Endothelial Lipase Is Synthesized by Hepatic and Aorta Endothelial Cells and Its Expression Is Altered in apoE Deficient Mice

Kenneth C-W. Yu†, Christopher David¶, Sujata Kadambi¶, Andreas Stahl†¶, Ken-Ichi Hirata†§, Tatsuro Ishida†§, Thomas Quertermous†, Allen D Cooper†¶ and Sungshin Y. Choi¶*
Palo Alto Medical Foundation-Research Institute¶, Palo Alto, CA, School of Medicine, Stanford University†, Palo Alto, CA.

*Corresponding author: Sungshin Y. Choi, Ph.D.
Research Institute, Palo Alto Medical Foundation
795 El Camino Real – Ames Bldg.
Palo Alto, CA 94301
650-853-2866
email address: chois@pamfri.org

§: Current Address: Division of Cardiovascular and Respiratory Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Short title: Tissue specific expression of endothelial lipase

Abbreviations:

EL = endothelial lipase, EC = endothelial cells, HL = hepatic lipase, LPL = lipoprotein lipase, EKO = apoE knockout, WT = wild-type, CA = cholic acid, HL = high fat, NC = normal chow, RT-PCR = real-time PCR, vWF = von Willbrand Factor, EC = endothelial cells.

ABSTRACT

Both LPL and HL are synthesized in parenchymal cells, secreted and bind to endothelial cells. To learn where endothelial lipase (EL) is synthesized in the adult animals the localization of EL in mouse and rat liver was studied by immunohistochemical analysis. Further, to test if EL could play a role in atherogenesis, expression of EL in the aorta and liver of apoE knockout mice was determined. EL, in both mouse and rat liver was colocalized with the vascular endothelial cells and not hepatocytes. In contrast, hepatic lipase (HL) was present in both hepatocytes and endothelial cells. By *in situ* hybridization EL mRNA was present only in endothelial cells in liver sections. EL was also present at low levels in the aorta of normal mice. We fed apoE knockout mice (EKO) and wild-type (WT) mice a variety of diets and determined EL expression in livers and aorta. EKO showed significant expression of EL in aorta. EL expression was lower in the liver of EKO than normal mice. Cholesterol feeding lowered EL in liver of both types of mice. In the aorta EL was higher in EKO than WT mice and cholesterol feeding had no effect. Together these data suggest that EL may be up regulated at the site of the atherosclerotic lesions and thus could supply lipids to the area.

INTRODUCTION

Two laboratories independently cloned an enzyme that belongs to the pancreatic lipase gene family (1, 2). The enzyme is now referred to as endothelial lipase (EL) because this enzyme was first identified at a high level in embryonic endothelial cells. It was also discovered in cholesterol loaded macrophages (1). The level seemed to decrease with maturation of endothelial cells and in the adult, the liver and thyroid gland are the tissues with the highest levels. Further, Northern blot analysis shows that it is present in HepG2 cells, a hepatoma cell line often used as a model of hepatocytes. Thus, its cell of origin in the adult is uncertain since the liver has both endothelial cells and macrophages as well as hepatocytes.

EL has a high molecular homology with HL and LPL (1,2). It contains about 500 amino acids with a molecular weight of approximately 55 kD with conservation of the catalytic triad as well as potential heparin and lipoprotein binding sites. However, a potential lid region is not well conserved when compared to the other lipases of its family. LPL is expressed in muscle, adipose, heart, mammary gland, brain and macrophages (7, 8). It is secreted and binds to endothelial cells in those organs. HL is synthesized by hepatocytes and remains there, adherent to both hepatocytes and endothelial cells or is transported to endothelial cells in the adrenal glands and ovaries (9, 10). Thus, both HL and LPL are synthesized by parenchymal cells, secreted and bind to endothelial cells. Whether EL is processed like HL and LPL is unknown.

As a lipase EL hydrolyzes water-soluble substrates (1) but does not have a high triglyceride lipase activity, at least in the presence of serum; rather, this enzyme has a readily detectable phospholipase A1 activity (1,2). In mice overexpression of EL using either a viral vector or transgenic animals results in a significantly lower plasma concentration of HDL cholesterol and apoA1 (1, 2, 3). Conversely, in animals with mutation of the EL gene (3) or infused with an anti-EL antibody HDL cholesterol levels are significantly elevated (4) suggesting that EL modulates plasma HDL levels *in vivo*. It has recently been reported that EL expression is increased in cultured human umbilical vein and coronary artery endothelial cells by inflammatory cytokines such as TNF-α and IL-1β (5, 6). This, along with its effect on HDL suggests that this enzyme could be involved in the development of atherosclerosis.

The present study was designed to identify the cellular origin of EL and to ascertain whether it moves from parenchymal cells to endothelial cells. Because liver is the major organ in lipoprotein metabolism and EL expression was detected at a high level in the liver by Northern blot analysis (1), we studied the localization of EL using rat and mouse liver sections. In addition, to learn if the inflammatory response and its ability to modulate plasma levels of HDL contribute to atherogenesis, we studied the regulation of EL expression in liver and aorta in response to a high fat diet in normal and apoE deficient animals, an animal model for atherosclerosis.

MATERIALS AND METHODS

Animals and diets: Both wild-type C57BL/J6 and apo E knockout mice (Jackson Laboratory, Bar Harbor, ME) were fed with the following diets for 4 weeks; normal chow (NC), chow plus 2% cholesterol (NC+Chol), a high fat (HF) (11) and a high fat supplemented with 1% cholic acid (HF+CA). High fat diets with or without cholic acid were purchased from Harlan Teklad (Madison, WI). Cholesterol was purchased from Sigma (St. Louis, MO).

Western blot analysis: Eighty ug of total liver membranes were subjected to 7% SDS-polyacrylamide gel electrophoresis followed by transferring to nitrocellulose membrane (BioRad, Hercules, CA). The membrane was then incubated with anti-EL antisera for 2 hours at room temperature. After washing and incubation with secondary antibodies conjugated with HRP the membrane was then developed with ECL (Amersham-Pharmacia Biotech).

Specificity of anti-EL antibodies: The specificity of polyclonal anti-EL antibodies was tested using Western blots as described above. Briefly, media collected from COS cells and CHO cells producing human EL and HL, respectively, was subject to electrophoresis followed by transfer to a nitrocellulose membrane. The membrane was then incubated with polyclonal anti-EL or anti-HL antibodies followed by secondary antibodies labeled with HRP. Anti-EL antibodies recognized only EL and not hepatic lipase (data not shown). Conversely, anti-hepatic lipase antibodies bound only to rat hepatic lipase and not EL. Thus, these antibodies are specific for the corresponding antigens and do not cross react with other lipases that are likely to be present on endothelial cells.

In Situ Hybridization: *In situ* hybridization was performed as described previously (1) using slides that were generated from paraformaldehyde-fixed, paraffin-embedded mouse liver sections according to the established methods or were purchased from Novagen (Madison, WI). A 611-base pair EcoRI mouse EL cDNA fragment encoding the carboxyl-terminal 52 amino acids and 3" untranslated region was cloned into pBluescript KS (+). This fragment was used for *in vitro* RNA probe transcription. Both anti-sense and sense cRNA probes were labeled with ^{35}S dUTP. Hybridization, washing, and probe detection were performed using SureSite II (Novagen, Madison, WI) according to the manufacturer's instructions.

Immunostaining: The tissues were fixed with PBS containing 4% paraformaldehyde and then placed in OCT embedding medium (12) and frozen over dry ice. Sections were cut to 8 μm thickness and place onto glass slides. The sections were incubated in PBS+0.1% Triton X100 for 5 minutes, in PBS+3% BSA for 30 minutes and then with antibodies. For staining endothelial cells, an anti-WF (von Willebrand Factor) antibody (Sigma Chemical Co., St. Louis. MO) was used followed by FITC-labeled secondary antibody (Molecular Probes Inc., Eugene, OR). For staining HL and EL, polyclonal anti-HL or EL

antibodies were used, respectively, followed by rhodamine-labeled secondary antibodies. Digital images of the stained sections were obtained using a Molecular Dynamics Multiprobe confocal laser microscope (Sunnyvale, CA).

Human adult thyroid tissue sections from thyroid tissues were purchased from Novagen (Madison, WI). Tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm thickness. Prior to use, paraffin was removed with 3x5 washes in xylene and 2x5 minutes in 100% ethanol as suggested by the manufacturer's instructions. The sections were washed in phosphate-buffered saline containing 1% bovine serum albumin, 10% fetal calf serum, and 1% normal donkey serum to block nonspecific binding. Primary and secondary antibodies were diluted in blocking solution and incubated for 1 hour. The sections were mounted in 90% glycerol/phosphate-buffered saline containing 1 mg/ml paraphenylinediamine. Monoclonal anti-CD31 antibodies were purchased from BD Pharmigen. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI).

Real Time-PCR: Total RNA was prepared from livers and aortas obtained from wild-type or apoE deficient mice using a kit from Qiagen and reverse transcriptase reaction was performed on 1 ug of RNA using random hexamers reverse transcriptase (Gibco BRL, Life Technologies, Vienna, Austria). The primers and probe for mouse EL were designed using Primer Express 1.5 (Applied Biosystems, Foster City, CA). The probe for EL was labeled with a reported dye (FAM) and quencher dye (TAMRA). The primers and TaqMan™ probe for GAPDH were purchased from ABI. Sequence of primers and probe will be available upon request. The probe for GAPDH was labeled with a reporter dye (VIC) and TAMRA. RT-PCR was performed using forty amplification cycles (95°C, 15s; 55°C, 1 min; 72°C, 30 s). The level of GAPDH RNA was quantified and used to normalize the concentration of EL in each sample.

RESULTS

Immunohistochemical Analysis of Liver Sections: The liver is one of the organs that expresses the highest levels of EL (1). It is also the most important organ in lipoprotein metabolism. To learn where EL is localized in the liver we performed a series of immunohistochemical experiments using anti-EL antibodies. For a reference mouse and rat liver sections were incubated with anti-hepatic lipase antibody followed by rhodamine conjugated secondary antibody. Endothelial cells were localized with anti-von Willebrand factor (WF) and an FITC conjugated secondary antibody. In rat liver, hepatic lipase localizes to both hepatocytes and endothelial cells (Figure 1A). In mouse liver, HL localizes with hepatocytes but not endothelial cells (Figure 1B). Liver sections obtained from mice transgenic for rat hepatic lipase have hepatic lipase on endothelial cells as well as parenchymal cells (Figure 1C).

Using the same technique rat and mouse liver sections were stained with anti-EL antibodies and anti-von Willebrand factor IgG. EL colocalizes with endothelial cells in the rat liver (Figure 2A) and mouse liver (Figure 2B). Unlike HL, EL is present only in endothelial cells and it is virtually absent in hepatocytes. To further confirm these results primary mouse hepatocytes and endothelial cells were isolated and stained for EL. Similar to the liver sections, EL was expressed only by the primary hepatic endothelial cells and not by the primary hepatocytes (data not shown).

Immunostaining of EL in other tissues: Northern blot analysis shows that EL is present in placenta, liver, lung, ovary, thyroid gland, testis and not in adrenal gland (1). The tissue distribution of EL was further assessed by immunostaining. EL was not present in heart or muscle (data not shown). It was detected in the lung as previously reported (data not shown) and adrenal glands (Figure 3A) but not in ovaries (Figure 3B). We also performed immunohistochemical analysis of thyroid sections since EL expression was quite high in thyroid (1). In thyroid as shown in Figure 3C EL is expressed in the in the endothelial cells of the large vessels (red). Nuclei were counterstained with DAPI (blue). Thus, EL protein colocalizes with organs where its mRNA is present and is generally found on endothelial cells in the organ.

In situ hybridization of EL in mouse liver: Immunostaining data show that EL is present in endothelial cells of the liver. In order to determine whether analogous to HL and LPL EL is synthesized and secreted by hepatocytes and then translocated to endothelial cells (EC) or whether EL is synthesized by endothelial cells, *in situ* hybridization with an EL cRNA probe was performed on mouse and rat liver sections. If it is synthesized in the hepatocyte and transferred to endothelial cells, then it would be analogous to HL and LPL. EL mRNA was readily identified in endothelial cells surrounding the large blood vessel in the mouse liver and could be seen in endothelial cells of the sinosoids. It was not detected in hepatocytes (Figure 4A). Similar results were obtained with rat liver

sections (Figure 4B). Thus, unlike HL and LPL, EL is synthesized by the endothelial cells and remains localized there.

Expression of EL in aorta and liver in apoE knockout mice: EL mRNA level is increased by inflammatory cytokines such as TNF-α and IL1-β in cultured endothelial cells (5, 6). EL modulates plasma HDL levels in transgenic and knock-out animals (3). Together these observations suggest that EL could play a role in the development of atherosclerosis which is an inflammatory condition. Using apoE knockout mice fed a high fat diet the expression of EL in aorta and liver was compared by immunohistochemistry. EL is expressed at a high level in the aorta of apoE knockout animals (upper panel, Figure 5A) while the expression of EL in the aorta of wild-type animals appears to be minimal (lower panel).

Using Western blotting we then determined the effect of an atherogenic phenotype on the level of EL expression in the liver. EL expression was significantly reduced in the liver of apoE knockout animals (lanes 6-9) as compared to the wild-type animals (lanes 1-5, Figure 5B). These data together suggest that the regulation of EL expression is tissue specific and the expression of the enzyme may be up-regulated at the site of atherosclerotic lesions.

Effect of fat, cholesterol and bile acid feeding on the expression of EL in apoE knockout (EKO) mice: To learn how the effect of the apoE$^{-/-}$ gene on the expression of EL in liver and aortic endothelial cells was determined, animals were fed diets of different cholesterol content and atherogenic potential. Animals were fed one of four diets; normal chow (NC), normal chow plus 2% cholesterol (NC+Chol), a diet containing saturated fat and cholesterol (HF) and another diet of saturated fat containing cholesterol and 1% cholic acid (HF+CA) for four weeks. Plasma triglyceride and cholesterol levels were measured (Table 1). Serum cholesterol levels rose significantly in apoE knockout animals but not in wild-type mice fed 2% cholesterol (NC+Chol) compared to those fed normal chow (NC) diet. The addition of saturated fat (HF) caused an increase in cholesterol in the wild-type mice and a further increase in apoE knockout mice. Serum triglyceride levels of the mice were not affected by cholesterol feeding.

The level of EL mRNA in the liver and aorta of those animals was determined using RT-PCR. On all of the diets EL expression in the liver of EKO mice was significantly lower than in livers of control mice ($p<0.05$) (Figure 6A). Cholesterol feeding, whether alone, or with saturated fat lowered EL in livers of both control and EKO mice. Interestingly, addition of cholic acid to a diet containing saturated fat (HF+CA) significantly increased EL expression in the liver compared to a diet containing saturated fat alone (HF) (Figure 6A). This is consistent with the notion that bile salts play a role in the hepatic inflammation (31, 32) seen in this model of atherosclerosis. In contrast to the liver, none of the diets affected EL levels in the aorta of either control or EKO mice (Figure 6B). On each diet EL expression was higher in endothelial cells of EKO than those of the control mice. This was not statistically significant because of the variability and small number in each group. However, when the data were pooled to

compare the two groups, EL expression was significantly higher in EKO mice compared to the control mice ($p<0.01$, data not shown). These data together demonstrate that there is a complex and tissue specific regulation of EL expression.

DISCUSSION

In the present study by use of *in situ* hybridization we demonstrated that EL is expressed in hepatic endothelial cells and not in hepatocytes. In addition, it was observed that the level of EL expression in hepatic endothelial cells is lower in apoE knockout (EKO) mice while it is higher in the aorta of EKO mice suggesting a tissue specific regulation of EL expression. This was confirmed by cholesterol feeding which reduced EL mRNA levels in hepatic endothelial cells and did not affect mRNA levels in aortic endothelial cells.

The tissue-specific expression of EL in the adult is different from that of LPL and hepatic lipase (HL). In rats and humans HL is synthesized by the hepatocytes and remains there, adherent to both hepatocytes and endothelial cells or is transported to the endothelial cells in the adrenal glands and ovaries. In mouse HL circulates in the plasma presumably due to a lack of heparin binding as a result of variation in the heparin binding region in this species. LPL is synthesized in parenchymal cells of muscle, adipose, heart, mammary gland and brain and functions while bound to the luminal surface of endothelial cells in these organs (13, 14). Thus, both HL and LPL are synthesized at one site and translocate to another site for function.

EL is found at a high level in embryonic endothelial cells but its level decreases with maturation. In the adult, it is detected in a number of tissues including placenta, lung, liver, testis, thyroid, and ovary. EL mRNA was detected at a high level on Northern blots of RNA from whole liver and HepG2 cells (1). Thus, it was possible that in the adult the enzyme was synthesized in parenchymal cells and secreted and bound to endothelial cells. Immunostaining data show that EL is present in the endothelial cells lining the vessels in the mouse liver but it was virtually undetectable in the hepatocytes. *In situ* hybridization revealed that unlike HL, EL mRNA is present in endothelial cells but not in hepatocytes. Thus, EL is different from HL and LPL since EL functions at the site where it is synthesized. The difference in distribution between cell types suggests nonoverlapping functions for HL and EL despite their similar localization and molecular sequences. Interestingly, all these lipases are expressed in macrophages. Indeed in the other tissues where it is abundant such as lung and thyroid it is localized to endothelial cells.

The role of LPL and HL in the development of atherosclerosis has been investigated by several laboratories. These enzymes appear to have both pro- and anti-atherogenic roles. LPL is antiathrogenic because the enzyme plays a role in clearing plasma chylomicron and VLDL remnant particles (15, 16) as well as LDL via bridging the lipoproteins to the cell surface receptors (17). However, in the vessel wall LPL may have proatherogenic properties. For instance, macrophages secrete significant amounts of LPL (18, 19, 20) and absence of macrophage-derived LPL reduced the formation of atherosclerotic lesions. However, LPL within the vessel wall increases lipoprotein retention in the subendothelial matrix (21, 22, 23) and it was shown that LPL may act as a monocyte adhesion protein (24). Together these properties could contribute to formation of atherosclerotic lesions.

HL is involved in the selective uptake of HDL (25, 26) and thus lowers HDL levels. There is an inverse relationship between HDL levels and the incidence of atherosclerotic coronary artery disease (27, 28). This may be because HL lowers HDL levels by converting HDL_2 to HDL_3 and facilitates selective uptake of HDL cholesteryl ester (25, 26). Transgenic animals as well as knock-out animals were used to study whether HL plays a role in the development of atherosclerosis *in vivo*. Busch et al. (29) reported that aortic cholesterol levels were reduced in mice that overexpress human HL. In contrast, HL deficiency also reduced the susceptibility to atherosclerosis in apoE knock-out mice (30). Thus, it is not clear yet whether HL contributes directly to the development of atherosclerosis or not although its level is related to susceptibility to atherosclerosis.

EL could potentially affect the development of atherogenesis through its effects on HDL levels and via local effects since it is synthesized by the endothelial cells and its expression is up regulated by the inflammatory cytokines such as TNF-α and IL-1β (5). Like LPL and HL, EL can bind apoB-containing lipoproteins, and thus, potentially facilitates their retention and cellular uptake and it can increase selective uptake of cholesteryl esters from HDL. Overexpression of EL reduces HDL levels (1, 2, 3) and conversely, animals deficient in EL showed significantly elevated levels of plasma HDL (3). Further, it is established that atherosclerosis is an inflammatory disease and that EL expression can be induced by inflammatory cytokines. In the present studies using apoE knockout mice, an animal model of atherosclerosis, it was found that EL expression is enhanced in the aorta and reduced in the liver of mice developing atherosclerosis. Thus, during atherogenesis EL in endothelial cells might contribute to cholesteryl ester uptake and EL from macrophages could contribute to retention of lipoproteins in the lesion. In animals lacking both EL and apoE there was a significant decrease in atherosclerotic lesion compared to apoE deficient mice (Ishida et el., unpublished data). Together, these data suggest that similar to LPL and HL, EL also has pro-atherogenic effects.

The change in EL expression in apoE deficient mice raises the question of what factors in addition to cytokines might alter EL expression and whether there may be organ specific regulation of its levels. Indeed, cholesterol feeding lowered EL mRNA level in hepatic endothelial cells. The effect was augmented somewhat by the addition of saturated fat to the diet but not further augmented by the addition of cholic acid. The latter not only further raises serum cholesterol levels and but also causes hepatic inflammation. Quantification of EL in the aorta is more difficult because of the small amount of tissue available but none of these regimes seemed to alter EL mRNA levels in aortic endothelial cells. The mechanism of the induction in apoE deficient mice is open to speculation but certainly could be due to the inflammation in the aorta of these animals that occurs as part of the atherosclerotic process.

In summary, the present studies demonstrated that EL is synthesized by hepatic endothelial cells and not by hepatocytes. It is present in the aorta and its expression in the aorta is higher in animals developing atherosclerosis compared

to normal animals. In contrast, its expression is down regulated in the liver of those animals. Additionally, in liver but not in aorta its expression may be regulated by cholesterol flux. Thus, EL could play a role in the development of the atherosclerosis by altering plasma levels of lipoproteins as well as by directly affecting cholesterol and lipid flux at the site of lesions

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FIGURE LEGENDS

Figure 1. Immunostaining of rat hepatic lipase in rat and mouse livers. Livers were prepared for immunostaining with antibodies against endothelial vWF and rat HL as described in *Materials and Methods*. **(A)** A rat liver section showing the colocalization of the HL (*red*) with hepatocytes and with endothelial cells (*green*). **(B**) A wild-type mouse liver section showing the colocalization of the HL (*red*) with hepatocytes but not with endothelial cells (*green*). **(C)** A liver section from a mouse transgenic for rat hepatic lipase showing the colocalization of the HL (*red*) with hepatocytes and with endothelial cells (*green*).

Figure 2. Immunostaining of endothelial lipase in rat and mouse livers. Livers were prepared for immunostaining with antibodies against endothelial vWF and EL as described in *Materials and Methods*. **(A)** A rat liver section showing the colocalization of the EL (*red*) with endothelial cells (*green*). **(B)** A wild-type mouse liver section showing the colocalization of the EL (*red*) with endothelial cells (*green*).

Figure 3. Immunostaining of endothelial lipase in adrenal glands, ovaries and thyroids. Wild-type mouse livers were prepared for immunostaining with antibodies against endothelial vWF and EL as described in *Materials and Methods*. **(A)** Presence of EL (*red*) colocalized with endothelial cells (*green*) in a section of the adrenal glands. **(B)** Absence of EL in a section of the ovary despite the presence of endothelial cells (*green*). **(C)** Presence of EL (*red*) in thyroid sections obtained from human adult. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; *blue*). Arrow bars indicate the presence of erythrocytes in the sections.

Figure 4. *In situ* hybridization of EL expression. ^{35}S-Labeled cRNA sense and antisense probes for EL were hybridized to sections of mouse liver (**4A**; 20X Objective). Similarly, rat liver sections were hybridized with the same probe and only the antisense probes showed a signal above background and rat liver (**4B**; 20X Objective).

Figure 5. **Expression of endothelial lipase in the aorta and liver of wild type and apoE-knockout mice.** Immunostaining and Western blotting were used to determine the expression of EL in livers of wild-type and apoE-knockout mice as described in *Materials and Methods*. (**A**) An apoE-knockout mouse aorta (upper panel) and wild-type mouse aorta (lower panel) section showing the colocalization of the EL (*red*) with endothelial cells (*green*). (**B**) Western blots showing the protein expression of EL in total liver membranes prepared from wild-type mice (lanes 1-5) and apoE knockout mice (lanes 6-9).

Figure 6. Effect of fat, cholesterol and bile acid feeding on the expression of EL in apoE knockout mice. Wild-type and apoE knockout mice were fed with normal chow (NC), NC+2% cholesterol (NC+Chol), high fat (HF) and HF+1% cholic acid (HF+CA) for four weeks. Real-time PCR was performed as described under "Materials and Methods" using total RNA prepared from aorta (**6A**) and liver (**6B**) of those animals. Data expressed as mean A.U. (arbitrary unit) $\pm$ S.D. (n=4). * $p<0.05$.

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Figure 1A

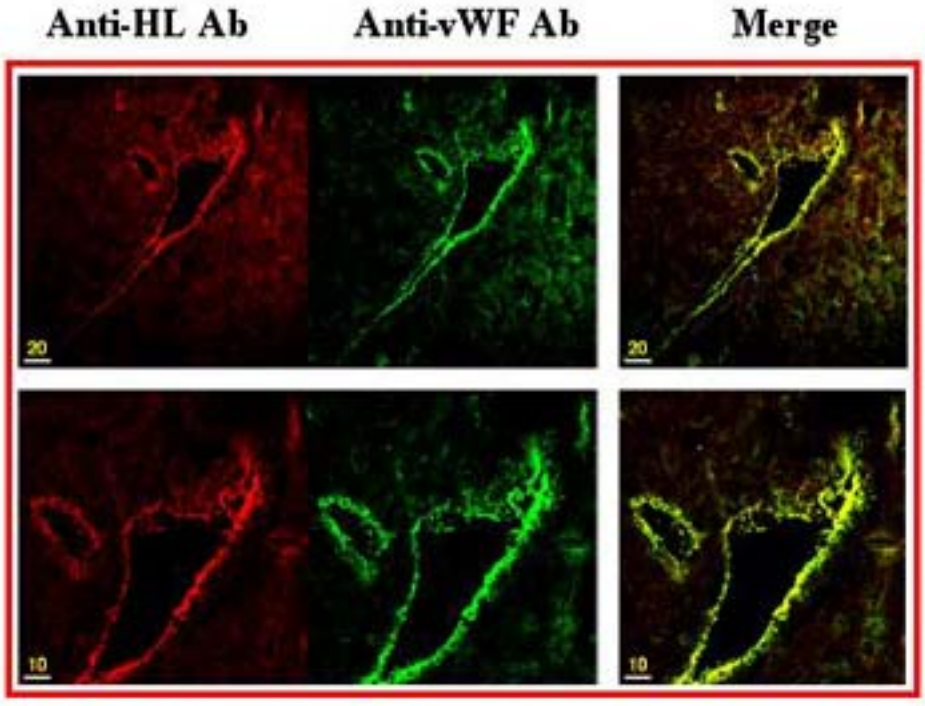


Figure 1B
Yu et al.

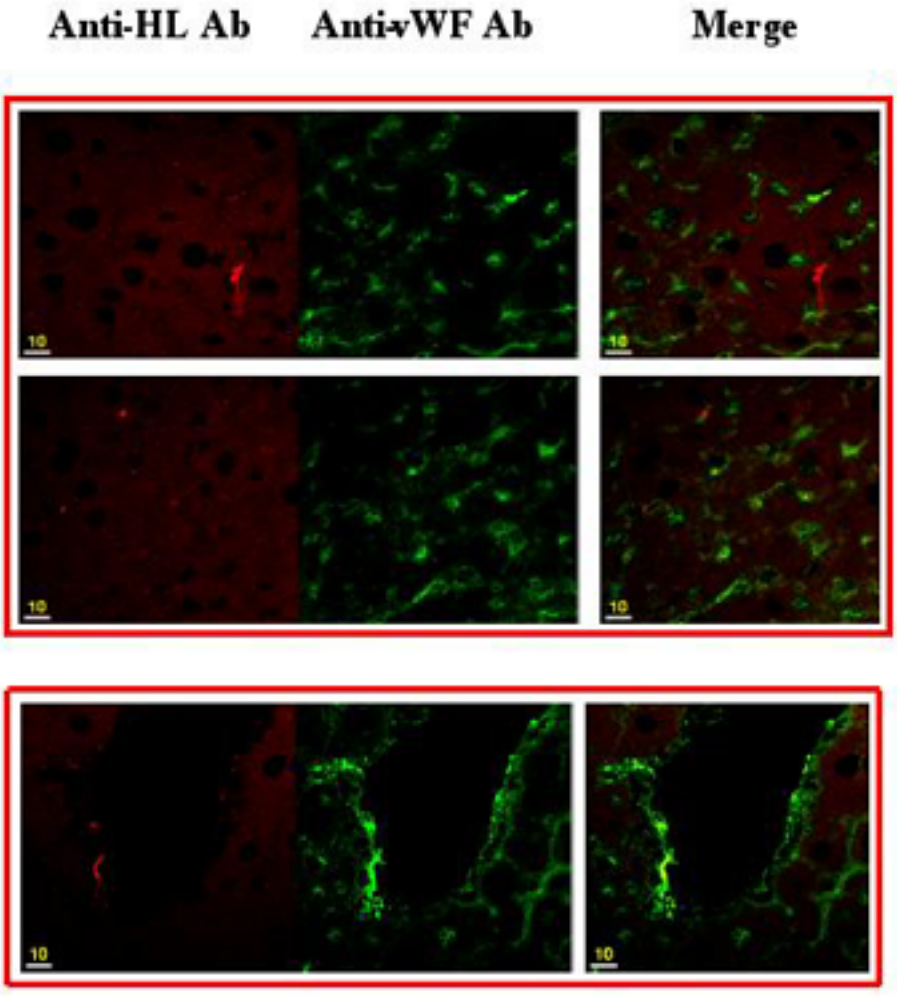


Figure 1C
Yu et al.

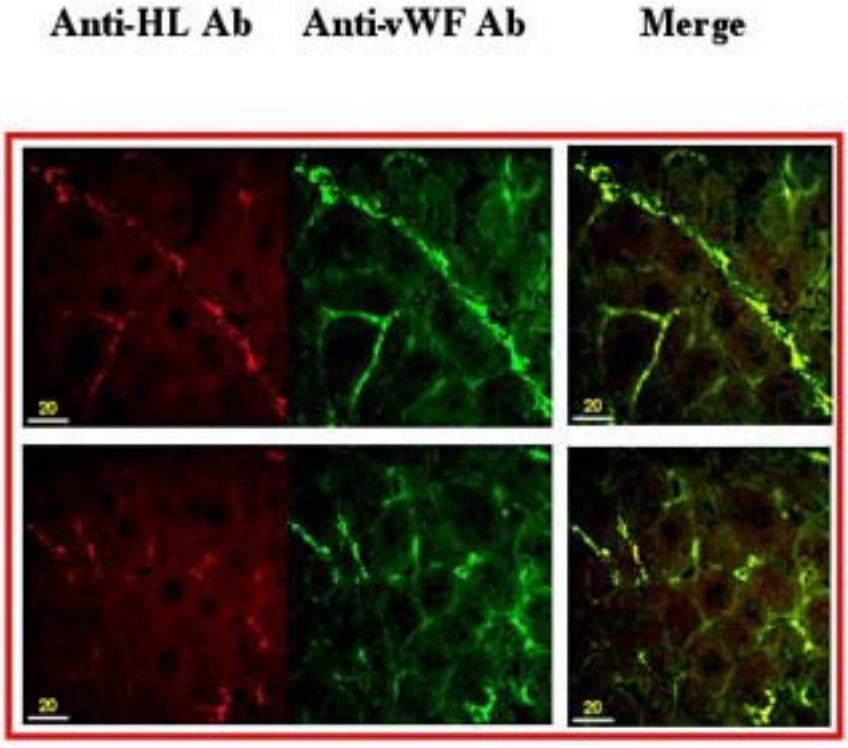


Figure 2A
Yu et al.

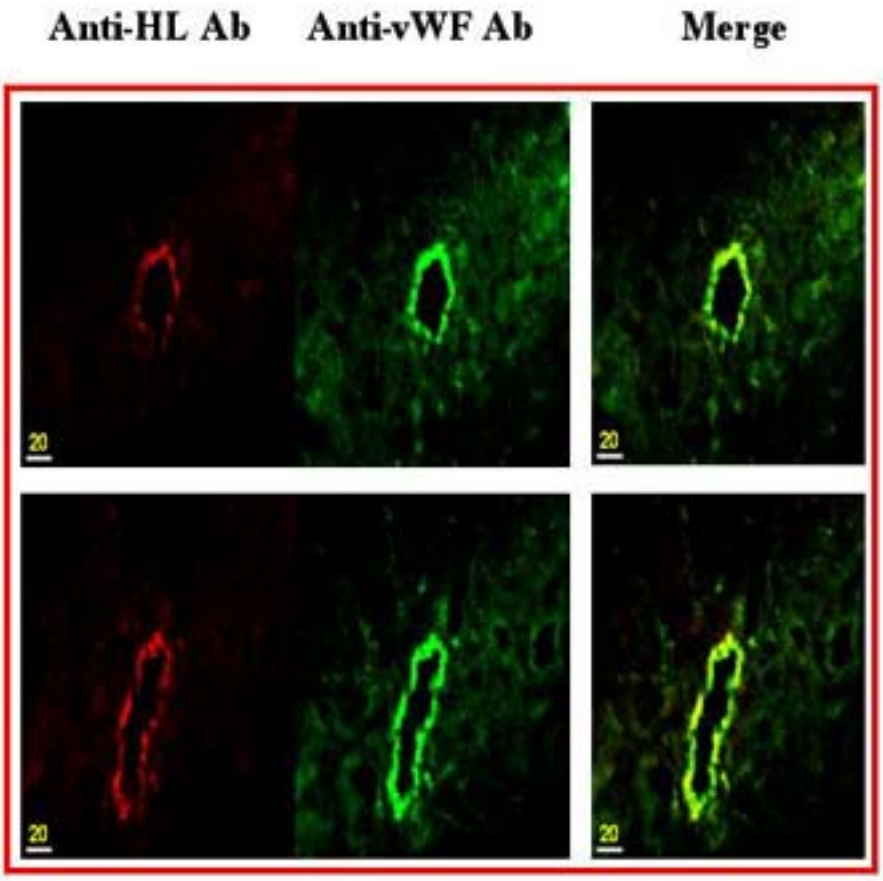


Figure 2B
Yu et al.

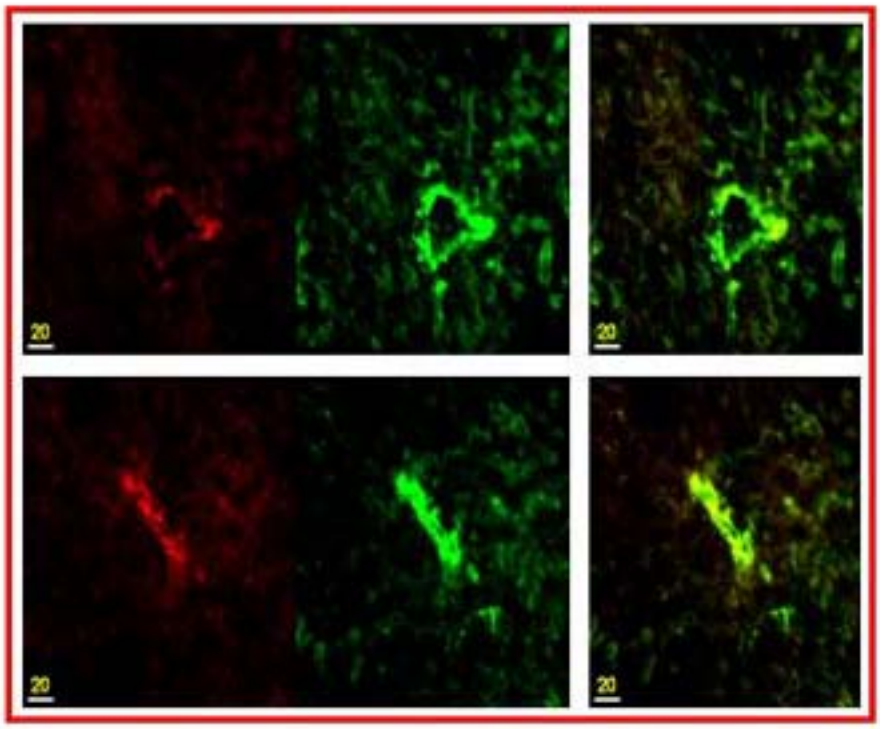


Figure 3A Yu et al.

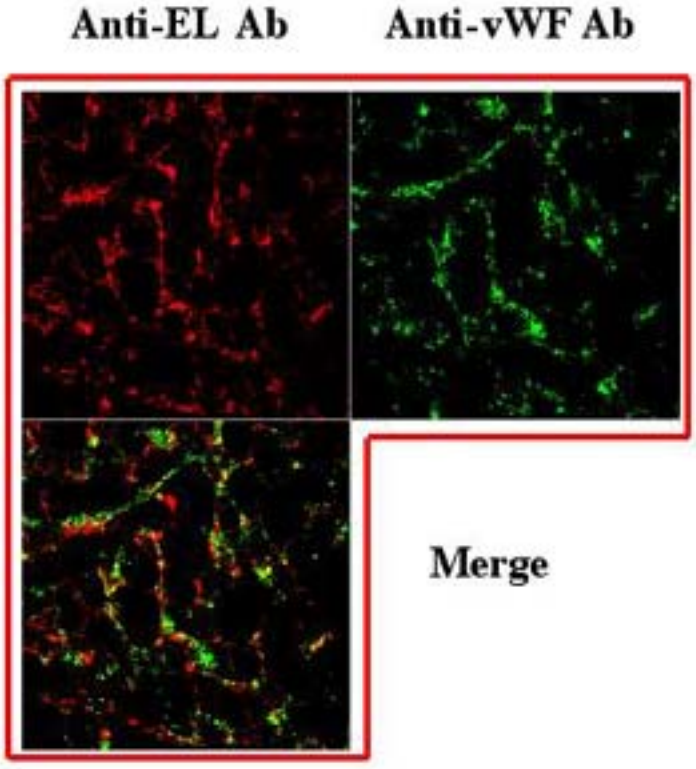


Figure 3B
Yu et al.

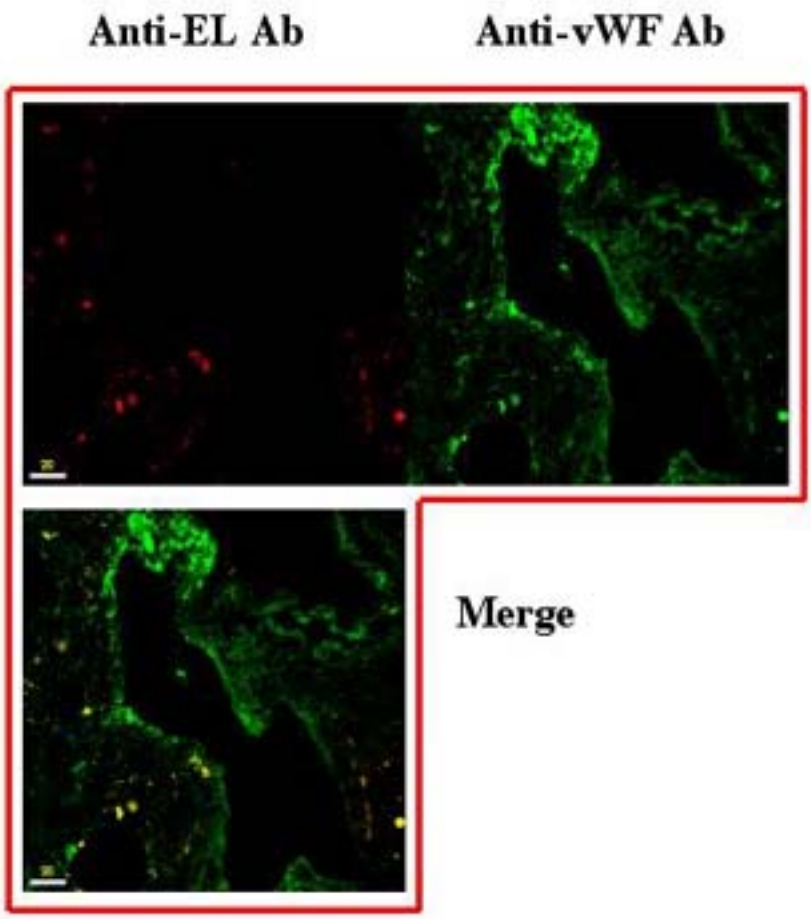
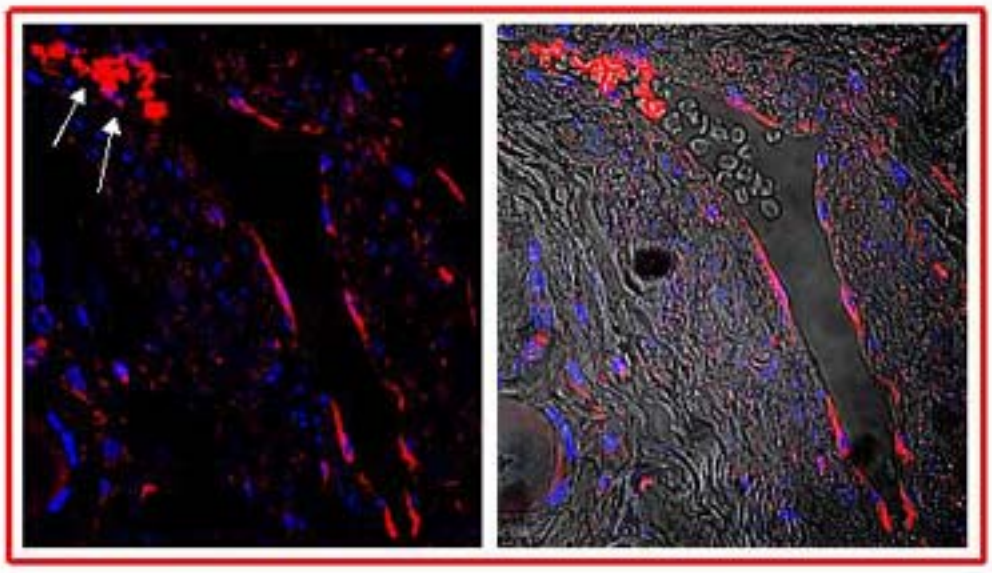


Figure 3C
Yu et al.



Sense cRNA

Figure 4A
Yu et al.

Anti-sense cRNA

20X Objective

Figure 4B
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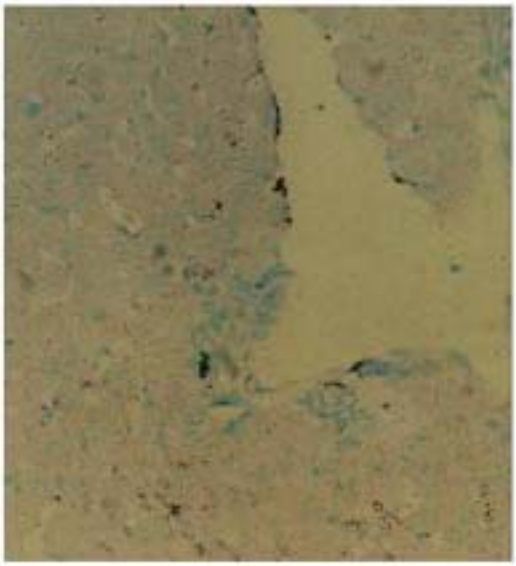

Figure 5A
Yu et al.

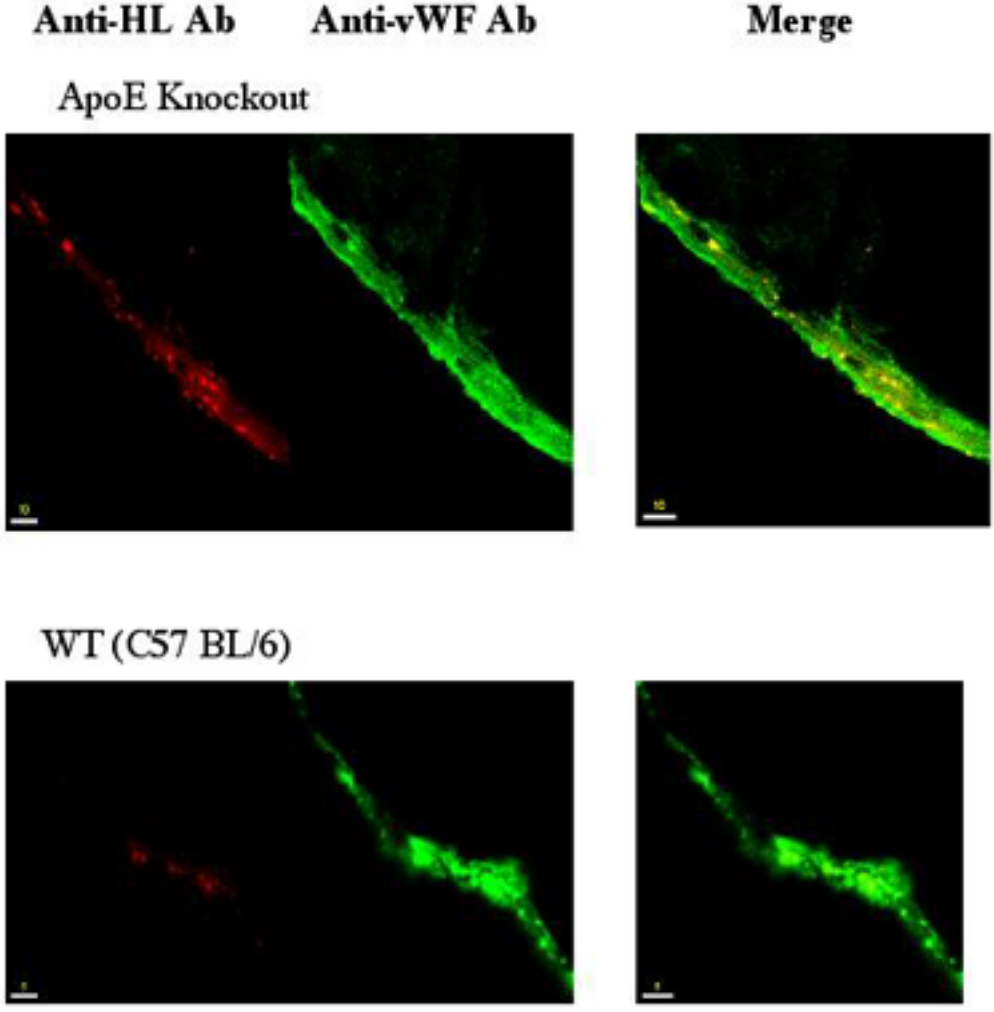

Figure 5B
Yu et al.

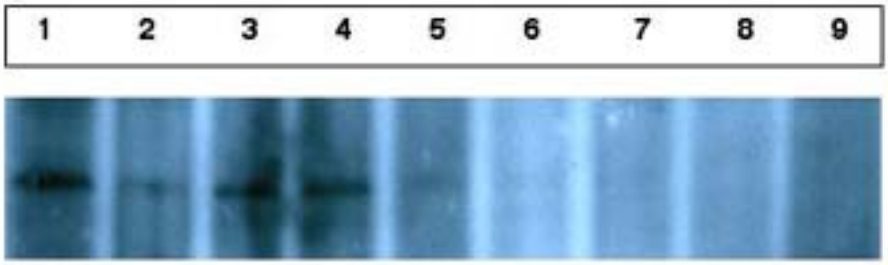

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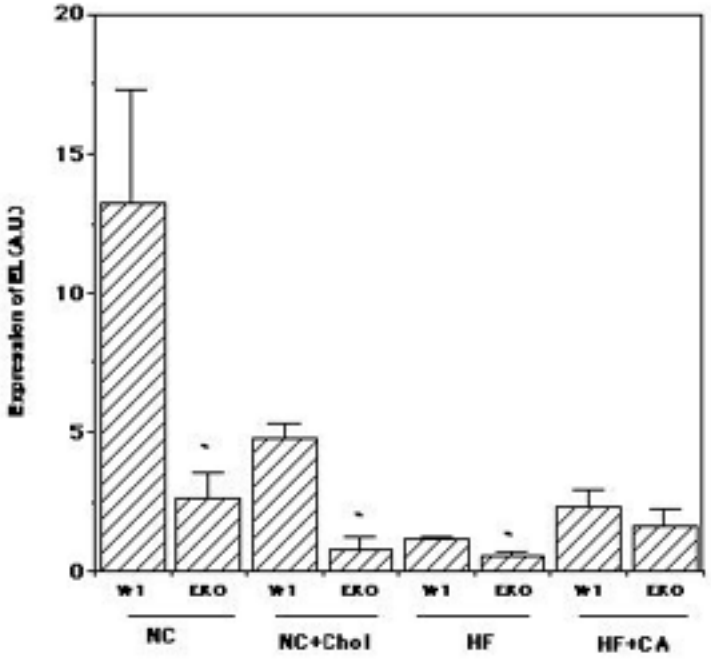
Figure 6A



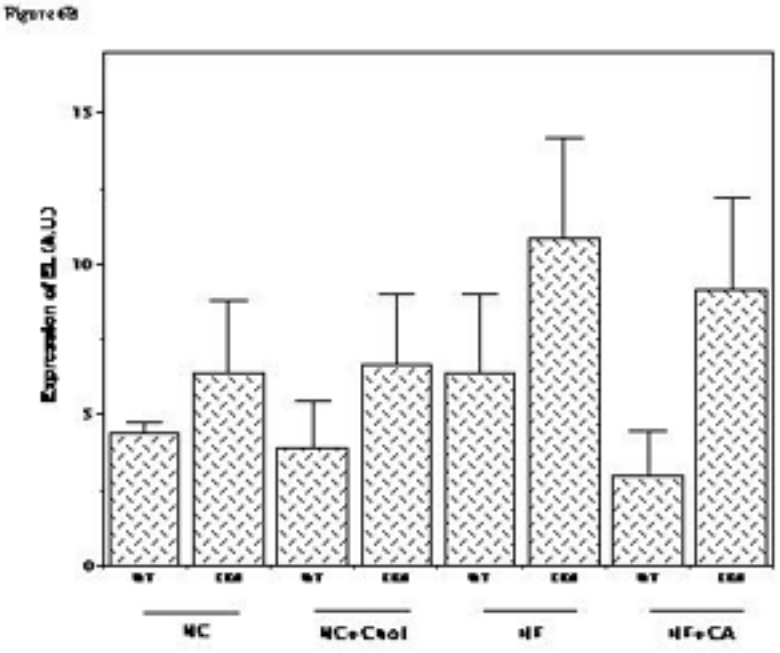

15
10
5
0
WT
CES1
WT
CES1
WT
CES1
WT
CES1
NC
NC+Cacl
HF
HF+CA

Table 1

	Cholesterol (n=3) (mg/dl)	Triglycerides (n=3) (mg/dl)
Normal Chow (NC)		
WT	77.7 ± 0.88	34.6 ± 2.3
EKO	684 ± 187	109.7 ± 16.7
NC+2% Cholesterol		
WT	59.0 ± 6.6	22.45 ± 2.6
EKO	1097 ± 59	52.49 ± 4.09
HF		
WT	146 ± 28.58	27.3 ± 7.7
EKO	1583 ± 18.9	74.7 ± 6.06
HF+2% Cholic Acid		
WT	150 ± 22.7	43.7 ± 5.78
EKO	1351 ± 131	65.1 ± 8.27