REFERENCES

1. A. R. Varfolomeev, *Intraosseous Infusions in the Complex Treatment of Lumbar Osteochondrosis*, Abstract of Ph. D. Dissertation, Tomsk (1974).
2. E. L. Sokov, *Zh. Nevropatol. Psikhiatr.*, № 4, 57-61 (1988).
3. O. A. Shevelev and E. L. Sokov, *Byull. Eksp. Biol. Med.*, **115**, № 5, 587-589 (1993).
4. G. A. Yankovskii, *Osteoreception* [in Russian], Riga (1982).

Adaptation to Periodic Hypoxia and a Diet Supplemented with Polyunsaturated Class ω-3 Fatty Acids Enhance the Resistance of Ca^{2+} Transport in the Myocardial Sarcoplasmic Reticulum to Free-Radical Oxidation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 7, pp. 42-45, July, 1995
Original article submitted June 27, 1994

The relationship between the level of accumulation of lipid peroxidation products and the status of the Ca^{2+}-transporting system in the sarcoplasmic reticulum of the rat myocardium is studied against the background of two cardioprotective factors, namely adaptation to periodic hypoxia and a diet enriched in polyunsaturated fatty acids of the ω-3 class. It is shown that the diet leads to an increase of level of lipid peroxidation products by 1.8 times in the heart and by 19 times in the liver, whereas a adaptation has no effect on the level of lipid peroxidation products in either of these organs. At the same time, the combined action of both factors considerably enhances the resistance of the myocardial Ca^{2+}-transporting system to free radical-induced oxidation. In *in vitro* experiments it is shown that adaptation to periodic hypoxia results in a more than twofold deceleration of Ca^{2+} transport inhibition during the oxidation induction by the Fe^{2+}/ascorbate system; the diet causes a 3.5-fold deceleration of such inhibition. The results show that the accumulation of a high level of lipid peroxidation products is not always followed by damage to the Ca^{2+}-transporting system in the myocardial sarcoplasmic reticulum.

Key Words: *polyunsaturated fatty acids; adaptation; lipid peroxidation; Ca^{2+} transport; myocardium*

The process of lipid peroxidation (LPO) which is constantly going on in the organism carries out a number of important physiological functions [1]. However, the LPO hyperactivation that is a feature of numerous pathological states causes damage to membranes and to membrane-associated enzymatic systems, in particular in the heart. In the last few decades it has come to be thought that LPO activation plays an important role in the pathogenesis of various diseases. LPO inhibition is associated with the protective action of various factors, while LPO activation is thought to be directly related to harmful effects on the membrane structures. In this context the effect of LPO on the

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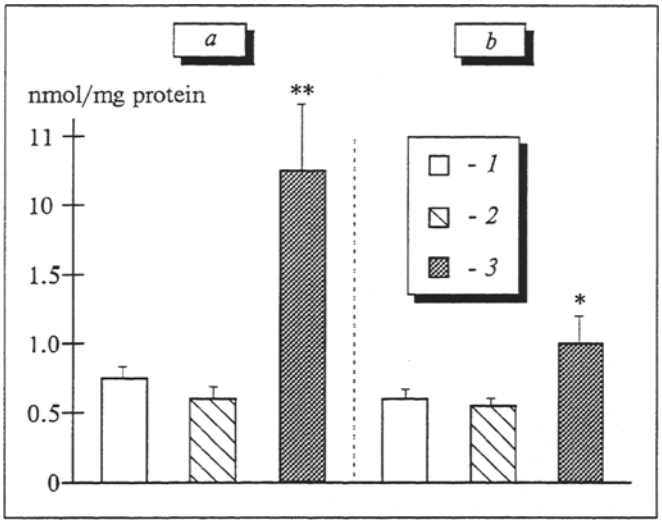

Fig. 1. Level of TBA-reactive LPO products in the liver (*a*) and heart (*b*); *1*) control group; *2*) after adaptation to periodic hypoxia; *3*) after consumption of a ω-3 PUFA-enriched diet; $^{*}p<0.01$; $^{**}p<0.001$ in comparison to the control.

ion-transporting membrane systems is of special importance. This includes the Ca-transporting system of the myocardial sarcoplasmic reticulum (SPR) that controls the intracellular Ca^{2+} ion concentration and exhibits a high sensitivity to the level of free-radical processes [2]. It is thus imperative that means of protection from free-radical damage be found. Among various possible approaches, special interest is being shown in non-drug prophylactic methods for the prevention of LPO-induced alterations of heart functioning.

The goal of our work was to study the status of the Ca^{2+}-transporting system of myocardial SPR and the intensity of the LPO process under the influence of two cardioprotective factors [3,5,6], namely a diet enriched with polyunsaturated fatty

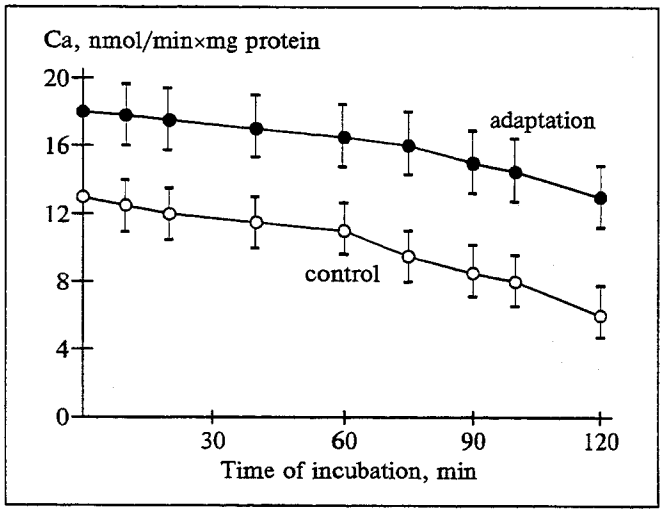

Fig. 2. Inhibition of Ca^{2+} transport by the induction of oxidation in the Fe^{2+}/ascorbate system in the control and after adaptation to periodic hypoxia.

acids (PUFA) of the ω-3 class and a course of periodic hypobaric hypoxia.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 300 g. Animals of one of the experimental groups during 60 days were fed a 5% diet enriched with w-3 PUFA isolated from the muscles of marine fish. The preparation contains (according to the data of *Trinita*, Moscow) up to 18% eicosapentaenoic acid, up to 12% docosahexaenoic acid, 18.1% oleic acid, 18.2% linoleic acid, and 18.3% linolenic acid (all PUFA), as well as saturated monoenic fatty acids. Adaptation to periodic hypobaric hypoxia was achieved by a 4-week course of daily 4-hour exposure of the animals in a pressure chamber under conditions simulating an altitude of 4500 m. The rats were gradually introduced to this regime by increasing the altitude by 1000 m every day. Rats were decapitated on the day after the last exposure to hypoxia or on the last day of consumption of the ω-3 PUFA-enriched diet. The hearts were extracted, washed free of blood, and placed in liquid nitrogen. The myocardial tissue was minced in an Ultra-Turrax homogenizer (25N-10 blade) at 12,000 rpm for 30 seconds in a medium containing 100 mM KCl, 25% glycerol, and 20 mM imidazole, pH 7.8, at a tissue/medium ratio of 1:4. The rate of Ca^{2+} transport in myocardial SPR was recorded according to the method of Kristensen *et al.* [8], by the rate of absorption of the added Ca^{2+} by the SPR vesicles. The reaction was conducted in a thermostatically controlled cell with stirring during 5 min. The homogenate (50-200 μl) in 5 ml of a medium containing 100 mM KCl, 15 mM K oxalate, 20 mM HEPES (pH 7), 4 mM $MgCl_2$, and 5 mM NaN_3 was added to the cell. ATP and Ca^{2+} were added to a final concentration of 4 and 2-20 mM, respectively, immediately before the start of the reaction. LPO intensity was estimated by the accumulation of products reactive with thiobarbituric acid (TBA) in the tissue homogenates [9]. LPO was induced by an Fe^{2+} (75 μM)/ascorbate(0.2 mM) system, and the kinetics of accumulation of TBA-reactive oxidation products was recorded.

RESULTS

The results of the study of the content of TBA-reactive products in the liver and heart are presented in Fig. 1. Adaptation to periodic hypoxia insignificantly alters the level of LPO products, while after a 60-day 5% diet enriched with ω-3

TABLE 1. Kinetic Parameters of the Functioning of the Ca^{2+} Transport System of the Myocardial SPR in the Control and after Adaptation to Periodic Hypoxia

Parameter	Control	Hypoxia
Rate of Ca^{2+} transport, μmol/g tissue/min	1.37±0.10 (100)	1.78±0.11* (129)
K_d, μM	0.21±0.01 (100)	0.13±0.01** (62)
V_{max}, μmol/g tissue/min	2.13±0.14 (100)	2.50±0.12 (117)

Note. *$p<0.05$; **$p<0.01$; in parentheses: %.

PUFA that are direct substrates of LPO a considerable activation of LPO takes place. The level of TBA-reactive products increases 1.8 times in the heart and 19 times in the liver, when compared to the control. Taking into account the high sensitivity of the SPR Ca^{2+}-transporting system to free-radical oxidation [2], one should expect the rise of the LPO intensity in the heart after the ω-3 PUFA-enriched diet to result in a harmful effect on the membrane-bound system of Ca^{2+} transport, while adaptation to periodic hypoxia, which practically does not alter the concentration of LPO products, should not affect Ca^{2+} transport. However, a study of the system of myocardial SPR Ca^{2+} transport after the two modalities of pretreatment showed this supposition to be erroneous.

In fact, against the background of the diet-caused accumulation of LPO products the initial rate of Ca^{2+} transport in SPR does not differ significantly from the control. Moreover, kinetic analysis and determination of the transport rate in the presence of different Ca^{2+} concentrations in the medium also failed to reveal any differences between the experimental and control groups (data not shown). It should be stressed that in both experimental groups the Ca^{2+} transport inhibition due to increasing ion concentrations in the medium takes place in the presence of the same Ca^{2+} concentrations as in the control, and does not shift to a zone of lower concentrations, as is the case in other situations, e.g., in of stress-induced damage of the Ca^{2+}-transporting system [7]. Overall, these results provide evidence of a complete preservation of the efficacy of Ca^{2+} transport in the myocardial SPR despite a simultaneous considerable LPO activation observed after 60 days on the diet enriched in ω-3 PUFA. After the adaptation to periodic hypoxia the intensity of LPO remained practically unchanged, and the parameters of Ca^{2+} pump functioning could also be expected to be equal to the control. However, here the nature of the effect of periodic hypoxia on the organism should be taken into account. A course of repeated long-term exposures to the hypobaria involves multiple returns to the conditions of normal partial pressure. For the organism this state (in com-

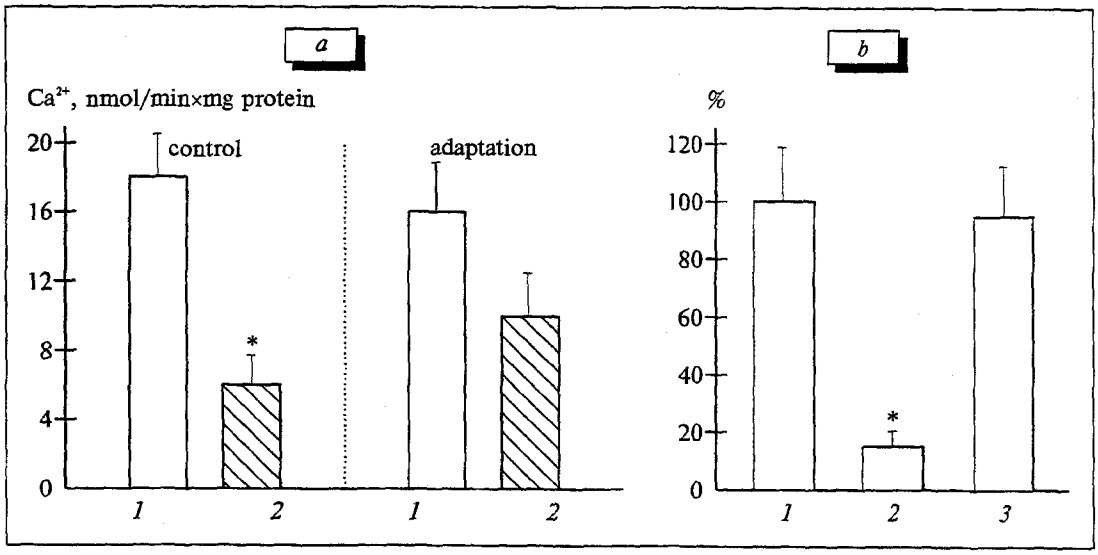

Fig. 3. Effect of *in vitro* induced accumulation of LPO products on the drop of the Ca^{2+} transport rate in SPR after a ω-3 PUFA-enriched diet and after adaptation to periodic hypoxia. *a*) reduction of the Ca^{2+} transport rate and increase of the initial level (*1*) of TBA-reactive products by the same magnitude (2 μmol/mg protein; *2*) in the control and after adaptation to periodic hypoxia. *$p<0.001$. *b*) drop in the rate of Ca^{2+} transport as compared to the initial rate (*1*) caused by an accumulation of TBA-reactive products (concentration equal to 5 μmol/mg protein) in the control (*2*) and after a ω-3 PUFA-enriched diet (*3*). *$p<0.001$.

parison with the hypobaric condition) represents a moderate reoxygenation which provokes the activation of LPO. Since this process is repeated many times and is of relatively moderate intensity, it may be effectively compensated for by an increase of the antioxidant system activity. This was shown earlier on a model of the enzymatic ensemble of antioxidant protection [4]. Thus, the lack of changes in the level of free-radical production after adaptation to periodic hypoxia is connected with an increased efficacy of the antioxidant system and, consequently, may influence the status of the Ca^{2+}-transporting system of the myocardial SPR. Indeed, analysis of this system showed (Table 1) that the rate of Ca^{2+} transport not only does not drop as a result of numerous oxygenations, but, on the contrary, is increased by 29%. The kinetic analysis revealed not only a change in V_{max}, but also a considerable (1.6-fold) drop of K_d (Table 1). This implies an increased affinity to Ca^{2+} and, correspondingly, a rise in the efficiacy of Ca^{2+} pump functioning. Thus, we have shown that, despite the considerable accumulation of LPO products owing to the ω-3 PUFA-enriched diet, the rate of Ca^{2+} transport in the myocardial SPR does not differ from the control, either quantitatively or qualitatively. Moreover, after the adaptation to periodic hypoxia, against the background of an unchanged level of TBA-reactive products the efficacy of the Ca^{2+} pump is even increased. These results directly point to a change in the resistance of the Ca^{2+}-transporting system to free-radical oxidation. In order to assess this phenomenon, we conducted a study of the system's resistance to LPO in a model system of LPO induction *in vitro* by the Fe^{2+}/ascorbate method. As is shown in Fig. 3, *a*, after the adaptation to periodic hypoxia the activation of free-radical oxidation *in vitro* causes a twofold drop in the rate of inhibition of the activity of Ca^{2+} transport in the rat myocardial SPR as compared to the control. This attests to a considerable increase of Ca^{2+} pump resistance to the LPO inducers. The ω-3 PUFA-enriched diet leads to a 1.8-fold rise of the initial level of TBA-reactive products in the heart, and to a 3-fold increase in the rate of accumulation of these products during LPO activation in the Fe^{2+}/ascorbate system *in vitro*. A comparison of the two simultaneous processes, namely accumulation of LPO products and inhibition of Ca^{2+} transport, demonstrated an analogous picture (Fig. 3, *b*). Thus, the accumulation of equal amounts of oxidized products caused a significantly lower (by 3.5 times) inhibitory effect on Ca^{2+} transport in the experimental (diet-consuming) animals as compared to the control. Moreover, the concentration of TBA-reactive products that was sufficient for total inhibition of Ca^{2+} transport in the myocardial SPR of the control animals failed to have a significant effect on the activity of the Ca^{2+}-transporting system of the experimental animals. Thus, both the PUFA-enriched diet bringing about an increased LPO level and adaptation to period hypoxia, which is not associated with a rise of the level of LPO products, boost the resistance of the Ca^{2+}-transporting system of the rat myocardial SPR to the harmful effect of free-radical oxidation. All this leads to an important conclusion, namely that accumulation of LPO products is not an obligatory factor inevitably exerting a harmful effect on the Ca^{2+}-transporting system of the myocardial SPR, since it may instigate factors producing a membrane-stabilizing effect.

REFERENCES

1. Yu. A. Vladimirov and A. I. Archakov, in: *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972), p. 249.
2. Yu. P. Kozlov, V. E. Kagan, and Yu. V. Arkhipenko, in: *Molecular Mechanisms of Oxygen-Caused Damage to the Ca-Transporting System in the Muscle Sarcoplasmic Reticulum* [in Russian], Irkutsk (1983), p. 134.
3. F. Z. Meerson, A. B. Shneider, and E. E. Ustinova, *Kardiologiya*, № 9, 67-69 (1990).
4. F. Z. Meerson, Yu. V. Arkhipenko, N. I. Rozhitskaya, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, № 7, 14-15 (1992).
5. F. Z. Meerson, L. M. Belkina, V. A. Isaev, *et al.*, *Kardiologiya*, № 3, 43-48 (1993).
6. F. Z. Meerson, L. M. Belkina, Hsian' Tzung, and V. A. Isaev, *Byull. Eksp. Biol. Med.*, **115**, № 2, 343-345 (1993).
7. T. G. Sazontova, *Ibid.*, **108**, № 9, 271-273 (1989).
8. S. D. Kristensen, E. B. Schmidt, H. R. Andersen, and Y. Dyerberg, *Atherosclerosis*, **64**, № 1, H13-H19 (1987).
9. P. L. McLennan, M. Y. Abeywardena, and Y. S. Charnock, *Amer. J. Clin. Nutr.*, **51**, 53-58 (1990).
10. F. Z. Meerson, *Adaptation, Stress, and Prophylaxis*, Berlin (1984), pp. 241-256.
11. F. Z. Meerson, T. G. Sazontova, and Yu. V. Arkhipenko, *Biomed. Sci.*, **1**, 73-378 (1990).
12. H. Ohkawa, W. Ohishi, and K. Yagi, *Analyt. Biochem.*, **95**, № 2, 351-358 (1979).
13. O. Poupa, Krofta, and Y. Prochazka, *Fed. Proc.*, **25**, 1234-1248 (1966).