

Aerobic exercise increases connexin43 expression in rat cardiac muscle

Fransisca Chondro*, Minarma Siagian**, and Dewi IS Santoso**

ABSTRACT

BACKGROUND

Intercellular communication in cardiac muscle is effected by connexin, particularly connexin43 (Cx43), forming gap junctions in cardiomyocytes. Aerobic physical exercise may result in increased left ventricular size and function. The purpose of the present study was to assess the effect of aerobics and detraining on C43 expression and distribution in rats.

METHODS

This was an in vivo experimental study on 32 young male Wistar rats. The animals were divided into the following 8 groups and their interventions: I : 4 weeks of aerobics (A4); II: exercise control for 4 weeks (C4); III: 4 weeks of aerobics plus 4 weeks of detraining (A4D4); IV: 8-week control (C8); V: 12 weeks of aerobics (A12); VI: 12-week control (C12); VII: 12 weeks of aerobics plus 4 weeks of detraining (A12D4); VIII: 16-week control (C16). Rat cardiac tissue was subjected to immunohistochemical assay to determine total Cx43, intercalated disc Cx43, and lateral Cx43. Independent t-test was used to compare all Cx43 parameters between control and treatment groups.

RESULTS

After aerobics, there were significant differences in total Cx43, intercalated disc Cx43, lateral Cx43, and intercalated disc Cx43 and lateral Cx43 percentages between control and treatment groups ($p < 0.05$). Between-group comparison in treatment groups did not find significant differences between exercise-only groups and groups with exercise plus detraining. Cx43 surface area tended to decrease after detraining.

CONCLUSIONS

Aerobics significantly increases Cx43 levels in rat hearts. Detraining decreases intercalated disc Cx43 and lateral Cx43.

Key words: Connexin43, gap junction, lateralization, aerobic physical exercise, rat.

*Department of Physiology,
Faculty of Medicine,
Trisakti University
**Department of Physiology,
Faculty of Medicine,
University of Indonesia

Correspondence

dr. Fransisca Chondro
Department of Physiology,
Faculty of Medicine,
Trisakti University,
Jl. Kyai Tapa No.260
Grogol - Jakarta
Phone : +6221-5672731
ext. 2804
Email:
fransisca.chondro@gmail.com

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Latihan fisik erobik meningkatkan ekspresi protein connexin43 pada sel otot jantung tikus

ABSTRAK

LATAR BELAKANG

Komunikasi antar sel otot jantung terjadi dengan bantuan protein connexin, terutama connexin43 (Cx43), yang merupakan protein utama penyusun gap junction pada sel otot jantung. Latihan fisik erobik dapat mengakibatkan peningkatan ukuran dan kerja ventrikel kiri. Penelitian ini bertujuan untuk menilai pengaruh latihan fisik erobik dan detraining terhadap ekspresi dan distribusi protein C43 pada tikus.

METODE

Studi eksperimental secara *in vivo* pada 32 ekor tikus jantan dewasa muda strain Wistar. Subjek penelitian dibagi menjadi 8 kelompok, yakni : kelompok I : tikus diberi perlakuan erobik selama 4 minggu; II kontrol 4 minggu; III erobik 4 minggu + detraining 4 minggu; IV kontrol 8 minggu; V erobik selama 12 minggu; VI kontrol 12 minggu; VII erobik 12 minggu + detraining 4 minggu; dan VIII kontrol 16 minggu. Pada organ jantung tikus dilakukan pemeriksaan imunohistokimia untuk mengukur kadar total Cx43, Cx43 diskus interkalaris, dan Cx43 lateral. Uji *t*-independen digunakan untuk membandingkan semua parameter Cx43 antara kedua kelompok perlakuan.

HASIL

Setelah diberikan latihan erobik, terdapat perbedaan bermakna pada parameter total Cx43, Cx43 diskus interkalaris, Cx43 lateral, dan presentase Cx43 diskus interkalaris dan lateral antara kedua kelompok perlakuan ($p < 0,05$). Pada perbandingan antara kelompok perlakuan, tidak terdapat perbedaan bermakna antara kelompok yang diberi latihan fisik saja dengan kelompok yang diberi latihan fisik dan detraining. Namun terjadi kecenderungan terjadi penurunan luas area Cx43 pada pemberian detraining.

KESIMPULAN

Latihan fisik erobik meningkatkan kadar Cx43 secara bermakna pada organ jantung tikus. Proses detraining mengakibatkan penurunan Cx43 diskus interkalaris dan lateral..

Kata Kunci : Connexin43, gap junction, lateralisasi, latihan fisik erobik, tikus

INTRODUCTION

In the human heart there are around 2 to 3 milliard cardiac muscle cells. The functioning of the heart is extremely complex. One of the factors enabling the functioning of the heart is intercellular communication, e.g. via gap junctions. Communication between cardiac muscle cells occurs through gap junction channels, allowing the rapid transmission of impulses and the synchronous contraction of the heart as a whole. Therefore the maintenance of gap junction functions is essential to guarantee

the normal functioning of the heart.^(1,2) The gap junction channels of the ventricular myocardium (especially in the intercalated discs) are formed mainly by connexin43 (Cx43), allowing electrical coupling and communication between adjacent cardiomyocytes.⁽³⁾ In postnatal development of the ventricle, the connexin proteins of the gap junctions, which in the prenatal period are laterally located, accumulate at the intercalated discs together with desmosomes and adherens junctions. The mean size of the gap junctions also increases with the postnatal ventricular development.^(4,5)

One of the characteristic cardiac structural abnormalities is the lateral redistribution of Cx43 proteins and decreased Cx43 protein expression. Decreased colocalization of Cx43 with *zonula occludens-1* (ZO-1) proteins and cadherins, and morphologic heterogeneity of the lateralized gap junctions indicates the occurrence of changes in their structure and function.⁽⁶⁾ Decreased Cx43 expression results in increased resistance at the gap junction, decreased transmission velocity of electrical impulses, and increased duration of action potential formation, all three of which lead to arrhythmia.⁽⁷⁾

The study by Molmen-Hansen et al. showed that regular high-intensity physical exercise is necessary to induce changes in substrate and energy source utilization by the heart.⁽⁸⁾ High-intensity physical exercise also increases mitochondrial respiration in the cardiomyocytes. High-intensity physical exercise is expected to induce cardiac adaptations in the form of increases in contractility, glucose oxidation, and mitochondrial functions.⁽⁹⁾ At the onset of hypertrophy, the amount of regulated connexin is increased. However, in longstanding hypertrophy with impending heart failure, the amount of connexin is decreased. To date the precise mechanism and the signalling pathway regulating connexin expression is still not known with certainty. Kostin et al. found that Cx43 expression is increased in the compensated hypertrophic stage, but decreases and becomes heterogeneous in decompensation.⁽¹⁰⁾ The decreased Cx43 protein expression is accompanied by decreased Cx43 mRNA, so that the decreased Cx43 expression presumably involves transcription factors.⁽¹¹⁾

Aerobic physical exercise results in two types of stress, i.e. oxidative stress and vascular strain. To date there have been a few studies to investigate the effect of aerobic physical exercise on the expression of connexin43. In the study conducted by Bearden et al., the investigators came to the conclusion that chronic physical exercise does cause changes

in connexin43 mRNA expression. However, it could not be concluded yet that chronic physical exercise causes changes in connexin protein expression.⁽¹²⁾

In view of the inconsistent study results, the present study was carried out to evaluate the effect of aerobic physical exercise and detraining on Cx43 protein expression in rat myocardiocytes.

METHODS

Design of the study

An experimental study conducted from November 2012 until March 2013 at the Pathological Anatomy Laboratory, University of Indonesia.

Subjects of the study

The subjects in this study consisted of young adult male Wistar rats weighing 150–250 grams, 8 weeks old at the start of the study, healthy, and fit to participate in the aerobic physical exercise program. The sample size was determined with the Federer formula: $(t-1)(n-1) \geq 15$, where t = number of experimental groups (8); n = number of animals per group. The calculated sample size was 4 animals per group, giving a total of 32 animals.

Intervention

The study subjects were divided into 8 groups, consisting of 4 control groups (coded C4, C8, C12, C16) and 4 treatment groups (A4, A12; A4D4, A12D4). The treatment groups were subjected to a single daily aerobics exercise of 20 minutes, at a treadmill velocity of 20 m/minute, with a rest period of 90 seconds every 5 minutes. As to the control exercise program, the animals were also put into the treadmill for 20 minutes, but with the treadmill inactive. In the detraining phase, the aerobics exercise program was stopped for a given period, during which the animals were left to themselves in their cages, without being placed in the treadmill. The treatment schema was as

follows: group I: 4 weeks of aerobics only (A4); group II: 4 weeks of control (C4); group III: 4 weeks of aerobics plus 4 weeks of detraining (A4D4); group IV: 8 weeks of control (C8); group V: 12 weeks of aerobics (A12); group VI: 12 weeks of control (C12); group VII: 12 weeks of aerobics plus 4 weeks of detraining (A12D4); group VIII: 16 weeks of control (C16).

Cx43 immunohistochemical assay

Detection of Cx43 protein in the intercalated discs and the lateral domains of cardiomyocytes was done by immunohistochemical assay. Paraffin-embedded rat cardiac tissue blocks were sectioned with a microtome and mounted on glass slides. The sections were then deparaffinized by immersing in xylol three times, each time for 5 minutes. Rehydration was done by immersion in graded alcohols (absolute, 96%, and 80%), for 4 minutes each. The rehydrated tissue sections were washed under running water for 5 minutes. Subsequently, endogenous peroxides were blocked with 0.5% H₂O₂ in methanol for 30 minutes. After again washing the slides under running water for 5 minutes, they were subjected to pretreatment with citrate buffer in a microwave oven and left to cool. The cooled slides were washed in phosphate buffered saline (PBS) at pH 7.4 for 5 minutes, followed by Background Sniper protein blocker (Biocare Medical) using donor horse serum 3% solution for 30 minutes at room temperature. After 30 minutes, the donor horse serum 3% solution was drained, and to the slides was added 200 μ l primary anti-connexin43/GJA1 antibody (Abcam Ab11370) at a dilution of 1/5000, and the slides incubated at room temperature for 1 hour. Then the slides were washed in PBS at pH 7.4 for 5 minutes, and 4 drops of Trekkie Universal Link biotinylated secondary antibody (part of Starr Trek detection system from Biocare Medical) was added to the slides, which were then incubated for 15 minutes at room temperature. Thereafter the slides were washed in PBS at pH 7.4 for 5 minutes, dried, and 5 drops of

horseradish peroxidase (HRP) labeled-streptavidin (TrekAvidin-HRP Label, Biocare Medical) were added and the slides again incubated at room temperature for 15 minutes. After washing in PBS at pH 7.4 for 5 minutes, the slides were dried and 200 μ l of chromogen (diaminobenzidine solution) was added to each slide and left to react for 2 minutes. After 2 minutes, the slides were washed under running water for 10 minutes, then counterstained with hematoxylin for 1 minute, followed by washing under running water for 5 minutes, to be reacted with a saturated solution of lithium carbonate (5% in distilled water) for 1 minute, and again washed under running water for 3 minutes. This was followed by dehydration of the slides in graded alcohols (80%, 96%, absolute) for 5 minutes each. The slides were cleared three times with xylol, for 5 minutes each. Entellan mounting fluid was added and the slides provided with a coverslip.

Image processing using ImageJ program

The immunohistochemical slides were subsequently examined under the light microscope, photographed, and the photographic images processed using the ImageJ program. For Cx43, all images were recorded using the same settings and the pixel intensities were quantified using ImageJ software. The whole surface area occupied by the Cx43 protein in the cardiomyocytes was calculated to determine the total Cx43 area and the intercalated disc Cx43 and lateral domain Cx43 (expressed in pixels).

Statistical analysis

For subgroup comparison of control versus treatment groups, data analysis was performed using the independent t-test, while for overall comparison of all control and treatment groups (C4, C12, C16, A4, A12, A4D4, A12D4), the data were analyzed by means of one-way Anova. The analysis was done at a significance level of 95% ($\alpha=0.05$), and differences were considered significant at $p<0.05$.

Ethical clearance

The protocol for the present study was approved by the Ethical Committee, Faculty of Medicine, University of Indonesia.

RESULTS

Initially the Cx43 levels of the 4-week aerobic exercise control group (C4) versus the treatment group (A4) were compared, as was also done with Cx43 levels of C8 and A4D4 (treatment group with 4 weeks of aerobics followed by 4 weeks of detraining) (Table 1). From the results it was concluded that there were significant differences between control and treatment groups with regard to levels of total Cx43, intercalated disc Cx43, lateral Cx43, and lateral Cx43 percentage. There was a 16.1% reduction in total Cx43 levels in group A4D4, in comparison with group A4 (4 weeks of aerobics without detraining) (Table 1).

After 12 weeks of aerobics and 12 weeks of aerobics followed by 4 weeks of detraining, there were significant differences in Cx43, intercalated disc Cx43, lateral Cx43, and intercalated disc Cx43 and lateral Cx43 percentages (Table 2).

After 12 weeks of aerobics followed by 4 weeks of detraining, there was a 7.1% decrease in total Cx43 levels, in comparison with Cx43 levels in the group performing only aerobics for 12 weeks.

In the comparison of the 4 control groups for total Cx43, there was only a significant difference between 8 weeks and 12 weeks of control exercise, at $p=0.002$ ($p<0.05$). Similarly for intercalated disc Cx43, there was only a significant difference between 8 weeks and 12 weeks of control exercise, at $p=0.001$ ($p<0.05$). For the parameters of lateral Cx43, intercalated disc Cx43 and lateral Cx43 percentages, there were no significant differences between the 4 control groups.

Regarding the between-group comparison of treatment groups, a comparison was carried out between group A4 and group A4D4. Also compared were group A12 and A12D4, as well as group A4 and group A12D4. For the parameter of total Cx43, a significant difference was found between group A4 and group A4D4. Total Cx43 between groups with A4 and A12 did not show significant differences (Table 3).

Table 1. Comparison of Cx43 levels between control and treatment groups after 4 weeks of aerobic physical exercise and 4 weeks of detraining

Parameter	Control group (n=4)	Treatment group (n=4)	p
After 4 weeks of aerobics	C4	A4	
Total Cx43 (pixels)	77006.35 ± 15027.75	173700.70 ± 21705.94	0.000
Intercalated disc Cx43 (pixels)	67944.83 ± 13249.02	136178.60 ± 15222.46	0.001
Lateral Cx43 (pixels)	9061.53 ± 1880.24	37522.10 ± 6820.15	0.001
Intercalated disc Cx43 (%)	88.24 ± 0.67	78.51 ± 1.50	0.000
Lateral Cx43 (%)	11.76 ± 0.67	21.49 ± 1.50	0.000
After 4 weeks of aerobics + 4 weeks of detraining	C8	A4D4	
Total Cx43 (pixels)	95007.85 ± 10294.72	145865.75 ± 17121.05	0.002
Intercalated disc Cx43 (pixels)	85461.63 ± 9417.55	117203.05 ± 17253.68	0.018
Lateral Cx43 (pixels)	9546.23 ± 1041.34	28662.70 ± 3876.59	0.000
Intercalated disc Cx43 (%)	89.93 ± 0.76	80.14 ± 3.51	0.011
Lateral Cx43 (%)	10.07 ± 0.76	19.86 ± 3.51	0.013

Cx = connexin; C4 = 4 weeks of control; A4 = 4 weeks of aerobics only ; A4D4 = 4 weeks of aerobics plus 4 weeks of detraining; C8 = 8 weeks of control

Table 2. Comparison of Cx43 levels between control and treatment groups after 12 weeks of aerobic physical exercise and 4 weeks of detraining

Parameter	Control group (n=4)	Treatment group (n=4)	p
After 12 weeks of aerobics	C12	A12	
Total Cx43 (pixels)	51726.68 ± 4418.09	169143.95 ± 21631.00	0.001
Intercalated disc Cx43 (pixels)	46369.15 ± 4683.57	140158.55 ± 17260.96	0.001
Lateral Cx43 (pixels)	5357.53 ± 332.62	28975.40 ± 6221.87	0.005
Intercalated disc Cx43 (%)	89.56 ± 1.43	82.94 ± 2.67	0.005
Lateral Cx43 (%)	10.44 ± 1.43	17.06 ± 2.67	0.005
After 12 weeks of aerobics and 4 weeks of detraining	C16	A12D4	
Total Cx43 (pixels)	75254.33 ± 15525.45	157285.10 ± 25728.50	0.002
Intercalated disc Cx43 (pixels)	67070.38 ± 14344.88	136530.15 ± 21759.99	0.002
Lateral Cx43 (pixels)	8183.95 ± 1382.24	20754.95 ± 4117.04	0.001
Intercalated disc Cx43 (%)	89.02 ± 1.14	86.86 ± 0.72	0.018
Lateral Cx43 (%)	10.98 ± 1.14	13.14 ± 0.72	0.018

Cx : connexin

C12 : 12 weeks of control; A12 : 12 weeks of aerobics only; C16 : 16 weeks of control; A12D4: 12 weeks of aerobics plus 4 weeks of detraining

DISCUSSION

From the results of an overall comparison between treatment groups and control groups, it may be concluded that although the experimental animals were of the same age, physical exercise had a substantial effect on the amount of Cx43 protein present in their cardiac tissues. In all aerobic physical exercise groups, there was an increase in the amounts of total Cx43, intercalated disc Cx43, and lateral Cx43, as well as an increase in lateral Cx43 percentage. For intercalated disc Cx43 percentage, lower values were found in the treatment groups than in the control groups. This reduction in intercalated disc Cx43 percentage may be due to the fact that although there was an increase in the amount of intercalated disc Cx43, there was also a significant increase in the amount of lateral Cx43, leading to a far greater increase in total Cx43. The intercalated disc Cx43 percentage was obtained by comparing intercalated disc Cx43 and total Cx43, and because the increase in total Cx43 was far higher than the increase in disc Cx43, the calculated Cx43 percentage was lower. Therefore, in spite of decreased

intercalated disc Cx43 percentage, there was an increase in the amounts of intercalated disc Cx43. This finding is in agreement with the results of the study by Bao et al.⁽¹²⁾ in vascular smooth muscle and endothelial cell cultures, stating that stress from aerobic physical exercise causes increases in Cx43 mRNA and protein. However, this is in contrast with the view of Bearden et al.⁽¹³⁾ who state that chronic physical exercise does not cause changes in Cx43 mRNA expression and that it cannot be concluded that chronic physical exercise causes changes in connexin protein expression.

From the subgroup comparison of all control groups by one-way Anova, it may be concluded that age differences between the control groups did not lead to significant differences in the amounts of lateral Cx43, intercalated disc Cx43 percentages, and lateral Cx43 percentages. Although significant age differences were found between total Cx43 and intercalated disc Cx43, these were present only in some groups, namely in the comparison between the groups with 8 weeks and 12 weeks of aerobics. Therefore it cannot be concluded that increasing age leads to increased Cx43 protein. This does not agree with the findings

Table 3. Analysis of variance and multiple comparisons of Cx43 levels between all control and treatment groups

	C4	C8	C12	C16	A4	A4D4	A12	A12D4	P
Total Cx43	77006,35 ±	95007,85 ±	51726,68 ±	75254,33 ±	173700,7 ±	145865,75 ±	169143,95 ±	157285,1 ±	
	15027,75 ^{abc} ±	10194,72 ^{cd} ±	4418,09 ^{abc} ±	15525,45 ^{abc} ±	21705,94 ^{abcd} ±	17121,05 ^{abcd} ±	21631 ^{abcd} ±	25728,5 ^{abcd} ±	0,000
Cx43 Diskus	67944,83 ±	85461,62 ±	46369,15 ±	67070,38 ±	136178,6 ±	117203,05 ±	140158,55 ±	136530,15 ±	
interkalate d	13249,02 ^{abc} ±	9417,55 ^{cd} ±	4683,57 ^{abc} ±	14344,88 ^{abc} ±	15222,46 ^{abcd} ±	17253,68 ^{abcd} ±	17260,96 ^{abcd} ±	21759,99 ^{abcd} ±	0,000
Cx43 lateral	9061,53 ±	9546,23 ±	5357,53 ±	8183,95 ±	37522,1 ±	28661,7 ±	28985,4 ±	20754,95 ±	
	1880,24 ^{abc} ±	1041,34 ^{cd} ±	332,62 ^{abc} ±	1382,24 ^{abc} ±	6820,15 ^{abc} ±	2876,59 ^{abcd} ±	6221,87 ^{abc} ±	4117,04 ^{abc} ±	0,000
Cx43 diskus	88,24 ±	89,93 ±	89,56 ±	89,02 ±	78,51 ±	80,14 ±	82,94 ±	86,86 ±	
interkalate d (%)	0,67 ^{abc} ±	0,76 ^{bc} ±	1,43 ^{bc} ±	1,14 ^{abc} ±	1,5 ^{abcd} ±	3,51 ^{abc} ±	2,67 ^{abc} ±	0,72 ^{abc} ±	0,000
Cx43 lateral (%)	11,76 ±	10,07 ±	10,44 ±	10,98 ±	21,49 ±	19,86 ±	17,06 ±	13,14 ±	
	0,67 ^{abc} ±	0,76 ^{bc} ±	1,43 ^{bc} ±	1,14 ^{abc} ±	1,5 ^{abcd} ±	3,51 ^{abc} ±	2,67 ^{abc} ±	0,72 ^{abc} ±	0,000

Cx : connexin

A4 : 4 weeks of control; A4D4 : 4 weeks of aerobics plus 4 weeks of detraining;; C8 : 8 weeks of control; A12 : 12 weeks of aerobics only;C12 : 12 weeks of control; A12D4 : 12 weeks of aerobics plus 4 weeks of detraining; C16 : 16 weeks of control. The difference in superscripts indicates a significant difference from the control (p<0.05)

of Barker et al., who stated that in post-natal development of the cardiac ventricles there is an increase in intercalated disc Cx43.⁽¹⁴⁾

On further consideration of the data on intercalated disc Cx43 and lateral Cx43 percentages, although no statistically significant differences were found between control groups, intercalated disc and lateral Cx43 values in the control tended to be stable. Therefore we may conclude that increasing age of the experimental animals in the control groups did not result in significant Cx43 differences between the control groups.

Comparison of the treatment groups was limited to find any effects of duration of physical exercise and detraining, therefore only the following pairs of groups were compared: groups A4 and A12; groups A4 and A4D4; and groups A12 and A12D4. In the comparison of groups A4 and A12 to find any effect of exercise dose on amount of Cx43, no significant differences were found between all parameters. After 12 weeks of aerobics plus 4 weeks of detraining, the reduction in total Cx43 (7.1%) was lower than after 4 weeks of aerobics plus 4 weeks of detraining (16.1%). With increasing duration of exercise, there was a proportionally diminishing effect of detraining on total Cx43 levels. The data also show that 12 weeks of aerobic physical exercise results in increased intercalated disc Cx43 in comparison with 4 weeks of exercise, so that we may conclude that long-term aerobics is very beneficial for health. In the comparisons to evaluate the effect of detraining on aerobic physical exercise, significant differences were found only in the comparison of total Cx43 between group A4 and A4D4. For the parameters intercalated disc Cx43 protein, lateral Cx43, intercalated disc Cx43 percentage, and lateral Cx43 percentage, no significant between-group differences were found. On the basis of these data it may be concluded that the 4-week detraining process after 4 weeks of aerobic physical exercise yielded significant differences in the amounts of total Cx43. This is consistent with the studies

by Benito et al.⁽¹⁵⁾ and Pelliccia et al.⁽¹⁶⁾ who stated that the cardiac structural and functional changes occurring during the adaptation process caused by physical exercise disappears within 8–13 weeks. Although for the other parameters no significant differences were found, from a consideration of the results on surface area and percentages of intercalated disc and lateral Cx43, we can see that the 4-week detraining process causes a reduction in the amounts of intercalated disc Cx43, lateral Cx43, and lateral Cx43 percentage. For the intercalated disc Cx43 there was a rebound, which is similar to the findings of Gamelin et al.⁽¹⁷⁾ and Sloan et al.,⁽¹⁸⁾ who revealed that chronic adaptation due to physical exercise disappears upon detraining. Our study results showed that after 12 weeks of aerobic physical exercise followed by 4 weeks of detraining, there were no significant differences in Cx43 levels as compared to 4 weeks of aerobic physical exercise followed by 4 weeks of detraining. Our study results are essentially in agreement with those of previous studies showing that the changes after detraining may be seen after 28 days.⁽¹⁹⁾ However, upon a closer look at the obtained data, it may be concluded that, although there were no significant differences among the parameters, 4 weeks of detraining still leads to a reduction in the areas of total Cx43, intercalated disc Cx43, lateral Cx43 lateral, and in lateral Cx43 percentage. As for intercalated disc Cx43 percentage, there is an increase after the detraining process. This is because the reduction in the amount of lateral Cx43 is proportionally greater than that in the amount of intercalated disc Cx43, so that in spite of a reduced amount of intercalated disc Cx43, its percentage is increased after the detraining process. This is in agreement with the conclusions of previous studies, revealing similar effects, in that chronic adaptation due to physical exercise disappears if the individual is subjected to the detraining process.^(15,20)

In this connection it is of interest to note that in postinfarction ischemic cardiomyopathy

and other types of heart failure, the expression of Cx43 is downregulated.⁽²¹⁾

One of the limitations of our study is the use of an IHC assay using anti-connexin43 only, so that all of the Cx43 proteins are detected, namely those active in gap junction formation as well as those that are inactive. Since active Cx43 proteins are those phosphorylated at their carboxyl terminal end, the accuracy of the Cx43 protein measurements may be increased by the addition of another antibody, that is capable of detecting phosphorylated Cx43. The use of two types of antibody allows us to find the amount of actively gap junction forming Cx43 protein, both in the intercalated discs and in the lateral domains of the cardiomyocytes. In this way we may ascertain whether physiological hypertrophy due to aerobic physical exercise is able to increase the amount of Cx43 protein in the lateral domains of the cardiomyocytes. In other words, whether it results in increased numbers of gap junctions in the lateral regions of the cardiomyocytes, thus causing a transversal propagation of electrical impulses that is one of the causes of arrhythmia.

CONCLUSIONS

This study demonstrates that Cx43 has an important and previously unknown modulatory effect in myocardial energy metabolism, as evidenced by the increased levels of protein expression after 4 and 12 weeks of aerobic physical exercise. These changes in Cx43 expression and distribution as a result of physical activity will diminish in the detraining process. The occurrence of these significant differences indicate the need for further in-depth investigation of the cardioprotective role of Cx43 proteins and its adaptation to ischemic conditions.



REFERENCES

1. Tirziu D, Giordano F, Simons M. Cell communications in the heart. *Circulation* 2010; 122:928-37.
2. Danik SB, Yu LF, Jie Z, Suk HJ, Morley GE, Fishman GI, et al. Modulation of cardiac gap junction expression and arrhythmic susceptibility. *Circ Res* 2004;95:1035-41.
3. Desplantez T, Dupont E, Severs NJ, Weingart R. Gap junction channels and cardiac impulse propagation. *J Membr Biol* 2007;218:13–28.
4. Hesketh GG, Shah MH, Halperin VL, Cooke CA, Akar FG, Yen TE, et al. Ultrastructure and regulation of lateralized connexin43 in the failing heart. *Circ Res* 2010;106:1153-63.
5. Sato T, Ohkusa T, Honjo H, Suzuki S, Yoshida M, Ishiguro YS, et al. Altered expression of connexin43 contributes to the arrhythmogenic substrate during the development of heart failure in cardiomyopathic hamster. *Am J Physiol Heart Circ Physiol* 2007;294:1164-73.
6. Palatinus JA, O'Quinn MP, Barker RJ, Harris BS, Jourdan J, Gourdie RG. ZO-1 determines adherens and gap junction localization at intercalated disks. *Am J Physiol Heart Circ Physiol* 2011;300:583-94.
7. Polontchouck L, Haefliger JA, Ebel B, Schaefer T, Stuhlmann D, Mehlhorn U, et al. Effects of chronic atrial fibrillation on gap junction distribution in human and rat atria. *J Am Coll Cardiol* 2001;38:883-91.
8. Molmen-Hansen HE, Stolen T, Tjonna AE, Aamot IL, Ekeberg IS, Tyldum GA, et al. Aerobic interval training reduces blood pressure and improves myocardial function in hypertensive patients. *Eur J Prev Cardiol* 2012;19:151-60.
9. Hafstad D, Boardman NT, Lund J, Hagve M, Khalid AM, Wislof U, et al. High intensity interval training alters substrate utilization and reduces oxygen consumption in the heart. *J Appl Physiol* 2011;111:1235-4
10. Kostin S, Dammer S, Hein S, Klovecorn WP, Bauer EP, Schaper J. Connexin 43 expression and distribution in compensated and decompensated cardiac hypertrophy in patients with aortic stenosis. *Cardiovasc Res* 2004;62: 426–36.
11. Teunissen BEJ, Jongasma HJ, Bierhuizen MFA. Review: Regulation of myocardial connexins during hypertrophic remodeling. *Eur Heart J* 2004;25:1979-8.
12. Bao X, Clark CB, Frangos JA. Temporal gradient in shear-induced signaling pathway: involvement of MAP kinase, c-fos, and connexin43. *Am J Physiol Heart Circ Physiol* 2000;278:1598–605.
13. Bearden SE, Linn E, Ashley BS, Looft-Wilson RC. Age-related changes in conducted vasodilation: effects of exercise training and role

- in functional hyperemia. *Am J Physiol Regul Integr Comp Physiol* 2007;293:1717-21.
14. Barker RJ, Price RL, Gourdie RG. Increased association of ZO-1 with connexin43 during remodeling of cardiac gap junctions. *Circ Res* 2002;90:317-24.
 15. Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC, et al. Cardiac arrhythmogenic remodeling in a rat model of a long-term intensive exercise training. *Circulation* 2011;123:13-22.
 16. Pelliccia A, Maron BJ, DeLuca R, DiPaolo FM, Spataro A, Culasso F. Remodeling of left ventricular hypertrophy in elite athletes after long-term deconditioning. *Circulation* 2002;105: 944-9.
 17. Gamelin FX, Berthoin S, Sayah H, Libersa C, Bosquet L. Effect of training and detraining on heart rate variability in healthy young man. *Int J Sports Med* 2007;28:1-7.
 18. Sloan RP, Shapiro PA, DeMeersman RE, Bagiella E, Brondolo EN, McKinley PS, et al. Impact of aerobic training on cardiovascular reactivity to and recovery from challenge. *Psychosom Med* 2011;73:134-41.
 19. Maass K, Shibayama J, Chase SE, Willecke K, Delmar M. C-Terminal truncation of connexin43 changes number, size, and localization of cardiac gap junction plaques. *Circ Res* 2007;101:1283-91.
 20. Bosquet L, Mujika I. Detraining. In: *Endurance training – science and practice*. Vitoria-Gasteiz: Inigo Mujika S.L.U.; 2012. p.99–106.
 22. Severs NJ, Coppen SR, Dupont E, Yeh HI, Ko YS, Matsushita T. Gap junction alterations in human cardiac disease. *Cardiovasc Res* 2004;62: 368–77.