Animal Models of Diabetic Complications Consortium
(U01 HL 70526)

Annual Report
(2005)

"Novel Models of Cardiovascular Complications of Diabetes"
UCLA

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PART A: Principal Investigators Summary

1. ***Program Accomplishments***

The EAC indicated that the consortium members concentrate on only 1 - 2 models. We have pursued the elderly LDLR-/- mouse on high fat diet as a model of accelerated diabetes and atherosclerosis. With 3-5 months of high fat diet there is a progressive worsening of glucose tolerance and atherosclerosis in the elderly animals. The rate of atherosclerosis progression is greater in the elderly (10-12 mos old) vs. young (3 mos old) LDLR-/- mice. Array analysis on vessels from young vs. old on chow vs. western diet was performed in order to identify potential mechanisms to explain the accelerated vascular injury. In exciting preliminary data we found that on chow diet enzymes which handle oxidative stress such as the family of glutathione peroxides are increased in elderly vs. young. In sharp contrast, after three months of western diet, these enzymes are increased in young animals, but actually *decrease* expression in vessels of older animals. These data suggest older animals have potential impaired capacity to deal with oxidative stress, possibly contributing to their accelerated atherosclerosis. We are also trying to determine whether this impaired capacity contributes to their worsening glucose tolerance. We also pursued the Apo A2 transgenic mouse which develops many features of the metabolic syndrome. This mouse was recently bred into hypercholesterolemic mice lacking apolipoprotein E (apoE-/-). Preliminary data suggests ApoA2tg enhances atherosclerosis by 2 fold. The mechanisms behind this acceleration are currently being investigated.

2. ***Collaboration within your group***

The elderly $LDLR^{-/-}$ animal reflects a concerted effort among investigators including Willa Hsueh, Alan Collins, Rajendra Tangirala and Christopher Lyon. The apoE -/- crossed with ApoA2tg is a product of the collaboration between Larry Castellani, Aldons Lusis, and Richard Davis.

3. ***Collaboration with other AMDCC groups***

The elderly mice were sent to Firouz Daneshgari and appear to have more extensive bladder dysfunction than younger animals. Control older animals are being sent to Dr. Daneshgari. The elderly animals will also be sent to Eva Feldman for neurological assessment.

4. ***Pertinent non-AMDCC Collaboration***

The Codelink array analysis was performed by Chris Glass and colleagues at UCSD through the auspices of the UCLA/UCSD Diabetes and Endocrinology Research Center (DERC). They are considered collaborators on this aspect of phenotyping the model.

5. ***Address EAC Comment***

None were received following the last meeting.

PART B: Project Reports by Responsible Investigators

Project 1: Elderly LDLR-/- on Western diet is a model of diabetes-accelerated atherosclerosis. As recommended by the EAC, we have studied the progression of both diabetes and atherosclerosis, as well as the inflammatory markers, during the three–five months of Western diet. As previously shown, there is a marked increase in lesion areas when elderly mice are placed on a Western diet compared to young (3 month old) mice (Figures 1A and 1B). The lesions are not only more extensive, but much more complex in the elderly mouse, consisting of necrotic lipid cores with cholesterol clefts covered by fibrous caps throughout the aorta. The lesion extent appears to also progress faster in the elderly mouse (Figure 2). The glucose tolerance worsens progressively on Western diet (Figure 3). Blood pressure levels remain normotensive, but there is a progressive increase in serum triglyceride and glucose and a decrease in HDLC (Table 1). There is also a progressive increase in leptin and PAI-1 (Figures 4 and 5). Resistin levels peaked at 2 months to 3000pg/ml and decline to 1500pg/ml at 5 mos., which are well above those on chow diet (Figure 6). Body weight was higher in the elderly vs. young animals at the start of the diet, but both increased at the same rate (Figure 7).

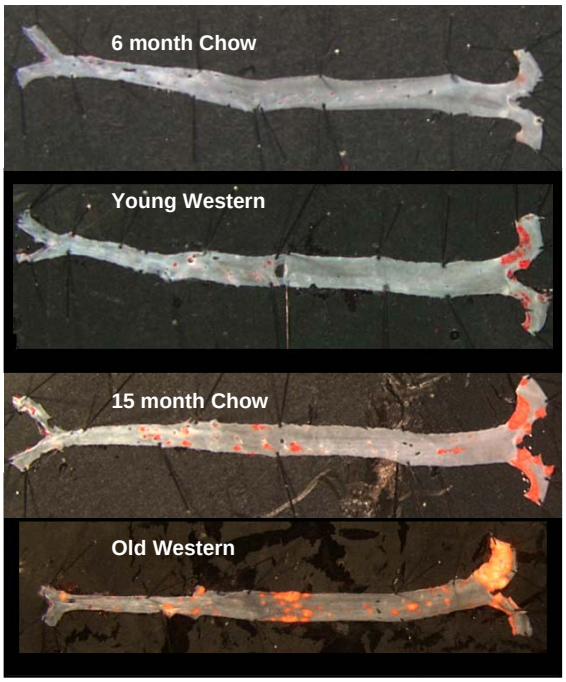


Figure 1A: Sudan red staining of aortae from LDLR$^{-/-}$ mice fed chow throughout or chow up to the last three months prior to sacrifice when they are given Western diet

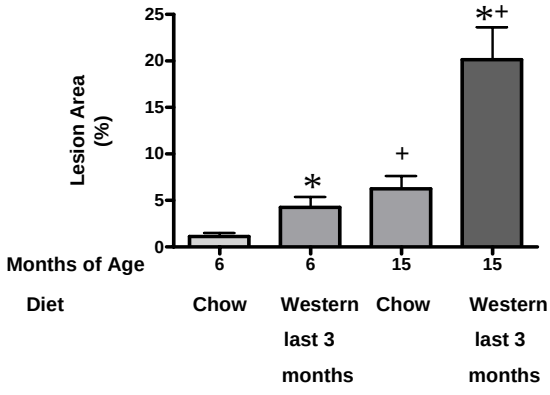


Figure 1B: En face analysis if atherosclerosis in the aortae

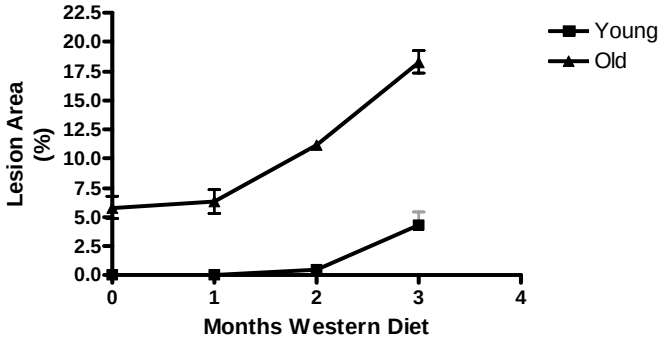
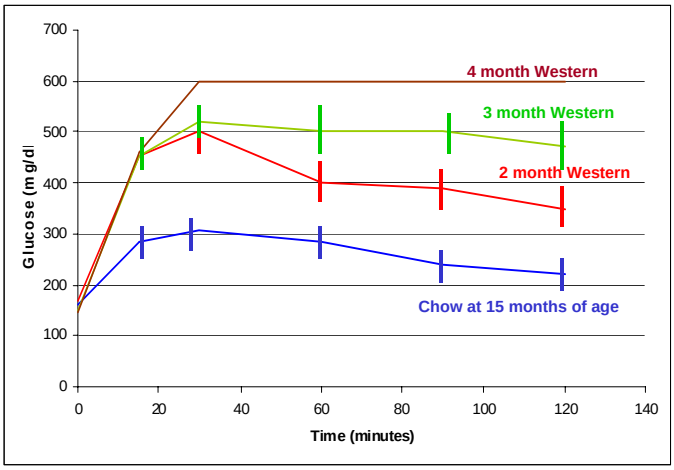


Figure 2: Atherosclerosis extent progresses faster in old vs young LDLR$^{-/-}$ mice on Western diet for 3 months



4 month Western diet mice exceeded glucometer reading at >600 mg/dl

Figure 3: See text for details

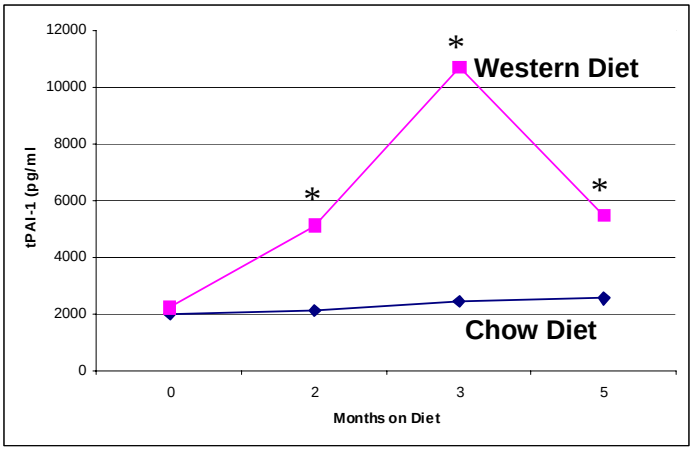


Figure 4: See text for details

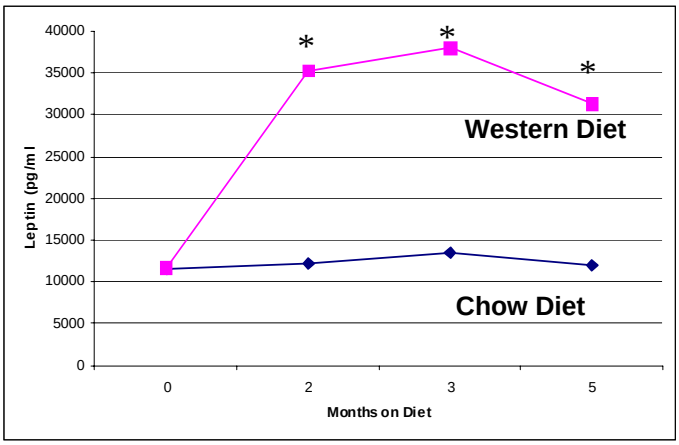
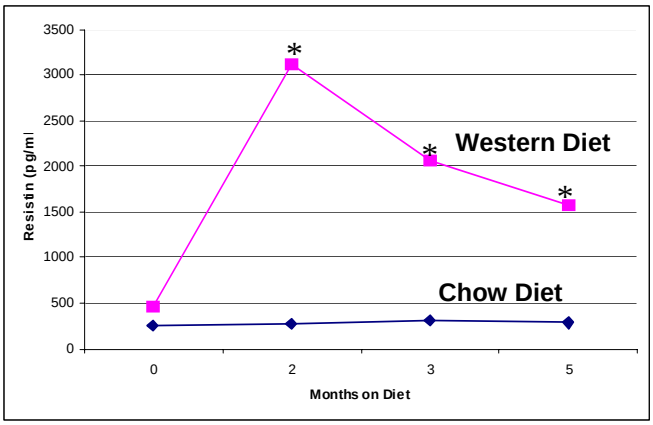


Figure 5: See text for details



p<0.05 vs Chow

Figure 6: See text for details

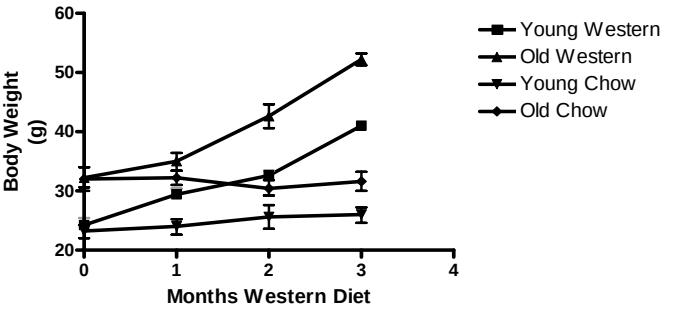


Figure 7: Western diet promotes weight gain at similar rates in both young and old LDLR$^{-/-}$ on Western diet compared to chow. Older mice start about 10 grams (g) heavier than young mice

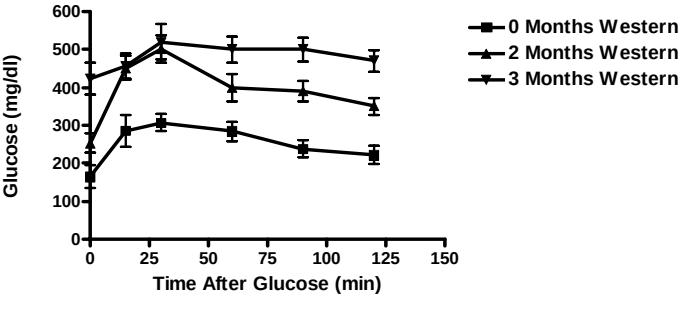


Figure 8: OGTT responses progressively worsen in older LDLR$^{-/-}$ mice from 0-3 months of Western diet

	Total Cholesterol (mg/dl)		Triglycerides (mg/dl)		HDL-cholesterol (mg/dl)		Glucose (mg/dl)	
	Young	Old	Young	Young	Old	Old	Young	Old
Chow	292±15	273±7	122±4	159±15	167±28	119±18	111±5	90±5
1 month Western Diet	583±72	654±82	124±7	190±11	251±26	157±21	108±2	82±5
2 month Western Diet	1307±110	1536±217	157±31	201±9	357±24	258±64	90±4	74±3
3 month Western Diet	1842±52	1928±154	147±11	285±25	423±27	371±60	105±8	67±5

Table 1: Lipid and glucose levels in LDLR$^{-/-}$ mice

To examine mechanisms involved in the accelerated vascular disease, array analysis using the Codelink system was performed in young vs. old LDLR-/- mice on chow diet and young vs. old on high fat diet. A prominent difference was found in oxidative stress systems, (Table 2). On chow diet, the older animals had higher levels of vascular expression of some antioxidant enzymes such as glutathiamine peroxidase (GPK) 1, 2, and 4. Upon administration of the Western diet, the expression of these enzymes increased in the young animals, but decreased in the older animals suggesting the vessels of the older animals could not handle oxidative stress. We are also investigating changes in system of inflammatory metabolic genes in immune and structural and matrix the vasculature. Array analyses are also being conducted on white fat obtained from the same animals.

Differential Regulation of Oxidative Stress Response Genes in the vasculature in Older Mice to Western and Chow Diet

Oxygen and reactive oxygen species metabolism				
Western Diet				
AccNo	**Description**	**Old/young**	**Young**	**Old**
NM_008161	glutathione peroxidase 3 (Gpx3)	-2.9	2341	808
NM_008162	glutathione peroxidase 4 (Gpx4)	-2.3	4625	1977
NM_008160	glutathione peroxidase 1 (Gpx1)	-2.5	2081	844
NM_013671	superoxide dismutase 2, mitochondrial (Sod2)	-2.3	780	338
NM_009804	catalase 1 (Cas1)	-1.8	4600	2613
NM_010497	isocitrate dehydrogenase 1 (NADP+), soluble (Idh1)	-4.3	173	40
NM_023719	thioredoxin interacting protein (Txnip)	-2.0	459	226
Chow Diet				
NM_023719	thioredoxin interacting protein (Txnip)	5.9	147	872
NM_008160	glutathione peroxidase 1 (Gpx1)	3.6	404	1437
AK021200	ES cells cDNA, RIKEN full-length enriched library, clone:C330014H07:cytochrome b-245, alpha	2.6	2892	7381
NM_030677	glutathione peroxidase 2 (Gpx2)	11.9	22	261
NM_010877	neutrophil cytosolic factor 2 (Ncf2)	2.8	234	664
NM_008162	glutathione peroxidase 4 (Gpx4)	2.2	1440	3167
NM_010497	isocitrate dehydrogenase 1 (NADP+), soluble (Idh1)	14.0	8	105

Western	**NM_011671**	**uncoupling protein 2, mitochondrial (Ucp2)**	**-2.9806**	**875.7**	**293.8**
Chow	NM_011671	uncoupling protein 2, mitochondrial (Ucp2)	8.5431	110.3	942.3
Western	**NM_009155**	**selenoprotein P, plasma, 1 (Sepp1)**	**-1.8077**	**2781**	**1539**
Chow	NM_009155	selenoprotein P, plasma, 1 (Sepp1)	3.8061	604	2298.9

Table 2: Coding for enzymes that handle oxidative stress are increased in old vs. young mice on chow diet, but do not increase in response to western diet in old compared to young mice!

The elderly LDLR-/- mice also appeared to have damage to other diabetes target organs. The older animals on Western diet had increased albumin/creatinine ratio compared to older animals on chow diet (Figure 8B). The hearts tended to display a greater profibrotic response with an increase in profibrotic genes including osteopontin (OPN), transforming growth factor β (TGFβ), fibronectin (FBN), and inflammation as measured by CD68, a marker of macrophage recruitment (Figure 9).

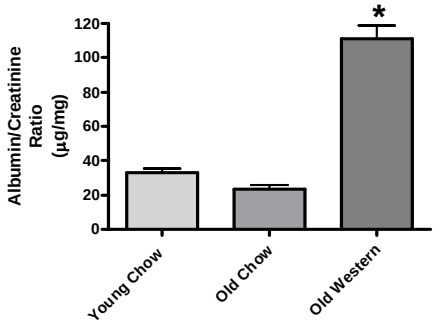


Figure 8A: Old diabetic mice (Western diet) develop significantly more albuminuria that nondiabetic mice (chow) of the same age

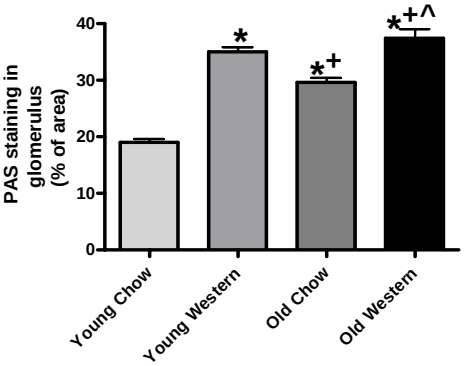


Figure 8B: Quantification of PAS staining in glomerular sections, per AMDCC protocol (www.amdcc.org)

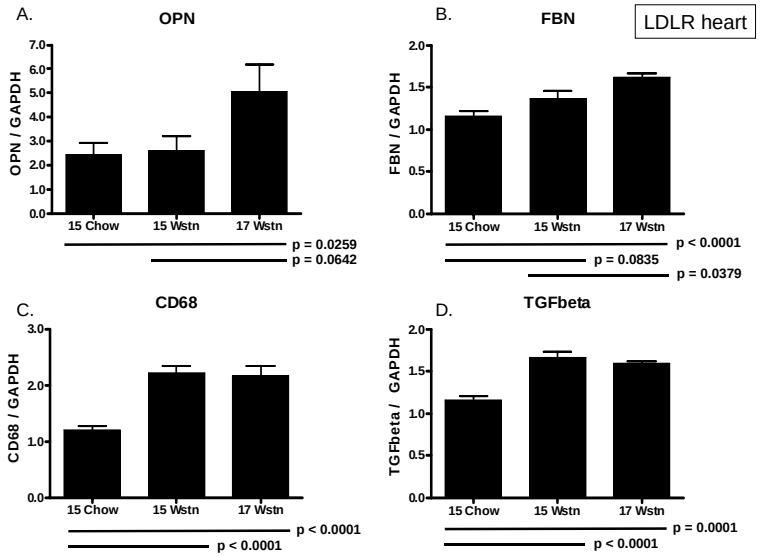


Figure 9: High Fat diet increases inflammatory gene expression in the hearts of LDLR$^{-/-}$ mice. Expression of osteopontin (OPN), CD68 (an idicator of macrophage infiltration), fibronectin (FBN) and transforming growth factor beta (TGFbeta) were measured by quantitative real-time PCR in cardiac ventricles of old LDLR$^{-/-}$ mice fed chow or western (Wstn) diet for the last 3 months (15 months old) or 5 months (17 months old) prior to sacrifice. Data was normalized to GAPDH, and all values are normalized to the lowest ratio among the groups.

Project 2: ApoE$^{-/-}$ x ApoA2tg mice

The apoA2tg crossed into the apoE$^{-/-}$ exhibited a 2-fold increase in the extent of atherosclerosis as determined by the *en face* method (Figure 10). The control apoE$^{-/-}$ mice had 12% of the aortic surface covered by lesions while the apoE$^{-/-}$ApoA2tg mice had 25% of the aorta as lesions. These changes were associated with greatly increased triglycerides and insulin resistance in the apoE$^{-/-}$ApoA2tg mice (Figure 11).

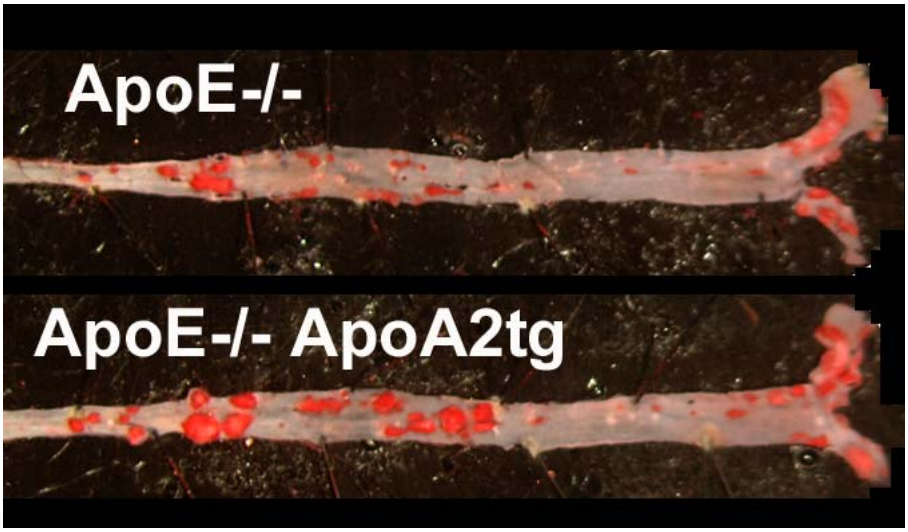
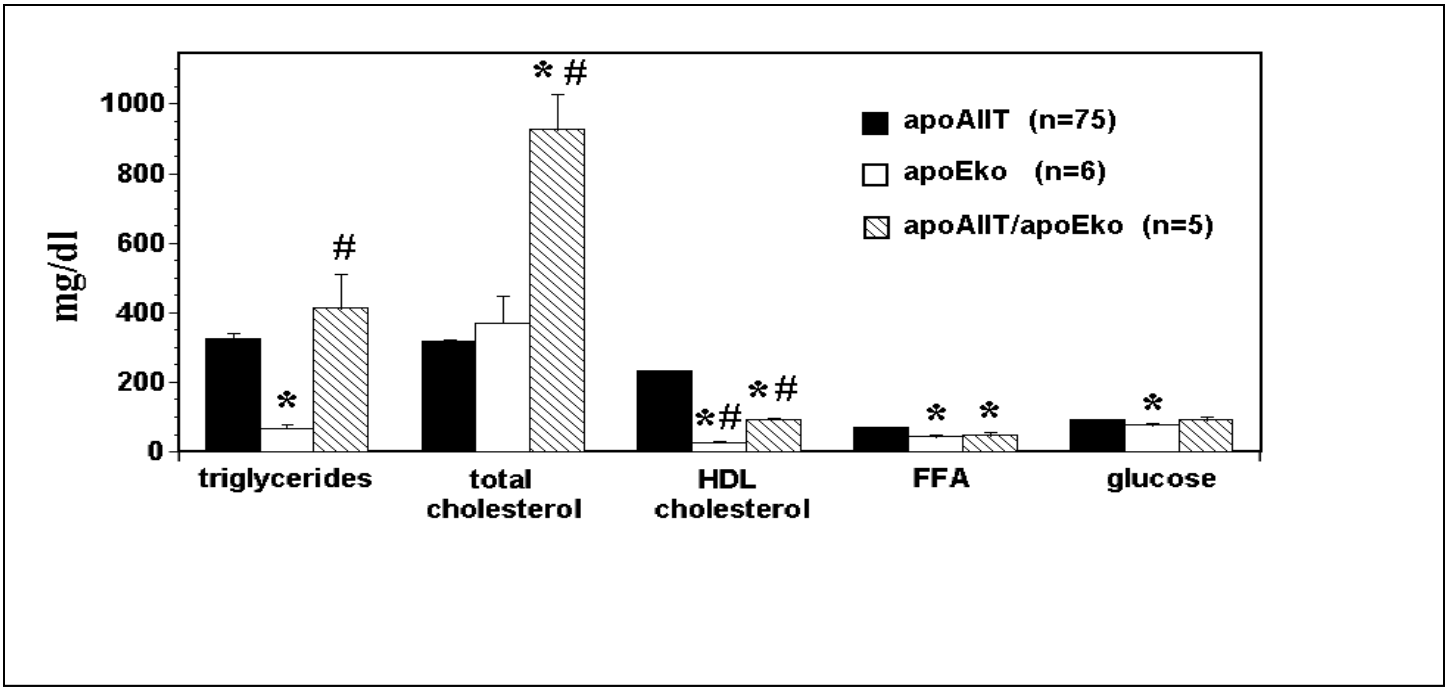


Figure10: See text for details


mg/dl
1000
800
600
400
200
0
apoAIIT (n=75)
apoEko (n=6)
apoAIIT/apoEko (n=5)
triglycerides
total cholesterol
HDL cholesterol
FFA
glucose