Postępy Nauk Medycznych, t. XXVIII, nr 6, 2015 DOI: 10.5604/08606196.1158006

*Michał Mączewski, Monika Duda, Mariusz Marciszek, Joanna Kołodziejczyk, Urszula Mackiewicz

The effect of omega-3 polyunsaturated fatty acids enriched diet on the contractile function, structure, Ca2+ handling and electrophysiology in the rat heart**

Wpływ diety wzbogaconej w wielonienasycone kwasy tłuszczowe omega-3 na funkcję skurczową i morfologię lewej komory oraz na wewnątrzkomórkowy obieg Ca2+ i elektrofizjologię w sercu szczura

Department of Clinical Physiology, Medical Center of Postgraduate Education, Warszawa
Head of Department: Michał Mączewski, MD, PhD

Key words

omega-3, fatty acids, fish oils, Ca^{2+} handling, action potential, heart contractile function

Słowa kluczowe

omega-3, wielonienasycone kwasy tłuszczowe, olej rybi, obieg Ca^{2+}, potencjał czynnościowy, czynność skurczowa lewej komory

Summary

Introduction. Omega-3 polyunsaturated fatty acids (ω-3 PUFA) are essential fatty acids that cannot be synthesized by mammals and must be delivered with food. Epidemiological observations suggest that supplementation of ω-3 PUFA reduces cardiovascular mortality, although results of clinical trials and animal studies are highly variable and inconsistent.

Aim. The aim of the study was to examine cardiovascular effects of ω-3 PUFA supplementation in healthy rats. Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), the two most commonly used ω-3 PUFAs, were used.

Material and methods. Rats were fed with normal chow or EPA- or DHA-enriched diet for 4 weeks. Epicardial monophasic action potentials (MAPs) were recorded, cardiomyocyte Ca^{2+} handling was assessed and eachocardiographic imaging was performed before and after 4 weeks of feeding.

Results. Neither EPA nor DHA affected left ventricular (LV) ejection fraction, wall thickness or diameters, indicating that 4 weeks of feeding had no effect on LV structure and function. While EPA enriched diet had no impact on intracellular Ca^{2+} handling, DHA-rich diet significantly increased amplitude of Ca^{2+} transient and SR Ca^{2+} content. Neither EPA nor DHA affected action potential duration, however EPA mildly increased action potential amplitude.

Conclusions. This indicates that ω-3 PUFAs are safe for the cardiovascular system, but the above mentioned cellular effects may be considered arrhythmogenic under specific conditions.

Streszczenie

Wstęp. Wielonienasycone kwasy omega-3 (ω-3 WNKT) należą do grupy tzw. niezbędnych kwasów tłuszczowych, których organizm ssaków nie może syntetyzować *de novo* i muszą być one dostarczane z pokarmem. Badania epidemiologiczne sugerują, że suplementacja ω-3 WNKT zmniejsza śmiertelność z przyczyn sercowo-naczyniowych. Jednakże przeprowadzone dotychczas badania kliniczne i podstawowe dostarczyły różnorodnych i często sprzecznych wyników.

Cel pracy. Celem tej pracy jest zbadanie wpływu suplementacji dwóch głównych przedstawicieli ω-3 WNKT: kwasu eikozapentaenowego (EPA) i dokozaheksaenowego (DHA), na układ sercowo-naczyniowy.

Materiał i metody. Szczurom podawano karmę wzbogaconą w EPA, DHA lub standardową dietę kontrolną. Po 4 tygodniach rejestrowano potencjały czynnościowe z powierzchni serca, badano wewnątrzkomórkowy obieg Ca^{2+} w izolowanych kardiomiocytach oraz echokardiograficznie oszacowano funkcję i wymiary lewej komory serca.

Wyniki. Dieta wzbogacona w EPA i DHA nie miała wpływu na frakcję wyrzucania, grubość ściany i średnicę lewej komory. Dieta wzbogacona w EPA nie miała wpływu na parametry wewnątrzkomórkowego obiegu Ca^{2+}, a dieta bogata w DHA zwiększała amplitudę

Address/adres:

*Michał Mączewski
Department of Clinical Physiology
Medical Center of Postgraduate Education
ul. Marymoncka 99/103, 01-813 Warszawa
tel. +48 (22) 569-38-41
fax +48 (22) 569-37-12
michal.maczewski@cmkp.edu.pl

**Contract grant sponsor: Ministry of Science and Higher Education, contract grant number: N N401 184039.

potencjału czynnościowego i zawartość Ca^{2+} w siateczce sarkoplazmatycznej. Obie diety nie wywierały wpływu na czas trwania potencjału czynnościowego, podczas gdy dieta wzbogacona w EPA nieznacznie zwiększała jego amplitudę.

Wnioski. Uzyskane wyniki wskazują, że diety wzbogacone w ω-3 WNKT są bezpieczne dla układu sercowo-naczyniowego, ale wpływ DHA na wewnątrzkomórkowy obieg Ca^{2+} może przyczynić się w sprzyjających okolicznościach do powstania wapniozależnych zaburzeń rytmu serca.

INTRODUCTION

Omega-3 polyunsaturated fatty acids (ω-3 PUFA) and omega-6 polyunsaturated fatty acids (ω-6 PUFA) belong to a group of so called essential fatty acids that cannot be synthesized by mammals due to lack of enzymes required to synthesize a double bond in a respective ω-3 or ω-6 position. Therefore ω-3 and ω-6 PUFA must be delivered with food.

Epidemiological observations suggest that diet rich in fatty marine fish (that are a rich source of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), two most common ω-3 PUFA) reduce cardiovascular mortality (Inuits from Grenland, Japanese from Okinawa) (1). Physicians Health Study demonstrated that males who consumed fish at least once weekly had lower risk of sudden cardiac death despite unchanged incidence of myocardial infarction (2). These observations were supported by clinical trials: in DART study increased fish consumption led to a 29% reduction of mortality of myocardial infarction patients (3). GISSI-Prevezione Trials demonstrated that ω-3 PUFA supplementation reduced sudden cardiac death by 45% (4). This and other evidence led to adoption of ω-3 PUFA supplementation by many organizations, such as American Heart Association, as a recommended measure to reduce cardiovascular risk.

However, there are emerging data on potentially detrimental effects of ω-3 PUFA supplementation. PUFAs also undergo random oxidation (also referred to as peroxidation or auto-oxidation) rapidly under the conditions of the human body and are a source of tissue-damaging free radicals. Omega-3 fatty acids can alter immune function sometimes in ways that may lead to a dysfunctional immune response to a viral or bacterial infection (5). Furthermore, experimental studies demonstrated that ω-3 PUFA can affect variety of cardiomyocyte-specific ion channels and transporters engaged in intracellular Ca^{2+} handling potentially affecting both contractility and susceptibility to arrhythmias (6).

AIM

The aim of the study was to examine cardiac safety of ω-3 PUFA supplementation, i.e. effects of 4-weeks of DHA and EPA feeding on left ventricular function, structure, action potential duration and parameters of cardiomyocyte Ca^{2+} handing to verify effects of ω-3 PUFA supplementation on both cardiac hemodynamic performance and pro/antiarrhythmic predisposition.

MATERIAL AND METHODS

Twenty four male Wistar-Kyoto rats, weighing 210-230 g, were used. All study animals were used in compliance with local and institutional regulations. The study conformed to the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the local ethics committee.

Study protocol

The rats were randomly assigned to 3 groups (8 rats per group) that received control chow, chow enriched with EPA or DHA in amount 3% of total energy intake for 4 weeks. EPA and DHA were a gift from KD Pharma, Germany.

At the end of the feeding period, all animals underwent echocardiography, the epicardial monophasic action potentials (MAPs) were recorded and hearts were processed for cardiomyocyte isolation and Ca^{2+} handling measurements.

Echocardiography

Echocardiography was performed using MyLab25 (Esaote, Italy) with 13 MHz linear array transducer. Under light anesthesia (ketamine HCl and xylazine, 75 mg and 3.5 mg/kg body weight, IP) wall thickness were determined from the short-axis view at the mid-papillary level. LV end-diastolic and end-systolic areas were planimetered from the parasternal long-axis view. LV ejection fraction (LVEF) was calculated as (LV diastolic area – LV systolic area)/LV diastolic area.

Myocyte isolation and Ca^{2+} transient recording

The LV myocytes were isolated by enzymatic digestion, as described previously (7) and superfused at 37°C with Tyrode's solution containing 1.8 mmol/l Ca^{2+}. Ca^{2+} transient was recorded using indo-1 fluorescence (excited at 365 and measured at 405 and 495 nm) (7).

Rate of Ca^{2+} transport by SERCA, NCX, PMCA and sarcoplasmic reticulum Ca^{2+} content

The rate of Ca^{2+} transport by sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA), Na^+/Ca^{2+} exchanger (NCX) and plasma membrane Ca^{2+}-ATPase (PMCA) was estimated from the rate constants (r1, r2, r3) of the single exponential curves fitted to electrically- and caffeine-evoked Ca^{2+} transients, as presented in figure 2A. The rate constants of the Ca^{2+} transient decay for SERCA and NCX was calculated according to formulas: r_{SERCA} = r1-r2 and r_{NCX} = r2-r3, respectively. The r3

was taken as an index of the rate of Ca^{2+} transport by PMCA (r_{PMCA} = r3). r_{SERCA}, r_{NCX} and r_{PMCA} describe average velocity of Ca^{2+} transport by SERCA and NCX and PMCA, respectively. SR Ca^{2+} content was estimated from the amplitude of caffeine-evoked Ca^{2+} transients in myocytes superfused with Na^+, Ca^{2+}-free (0Na0Ca) solution (fig. 2A) (7).

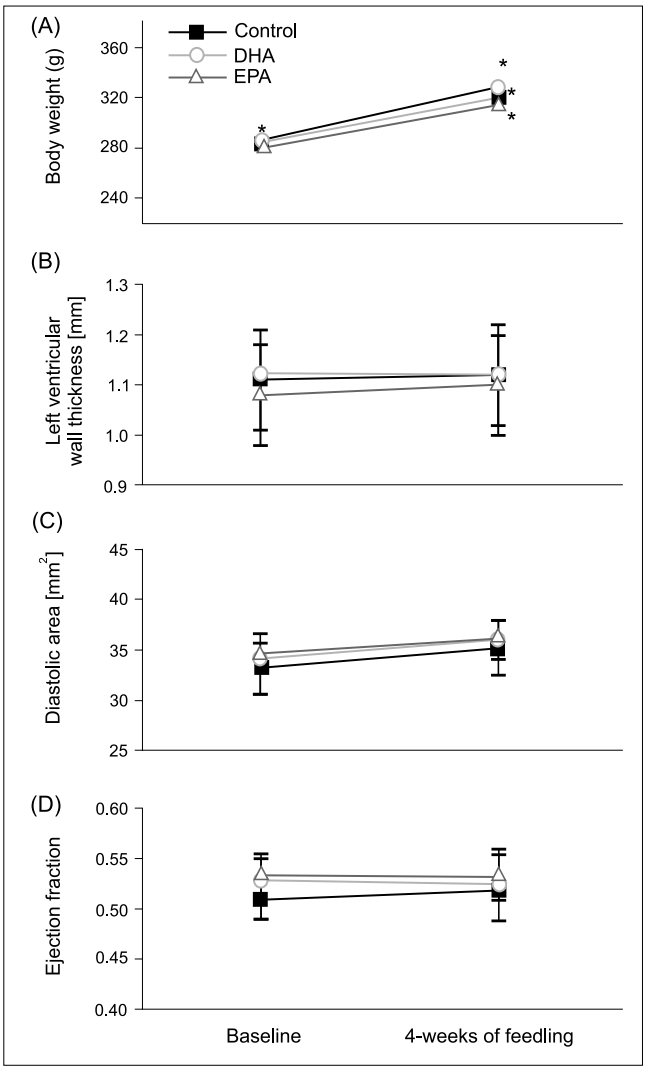

Fig. 1. Body mass, LV function and morphology in rats feeding with chaw and EPA or DHA enriched diet. Mean body mass (A), left ventricular wall thickness (B) left ventricular end-diastolic area (C) and left ventricular ejection fraction (D) in rats before and after 4 weeks of feeding with chaw and EPA or DHA enriched diets. Means ± SEM, n = 8 rat in each group.

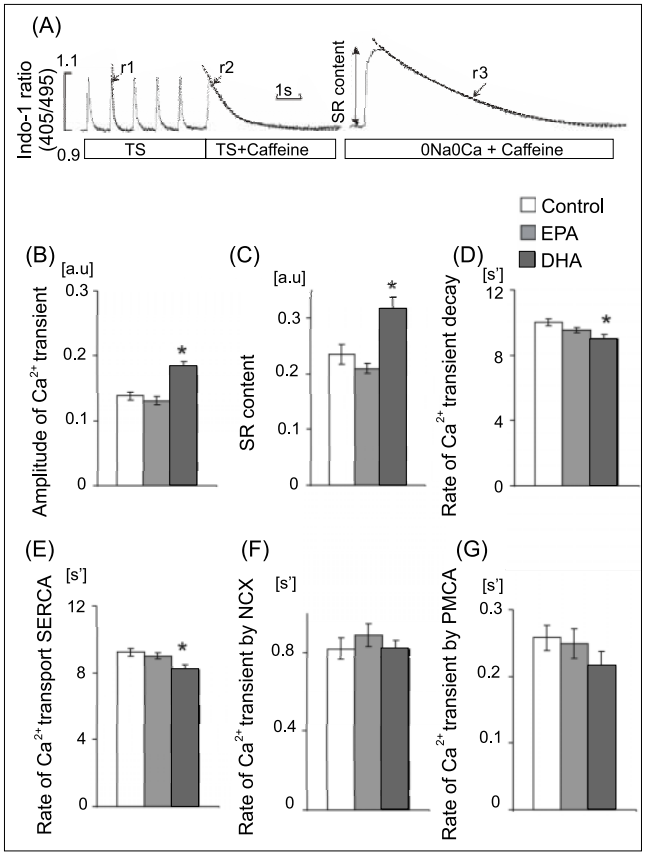

Fig. 2. Ca^{2+} handling parameters in rats receiving standard and EPA or DHA enriched diet. (A) Experimental protocols: cardiomyocytes were stimulated at 1Hz. Caffeine was applied in cardiomyocytes superfused with Tyrode solution (TS) or Na^+/Ca^{2+} free solution (0Na-0Ca). Single exponential curves were fitted to decaying part of electrically- or caffeine-evoked Ca^{2+} transients and rate constants of their decay (r1, r2 and r3) were calculated. The rate of Ca^{2+} transport by sarcoplasmic recticulum Ca^{2+}-ATPase (SERCA) and by sarcolemmal transporters: Na^+/Ca^{2+} exchanger (NCX) and sarcolemmal Ca^{2+}-ATPase (PMCA) was calculated according to formulas: r_{SERCA} = r1-r2 and r_{NCX} = r2-r3, r_{PMCA}=r3, respectively. Amplitude of Ca^{2+} transients evoked by caffeine in myocytes superfused with 0Na0Ca solution was taken as an index of sarcoplasmic reticulum (SR) Ca^{2+} content. Amplitude of Ca^{2+} transient (B), SR Ca^{2+} content (C), the rate of Ca^{2+} transient decay (D) and the rate of Ca transport by SERCA (E) NCX (F) and PMCA (G) in cardiomyocytes from rats receiving standard diet (Control) and EPA or DHA enriched diet. Means ± SEM, n = 32-120 measurements in myocytes isolated from 8 rat in each group.
*p < 0.05 EPA or DHA enriched diet vs. control diet

Monophasic action potentials recording

The local subepicardial monophasic action potentials (MAPs) were recorded using a miniature suction electrode consisting of the measuring electrode (silver wire, ∅ = 0.5 mm) protruding from the small plastic tube connected to the vacuum source, and the reference electrode (the silver wire was attached to the outer surface of the plastic tube). To record MAPs, the surface of the heart was gently touched with the measuring electrode and suction was applied. MAP reflects the course of action potentials of a small group of myocytes.

MAPs were recorded from two points of the LV wall: near the base and halfway between the base and the apex. Duration of five MAPs were averaged for each point and subsequently averaged from two points in each area. Eight rats per group were studied. Representative MAP recording are shown in figure 3A.

Statistical analysis

Data are presented as means ± SEM. Normal data distribution was verified by Shapiro-Wilk test while homogeneity of variances by Levene's test. Student t-test, one-way or two-way ANOVA followed by Tukey's post-hoc test or Kruskal–Wallis test (Statistica software 8.0)

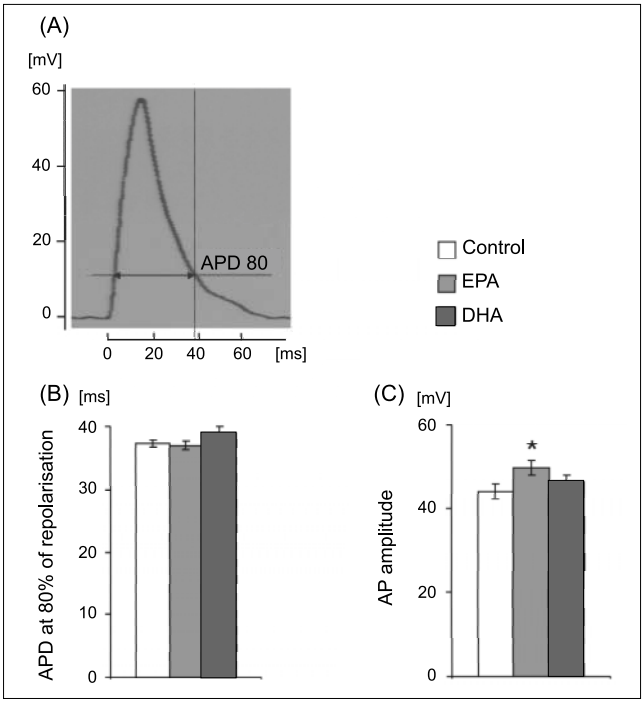

Fig. 3. Monophasic action potentials in rats receiving standard and EPA or DHA enriched diet. (A) Representative example of monophasic action potentials (MAPs) in rat receiving control diet recorded from left ventricular free wall. (B) Monophasic action potential duration at 80% of repolarization recorded from left ventricular free wall. (C) Amplitude of monophasic action potential. Means ± SEM, n = 8 hearts in each group.
*p < 0.05 EPA or DHA enriched diet vs. control diet

were used when appropriate. P < 0.05 was accepted as a level of significance.

RESULTS

There were no changes in the gain of body weight between rats receiving the control chow and DHA or EPA enriched chow after 4 weeks of feeding (fig. 1 A-D).

Left ventricular function and dimensions

As figure 1 demonstrates, ω-3 PUFA feeding did not affect LV function, wall thickness or diameters, indicating that 4 weeks of feeding has no effect on LV structure and function.

Calcium handling

Intracellular Ca^{2+} handling determinates amplitude of the cardiomyocyte contractions as well as the rate of relaxation. Moreover intracellular Ca^{2+} disturbances may promote Ca^{2+}-dependent arrhythmias.

While EPA enriched diet had no impact on intracellular Ca^{2+} handling, DHA introduced to the diet significantly increased amplitude of Ca^{2+} transient and SR Ca^{2+} content as compared to these parameters in cardiomyocytes from the rats feeding with the chow diet (fig. 2B and C). Moreover, DHA slightly decreased the rate of Ca^{2+} transient decay (fig. 2D) probably decreasing average rate of Ca^{2+} transport to SR by SERCA (fig. 2E). There was no statistically significant impact of incorporated DHA on NCX (fig. 2F) and PMCA (fig. 2G) function, however the trend to decrease of PMCA function was noticeable (p = 0.045).

Monophasic action potentials

The normal shape of AP in cardiomyocytes ensures electrical stability of the heart. Decrease of the amplitude as well as the changes of AP duration influences the propensity to arrhythmias. Additionally, the AP duration has impact on the contractility, modulating the Ca^{2+} influx.

We did not found any effect of both EPA and DHA introduced to the diet on AP duration (fig. 3B), while the slight increase of AP amplitude was observed in harts of rats receiving EPA enriched diet (fig. 3C).

DISCUSSION

We show that PUFAs, EPA and DHA, given to rats with diet for 4 weeks, do not affect left ventricular function or structure and have only mild effect on electrophysiological parameters and Ca^{2+} handling in the isolated LV cardiomyocyets. This indicates that omega-3 PUFAs are safe for the cardiovascular system. Nevertheless, some of these cellular effects may be considered arrhythmogenic under specific conditions.

Cellular effects of omega-3 PUFAs

Omega-3 PUFAs can exert cellular effects through incorporation to the cellular membranes and through direct effects of their free form circulating in the plasma. The effect of free PUFAs on cardiomyocyte function has been widely studied in vitro and results suggest that PUFAs may exert depressing effect on cardiac contractility (6). In isolated cardiomyocytes addition of free PUFAs to the superfusion resulted in reduction of L-type Ca^{2+} current, inhibition of ryanodine receptors (RyRs) and diminution of sarcoplasmic reticulum (SR) Ca^{2+} content (8). Moreover, it has been shown that PUFAs have dose dependent impact on AP duration in isolated cardiomyocytes. Addition of low PUFAs concentration to the perfusion solution resulted in prolongation of AP duration, while higher concentration shortened it (8, 9). The inhibition of Ca^{2+} current and RyRs as well as the shortening of AP duration at higher PUFAs concentration produced marked reduction in Ca^{2+} transient amplitude and cell shortening (10, 11), which should depress heart function *in vivo*.

However, dietary PUFAs supplementation does not only result in increase of the concentration of free PUFAs circulating in the blood, the impact of which was examined of the above mentioned experiments, but also leads to DHA and EPA incorporation in the cellular membranes. Leifert et al. have shown that in rats fed with PUFAs enriched diet for 3 weeks the level of DHA and EPA in myocytes membranes increased from 6 to 20% and from 0 to above 3% of total phospholipids, respectively (12). It is conceivable, that changed phospholipids membrane composition may influence the function of the membrane channels and transporters an thus myocyte function. Indeed, in several stud-

ies the PUFAs incorporated to the cellular membranes due to prolonged dietary supplementation exerted some effects on cardiomyocyte function, however less pronounced than those induced by free PUFAs introduced to the perfusion solution. Moreover, the results of these studies were inconsistent. In Leifert et al. study, fish oil enriched diet given to rats for 3 weeks, did not affect Ca^{2+} transient amplitude and SR Ca^{2+} content, however, decreased the rate of Ca^{2+} transient decay due to decrease of NCX-mediated Ca^{2+} removal (12). On the other hand, Verkerk et al. showed in pigs fed a diet of fish oil for 8 weeks, faster rate of Ca^{2+} transient decay, lack of impact on the NCX function, inhibition of Ca^{2+} current and unchanged amplitude of Ca^{2+} transient (13). Moreover, Billman et al. did not find any effect of incorporated PUFAs on Ca^{2+} current, Ca^{2+} transient and cell shortening in dogs receiving fish oil in the diet for 3 months (14). The results of the above studies suggest that the effect of incorporated PUFAs is different from that exerted by free PUFAs and strictly depends on the species used along the experiments, the time of fish oil supplementation and the amount of EPA and DHA incorporated to mtyocytes membranes.

To add the new information in this area we investigated separately the effect of EPA and DHA enriched diet and we showed that in rats fed for 4 weeks only DHA had some impact on Ca^{2+} handling parameters, increasing amplitude of Ca^{2+} transient and SR Ca^{2+} content. The rate of Ca^{2+} transient was slightly decreased due to diminished SERCA function. These changes should promote increase of myocyte contraction and cardiac function.

Effects of omega-3 PUFAs on the left ventricular function

Effects of omega-3 PUFAs on LV function *in vivo* have not been studied systematically. To date the impact of PUFAs dietary supplementation was assessed only in the two studies in marmosets (15) and dogs (16). In marmosets fed in fish oil there was no evidence of cardiac hypertrophy, however, mild increase of ejection fraction associated with greater end diastolic volume was found (15). On the other hand in dogs receiving fish oil enriched diet in spite of significant increase in the DHA and EPA incorporation in the myocytes membranes and exposition of myocytes to increased level of free PUFAs LV function was not changed (16). However, it should be noticed that dietary supplementation of PUFAs was much longer in marmosets as compare to dogs (24 vs. 3 months). Similarly to Billman et al., here we demonstrate that neither DHA nor EPA had any effect on the left ventricular diastolic area (as a marker of LV volume), ejection fraction (as a marker of LV contractility) or LV wall thickness (as a marker of potential hypertrophy) (16). We found no changes in LV function in spite of increased SR Ca^{2+} content and amplitude of Ca^{2+} transients, which should result in increase of contractility of individual cardiomyocytes. However, as was mentioned above in our cellular experiments we investigated the effect of incorporated PUFAs only. *In vivo* this effect is influenced by the free PUFAs circulating in the blood. The experiments with myocytes superfused with free PUFAs have shown that PUFAs in this form rather diminish the amplitude of Ca^{2+} transient through decreasing of Ca^{2+} current. It is conceivable, that this two opposite effects of incorporated and free PUFAs superimposed in vivo and resulted in no impact on LV contractile function, at least after rather short period of PUFAs supplementation.

Potential effect of omega-3 PUFAs on arrhythmogenesis

Dangerous ventricular arrhythmias, the main cause of sudden death, are predominantly of reentrant nature and occur when a premature ventricular extrasystolic beat coexists with a heterogeneity of refractoriness. Under this condition the propensity to unidirectional block of conduction and induction of reentrant circuit is high. Additionally, the maintenance of reentrant arrhythmias is promoted by the short refractory period (due to shortening of APD) and by decreased velocity of impulse conduction (17).

Premature ventricular beats most commonly are initiated by the spontaneous Ca^{2+} release from SR promoted by Ca^{2+} overload. *In vitro* experiments showed that addition of PUFAs to the superfusion solution, the condition resemble the influence of free PUFAs circulating in the blood, reducing Ca^{2+} current and activity of RyRs, diminish SR Ca^{2+} content. Consequently, threshold for spontaneous Ca^{2+} release from SR is increased and propensity to premature beats and thus arrhythmias reduced (10, 18). Incorporated PUFAs also seem to have some anti-arrhytmogenic effects. In pigs fed a diet of fish oil for 8 weeks incidence of spontaneous Ca^{2+} release, subsequent afterdepolarizations and triggered AP were much rarer as compared to control group (19). In fed animals augmentation of Ca^{2+} current, SR Ca^{2+} content and NCX function in response to adrenergic stimulation was much weaker than in control group. On the other hand we show, that DHA enriched diet increased Ca^{2+} SR content and amplitude of Ca^{2+} transient which may promote spontaneous Ca^{2+} release from SR. Probably *in vivo* this potentially pro-arrhythmic affect is counterbalanced by the anti-arrhythmic effect of circulating free PUFAs. Additionally, we found that EPA enriched diet increased AP amplitude, which promotes faster propagation of the excitation pulses and diminishes propensity to reentrant arrhythmias.

CONCLUSIONS

In summary, our results indicate that DHA and EPA enriched diets given to the rats for 4 weeks are safe and do not impair cardiac function and do not induce detrimental left ventricular remodeling. The augmentation of Ca^{2+} handling by DHA enriched diet needs to be further elucidated especially in contest of arrhythmogenesis in pathological conditions such as ischemia or myocardial infarction, which per se promote myocyte Ca^{2+} overload.

BIBLIOGRAPHY

1. London B, Albert C, Anderson ME et al.: Omega-3 Fatty Acids and Cardiac Arrhythmias: Prior Studies and Recommendations for Future Research: A Report from the National Heart, Lung, and Blood Institute and Office of Dietary Supplements Omega-3 Fatty Acids and Their Role in Cardiac Arrhythmogenesis Workshop. Circulation 2007; 116(10): e320-e335.
2. Albert CM, Hennekens CH, O'Donnell CJ et al.: Fish Consumption and Risk of Sudden Cardiac Death. JAMA 1998; 279(1): 23-28.
3. Burr ML, Fehily AM, Gilbert JF et al.: Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989; 2(8666): 757-761.
4. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999; 354(9177): 447-455.
5. Fenton JI, Hord NG, Ghosh S, Gurzell EA: Immunomodulation by dietary long chain omega-3 fatty acids and the potential for adverse health outcomes. Prostaglandins Leukot Essent Fatty Acids. 2013; 89(6): 379-390.
6. Sankaranarayanan R, Venetucci L: Are the anti-arrhythmic effects of omega-3 fatty acids due to modulation of myocardial calcium handling? Front Physiol 2012. doi:10.3389/fphys.2012.00373.
7. Mączewski M, Mackiewicz U: Effect of metoprolol and ivabradine on left ventricular remodelling and Ca2+ handling in the post-infarction rat heart. Cardiovasc Res 2008; 79: 42-51.
8. Macleod JC, Macknight AD, Rodrigo GC: The electrical and mechanical response of adult guinea pig and rat ventricular myocytes to omega3 polyunsaturatedfatty acids. Eur J Pharmacol 1998; 356: 261-270.
9. Rodrigo GC, Dhanapala S, Macknight AD: Effects of eicosapentaenoic acid on the contraction of intact, and spontaneous contraction of chemically permeabilized mammalian ventricular myocytes. J Mol Cell Cardiol 1999; 31: 733-743.
10. Negretti N, Perez MR, Walker D, O'Neill SC: Inhibition of sarcoplasmic reticulum function by polyunsaturatedfatty acids in intact, isolated myocytes from rat ventricular muscle. J Physiol 2000; 523: 367-375.
11. O'Neill SC, Perez MR, Hammond KE et al.: Direct and indirect mod- ulation of rat cardiac sarcoplasmic reticulum function by n-3 polyunsaturatedfatty acids. J Physiol 2002; 538: 179-184.
12. Leifert WR, Dorian CL, Jahangiri A, McMurchie EJ: Dietary fish oil prevents asynchronous contractility and alters Ca(2+) handling in adult rat cardiomyocytes. J Nutr Biochem 2001; 12: 365-376.
13. Verkerk AO, vanGinneken AC, Berecki G et al.: Incorporated sarcolemmal fish oil fatty acids shorten pig ventricular action potentials. Cardiovasc Res 2006; 70: 509-520.
14. Billman GE, Harris WS, Carnes CA et al.: Dietary omega-3 fatty acids and susceptibility to ventricular fibrillation: lack of protection and a pro-arrhythmic effect. Circ Arrhythm Electrophysiol 2012; 5: 553-560.
15. McLennan PL, Barnden LR, Bridle TM et al.: Dietary fat modulation of left ventricular ejection fraction in the marmoset due to enhanced filling. Cardiovasc Res 1992; 26: 871-877.
16. Billman GE, Nishijima Y, Belevych AE et al.: Effects of dietary omega-3 fatty acids on ventricular function in dogs with healed myocardial infarctions: *in vivo* and *in vitro* studies. Am J Physiol Heart Circ Physiol 2010; 298: H1219-H1228.
17. Nattel S, Maguy A, Le Bouter S, Yeh YH: Arrhythmogenic Ion-Channel Remodeling in the Heart: Heart Failure, Myocardial Infarction, and Atrial Fibrillation. Physiol Rev 2007; 87: 425-456.
18. DenRuijter HM, Berecki G, Verkerk AO et al.: Acute administration of fish oil inhibits triggered activity in isolated myocytes from rabbits and patients with heart failure. Circulation 2008; 117: 536-544.
19. Berecki G, DenRuijter HM, Verkerk AO et al.: Dietary fish oil reduces the incidence of triggered arrhythmias in pig ventricular myocytes. Heart Rhythm 2007; 4: 1452-1460.

received/otrzymano: 04.05.2015
accepted/zaakceptowano: 22.05.2015