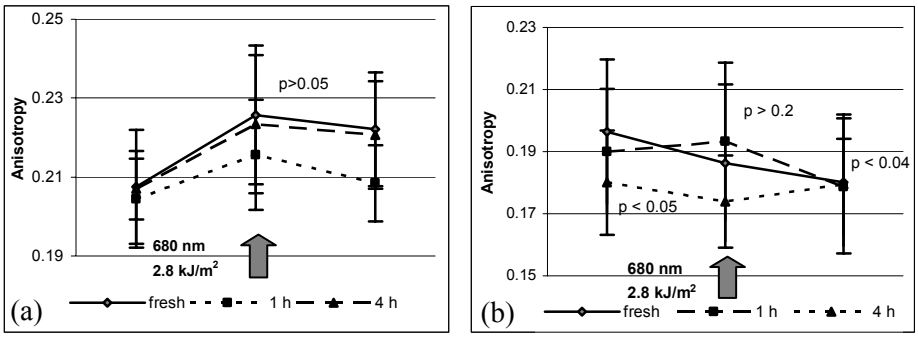


Fig. 3. – DPH fluorescence anisotropy (*r*) data, demonstrating apparently significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) sorted in different groups according to the length of previous serum starvation periods (fresh – measurements made immediately after the transfer of cells in serum-free buffer; 1h, 4h – cells kept the respective hours at room temperature in serum-free buffer).

Laser irradiation induced changes, reported by the TMA-DPH probe in the polar headgroup region of the plasma membrane bilayer, are more substantial (Fig. 4). Systematic decrease of anisotropy coefficients during laser irradiation is apparent in both platelets and lymphocytes. In peripheral lymphocytes late effects of laser irradiation are even more substantial. Statistical significances of the observed fluorescence anisotropy differences are also higher.

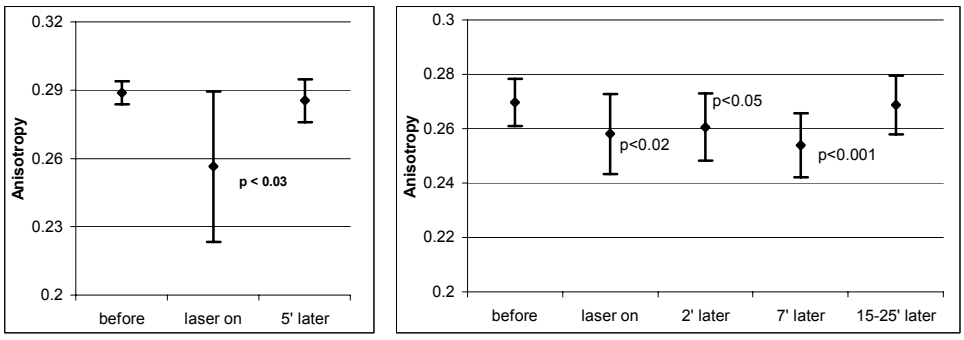


Fig. 4. – TMA-DPH fluorescence anisotropy (*r*) data, demonstrating significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) exposed to various periods (0–4h) of previous serum starvation.

Data concerning the corresponding lipid order parameter and membrane fluidity changes (Fig. 5) illustrate the same increased sensitivity to laser irradiation of the lipid headgroup region of the plasma membrane.

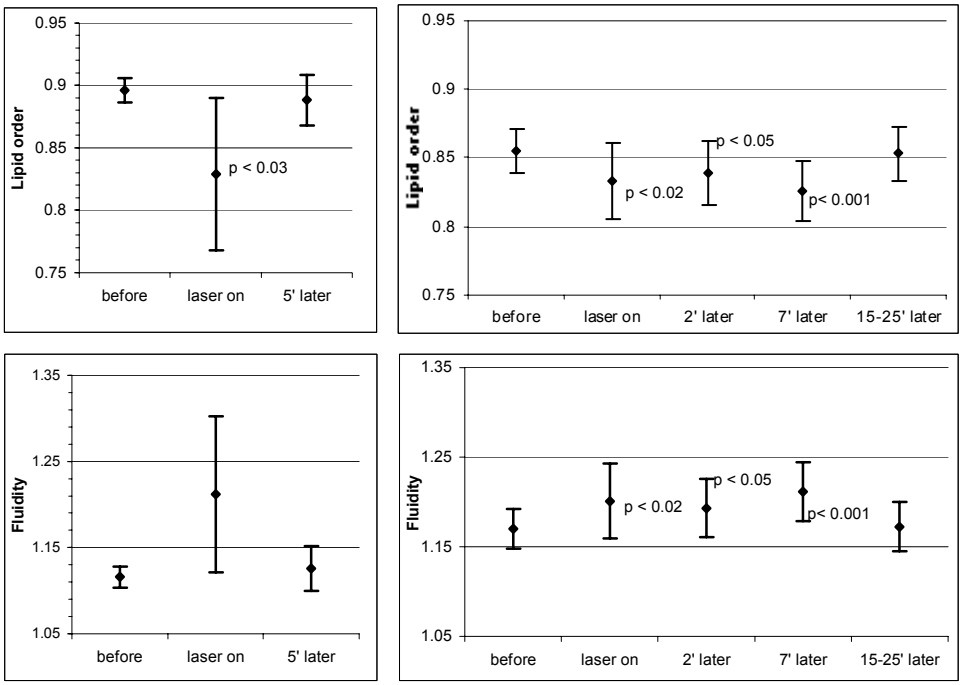


Fig. 5. – Data concerning membrane lipid order (*S*) and fluidity (*f*) in the headgroup region of the plasma membrane lipid bilayer, demonstrating significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) exposed to various periods (0–4h) of previous serum starvation.

More thorough examination of laser effects on "fresh" samples demonstrates a relatively low sensitivity to irradiation of metabolically intact cells (Fig. 6) and significant changes in time of laser effects during repetitive irradiation (Fig. 7). Use of cell populations, homogeneous as concerns the metabolic status and treatment parameters, allows disclosure of significant differences between the characteristics of groups exposed to different serum starvation periods, and significant group-dependent differences in the observed laser irradiation effects (Figs. 7–9, Tables 1 and 2). As a general rule, the 680 nm laser induced late effects are more substantial and significant in lymphocytes as compared to platelets – at least at the time scale covered by us. Furthermore, mild metabolic impairment due to shorter periods of serum starvation makes cells more sensitive to red laser radiation, repetitive laser irradiation being able to – at least partially – restore initial membrane parameters, while serious metabolic impairment due to long period serum starvation (≥ 24h) causes loss of cells sensitivity to laser irradiation in case of both platelets (Figs. 7–9, Tables 1 and 2) and lymphocytes (Fig. 8b, Fig. 9b).

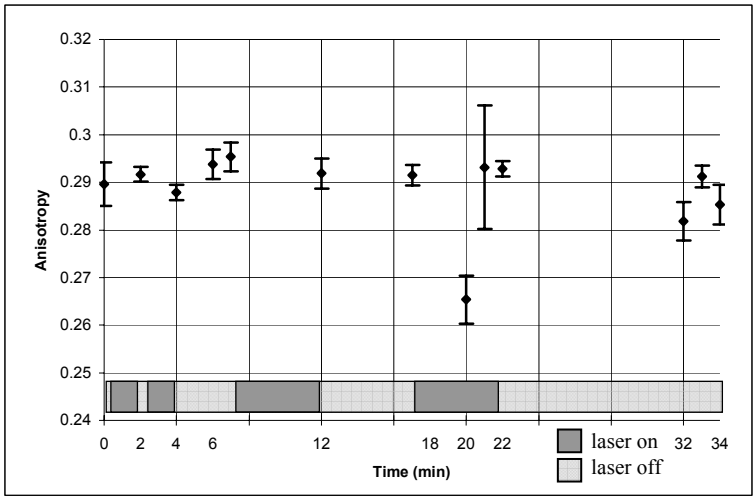


Fig. 6. – TMA-DPH fluorescence anisotropy (*r*) data, demonstrating relative insensitivity to laser irradiation of "fresh" samples (measured immediately after cell transfer in serum-free buffer), and time and treatment parameter dependence of the observed effects during repetitive irradiations, as seen in human platelets.

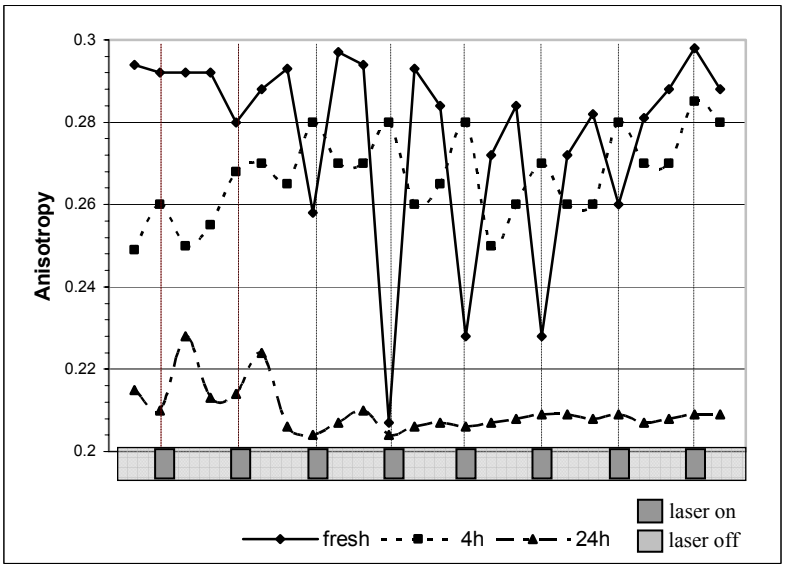


Fig. 7. – TMA-DPH fluorescence anisotropy (*r*) data, demonstrating relative insensitivity to single laser irradiation of metabolically intact cells, and time and treatment parameter dependence of the observed effects during repetitive irradiations, as seen in metabolically intact and impaired human platelets (fresh - metabolically intact, measured immediately after cell transfer in serum-free buffer; 4h, 24h – metabolically impaired due to cell exposure to a serum starvation regime 4h and 24h, respectively).

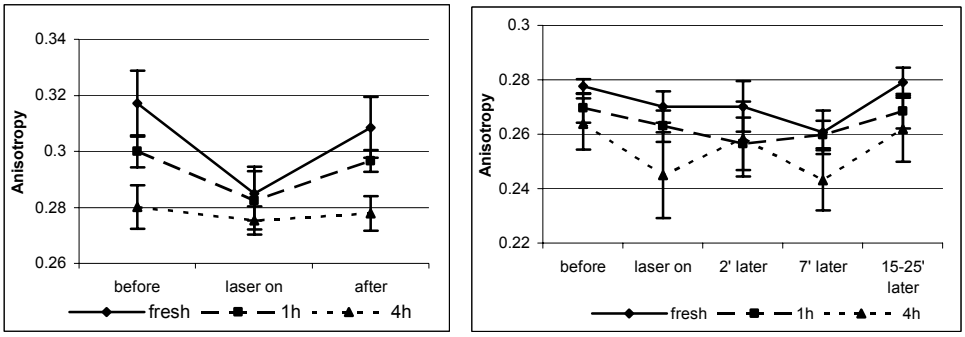


Fig. 8. – TMA-DPH fluorescence anisotropy (*r*) data, demonstrating significant laser irradiation effects in human platelets (a) and peripheral lymphocytes (b) sorted in different groups according to lengths of previous serum starvation periods (fresh –immediately after the transfer of cells in serum-free buffer; 1h, 4h – cells kept the respective hours at room temperature in serum-free buffer).

Early effects are more important in platelets as compared to lymphocytes. One hour of metabolic impairment does not lead to significant changes in the followed plasma membrane parameters in peripheral lymphocytes, but causes significant decrease in TMA-DPH fluorescence anisotropy, that means in the lipid order parameter in the headgroup region of the plasma membrane, in platelets. In contrast sensitivity to laser irradiation of these platelets is higher, as compared to lymphocytes. Four hours of metabolic starvation make peripheral lymphocytes more sensitive to red laser irradiation, while platelets turn out to be relatively insensitive in these conditions.

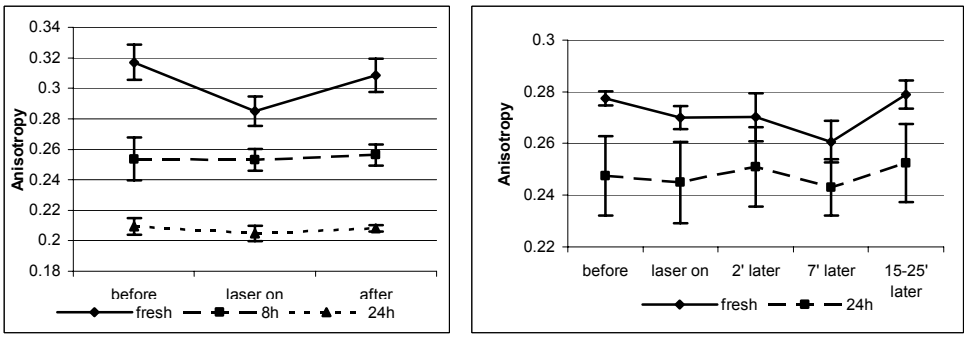


Fig. 9. – TMA-DPH fluorescence anisotropy (*r*) data, demonstrating apparently significant laser irradiation effects in human platelets(a) and peripheral lymphocytes (b) sorted in different groups according to the lengths of previous serum starvation periods (fresh – measurements made immediately after the transfer of cells in serum-free buffer; 8h, 24h – cells kept the respective hours at room temperature in serum-free buffer, * – platelets after an additional 20 min of repetitive laser exposure period in a 4 min rest / 2 min irradiation regime).

24 hours of serum starvation effects are similar in human platelets and peripheral lymphocytes (Fig. 9); however decrease in membrane lipid order is less substantial in lymphocytes than in platelets. After 8 hours of serum starvation platelets membrane parameters are significantly changed, and red laser effects are not statistically significant at the level $p = 0.05$.

*Table 1*

TMA-DPH fluorescence anisotropy (r) data reporting membrane lipid order (S) and fluidity (f) in the lipid headgroup region of plasma membrane of metabolically intact and impaired human platelets, before, during and after irradiation with a 680 nm laser light (Fluence during individual data point: 2.8 kJ/m$^2$; fluence rate: ~234 W/m$^2$; t – length of serum starvation period).

| | t (h) | 0 | 1 | 4 | 8 | 24 |
|---|---|---|---|---|---|---|
| $10^7$ platelets/ml in serum-free Hepes buffer before | r | 0.317±0.012 | 0.300±0.006 | 0.280±0.008 | 0.263±0.017 | 0.209±0.006 |
| | S | 0.950±0.022 | 0.918±0.011 | 0.873±0.013 | 0.843±0.031 | 0.740±0.011 |
| | f | 1.053±0.024 | 1.090±0.013 | 1.145±0.017 | 1.188±0.044 | 1.351±0.020 |
| during a 2.8 kJ/m$^2$ 680 nm laser irradiation | r | 0.285±0.010 | 0.283±0.010 | 0.275±0.005 | 0.247±0.005 | 0.206±0.005 |
| | S | 0.888±0.019 | 0.884±0.021 | 0.865±0.009 | 0.814±0.010 | 0.730±0.010 |
| | f | 1.126±0.025 | 1.132±0.026 | 1.156±0.012 | 1.228±0.015 | 1.369±0.020 |
| 5 min after laser irradiation | r | 0.309±0.011 | 0.297±0.004 | 0.278±0.006 | 0.253±0.005 | 0.208±0.002 |
| | S | 0.934±0.020 | 0.911±0.007 | 0.869±0.011 | 0.825±0.009 | 0.738±0.004 |
| | f | 1.071±0.023 | 1.097±0.009 | 1.151±0.014 | 1.213±0.013 | 1.355±0.008 |

Previous exposure to a repetitive irradiation regime of metabolically impaired platelets, not significantly sensitive to red laser irradiation, may partially restore both sensitivity and initial membrane parameters (Fig. 7, Fig. 9 and Table 2).

*Table 2*

TMA-DPH fluorescence anisotropy (r) data reporting membrane lipid order (S) and fluidity (f) in the lipid headgroup region of plasma membrane of metabolically intact and impaired human platelets, before, during and after irradiation with a 680 nm laser light (Fluence during individual data point: 2.8 kJ/m$^2$; fluence rate: ~234 W/m$^2$; t – length of serum starvation period; * - sample kept before measurement an additional 10 min at 37$^o$C in a repetitive irradiation regime).

| | t (h) | 0 | 0* | 8 | 8* |
|---|---|---|---|---|---|
| $10^7$ platelets/ml in serum-free Hepes buffer before | r | 0.317±0.012 | 0.294±0.013 | 0.263±0.017 | 0.247±0.007 |
| | S | 0.950±0.022 | 0.906±0.024 | 0.843±0.031 | 0.813±0.013 |
| | f | 1.053±0.024 | 1.103±0.028 | 1.188±0.044 | 1.230±0.020 |
| during a 2.8 kJ/m$^2$ 680 nm laser irradiation | r | 0.285±0.010 | 0.297±0.017 | 0.247±0.005 | 0.259±0.002 |
| | S | 0.888±0.019 | 0.912±0.031 | 0.814±0.010 | 0.836±0.004 |
| | f | 1.126±0.025 | 1.096±0.044 | 1.228±0.015 | 1.196±0.006 |
| 5 min after laser irradiation | r | 0.309±0.011 | 0.308±0.012 | 0.253±0.005 | 0.260±0.004 |
| | S | 0.934±0.020 | 0.933±0.022 | 0.825±0.009 | 0.838±0.006 |
| | f | 1.071±0.023 | 1.072±0.024 | 1.213±0.013 | 1.193±0.009 |

## DISCUSSION

Light absorption by chromophores in the respiratory chain of mitochondria – presumed to cause short-term oxidation of NADH pool and a shift in redox state of cytochrome c oxidase molecule into the more oxidized direction – also causes changes in the redox state of the cytoplasm, transiently shifting the overall redox state of a cell toward the oxidized direction [8–10, 12].The photosignal transduction and amplification chain is supposed to start with the respiratory chain further including the cytoplasm, the plasma membrane, and the nucleus. The way the signal is transmitted is assumed to be a cascade of rapid changes in cellular homeostasis parameters, crucial among them being the redox potential and ATP content of the cell [10–14]. The redox regulatory mechanism in many cells is more important than the photoacceptor control over the level of intracellular ATP, modulation of cellular redox state affecting gene expression *via* redox dependent transcription factors, as nuclear factor kappa B (NF-kB) and activator protein (AP)-l [6].

The magnitude of cellular response to low power laser light is determined by the cellular redox potential , intracellular pH and the amount of cellular ATP at the moment of irradiation. The cells with a lowered $pH_i$, with redox state shifted to the reduced side or decreased energy charge, respond more strongly than the cells with normal or close-to-normal parameter values [13–14].

As a rule, oxidants stimulate cellular signalling systems and reductants generally suppress the upstream signalling cascade resulting in the suppression of transcription factors [3]. Recent investigations unambiguously demonstrated involvement of cytokine and growth factors beneficial effects on wound healing, on the one hand [19], and enhancement of the production and release of these factors under the influence of soft laser irradiation at appropriate incident energy density and fluence rate, on the other hand [20, 28–30]. Cytokine and growth factor production and release are end-events of the signal transduction cascades in cells. These cascades include enzyme activities, phosphorylation/dephosphorylation events, ion transport and translocation of various other components between the membrane, cytosol and cytoskeleton, as well as changes in cell membrane properties. Thorough knowledge of low power laser effects on all these particular steps of signalling cascades is not available yet.

Our results demonstrating a significant dependence of the 680 nm laser light membrane effects on the cells actual metabolic state provide further support to the hypothesis of regulative role of redox homeostasis and cellular energy status in the photosignal transduction and amplification chain involved in the low power lasers action mechanism.

Systematic studies concerning membrane effects of low power infrared laser [15], and of various combinations of red and infrared laser lights, as well as low power lasers effects on various steps of cellular signalling are currently in progress

in our laboratory with the final goal to understand low power, long wavelength laser effects on cell function and to contribute to the development of the scientific basis necessary to effectively elaborate optimal treatment parameters/regimes.

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