Warning: not all carriers of pathogenic mutations in desmosomal genes should follow the same medical advices!

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The study by Cheedipudi et al.[1] published in this issue of the Journal provides very provocative and thought-generating data that highlight the existence of a major adaptive response of the murine heart to environmental stressors and particularly to exercise.

The team of authors led by Dr. Marian have provided in the past enlightening contributions to the understanding of the pathogenesis of the Arrhythmogenic Cardiomyopathy (ACM)[2-4] and in this investigation they explore the interesting question of whether physical training may impact cardiac transcriptomic in wild type mice and in ACM mice.

The first novel contribution provided by the study comes from the idea of assessing transcriptional changes in cardiac cells of wild-type mice exposed to regular physical exercise up to six months of age and showing that physical training triggers transcriptional changes in healthy animals. Despite it is well known that cardiac remodeling occurs in athletes, the underlying molecular mechanisms are largely undefined as data on exercise-related transcriptional changes in cardiac cells in humans are missing. The study by Cheedipudi et al.[1] nicely demonstrates that, at least in young rodents, physical training modifies the transcriptome and pinpoints to some pathways that are modulated. Overall, the authors report changes in transcript levels of 2,529 genes in wild type mice, of which 1,390 are upregulated and 1,139 downregulated. Looking at the pathways implicated in this process, the authors noted the upregulation of genes that regulate protein secretion, mitotic spindle and PI3K/AKT/mTOR signaling. Interestingly, downregulated genes were involved in inflammation and oxidative phosphorylation. These observations are most valuable, as they may guide human studies to better define the mechanisms for the health promoting role of exercise in athletes and in the population with an active lifestyle.

The most interesting part of the study, however, concerns the results of the investigations conducted in mice carriers of a heterozygous deletion in the desmoplakin gene and therefore affected by ACM. The observations are apparently in sharp departure from the current view, supported by pre-clinical and clinical data, that exercise is deleterious for ACM patients who carry

desmosomal mutations. Let us therefore step back and put the data by Cheedipudi and coworkers[1] in the context of current knowledge about exercise modification of cardiac physiology.

The observation that exercise could be a specific trigger for arrhythmic events in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) was originally reported in 1982, by Marcus et al.[5] Subsequently, evidence that competitive athletes were particularly susceptible to deaths associated with ARVC emerged[6] and further supported the hypothesis that exercise is a trigger for arrhythmic events in ACM.

Interestingly, after these initial clinical observations highlighting the detrimental effect of training in ACM patients, a very interesting pre-clinical observation was made by Paulus Kirchhof and coworkers in 2006.[7] In this work the authors investigated the consequences of exercise in ten-month-old heterozygous plakoglobin-deficient mice and compared them with healthy mice exposed to a similar training. Data showed that knock-in mice developed a clear right ventricular phenotype, characterized by increased right ventricular volume, reduced right ventricular function, and manifested spontaneous ventricular arrhythmias. Interestingly, the authors specifically highlighted that "left ventricular size and function were not altered", thus pointing out the presence of a selective right ventricular form of ACM.

For several years this pre-clinical observation remained confined to the basic science arena and it was only 7 years later, in 2013, that Hugh Calkins and his team published a seminal paper[8] that confirmed in the clinical arena the concept that exercise can accelerate the development of an abnormal cardiac morphology and depress the cardiac function in patients with ACM. In support that life-threatening arrhythmias development in facilitated in individuals who perform strenuous exercise, the authors also showed that among ACM patients who had never experienced an episode of ventricular tachycardia or ventricular fibrillation, those who developed a first life-threatening arrhythmic episode over a mean follow-up of 8 years were 13 endurance athletes (p=0.002).

After the publication of the data by James et al.,[8] several clinical studies have confirmed the association between sport and aggressive forms of right ventricular arrhythmogenic

cardiomyopathy.[9, 10] We recently observed a suggestive clinical case of two sisters, both carriers of a nonsense mutation on the plakophillin-2 (PKP-2) gene, where the one who actively practiced competitive sport shows a severe phenotype, while the sister who never practiced sport has a normal cardiac phenotype (Figure 1). Accordingly, the concept that endurance training should be avoided in ARVC is now part of clinical practice recommendations: in the recent 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy[11] there is a Class III recommendation that reads as follows; *"Individuals with ARVC should not participate in competitive or frequent high-intensity endurance exercise, as this is associated with increased risk of ventricular arrhythmias and promoting progression of structural disease"*.

In this context, it is quite surprising that Cheedipudi et al.[1] presents now pre-clinical data that deviate from previous data, showing that physical training restores normal transcript levels of most genes with altered expression in mice carrying an heterozygous desmoplakin deletion. The conclusion of the authors is that "treadmill exercise has potential beneficial effects in a subset of cardiac phenotypes in ACM". Furthermore, in the "translational perspective" of their paper[1] the authors directly challenge the current recommendation to ACM patients to avoid strenuous physical exercise and state that "the findings suggest that treadmill exercise might have partial salutary phenotypic effects in ACM".

How can we explain the data by Cheedipudi et al.[1] and how can we now mediate between the two opposite points of view, supporting on one side that exercise is dangerous to ACM patients and on the other side that it may recover the abnormal transcript profile in ACM patients?

First of all, it should be kept into consideration that despite the transcriptional signature of ACM identified in mice carriers of the desmoplakin mutation is substantially ameliorated after exercise, the animals do not show recovery of the phenotype and in particular no recovery of systolic function nor reduction of fibrosis is observed after exercise: accordingly, the changes at the mRNA level might only partially lead to an improvement of the clinical phenotype.

However, we might have other considerations that account for the different response to exercise of the mice studied by Cheedipudi et al.[1] and the mice with heterozygous plakoglobin deficiency studied by Kirchhof.[7]

When looking at the results of the transcriptomic study by Cheedipudi et al.,[1] we should keep in mind that they pertain to an animal model of a special type of ACM that is caused by desmoplakin mutations. It is known that this variant of ACM has peculiar clinical manifestations.[12]

Desmoplakin is a is a large protein localized to desmosomes in intercalated discs, where it anchors the intermediate filament desmin to the desmosomal plaque. Mutations in desmoplakin in humans are associated with severe cardiomyopathies.[12] Being a protein of the desmosomes, it was initially associated with the so called arrhythmogenic right ventricular cardiomyopathy (ARVC) that is caused by loss-of-function mutation in a variety of desmosomal proteins.[11] However, it has become evident over the years that carriers of pathogenic loss-of-function DNA variant in the desmoplakin gene, often do not manifest with a disease of the right ventricle, but rather they manifest with the so called "left-dominant arrhythmogenic cardiomyopathy", a disease that resemble non-ischemic dilated cardiomyopathy[12] and even ischemic dilated cardiomyopathy.[13] This observation suggests that desmoplakin-related cardiomyopathy may present phenotypical differences, as compared to other genetic variants of ACM and that its response to physical exercise might therefore be different from the response in patients carriers of mutations in plakophilin or plakoglobin that typically manifest with a predominant right ventricular disease.

In line with this consideration is the observation that, while the mice by Kirchhof et al.[7] exclusively presented right ventricular dysfunction and arrhythmias originating from the right ventricle, the mice of Cheedipudi et al.[1] showed only left ventricular dysfunction and dilatation. Despite no transcriptomic data are available for the plakoglobin deficient mice studied by Kirchhof et al.[7] and therefore we do not know whether exercise would ameliorate the abnormal transcript profile induced by the disease, it is tempting to hypothesize that in analogy with the different

phenotypical response to exercise, mice with a right ventricular disease would also show worsening of detrimental transcriptomic changes during exercise.

In support of this view is the excellent review by La Gerche and coworkers[14] published in Cardiovascular Research in 2017 that elegantly describes why and how strenuous exercise is likely to impact adversely the right ventricle but not the left ventricle. The review is suggestively entitled "Exercise and the right ventricle: a potential Achilles' heel" and highlights how right ventricular stress is lower at rest when the right ventricle works against a very low resistance and a highly compliant pulmonary circulation.[14] However, according to the authors, during exercise the wall stress of the right ventricle becomes similar to that of the left ventricle because of three factors: 1) a greater increase in pulmonary artery pressures relative to systemic vascular pressures, 2) a slightly greater increase in ventricular volumes and, 3) a lesser compensation for wall stress through myocardial thickening.[14] It is therefore logical to consider that, in the presence of desmosomal mutations that predominantly alter the architecture of the right ventricle, the exposure to strenuous physical exercised may further accentuate right ventricular remodeling toward dilatation and mechanical dysfunction.[15] Defective desmosomal proteins are in fact likely to disrupt cellular adhesions, to alter signaling pathways and to modify transcriptomic, leading to cells' death and fibrosis, and making the right ventricle more susceptible to the adverse consequences of endurance sport activity.[15]

On the contrary, mutations such as the desmoplakin variants that cause arrhythmogenic left-dominant ventricular cardiomyopathy may well be more resistant to the exposure to exercise, as shown in the mice model studied by Cheedipudi.[1] Overall, the evidence that endurance training in desmoplakin haploinsufficient mice may counteract the adverse consequences of exercise at the level of transcriptomic, may open the doors to a new set of considerations about the differential impact of genetic variants and environmental triggers among the different diseases grouped under the acronym of ACMs.

For the time being, however, it seems adequate to continue and follow the recommendations of scientific societies that, based on a large platform of clinical data, advise that ACM patients with right ventricular disease should refrain from intensive exercise.[11] Meanwhile, fundamental studies on how exercise may modulate cardiac remodeling in the presence of different mutations of desmosomal proteins should shed more light on the dynamic response of the hearts to environmental and lifestyle challenges that modulate phenotypical manifestations of ACM.

Figure 1

Strenuous exercise leads to the development of phenotype in arrhythmogenic right ventricular cardiomyopathy (ARVC). The image shows the effect that the prolonged practice of strenuous physical activity has on the development of the electrocardiographic (ECG), arrhythmic and structural manifestations of ARVC. The cartoon refers to two sisters, both carriers of a nonsense mutation on the plakophilin-2 (PKP-2) gene without other variants on the main desmosomal genes.

Sister A, born in 1962, has never practiced intense sports and shows normal ECG and cardiac magnetic resonance. She is currently asymptomatic and has never experienced arrhythmic episodes.

Sister B, born in 1960, has been practicing endurance running from early adulthood, training 12 hours per week for over 35 years and participating in several marathons. Basal 12-lead ECG presents the typical changes of ARVC (epsilon wave, indicated by the arrow, and negative T waves on precordial leads V1-V4). Cardiac magnetic resonance showed a marked dilation and severe contractile dysfunction of the right ventricle (RV in the pictures), as compared to the left ventricle (LV). She also presented with one episode of sustained monomorphic ventricular tachycardia (left bundle branch block morphology and superior axis) with a rate of 170 bpm that she tolerated well (she continued working).

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