Salutary effects of red palm oil intake on post-ischemic heart recovery and incidence of malignant arrhythmias demonstrated in hypertensive rats

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Abstract. Cardiac connexin-43 (Cx43) channels at the gap junctions play a crucial role in synchronizing the myocardium, allowing electrical and molecular signals propagation amongst cardiomyocytes. Previously we have shown that up-regulation of Cx43 is most likely involved in the antiarrhythmic mechanisms of omega-3 fatty acids. The aim of this study was to explore whether antioxidant rich red palm oil (RPO) may offer protection from malignant arrhythmias and/or attenuate an abnormal myocardial Cx43 expression in spontaneously hypertensive rats (SHR). Untreated hypertensive and age-matched normotensive rats were compared with those supplemented by 200microL/day RPO for 5 weeks. Left ventricular tissue was used for real time PCR analysis, immunoblotting and in situ immunostaining to determine Cx43 gene and protein expression as well as Cx43 distribution. The isolated perfused heart was used to examine post-ischemic reperfusion-induced arrhythmias, electrically inducible ventricular fibrillation (VF) and heart function. Results showed that the expression of Cx43-mRNA and total Cx43 proteins as well as its functional phosphorylated forms were increased and disordered myocardial localization of Cx43 was attenuated in the left ventricles of RPO-fed SHR compared to those untreated. This was associated with suppression of post-ischemic reperfusion-related ventricular tachycardia and electrically-inducible VF, as well as with better post-ischemic heart recovery. However, the treatment dose of RPO caused down-regulation of myocardial Cx43 in normotensive rats, and was associated with poor arrhythmia protection and cardiodepression suggesting overdose. In conclusion, our results indicate that SHR benefit from RPO intake particularly due to better post-ischemic heart recovery and protection from malignant arrhythmias. This cardioprotection can be partially attributed to up-regulation of myocardial Cx43. It appears that consumption of RPO might be salutary in hypertensive individuals, and should be examined by clinicians. Tab. 1, Fig. 7, Ref. 37. Online full text (Free, PDF) www.cardiology.sk

Key words: hypertensive rats – red palm oil – myocardial connexin43 – malignant arrhythmias – heart function

Viczenczová C, Bačová B, Radošinská J, Knezl V, Beňová T, Gonçalvesová E, Tribulová N. **Protektívny účinok konzumácie červeného palmového oleja na obnovu funkcie ischémiou postihnutého srdca a výskyt malígnych arytmií u hypertenzného potkana.** Cardiology Lett. 2012;21(5):388–396

Abstrakt. Srdcové neselektívne kanály v nexoch tvorené konexínom-43 (Cx43) umožňujú propagáciu elektrických a molekulových signálov medzi kardiomyocytmi, a preto majú kľúčovú úlohu pri synchronizácii myokardu. Naše predchádzajúce štúdie ukázali, že up-regulácia Cx43 sa veľmi pravdepodobne podieľa na

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Bacova B, Radosinska J, Viczenczova C, Knezl V, Dosenko V, Benova T, Navarova J, Gonçalvesova E, Van Rooyen J, Weismann P, Slezak J, Tribulova N. Up-regulation of myocardial connexin-43 in spontaneously hypertensive rats fed red palm oil is most likely implicated in its anti-arrhythmic effects.

antiarytmických účinkoch omega-3 mastných kyselín. Cieľom tejto štúdie bolo preskúmať, či na antioxidanty bohatý červený palmový olej (RPO) má antiarytmické účinky a/alebo zmierni abnormálnu expresiu Cx43 v myokarde spontánne hypertenzných potkanov (SHR). Neovplyvnené hypertenzné a rovnako staré nehypertenzné potkany sa porovnávali s tými, ktoré dostávali denne počas piatich týždňov 200 RPO microL. Tkanivo z ľavej komory srdca bolo použité na analýzu génovej a proteínovej expresie Cx43, ako aj na detekciu jeho in situ myokardiálnej lokalizácie. Izolované perfundované srdce sa použilo na vyšetrenie post-ischemicko reperfúznych arytmií, elektricky indukovanej komorovej fibrilácie (VF) a funkcie srdca. Zistilo sa, že pri porovnaní s neovplyvnenými SHR je expresia Cx43-mRNA, jeho celkového proteínu a funkčnej fosforylovanej formy zvýšená a jeho abnormálna lokalizácia je výrazne eliminovaná v ľavej komore srdca SHR potkanov suplementovaných RPO. Tieto zmeny boli sprevádzané zníženou incidenciou post-ischemicko-reperfúznych tachykardií a elektricky indukovanej VF, ako aj lepšou obnovou funkcie srdca. Na rozdiel od hypertenzných potkanov použitá dávka RPO mala za následok down-reguláciu mykoardiálneho Cx43 u nehypertenzných potkanov. To bolo sprevádzané nedostatočnou ochranou proti arytmiám a kardiodepresiou pravdepodobne v dôsledku predávkovania. Výsledky štúdie poukazujú na benefit suplementácie spontánne hypertenzných potkanov červeným palmovým olejom, ktorý sa prejavil lepším post-ischemickým zotavením srdca a ochranou pred malígnymi arytmiami. Na tomto kardioprotektívnom účinku sa môže podieľať up-regulácia myokardiálneho Cx43. Zdá sa, že konzumácia RPO by mohla byť užitočná pre hypertonikov, čo je potrebné otestovať v klinike. Tab. 1, Obr. 7, Lit. 37. Online full text (Free, PDF) www.cardiology.sk

Kľúčové slová: hypertenzný potkan – červený palmový olej – myokardiálny konexín-43 – malígne arytmie – funkcia srdca

It is well known that healthy diet and lifestyle are the cornerstones of cardiovascular disease prevention. This approach has created a growing interest in dietary red palm oil (RPO) research. In spite of its high level of saturated fatty acid content (50%), RPO intake has not been found to promote vascular disease. On the contrary, the benefits of RPO to health includes reduction in the risk of arterial thrombosis and/or atherosclerosis, inhibition of endogenous cholesterol biosynthesis and platelet aggregation, a reduction in oxidative stress and a reduction in blood pressure (1). Oxidative stress and the severity or progression of disease has stimulated further interest in the potential role of RPO (a cocktail of natural antioxidants) to improve redox status. The latter is probably due to the ratio of saturated (50%) to unsaturated fatty acids (40% mono- and 10% poly-unsaturated) and high concentration of antioxidants such as beta-carotene, tocotrienols, tocopherols and vitamin E (1, 2). Thus, the consumption of moderate amounts of palm oil and reduction in the level of oxidation may reduce the health risk believed to be associated with the consumption of palm oil. Recent experimental studies have demonstrated that dietary RPO supplementation offers protection against ischaemia- and/or reperfusion-related injury by improved cardiac output recovery (3 – 5). Evidence strongly suggests that improved heart function was due to modulation of cellular signalling via MAPKs, NO-cGMP, PI3-K and pro-survival PKB/Akt pathways (1, 3, 4, 6 – 9). Nevertheles, the other molecular mechanims involved in RPO-related cardioprotection have still not been fully elucidated. Moreover, data about antiarrhythmic effects of RPO supplementation are missing.

It has been demonstrated by our and other studies (10 – 14) that impaired cell-to-cell communication due to alterations in distribution and/or expression in myocardial intercellular connexin-43 (Cx43) promotes development of malignant arrhythmias and contractile dysfunction. Cardiac Cx43 channels at the gap junctions play a crucial role in synchronizing the myocardium allowing electrical and molecular signal propagation amongst cardiomyocytes (15). We have previously shown that up-regulation of Cx43 is most likely involved in the antiarrhythmic mechanisms of omega-3 fatty acids (16 – 18). Therefore, the purpose of this study was to explore whether RPO intake may offer protection from malignant arrhythmias and/or attenuate an abnormal myocardial Cx43 expression in spontaneously hypertensive rats.

Material and Methods

Animals monitoring and samples collections

All animal experiments were performed in accordance with the rules issued by the State Veterinary Administration of the Slovak Republic, legislation No 289/2003, and they conform to the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985). Male, three-month-old spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) fed a standard rat chow plus RPO (200microL/day) for 5 weeks were compared with untreated controls. Systolic blood pressure (BP) was measured non-invasively by tail-cuff plethysmography using the Statham Pressure Transducer P23XL (Hugo Sachs, Germany) at the beginning and the end of the experiment, and body weight (BW) was monitored as well. Fasting blood glucose (BG), triglycerides (TG) and cholesterol (CH) were registered at the end of the experiment using commercial kit from Biolab Diagnostics. The hearts were rapidly excised into ice-cold saline from the anaesthetized rats, whereby whole heart and left ventricular weights were registered.

Left ventricular tissue was used for real time PCR and immunoblotting to determine Cx43 mRNA and protein expressions as previously described (19). Left ventricular cryostat sections were used to detect in situ Cx43 distribution and quantitative image analysis (in details see 16). Isolated Langendorff-perfused rat heart was used for examination of post-ischemic reperfusion-induced arrhythmias, electrically inducible ventricular fibrillation (VF) and heart function.

Experimental protocol of isolated Langendorff-perfused heart

The heart was perfused via canulated aorta in Langendorff mode with oxygenated Krebs-Henseleit solution at constant pressure of 80mmHg and temperature of 37 ^{0}C. Left ventricular pressure (LVP) was measured isovolumetrically by a water-filled latex balloon, which was introduced into the left ventricle through the mitral orifice and connected to a pressure transducer. Bipolar ECG, LVP and the coronary flow (CF) were continuously monitored during experiment for the evaluation of cardiac arrhythmias and heart function. Left ventricular systolic function was assessed by recording LVP and the positive and negative first derivatives of LVP, +dp/dt (mmHg/s), -dp/dt (mmHg/s). The last are sensitive indices of contractile function with respect to the rate of increase and rate of decrease of intraventricular pressure, respectively. Upon 20 min of equilibration the heart was subjected to 30 min global ischemia followed by 5 min reperfusion to test incidence and duration of reperfusion-induced arrhythmias. Thereafter the susceptibility of the heart to electrically-induced sustained (2 min lasting) VF was examined and the ability of the heart to restore sinus rhythm was registered.

Statistical analysis

The data are expressed as means ± standard deviations (SD). One-way ANOVA followed by Duncan's post hoc test was used to analyze statistical significance between means. Comparison between the two groups was performed by the two-tailed Student's t-test. Values were considered to differ significantly when $p<0.05$.

Results

General characteristics of animals

RPO intake significantly reduced systolic BP by 13% in SHR and BG in both SHR and WKY rats by 20% and 14% respectively. There were no significant differences in plasma CH and TG between untreated and RPO-treated groups. Neither body and heart weights nor LVW/BW index was affected by RPO supplementation. Data are summarized in **Table 1**.

Myocardial Cx43-mRNA and protein expression and Cx43 distribution

Real time PCR showed that there is no difference in myocardial Cx43-mRNA level between WKY and SHR. However, RPO supplementation resulted in a significant increase of Cx43-mRNA in SHR unlike WKY rats, in which Cx43 gene transcription was suppressed **(Figure 1)**. Immunoblotting revealed three obvious forms of myocardial Cx43, i.e. two functional phosphorylated (P-Cx43) and one un-phosphorylated form in all examined rats **(Figure 2A).** Compared to WKY rats the total Cx43 expression as well as P-Cx43 was significantly reduced in untreated SHR **(Figures 2B, 2C)**. The RPO diets of WKY and SHR resulted in a differential response. There was a significant increase of both total and P-Cx43 in SHR heart ventricles, while these parameters were suppressed in WKY hearts **(Figures 2B, 2C)**. In ventricular tissues of WKY rats the immunostaining of Cx43 **(Figure 3)** was uniform and distributed predominantly at intercalated disk-related gap junctions (end-to-end orientation) and sporadically at the side-to-side oriented gap junctions. In contrast, an abnormal and heterogeneous distribution was

Table 1 Main characteristics of untreated and red palm oil treated rats

	WKYc	WKYrpo	SHRc	SHRrpo
BP (mmHg)	123.6±11,6	117,8±12,2	184,9±20,2 *	160,6±13,8 #
BW (g)	404,3±27,3	417,5±47,8	322,7±20,3*	321,3±25,1
HW (g)	1,25±0,09	1,21±0,16	1,31±0,1	1,23±0,1
LVW (g)	0,765±0,07	0,789±0,07	0,848±0,05	0,810±0.06
HW/BW (mg/g)	3,11±0.3	2,9±0,29	3,66±0,19 *	3,3±1,34
LVW/BW (mg/g)	2,15±0,19	1,92±0,18	2,63±0,14*	2,8±0,1
BG (mmol/l)	6,38±0,84	5,5±0,43*	5,6±1,13	4,48±0,24#
TG (mmol/l)	0,53±0,06	0,462±0,09	0,360±0,03 *	0,417±0,07
CHOL (mmol/l)	2,39±0,27	2,20±0,13	2,19±0,11	2,18±0,1

Values are means ± SD of 13 rats in each group.
WKYc – Wistar Kyoto control rats, WKYrpo – WKY rats supplemented with red palm oil, SHRc – SHR control rats, SHRrpo – SHR rats supplemented with red palm oil, BP – blood pressure, BW – body weight, HW – heart weight, LVW – left ventricular weight, BG – blood glucose, TG – triglycerides, CH – cholesterol. Significant difference from WKYc: * $p<0.05$, significant difference from SHRc: # $p<0.05$.

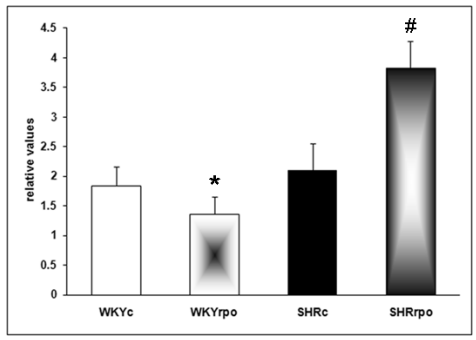

Figure 1 Cx43 mRNA expression normalized to actin in left ventricles of untreated and RPO-treated SHR and WKY rat hearts
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, *significant difference ($p<0.05$) from WKYc and # from SHRc.

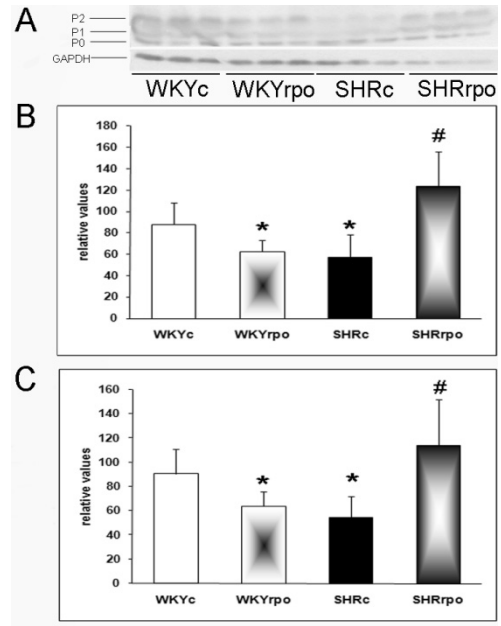

Figure 2 Representative immunoblot (A) showing three forms of Cx43 and densitometric quantification of total Cx43 expression (B), its phosphorylated form (C) normalized to GAPDH in left ventricles of untreated and RPO-treated SHR and WKY rat hearts
P0 – unphosphorylated, P1,P2 – phosphorylated forms of Cx43, WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, *significant difference ($p<0.05$) from WKYc and # from SHRc.

detected in SHR heart ventricle, characterized by enhanced lateral staining of Cx43-positive gap junctions and disordered intercalated disk-related staining when compared to WKY rats. This pattern of Cx43 distribution was partially eliminated in the RPO-treated SHR heart.

Incidence and duration of post-ischemic arrhythmias

Compared to WKY rats the SHR hearts exhibited significantly shorter duration of reperfusion-related bradycardia. On the contrary, duration of bradycardia was markedly prolonged due to RPO supplementation of SHR but was not in WKY rats **(Figure 4A)**. Furthermore, RPO intake almost eliminated reperfusion-induced ventricular tachycardia (VT) in SHR while it did not affect it in WKY rat hearts **(Figure 4B)**. There was no difference in the incidence of electrically-inducible sustained VF between WKY and SHR. However, more stimuli for induction of sustained (two min lasting) VF was needed in WKY compared to SHR (not shown). RPO diet resulted in a clearcut reduction of sustained VF occurrence in SHR and to a lesser extent in WKY rats **(Figure 5A)**. Moreover, stop-flow-induced reversion of sustained VF into sinus rhythm was significantly faster in the hearts of RPO supplemented animals, namely in SHR and to a lesser extent in WKY rats **(Figure 5B)**.

Post-ischemic heart function

HR was lower in SHR compared to WKY under basal condition and RPO supplementation supressed it markedly in WKY, though not in SHR **(Figure 6A)**. Moreover, RPO intake caused a significant decline of HR during postischemic reperfusion in SHR (by 42% in SHR) but not in WKY. Furthermore, RPO enhanced CF significantly during reperfusion in both WKY and SHR by 39% and 43% **(Figure 6B)**. There was

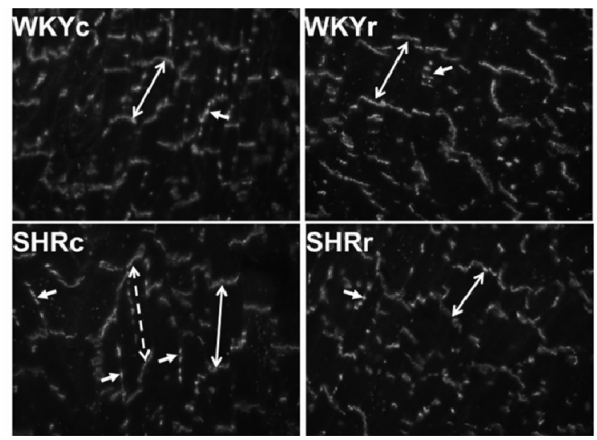

Figure 3 Representative images of Cx43 immunolabelling in the left ventricles of untreated and RPO-treated WKY and SHR rat hearts. Note conventional distribution of Cx43-positive gap junctions predominantly at the intercalated discs (thin arrows) and sporadically on lateral surfaces (thick arrows) of the cardiomyocytes in both groups of WKY rats. The lateralization is enhanced in SHR hearst that, in addition, exhibit disordered expression of Cx43 at the intercalated disc (broken arrow). RPO diet partially eliminated abnormal Cx43 distribution in the SHR heart (SHRr). Magnification, objective 40x.

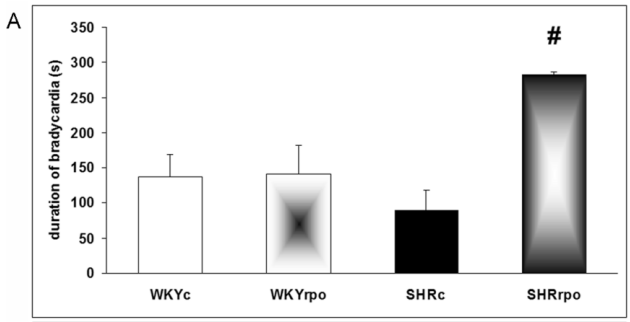

Figure 4A Duration of postischemic repefusion-induced bradycardia in untreated and RPO-treated SHR and WKY rat hearts
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, # significant difference (p<0.05) from SHRc.

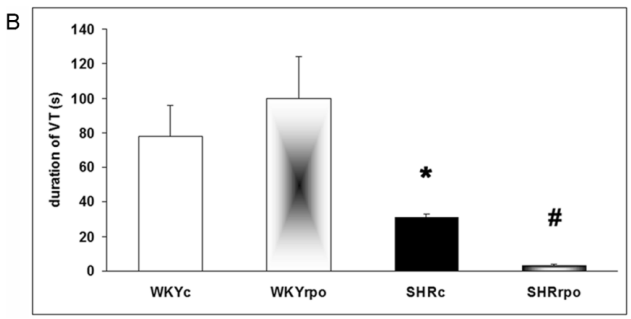

Figure 4B Duration of postischemic repefusion-induced ventricular tachycardia (VT) in untreated and RPO-treated SHR and WKY rat hearts
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, *significant difference (p<0.05) from WKYc and # from SHRc.

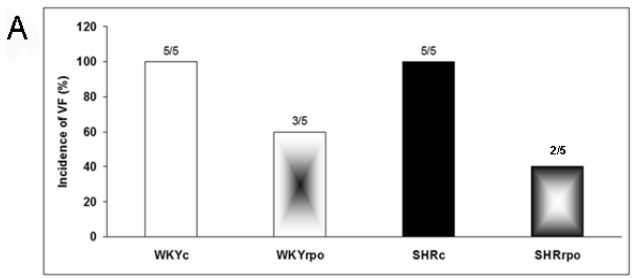

Figure 5A Incidence of electrically-induced ventricular fibrillation (VF) in untreated and RPO-treated SHR and WKY rat hearts
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Number of fibrillating hearts to total number of hearts per group are demonstrated.

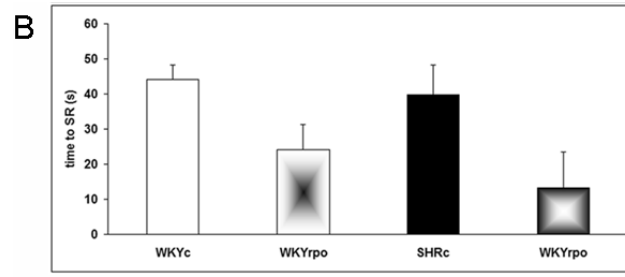

Figure 5B Time to reversion of VF into sinus rhythm of untreated and RPO-treated SHR and WKY rats
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, *significant difference (p<0.05) from WKYc and # from SHRc.

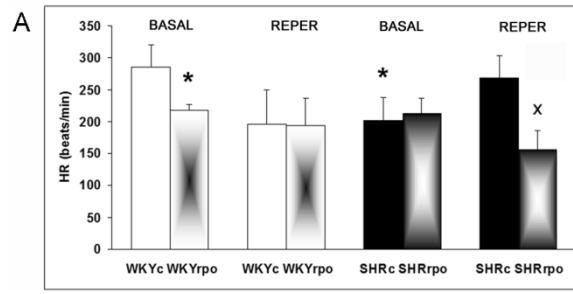

Figure 6A Heart rate in untreated and RPO-treated SHR and WKY rat hearts during basal condition and postischemic reperfusion
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, *significant difference (p<0.05) from WKYc basal condition and x from SHRc during postischemic reperfusion.

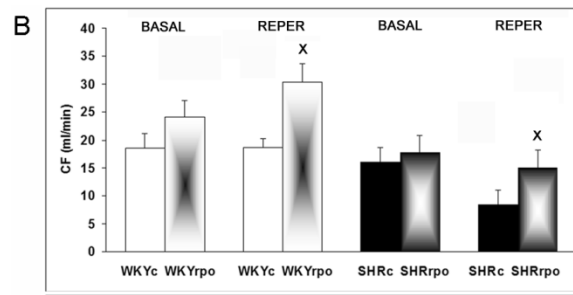

Figure 6B Coronary flow in untreated and RPO-treated SHR and WKY rat hearts during basal condition and postischemic reperfusion
WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, x significant difference (p<0.05) from WKYc and SHRc during postischemic reperfusion.

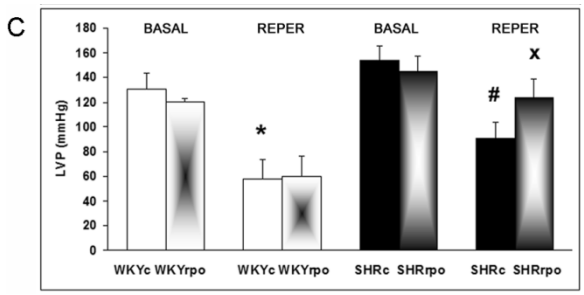

Figure 6C Left ventriclular pressure in untreated and RPO-treated SHR and WKY rat hearts during basal condition and postischemic reperfusion

WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, x significant difference ($p<0.05$) from SHRc during postischemic reperfusion, *significant difference ($p<0.05$) from WKYc basal condition, # significant difference ($p<0.05$) from SHRc basal condition.

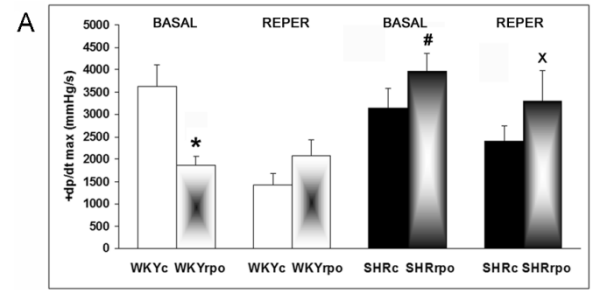

Figure 7A Contractile function assessed by (+)dp/dt max in untreated and RPO-treated SHR and WKY rat hearts during basal condition and postischemic reperfusion

WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, x significant difference ($p<0.05$) from SHRc during postischemic reperfusion, *significant difference ($p<0.05$) from WKYc basal condition, # significant difference ($p<0.05$) from SHRc basal condition.

a differential strain-related response to RPO supplementation on the parameters of contraction and relaxation. Suppresion of +dp/dt max was observed in the RPO-treated WKY rat heart under basal conditions and there was no change during postischemic reperfusion **(Figure 7A)**. In contrast, RPO supplementation enhanced this parameter under basal conditions as well as during postischemic reperfusion in the SHR heart **(Figure 7A)**. Index of relaxation, -dp/dt max, was supressed in RPO-treated WKY but not SHR heart under basal contition and enhanced during postischemic reperfusion in the SHR heart only **(Figure 7B)**.

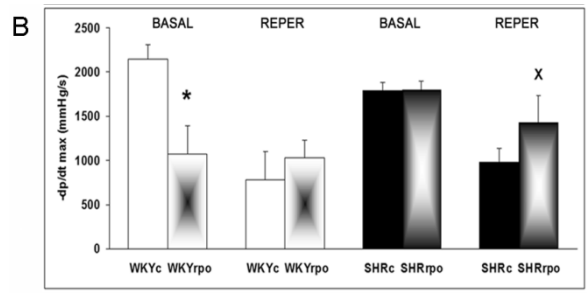

Figure 7B Contractile function assessed by (-)dp/dt max in untreated and RPO-treated SHR and WKY rat hearts during basal condition and postischemic reperfusion

WKYc – untreated WKY rats, WKYrpo – WKY rats treated with red palm oil, SHRc – untreated SHR, SHRrpo – SHR treated with red palm oil. Results are mean ± SD, x significant difference ($p<0.05$) from SHRc during postischemic reperfusion, *significant difference ($p<0.05$) from WKYc basal condition.

Discussion

We have demonstrated in this study, for the first time, that RPO supplementation protects SHR heart from life-threatening arrhythmias and post-ischemic reperfusion-related dysfunction. This was linked with up-regulation of myocardial Cx43. In addition, RPO supplementation reduced blood pressure in SHR and blood glucose in both SHR and WKY rats. The cardioprotective effect of RPO intake in healthy WKY rat heart was, however, less pronounced and most likely related to down-regulation of Cx43.

We used SHR as a model to mimic human essential hypertension, known to be associated with increased risk for arrhythmias due to structural remodelling of the heart. Left ventricular hypertrophy (LVH), i.e. increase of the cardiomyocyte size and consequently myocardial muscle mass, can be considered as an adaptive process serving to compensate for heamodynamic load. However, LVH is accompanied by abnormal distribution of Cx43, which can contribute to the development of arrhythmogenic substrate (16, 17, 18, 20, 21). Indeed, as we have shown in this study and previous studies (22 – 24) LVH in SHR was accompanied by down-regulation of gap junction Cx43 as well as by its abnormal topology (remodelling). This was associated with increased susceptibility of the heart to malignant VT or VF. In course of time LVH in humans, as well as SHR, has become a disease process (including displacement fibrosis) with endpoints such as acute myocardial infarction, heart failure and/or sudden cardiac death (25, 26). Noteworthily, this process includes the deterioration of myocardial Cx43 mediated cell-to-cell communication, contributing not only to the occurrence of life-threatening arrhythmias, but also heart failure (20, 21, 23, 27). Although it is difficult to prove that altered Cx43 expression causes arrhythmias and heart failure the substantial

evidence, including Cx43 knockout models in mice, indicates that Cx43 remodelling plays an important pathogenic role (13, 16, 28, 29). Results of this study further support this view.

Despite the progress in management of hypertension by prevention or regression of myocardial remodelling there is still the need for novel therapeutic targets and approaches for cardiovascular risk reduction. Diet is very important in controlling blood lipids, in which the amount and composition of fats present in a diet is one of the factors that plays a role in the regulation of BP and incidence of hypertension. Accordingly, saturated fats and oxidized oil cause an increase in BP, while ingestion of unsaturated fats has the opposite effect. Palm oil does not contain much lauric acid, which is known to raise plasma lipid saturated fatty acid levels (1). It has been shown that palm oil diets containing 15% (w/w) RPO for 18 weeks did not raise BP (30). We have demonstrated both antihypertensive and hypoglycemic effects resulting from short-term RPO supplementation. The latter suggests that RPO may affect insulin resistance and/or glucose metabolism.

Noteworthily, feeding of SHR with RPO for five weeks resulted in significant increase of both myocardial Cx43-mRNA and Cx43 protein levels. Up-regulation of Cx43 has also been recently found due to omega-3 fatty acids supplementation of spontaneously diabetic rats (31). Various cardioprotective drugs and fatty acids (e.g. statins, sartans, antidiabetics and omega-3 FA) are ligands for transcription factor, peroxisome proliferator-activated receptor (PPAR), and consequently activate specific gene expression (32 – 34). Accordingly, we hypothesize that some compound present in RPO may, via PPAR, affect Cx43 gene transcription. Indeed, direct transcriptional activation of Cx43 mRNA by carotenoids (components of RPO) has been reported in an experimental model of cancer (35). Further study should prove this hypothesis. Perhaps it might also elucidate why myocardial mRNA was decreased in normotensive rats after RPO intake. Indeed, our results point out that RPO supplementation in dose 200microL/day for 5 weeks was beneficial for hypertensive but not normotensive rats. The dose-response effects of tocotrienols have been recently shown (8). Thus, it may be argued that that "overdose" of antioxidants may have undesirable effects in healthy individuals and that the "treatment" dose of RPO may be adverse for healthy subjects.

Furthermore, we have demonstrated that not only total but also phosphorylated forms of Cx43 were enhanced due to RPO supplementation in SHR heart ventricle, while both were suppressed in WKY rats. In this context it should be noted that turnover and function of Cx43, including channel gatings are controlled by phosphorylation (reviewed in 11). In the normal heart, Cx43 is phosphorylated at multiple sites, resulting in electrophoretic migration at 45 kDa, while fully dephosphorylated Cx43 at 41 kDa. Factors and conditions altering the phosphorylation pattern of Cx43 can also alter its properties and consequently affect both heart function and its susceptibility to arrhythmias. Our results are consistent with the study showing that up-regulation of relative levels of phosphorylated Cx43 was associated with preservation of Cx43 signalling and cardioprotection (36). Thus, it is most likely that myocardial up-regulation of Cx43 due to RPO supplementation of SHR confers cardioprotection. In turn, the latter was much less pronouced in normotensive rats in which RPO supplementation down-regulated Cx43 (probably due to overdose). Likewise as in RPO supplementation, omega-3 fatty acids intake improved Cx43 phosphorylation in SHR hearts. This was associated with an antiarrhythmic effect, while deficient phosphorylation of Cx43 and/or its abnormal distribution has been shown proarrhythmic (16, 18).

Furthermore, in situ immunolabelling of Cx43 revealed that its abnormal distribution was attenuated in the left ventricle of SHR fed with RPO. Similarly, omega-3 FA and aliskiren restored normal localization of Cx43 to the intercalated disks, enhanced Cx43 expression and attenuated electrical remodelling and arrhythmia induction in a transgenic rat model of high renin hypertension (37). Altogether, the attenuation of abnormal Cx43 expression and distribution indicate that RPO intake can be beneficial for myocardial cell-to-cell communication in the SHR heart to improve its synchronization. This may explain, at least partially, reduction of electrically-induced sustained VF and duration of post-ischemic reperfusion-induced VT. In addition, up-regulation of Cx43 in SHR most likely contributed to enhanced ability of the heart to terminate VF and to restore sinus rhythm. The link between up-regulation of Cx43 and decreased propensity of the heart to malignant arrhythmias is supported by the fact that down-regulation of Cx43 in normotensive WKY rats was associated with much lesser arrhythmia protection. It is noteworthy that RPO-related up-regulation of myocardial Cx43 and improvement of its normal distribution (in SHR), unlike its down-regulation (in WKY), was linked with improved functional recovery, improved CF and increased contraction and relaxation during reperfusion.

Taken together, the results indicate that SHR benefit from RPO supplementation, particularly due to its apparent anti-arrhythmic and postischemic-reperfusion-related cardioprotective effects that can be, in part, explained by up-regulation of myocardial Cx43. Moreover, it appears that RPO intake should be adjusted according to either treatment or prevention of heart disease.

Clinical implication

Findings of this study support the idea that RPO supplementation, like omega-3 fatty acids intake, in combination with antihypertensive drugs may be a novel strategy to reduce risk from malignant arrhythmias and heart failure in patients suffering from hypertension.

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