

EXPERIMENTAL Article

Heart function assessment during aging in apolipoprotein E knock-out mice

Elisa A. Liehn^{1,2,3,*}, Ana-Mihaela Lupan⁴, Rodica Diaconu^{1,2}, Mihai Ioana¹, Ioana Streata¹, Catalin Manole³, Alexandrina Burlacu⁴

¹Human Genetic Laboratory, University of Medicine and Pharmacy of Craiova, Craiova, Romania

²Department of Cardiology, Angiology and Intensive Care, Medical Faculty, University Hospital Aachen, Aachen, Germany

³Victor Babes National Institute of Pathology, Bucharest, Romania

⁴Nicolae Simionescu Institute of Cellular Biology and Pathology, Bucharest, Romania

*Correspondence to: Dr. Elisa A. Liehn, Department of Cardiology, Angiology and Intensive Care, Medical Faculty, University Hospital Aachen, Germany; Phone: +49-241-80 35983; Email: eliehn@ukaachen.de

Submitted: Sept. 8, 2021; Revised: Sept. 23, 2021; Accepted: Sept. 28, 2021; Published: Sept. 28, 2021;

Citation: Liehn EA, Lupan AM, Diaconu R, Ioana M, Streata S, Manole C, Burlacu A. Heart function assessment during aging in apolipoprotein E knock-out mice. *Discoveries* 2021, 9(3): e136. DOI: 10.15190/d.2021.15

ABSTRACT

BACKGROUND: Apolipoprotein (apo) E isoforms have strong correlations with metabolic and cardiovascular diseases. However, it is not clear if apoE has a role in development of non-ischemic cardiomyopathy. Our study aims to analyze the involvement of apoE in non-ischemic cardiomyopathy.

METHODS AND RESULTS: Serial echocardiographic measurements were performed in old wildtype and apoE deficient (apoE^{-/-}) mice. Morphological and functional cardiac parameters were in normal range in both groups at the age of 12 month. At the age of 18 months, both groups had shown ventricular dilation and increased heart rates. However, the apoE^{-/-} mice presented signs of diastolic dysfunction by hypertrophic changes in left ventricle, due probably to arterial hypertension. The right ventricle was not affected by age or genotype.

CONCLUSION: Even in the absence of high fat diet, apoE deficiency in mice induces mild changes in the cardiac function of the left ventricle during aging, by developing diastolic dysfunction, which leads to heart failure with preserved ejection fraction. However, further studies are necessary to conclude over the role of apoE in cardiac physiology and its involvement in development of heart failure.

Keywords: apoE, cardiovascular models, cardiomyopathy.

INTRODUCTION

Apolipoprotein E (apoE) is a ubiquitous protein, essential for the lipid metabolism in the whole body¹⁻². The role of apoE in lipid metabolism, ensuring the fulfillment of cholesterol homeostasis and adequate clearance to circulating lipids³⁻⁴ is the best known. Numerous studies have shown new other functions of this protein, independent of lipid metabolism, thus highlighting the polymorphism and multifunctionality of apoE⁵. Therefore, the deep understanding of all functions of this lipoprotein is for a great interest for the current research community, especially in the field of cardiovascular and metabolic diseases.

There are three different *apoE* alleles in humans: ε2, ε3, ε4. ε3 allele is the most common with a prevalence of 62% in the population, being considered the wild-type isoform⁶. Current clinical knowledge of apoE is limited to statistical correlations of certain alleles and/or genotypes with various chronic conditions⁶⁻¹⁰. The progression of cardiovascular diseases such as atherosclerosis is more accelerated in carriers of certain apoE alleles⁵.

Recently, a strong correlation was observed between the presence of apoE isoforms and severe Covid-19 infections¹¹⁻¹⁵, due to exaggerated lung inflammation in these patients. Increased susceptibility to inflammation seems also responsible for development of dilatative cardiomyopathy¹⁶⁻¹⁷ in patients carrying *e4* allele. However, a direct link between the apoE defects and development of cardiomyopathy is still under debate.

The current study aims to investigate the effect of apoE deficiency on the heart function.

MATERIAL AND METHODS

Animal model

All animal experiments were performed after approval (No. 359 from 19.06.2017), according to the European legislation and according to the FELASA guideline for care and use of experimental animals. Twelve-month old C57BL/6 mice (Mus Musculus, wild-type) and apoE knockout mice with matched background (apoE^{-/-}) were fed normal diet (3.3% crude fat, cholesterol-free). Echocardiography was performed at the beginning of experiments and 6 months later (at the age of 18 months). Mice were kept during all period in standardized conditions (21° ± 2 °C, 60% ± 5% humidity, and a 12-hour light/dark cycle, free access to food and water ad libitum).

Study design

Nineteen mice, females and males, were included in the study as presented in the Table 1.

Echocardiography

Mice were sedated with 1.5% isoflurane, placed on a warm, ECG-monitoring table. Echocardiographic measurements were performed using a small-animal ultrasound imager (Vevo 2100, FUJIFILM Visualsonics, Toronto, Canada). Measurements in short and long parasternal axis were taken in B-Mode (2D-realttime) and M-Mode using a 40 MHz transducer, as described before¹⁸⁻¹⁹. The left ventricular (LV) ejection fraction (EF) was assessed and analyzed in the long and short axes obtained at the level of papillary muscles. The systolic and diastolic LV volumes, LV wall thickness, LV mass, heart rate (HR), as well as the valve gradients and velocity were also recorded and calculated.

Statistical analysis

Data represent mean ± SEM. Statistical analysis was performed with Prism 6.1 software (GraphPad). Means of more than two groups were compared with 1-way ANOVA followed by Tukey's post-hoc-test, as indicated. p-values of <0.05 were considered significant.

RESULTS

Nineteen mice, females and males, were included in the study (Table 1). Four mice died during observation period. No correlation was made between the survival rate and the mouse's genotype. There were no differences between the males and female's echocardiographic parameters within each group, therefore, the results included all mice, independent of gender.

GENOTYPE	SEX	12 MONTHS	18 MONTHS	DIED DURING THE OBSERVATION PERIOD
WILD-TYPE	female	n=4	n=3	1
	male	n=3	n=1	2
APOE ^{-/-}	female	n=7	n=6	1
	male	n=5	n=5	0

Table 1. Mice included in the study.

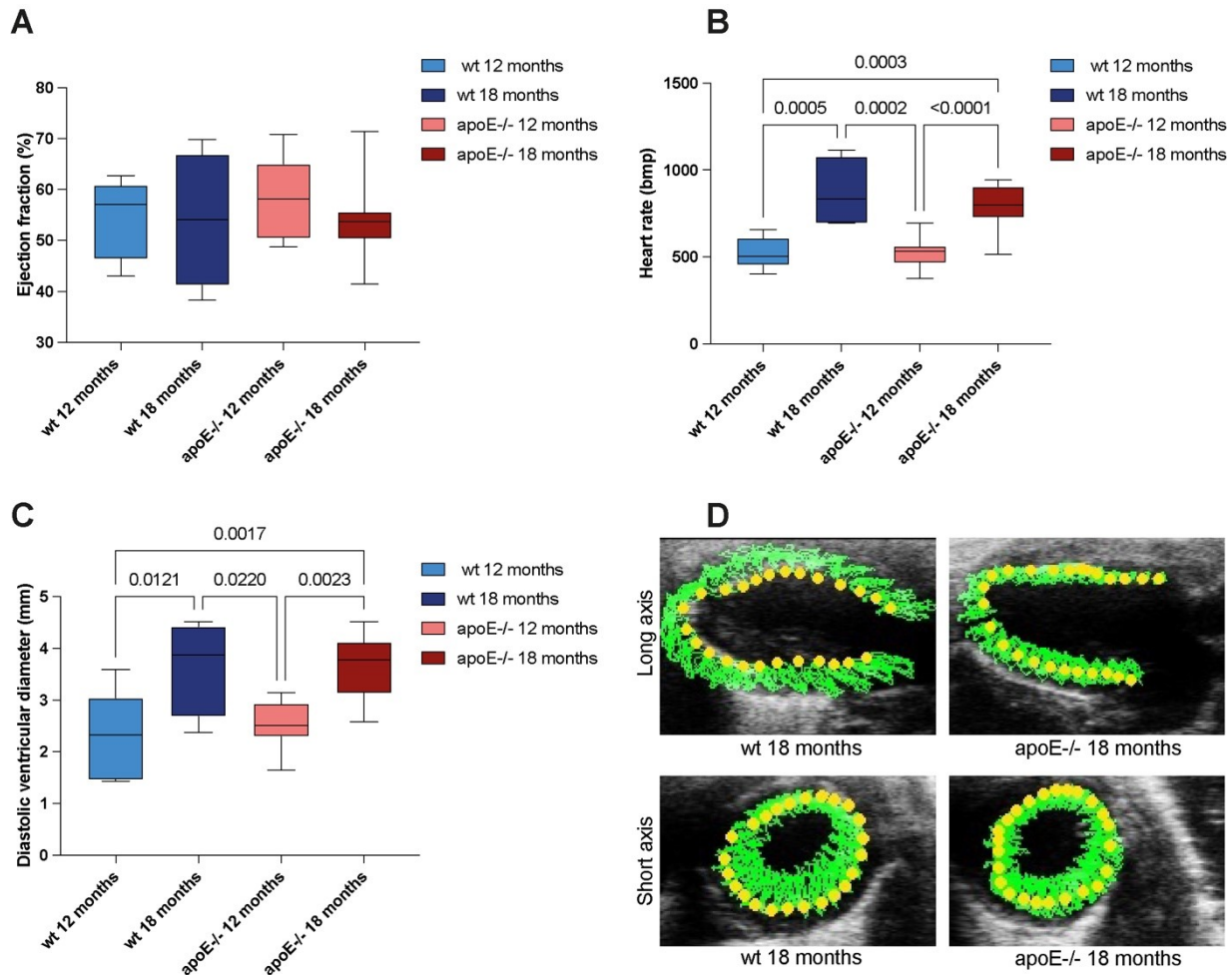


Figure 1. Morphological and functional assessment of the heart. (A) Ejection fraction, (B) Heart rate and (C) Diastolic ventricular diameter of the mice measured at 12 and 18-months old. (D) Representative strain ultrasound images in long axis (upper panel) and short axis (lower panel). Significant p values are depicted in each figure.

During aging, both the wildtype and apoE^{-/-} mice have lost weight, but this was not statistically different. The EF also remained preserved over the time in both groups (Figure 1A). However, the mice present a significant increase in the heart rate during aging (Figure 1B) and significant increase in diastolic ventricular diameter (Figure 1C), as signs of heart deterioration. Representative strain ultrasound images showing the movement of the left ventricle wall is showing (Figure 1D).

Interestingly, only the old apoE^{-/-} mice developed ventricular hypertrophy during aging, demonstrated by increased left ventricular mass (Figure 2A) and left ventricular wall thickness (Figure 2B). Furthermore, the mean and peak gradients (Figure 2C,D) and the mean and peak velocities (Figure 2 E,F) over aortic valve were significantly increased in old apoE^{-/-} mice. Since there were no signs of

calcification or obstruction of valve leaflets, these changes can be explained by increased blood pressure over time in these mice. The E/A ratio was not significantly different between the groups and no clear sign of diastolic dysfunction was noted in these mice (Figure 3A). However, isovolumetric ventricular relaxation time (IVRT) was decreased in old apoE^{-/-} compare wildtype mice (Figure 3B), as a sign of heart failure with preserved heart function. The gradients and velocity over pulmonary artery, as predicted by doppler echocardiographic pulmonary artery pressure, did not differ between the two groups or during aging (Figure 4), corresponding to a normal and unchanged right ventricular function. Overall, our data showed that apoE deficient mice develop mild ventricular dysfunction during aging, even in the absence of high fat diet, which progresses towards heart failure with preserved EF.

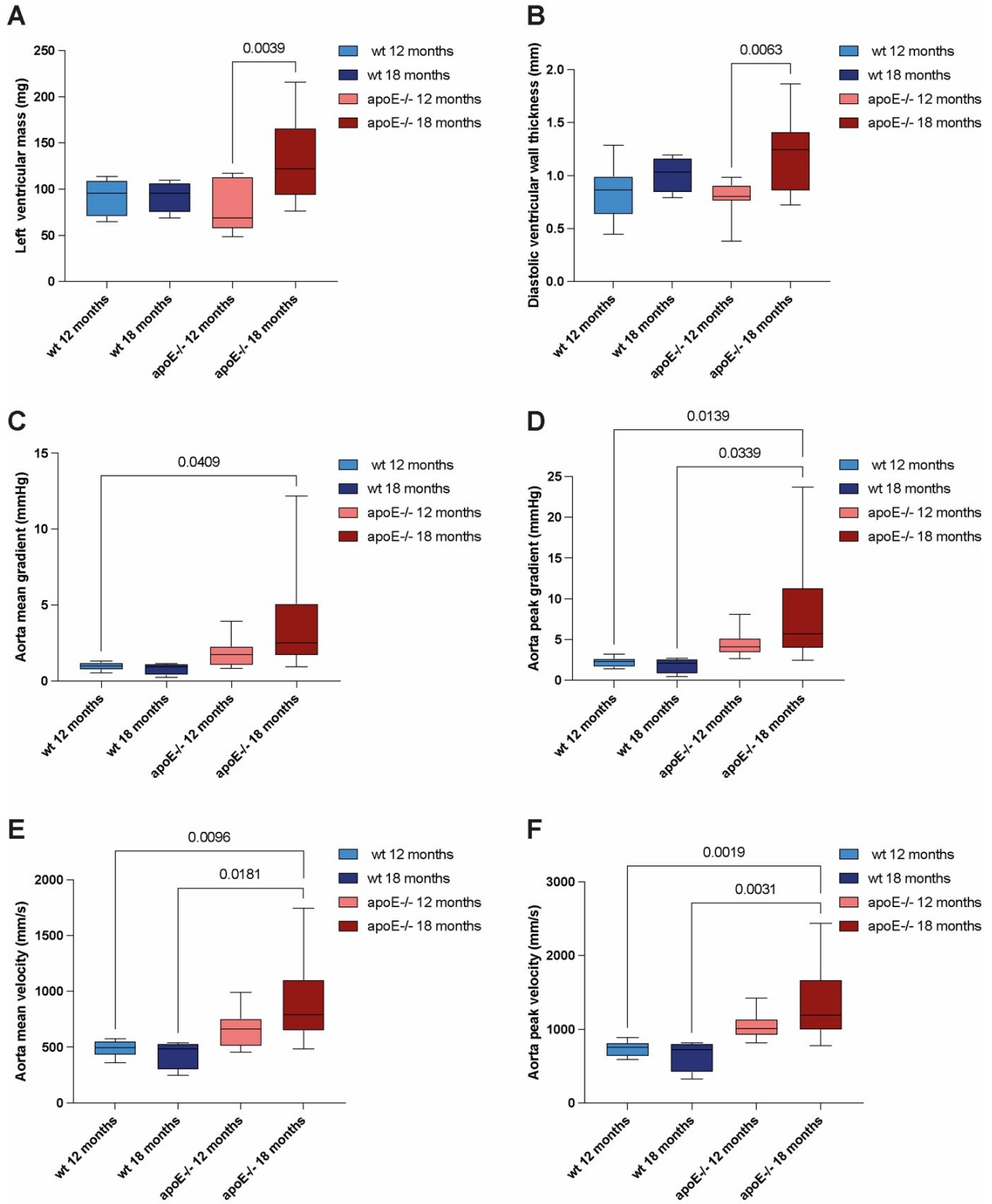


Figure 2. Hypertrophic changes in the heart of old apoE^{-/-} mice. (A) Left ventricular mass, (B) Diastolic ventricular wall thickness, (C) Aortic mean gradient, (D) Aortic peak gradient, (E) Aortic mean velocity and (F) Aortic peak velocity in mice measured at 12 and 18-months old. Significant *p* values are depicted in each figure.

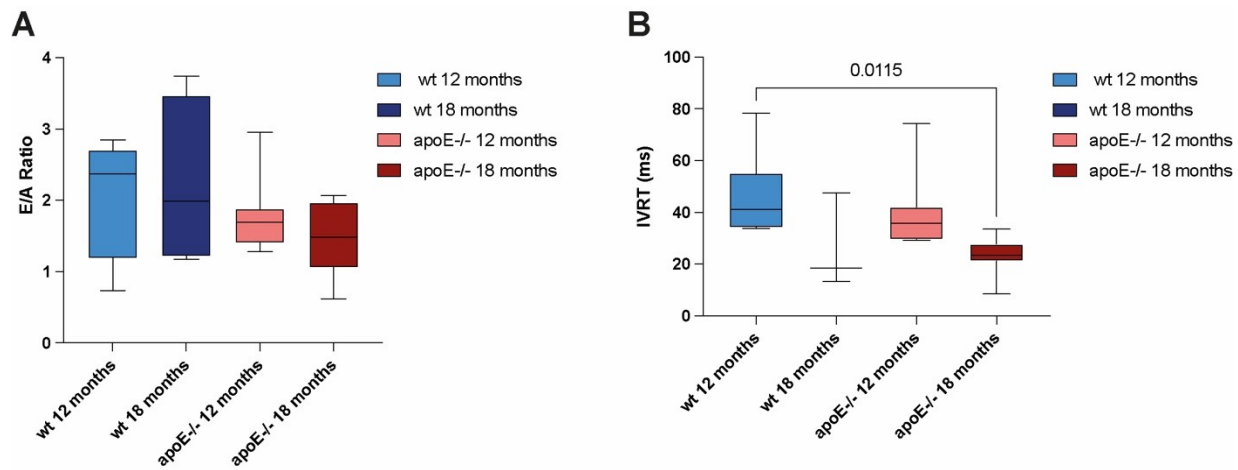


Figure 3. Assessment of diastolic dysfunction. (A) E/A ratio and (B) IVRT measured in mice at 12 and 18-months old. Significant *p* values are depicted in each figure.

DISCUSSION

In this study, we have shown that deficiency of apoE could induce heart failure with preserved heart function by development of hypertrophic cardiomyopathy during ageing. Interestingly, we did not observe any changes in right ventricular function, despite the recent data in this regard²⁰.

In the context of the cardiovascular diseases, the generation of genetically engineered mouse models became necessary to understand disease mechanisms in humans. Due to its role in lipid metabolism, apoE deficient mice (apoE^{-/-}) became the main animal model to study atherosclerosis in cardiovascular research²¹⁻²⁴. While the apoE knock-out mouse is an excellent model of atherosclerosis, the lack of apoE is extremely rare in the human population. As wild-type mouse apoE is equivalent to human apoE3 isoform, it is reasonable to speculate that human missing apoE3 (the presence of apoE2 and apoE4 isoforms), shares similar effects with ApoE deficient mice^{2,6}.

Recently, many studies showed that the frequency of the ϵ 4 allele is significantly increased in patients with dilated cardiomyopathy^{16,20,25}, so the ϵ 4 allele can be considered a novel risk factor for this non-ischemic cardiomyopathy. The possible mechanisms for inducing severe forms of dilated cardiomyopathy in ϵ 4 allele carriers still need to be investigated. It can be speculated that repetitive inflammation²⁶,

apoptotic processes⁸, reduced antioxidant activity⁹, increased cell proliferation⁷ or excessive intracellular lipid accumulation^{20, 27-30} induced by apoE4 modify structurally and functionally the heart, thus inducing cardiomyopathy and heart failure.

In the present study we have found changes in ventricular morphology, such as mass and thickness, which were associated with signs of diastolic dysfunction, but preserved ejection fraction. This is in accordance with recent studies which demonstrated that lipid accumulation into the heart induced increased catecholamines and angiotensin II release³¹⁻³³, thus increasing the blood pressure and inducing ventricular hypertrophy. Since the ejection fraction was preserved, and no signs of diastolic dysfunction were seen,

we assumed that this could also be considered as a model of heart failure with preserved ejection

fraction³⁴⁻³⁶. As a limitation of our study, we didn't use high fat diet, which would have increased lipid accumulation and tissular damages and thus, aggravated the heart failure.

Further, in our recent study we have observed a different pattern in structural changes in right and left ventricles induced in apoE^{-/-} rats during aging and high fat diet²⁰. Similar, humans carrying ϵ 4 allele presented severe decrease in right ventricular function and increase in pulmonary arterial hypertension²⁰. Other experimental studies described

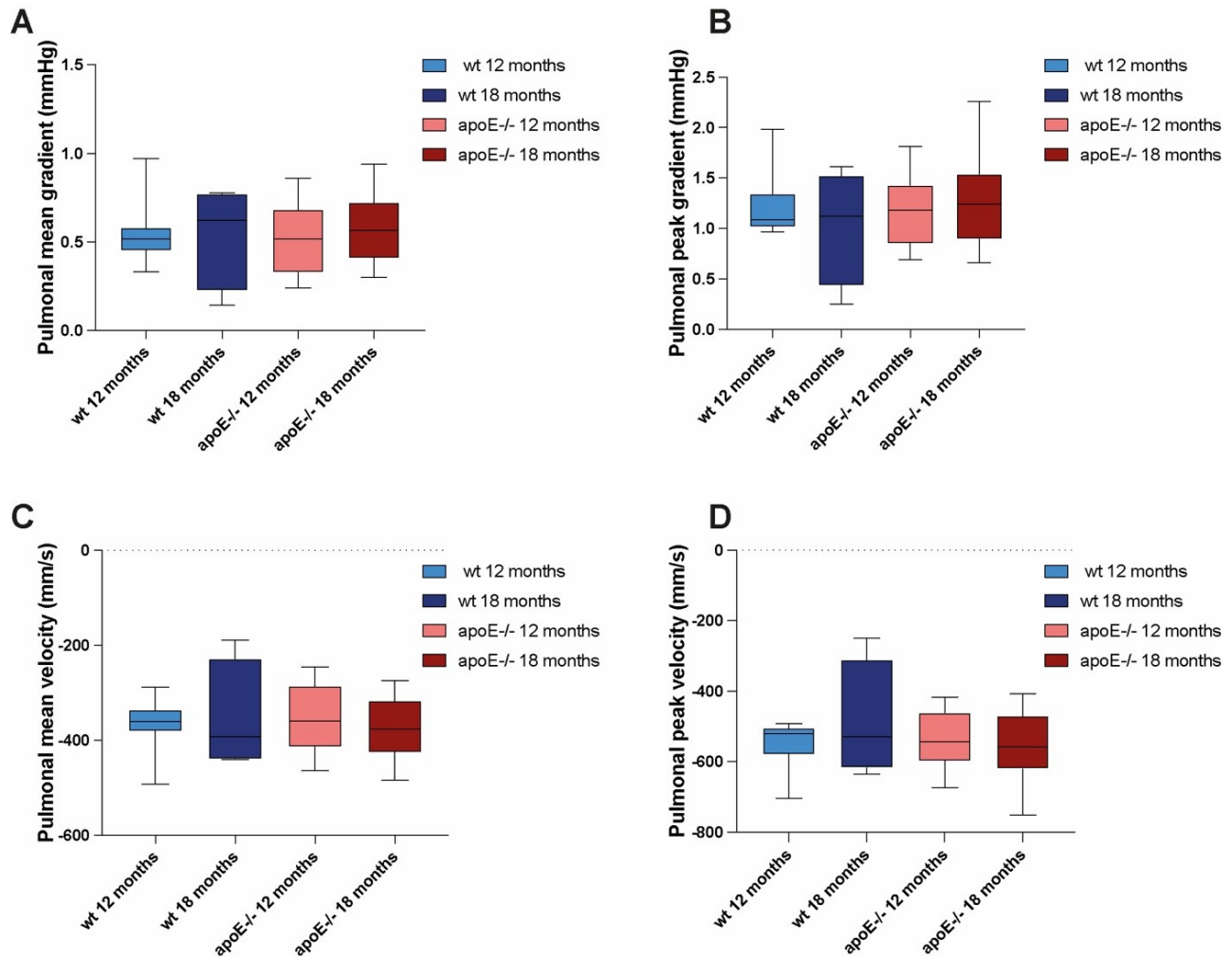


Figure 4. Assessment of pulmonary artery valve gradients. (A) Pulmonary mean gradient, (B) Pulmonary peak gradient, (C) Pulmonary mean velocity and (D) Pulmonary peak velocity measured at 12 and 18-months old. Significant p values are depicted in the respective figures.

the development of atherosclerosis with severe pulmonary arterial hypertension in apoE^{-/-} mice feeding high fat diet³⁷⁻³⁹. In the present study we didn't observe any changes in the right ventricular function, due probably to the still young age of the mice and normal diet during the experimental period. In conclusion, apoE deficiency can induce ventricular dysfunction. However, in the absence of high fat diet, the mild changes are followed by development of heart failure with preserved ejection fraction. This is an interesting observation, since the cardiovascular scientific community is missing an established experimental animal model for this kind of pathology⁴⁰. However, other studies are necessary to conclude over the role of apoE in cardiac physiology and its involvement in development of heart failure.

Acknowledgments

The study was supported by the Interdisciplinary Center for Clinical Research IZKF Aachen (research group E.A.L.) and Romanian Academy (research group A.B.).

Conflict of Interest

The authors declare no conflicts of interest.

REFERENCES

1. Mahley, R. W., Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988, 240 (4852), 622-30.
2. Liehn, E. A.; Ponomariov, V.; Diaconu, R.; Streat, I.; Ioana, M.; Crespo-Avilan, G. E.; Hernandez-Resendiz, S.; Cabrera-Fuentes, H. A., Apolipoprotein E in Cardiovascular Diseases:

- Novel Aspects of an Old-fashioned Enigma. *Archives of medical research* 2018, 49 (8), 522-529.
3. Cornelissen, A.; Simsekyilmaz, S.; Liehn, E.; Rusu, M.; Schaaps, N.; Afify, M.; Florescu, R.; Almalla, M.; Borinski, M.; Vogt, F., Apolipoprotein E deficient rats generated via zinc-finger nucleases exhibit pronounced in-stent restenosis. *Sci Rep* 2019, 9 (1), 18153.
 4. Castano, D.; Rattanasopa, C.; Monteiro-Cardoso, V. F.; Corliano, M.; Liu, Y.; Zhong, S.; Rusu, M.; Liehn, E. A.; Singaraja, R. R., Lipid efflux mechanisms, relation to disease and potential therapeutic aspects. *Adv Drug Deliv Rev* 2020, 159, 54-93.
 5. Wang, X. L.; McCredie, R. M.; Wilcken, D. E., Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. *Arterioscler Thromb Vasc Biol* 1995, 15 (8), 1030-4.
 6. Abondio, P.; Sazzini, M.; Garagnani, P.; Boattini, A.; Monti, D.; Franceschi, C.; Luiselli, D.; Giuliani, C., The Genetic Variability of APOE in Different Human Populations and Its Implications for Longevity. *Genes (Basel)* 2019, 10 (3).
 7. Vogel, T.; Guo, N. H.; Guy, R.; Drezlich, N.; Krutzsch, H. C.; Blake, D. A.; Panet, A.; Roberts, D. D., Apolipoprotein E: a potent inhibitor of endothelial and tumor cell proliferation. *J Cell Biochem* 1994, 54 (3), 299-308.
 8. Lynch, J. R.; Tang, W.; Wang, H.; Vitek, M. P.; Bennett, E. R.; Sullivan, P. M.; Warner, D. S.; Laskowitz, D. T., APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003, 278 (49), 48529-33.
 9. Zito, G.; Polimanti, R.; Panetta, V.; Ventriglia, M.; Salustri, C.; Siotto, M. C.; Moffa, F.; Altamura, C.; Vernieri, F.; Lupoi, D.; Cassetta, E.; Rossini, P. M.; Squitti, R., Antioxidant status and APOE genotype as susceptibility factors for neurodegeneration in Alzheimer's disease and vascular dementia. *Rejuvenation Res* 2013, 16 (1), 51-6.
 10. Asaro, A.; Carlo-Spiewok, A. S.; Malik, A. R.; Rothe, M.; Schipke, C. G.; Peters, O.; Heeren, J.; Willnow, T. E., Apolipoprotein E4 disrupts the neuroprotective action of sortilin in neuronal lipid metabolism and endocannabinoid signaling. *Alzheimers Dement* 2020, 16 (9), 1248-1258.
 11. Wang, C.; Zhang, M.; Garcia, G., Jr.; Tian, E.; Cui, Q.; Chen, X.; Sun, G.; Wang, J.; Arumugaswami, V.; Shi, Y., ApoE- Isoform-Dependent SARS- CoV-2 Neuro-tropism and Cellular Response. *Cell Stem Cell* 2021, 28 (2), 331-342 e5.
 12. Gkouskou, K.; Vasilogiannakopoulou, T.; Andreakos, E.; Davanos, N.; Gazouli, M.; Sanoudou, D.; Eliopoulos, A. G., COVID-19 enters the expanding network of apolipoprotein E4-related pathologies. *Redox Biol* 2021, 41, 101938.
 13. Hubacek, J. A.; Dlouha, L.; Dusek, L.; Majek, O.; Adamkova, V., Apolipoprotein E4 Allele in Subjects with COVID-19. *Gerontology* 2021, 67 (3), 320-322.
 14. Bhatt, K.; Agolli, A.; Patel, M. H.; Garimella, R.; Devi, M.; Garcia, E.; Amin, H.; Domingue, C.; Guerra Del Castillo, R.; Sanchez-Gonzalez, M., High mortality co-infections of COVID-19 patients: mucormycosis and other fungal infections. *Discoveries (Craiova)* 2021, 9 (1), e126.
 15. Waleed, R. M.; Sehar, I.; Iftikhar, W.; Khan, H. S., Hematologic parameters in coronavirus infection (COVID-19) and their clinical implications. *Discoveries (Craiova)* 2020, 8 (4), e117.
 16. Jurkovicova, D.; Goncalvesova, E.; Sedlakova, B.; Hudecova, S.; Fabian, J.; Krizanova, O., Is the ApoE polymorphism associated with dilated cardiomyopathy? *Gen Physiol Biophys* 2006, 25 (1), 3-10.
 17. Alameddine, F. M.; Zafari, A. M., Genetic polymorphisms and oxidative stress in heart failure. *Congest Heart Fail* 2002, 8 (3), 157-64, 172.
 18. Schumacher, D.; Curaj, A.; Staudt, M.; Cordes, F.; Dumitrascu, A. R.; Rolles, B.; Beckers, C.; Soppert, J.; Rusu, M.; Simsekyilmaz, S.; Kneizeh, K.; Ramachandra, C. J. A.; Hausenloy, D. J.; Liehn, E. A., Phosphatidylserine Supplementation as a Novel Strategy for Reducing Myocardial Infarct Size and Preventing Adverse Left Ventricular Remodeling. *Int J Mol Sci* 2021, 22 (9).
 19. Schumacher, D.; Curaj, A.; Simsekyilmaz, S.; Schober, A.; Liehn, E. A.; Mause, S. F., miR155 Deficiency Reduces Myofibroblast Density but Fails to Improve Cardiac Function after Myocardial Infarction in Dyslipidemic Mouse Model. *Int J Mol Sci* 2021, 22 (11).
 20. Diaconu, R.; Schaaps, N.; Afify, M.; Boor, P.; Cornelissen, A.; Florescu, R.; Simsekyilmaz, S.;

- El-Khoury, T.; Schumacher, D.; Ioana, M.; Streata, I.; Militaru, C.; Donoiu, I.; Vogt, F.; Liehn, E. A., Apolipoprotein E4 is Associated with Right Ventricular Dysfunction in Dilated Cardiomyopathy-An Animal and In-Human Comparative Study. *Int. J. Mol. Sci.* 2021, 22.
21. Buzello, M.; Tornig, J.; Faulhaber, J.; Ehmke, H.; Ritz, E.; Amann, K., The apolipoprotein e knockout mouse: a model documenting accelerated atherogenesis in uremia. *J Am Soc Nephrol* 2003, 14 (2), 311-6.
 22. Lo Sasso, G.; Schlage, W. K.; Boue, S.; Veljkovic, E.; Peitsch, M. C.; Hoeng, J., The Apoe(-/-) mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. *J Transl Med* 2016, 14 (1), 146.
 23. Getz, G. S.; Reardon, C. A., ApoE knockout and knockin mice: the history of their contribution to the understanding of atherogenesis. *J Lipid Res* 2016, 57 (5), 758-66.
 24. Vasquez, E. C.; Peotta, V. A.; Gava, A. L.; Pereira, T. M.; Meyrelles, S. S., Cardiac and vascular phenotypes in the apolipoprotein E-deficient mouse. *J Biomed Sci* 2012, 19, 22.
 25. Peng, W.; Liu, J. A.; Chen, G.; Zheng, M.; Zhang, L.; Li, A.; Zhou, Y.; Li, J.; Li, G.; Wang, Y., The correlation between ApoE gene polymorphism and non-ischemic chronic heart failure. *Int. J. Clin. Exp. Med.* 2017, 10 (12), 16809-16814.
 26. DeKroon, R. M.; Mihovilovic, M.; Goodger, Z. V.; Robinette, J. B.; Sullivan, P. M.; Saunders, A. M.; Strittmatter, W. J., ApoE genotype-specific inhibition of apoptosis. *J Lipid Res* 2003, 44 (8), 1566-73.
 27. Diaconu, R.; Donoiu, I.; Mirea, O.; Balseanu, T. A., Testosterone, cardiomyopathies, and heart failure: a narrative review. *Asian J Androl* 2021, 23 (4), 348-356.
 28. Zachowski, A., Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. *Biochem J* 1993, 294 (Pt 1), 1-14.
 29. Wood, W. G.; Igbaybova, U.; Muller, W. E.; Eckert, G. P., Cholesterol asymmetry in synaptic plasma membranes. *J Neurochem* 2011, 116 (5), 684-9.
 30. Cappelletta, D.; Esposito, G.; Piegari, E.; Russo, R.; Ciuffreda, L. P.; Rivellino, A.; Berrino, L.; Rossi, F.; De Angelis, A.; Urbanek, K., SIRT1 activation attenuates diastolic dysfunction by reducing cardiac fibrosis in a model of anthracycline cardiomyopathy. *Int J Cardiol* 2016, 205, 99-110.
 31. Abdalla, S.; Fu, X.; Elzahwy, S. S.; Klaetschke, K.; Streichert, T.; Qwitterer, U., Up-regulation of the cardiac lipid metabolism at the onset of heart failure. *Cardiovasc Hematol Agents Med Chem* 2011, 9 (3), 190-206.
 32. Kim, S.; Dugail, I.; Standridge, M.; Claycombe, K.; Chun, J.; Moustaid-Moussa, N., Angiotensin II-responsive element is the insulin-responsive element in the adipocyte fatty acid synthase gene: role of adipocyte determination and differentiation factor 1/sterol-regulatory-element-binding protein 1c. *Biochem J* 2001, 357 (Pt 3), 899-904.
 33. Hongo, M.; Ishizaka, N.; Furuta, K.; Yahagi, N.; Saito, K.; Sakurai, R.; Matsuzaki, G.; Koike, K.; Nagai, R., Administration of angiotensin II, but not catecholamines, induces accumulation of lipids in the rat heart. *Eur J Pharmacol* 2009, 604 (1-3), 87-92.
 34. Gazewood, J. D.; Turner, P. L., Heart Failure with Preserved Ejection Fraction: Diagnosis and Management. *Am Fam Physician* 2017, 96 (9), 582-588.
 35. Harper, A. R.; Patel, H. C.; Lyon, A. R., Heart failure with preserved ejection fraction. *Clin Med (Lond)* 2018, 18 (Suppl 2), s24-s29. doi: 10.7861/clinmedicine.18-2-s24.
 36. Ahmed, S. N.; Jhaj, R.; Sadasivam, B.; Joshi, R., Regression of the Left Ventricular Hypertrophy in Patients with Essential Hypertension on Standard Drug Therapy. *Discoveries (Craiova)* 2020, 8 (3), e115.
 37. Umar, S.; Partow-Navid, R.; Ruffenach, G.; Iorga, A.; Moazeni, S.; Eghbali, M., Severe pulmonary hypertension in aging female apolipoprotein E-deficient mice is rescued by estrogen replacement therapy. *Biol Sex Differ* 2017, 8, 9.
 38. Yao, X.; Gordon, E. M.; Figueroa, D. M.; Barochia, A. V.; Levine, S. J., Emerging Roles of Apolipoprotein E and Apolipoprotein A-I in the Pathogenesis and Treatment of Lung Disease. *Am J Respir Cell Mol Biol* 2016, 55 (2), 159-69.
 39. Renshall, L.; Arnold, N.; West, L.; Braithwaite, A.; Pickworth, J.; Walker, R.; Alfaidi, M.; Chamberlain, J.; Casbolt, H.; Thompson, A. A. R.; Holt, C.; Iglarz, M.; Francis, S.; Lawrie, A., Selective improvement of pulmonary arterial hypertension with a dual ETA/ETB receptors antagonist in the apolipoprotein E(-/-) model of PAH and atherosclerosis. *Pulm Circ* 2018, 8 (1).

40. Conceicao, G.; Heinonen, I.; Lourenco, A. P.; Duncker, D. J.; Falcao-Pires, I., Animal models of heart failure with preserved ejection fraction. *Neth Heart J* 2016, 24 (4), 275-86.

DISCOVERIES is a peer-reviewed, open access *This article is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited and it is not used for commercial purposes; 2021, Liehn EA et al., Applied Systems and Discoveries Journals.*