



Artery Research

ISSN (Online): 1876-4401

ISSN (Print): 1872-9312

Journal Home Page: <https://www.atlantis-press.com/journals/artres>

Chronological changes of aortic and hepatic lesions in apolipoprotein E deficient mice[☆]

Matilde Otero-Losada, Santiago McLoughlin, Gastón A. Rodríguez-Granillo, Angélica Muller, Graciela Ottaviano, José Milei

To cite this article: Matilde Otero-Losada, Santiago McLoughlin, Gastón A. Rodríguez-Granillo, Angélica Muller, Graciela Ottaviano, José Milei (2011) Chronological changes of aortic and hepatic lesions in apolipoprotein E deficient mice[☆], Artery Research 5:3, 109–111, DOI: <https://doi.org/10.1016/j.artres.2011.05.001>

To link to this article: <https://doi.org/10.1016/j.artres.2011.05.001>

Published online: 7 December 2019



SHORT COMMUNICATION

Chronological changes of aortic and hepatic lesions in apolipoprotein E deficient mice[☆]

Matilde Otero-Losada, Santiago McLoughlin, Gastón A. Rodríguez-Granillo, Angélica Muller, Graciela Ottaviano, José Milei*

Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" (ININCA), Facultad de Medicina, Universidad de Buenos Aires (UBA) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

Received 28 March 2011; received in revised form 3 May 2011; accepted 10 May 2011
Available online 2 July 2011

KEYWORDS

Apolipoprotein E;
Atherosclerosis;
Aortic arch;
Liver injury;
Time-course

Background

Apolipoprotein E deficiency impairs lipid metabolism and accelerates atherosclerosis development in a genetically altered mouse model.¹ Apo E deficiency is related to the hepatic expression of proinflammatory mediators² and to accelerated aging in heart, kidneys and liver.³ In relation with hepatic damage, increased susceptibility to develop severe liver injury⁴ and a 5-lipoxygenase mediated inflammatory mechanism have been reported.⁵ Yet, despite the wide utilization of apo E-deficient (knock-out, null or ^(-/-)) mice for multiple purposes, the relationship between

hepatic injury and atherosclerotic development is still unclear.⁶

Aims

This work was conducted in order to explore a possible temporal association between hepatic damage and atherosclerotic evolution in Apo E-deficient mice and to characterize the lesions as well.

Methods

Fifteen newborn apolipoprotein E-deficient male mice (on a C57BL/6J background, The Jackson Laboratory, Bar Harbor, Maine, USA) were fed a standard chow diet. At 18, 20 and 30 weeks of age, consecutive groups of 6 randomly selected mice were euthanized. Procedures were approved by the Animal Care and Use Committee of the

[☆] This work was partially supported by an educational grant of the Fundación Florencio Fiorini and by funds from Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" (UBA-CONICET).

* Corresponding author. Tel./fax: +54 +11 4508 3836.

E-mail address: ininca@fmed.uba.ar (J. Milei).

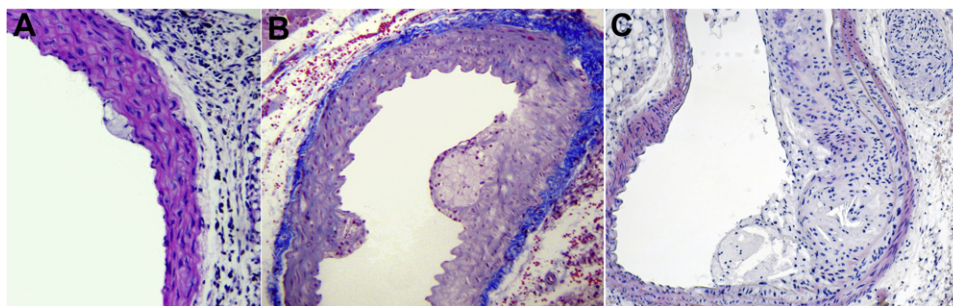


Figure 1 Chronological evolution of atherosclerotic lesion in Apo E-deficient mice. Age in weeks: 16 (A), 20 (B), 30 (C). For description see text.

University of Buenos Aires. Aortic arch and liver were dissected and immersed in 10% buffered formaldehyde (Formalin 10% buffered solution, pH = 7.0) at room temperature for at least 24 h fixation. After dehydration (graded ethanol series of 50%, 70%, and 100%), tissues were embedded in paraffin blocks and serially cut. Blocks were oriented to allow transverse serial sectioning and localization of the aortic arch. Hematoxylin-eosin and Heidenhain's Azan trichrome staining were used for histological examination. Acetic orcein stain was used to identify elastic fibers. A qualitative description of plaque histological features included the internal elastic membrane, lipid deposition, plaque inflammation and deposition of calcified material.

Tissue histomorphometry and planimetry were performed using a software-coupled (Image Pro Plus for

Windows, v3) Nikon Eclipse E400 microscope. Plaque area and tunica media' length were measured in digitized magnified sections. Intimal layer and intima/media ratio were considered when atherosclerotic lesions were observed. For liver histological analysis, non-alcoholic steato hepatitis (NASH)⁷ features were scored (0 = lowest, 4 = highest) including: steatosis, hepatocellular injury, parenchymal inflammation, portal inflammation and fibrosis.

Data were submitted to MANOVA, zero-order and partial bivariate correlations (Pearson's *r* coefficient). Alpha level of statistical significance was set at 0.05.

Results

Minute focal accumulations of lipid-laden macrophages were observed at 16 weeks (Fig. 1A). By the 20th week an increased number of macrophages were clustered in globular accumulations. These were covered by a thin fibrous

Table 1 Time course of changes in aorta and liver in apoE deficient mice.

	Age (weeks)		
	16	20	30
(n)	(4)	(6)	(5)
Plaque area (μm^2)	—	20531 \pm 6961	111536 \pm 26131 ^b
Stenosis (%)	—	13 \pm 4	31 \pm 9 ^c
Intima (μm)	11 \pm 1	139 \pm 33 ^a	164 \pm 20 ^a
Media (μm)	—	56.5 \pm 8.0	23.9 \pm 4.9 ^c
Intima-media	—	3.2 \pm 0.7	8.3 \pm 2.2 ^e
(n)	(4)	(4)	(4)
Steatosis (0–4)	3.8 \pm 0.3	2.8 \pm 0.6	3.0 \pm 0.0
Hepatocellular injury (0–4)	2.5 \pm 0.3	2.5 \pm 0.7	2.8 \pm 0.3
Parenchymal Inflammation (0–4)	—	0.3 \pm 0.3	1.5 \pm 0.3 ^c
Portal Inflammation (0–4)	0.5 \pm 0.3	0.8 \pm 0.3	1.8 \pm 0.3 ^d
Fibrosis	—	—	—

Data are shown as media \pm SE.

^a $p < 0.01$ vs. 16 weeks.

^b $p < 0.0001$.

^c $p < 0.01$.

^d $p < 0.03$.

^e $p < 0.05$ vs. 20 weeks.

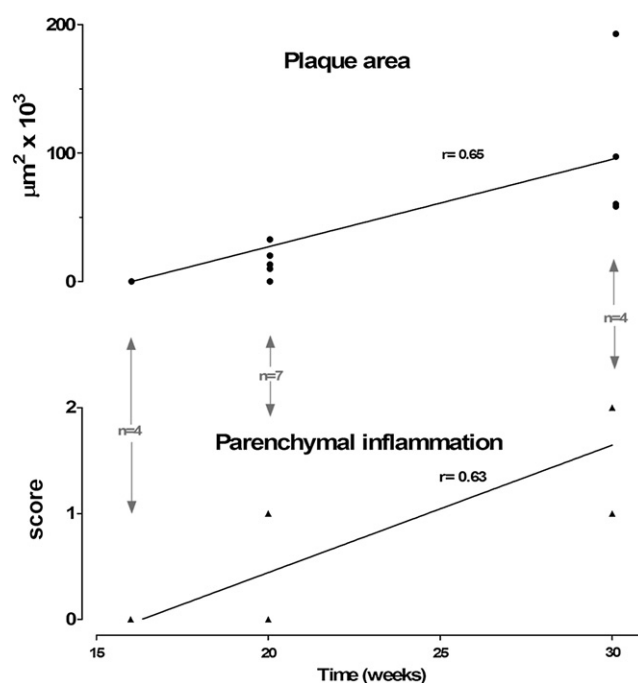


Figure 2 Plaque area and liver inflammation in Apo E^(-/-) mice at 16, 20 and 30 weeks of life.

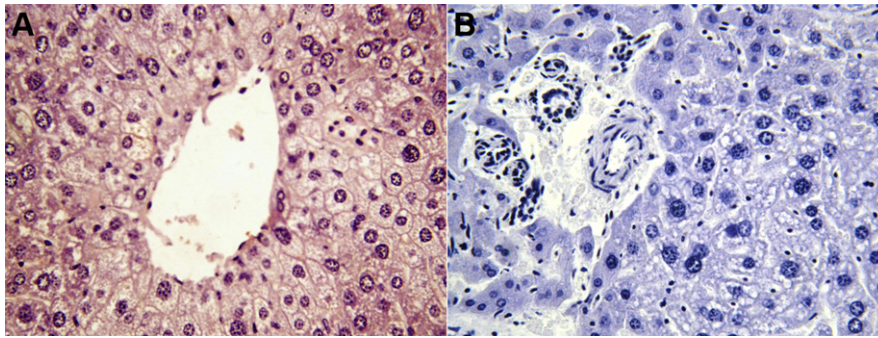


Figure 3 Hepatic steatosis (left) and parenchymal inflammation (right) in 30 weeks' Apo E-deficient mice.

cap and prolapsed in the arterial lumen. The integrity of the internal elastic membrane was spared (Fig. 1B). By the 30th week large acellular necrotic xanthomas formed a fibro-fatty nodule (cholesterol and connective tissue) which extended from the lumen to the internal elastic membrane. Along with this, luminal caliber was largely reduced with thinning and loss of the fibrous cap. Extensive atrophy of the media layer was observed being replaced with plaque components. Internal and external elastic membranes were disrupted and discontinued at the site of plaque development (Fig. 1C).

Plaque area ($F_{1,8} = 10.34$, $p < 0.012$), media thickness ($F_{1,8} = 11.88$, $p < 0.009$) and intima-media ratio ($F_{1,8} = 5.32$, $p < 0.05$) were time-dependent. Increase in plaque area (5-fold, $p < 0.0001$), stenosis percentage (2.4 fold, $p < 0.01$) and intima-media ratio (2.6 fold, $p < 0.05$) along with media thinning (-58% thickness, $p < 0.01$) were observed from weeks 20–30 (Table 1, Fig. 2).

Unlike steatosis or hepatocellular injury, liver parenchymal inflammation ($F_{2,9} = 13.29$, $p < 0.002$) and portal inflammation ($F_{2,9} = 6.30$, $p < 0.02$) were time-dependent and increased by 5-fold ($p < 0.01$) and 2-fold ($p < 0.03$) respectively from weeks 20–30 (Table 1). Fibrosis was not observed at any time (Fig. 3).

Interestingly not only time accounted for 62% of plaque area variation and 53% of changes in liver parenchymal inflammation but both variables mirrored each other's time-course as well (Fig. 2).

Conclusion

High variability of plaque area and liver parenchymal inflammation found around 20 weeks could not be explained by time and exceeded interindividual variability observed at any other time. An evolving period characterized by rapid changes might alternatively explain high variability in plaque area and parenchymal inflammation observed at this time. The estimated half-time for severe lesions development as predicted by a sigmoidal data fitting equation (~ 20.4 weeks) is in agreement with previous reports showing full development of lesions around 40 weeks.⁸

A complex interplay of adhesive cellular interactions, chemotactic factors, proinflammatory chemokines and

growth-regulatory molecules participates in atherogenesis.⁹ Knowledge of the temporal course of putative atherosclerosis-related inflammation mediators across lifespan might help to identify key candidates responsible for atherosclerosis evolution in this mouse model.

Atherosclerotic evolution might be associated with the progression to a severe inflammatory form of liver damage in apolipoprotein E-deficient mice. Present findings may suggest that a series of rapid and transient changes might take place around 20 weeks of life. These might be critical in determining the progression of tissue damage in this mouse model of atherosclerosis.

References

1. Meir KS, Leitersdorf E. Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress. *Arterioscler Thromb Vasc Biol* 2004;**24**:1006–14.
2. Yin M, Zhang L, Sun XM, Mao LF, Pan J. Lack of apoE causes alteration of cytokines expression in young mice liver. *Mol Biol Rep* 2010;**37**:2049–54.
3. Bonomini F, Filippini F, Hayek T, Aviram M, Keidar S, Rodella LF, et al. [polipoprotein E and its role in aging and survival. *Exp Gerontol* 2010;**45**:149–57.
4. Ferré N, Martínez-Clemente M, López-Parra M, González-Pérez A, Horrillo R, Planagumà A, et al. Increased susceptibility to exacerbated liver injury in hypercholesterolemic ApoE-deficient mice: potential involvement of oxysterols. *Am J Physiol Gastrointest Liver Physiol* 2009;**296**:G553–562.
5. Martínez-Clemente M, Ferré N, González-Pérez A, López-Parra M, Horrillo R, Titos E, et al. 5-lipoxygenase deficiency reduces hepatic inflammation and tumor necrosis factor alpha-induced hepatocyte damage in hyperlipidemia-prone ApoE-null mice. *Hepatology* 2010;**51**:817–27.
6. Alkhoury N, Tamimi TA, Yerian L, Lopez R, Zein NN, Feldstein AE. The inflamed liver and atherosclerosis: a link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk. *Dig Dis Sci* 2010;**55**:2644–50.
7. Collier J. Non-alcoholic fatty liver disease. *Medicine* 2007;**35**: 86–8.
8. Martin G, Dol F, Marés AM, Berezowski V, Stael B, Hum DW, et al. Lesion progression in apoE-deficient mice: implication of chemokines and effect of the AT1 angiotensin II receptor antagonist ibesartan. *J Cardiovasc Pharmacol* 2004;**43**:191–9.
9. Mach Francois. The role of chemokines in atherosclerosis. *Curr Atheroscler Rep* 2001;**3**:243–51.