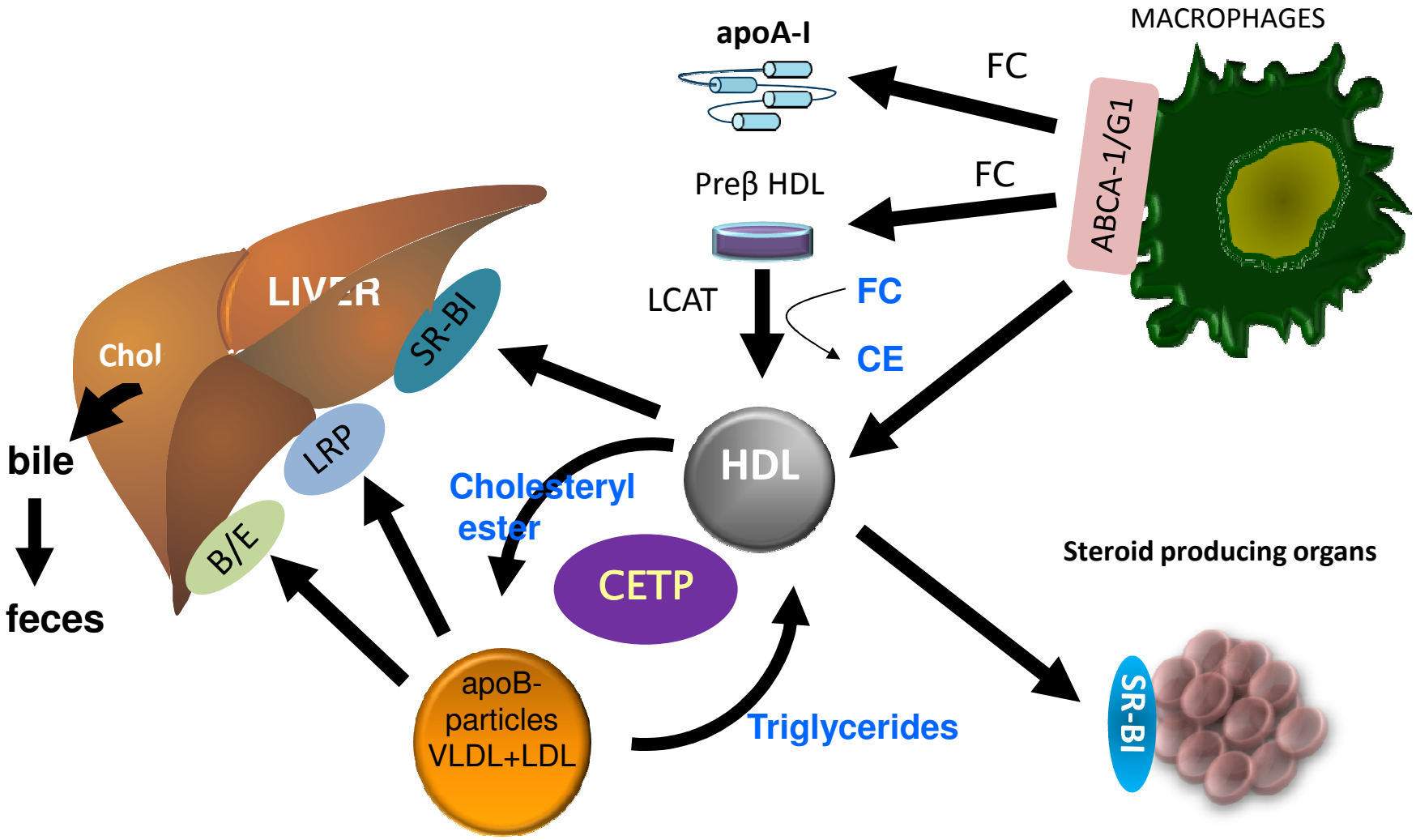
Cholesteryl ester transfer protein (CETP): Its role in atherosclerosis, inflammation and infection

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Cholesteryl ester transfer protein (CETP) transfers neutral lipids among plasma lipoproteins



An increase of the plasma HDL-C concentration is the most remarkable pharmacological effect of CETP inhibition

How does it relate to human atherosclerosis?

Advantages of having elevated plasmaCETP concentration

In cells: **transient transfection of CETP cDNA into COS-7 cells showed increased high density lipoprotein (HDL)-mediated efflux of free cholesterol** *(Zhang Z. Atherosclerosis 159: 67, 2001)*

In mice:

- **although not altering the biliary and fecal steroid excretion huCETP expression enhances the liver HDL-cholesteryl ester uptake** *(Harada LM, Cazita PM, Quintao E. Atherosclerosis 191:313, 2007)*

- **systemic overexpression of CETP (adenoviral infection) stimulates the egress of ^{3}H-cholesterol from cells to plasma and liver** *(Tanigawa H. Circulation. 116:1267, 2007)*

Advantages of having elevated CETP concentration

Against the use of CETP inhibitors = Low plasma CETP could be a drawback

In humans:

- **Plasma from subjects with low CETP mass has a diminished ability to stimulate cholesterol efflux from fibroblasts** *(Borggreve SE. Biochim Biophys Acta 1781: 10, 2008)*
- **Efflux of free cholesterol from macrophages obtained from CETP deficient cases is significantly decreased** *(Zhang Z. Atherosclerosis 159: 67, 2001)*
- **Torcetrapib does not modify the fecal sterol excretion or serum markers of cholesterol synthesis and also lowers the fractional catabolic rate of HDL apoA1** *(Brousseau ME. ATVB. 25: 1057, 2005)*

Advantages of having low CETP concentration

Favoring the use of CETP inhibitors:

- **In humans: increases the ABCG1-dependent cholesterol efflux in CETP deficient patients and in Torcetrapib treated subjects when matched for unit of HDL in medium** *(Tall A. Am. J. Cardiol. 104:39E, 2009)*

- **In mice: Torcetrapib increases macrophage ^{3}H-cholesterol fecal excretion in experimental diabetes** *(Briand F. Vlin. Transl. Sci 4(6):414, 2011)*

- **In hamsters: Torcetrapib increases egress of ^{3}H-cholesterol from macrophages to plasma and feces** (*Tchoua U. Cardiovasc. Res. 77:732, 2008; Niesor EJ, J Lipid Res. 51:3443, 2010)*

CETP inhibition increases plasma HDL-C concentration and protects against atherosclerosis in rabbits:

- **liver targeted antisense oligonucleotides against CETP:** *Sugano M. JBC. 273:5033 , 1998*
- **vaccination against CETP :** *Rittershaus CW. ATVB. 20:2106, 2000*
- **drug that inhibits CETP (JTT-705):** *Okamoto H. Nature 406:203, 2000*

In spite of several population studies there has been considerable controversy on the role of CETP in human atherosclerosis

In humans - CETP deficiency has been reported as:

- **Increasing longevity or lowering CVD event rates (4 studies):**

- Koizumi J. Atherosclerosis. 58:175 (1985)
- Moriyama Y. (participants with HDL>80mg/dL). Prev. Med. 27:659 (1998).
- Curb JD, Tall AR.: Japanese-Americans in Honolulu JLR. 45:948 (2004).
- Barzilai N. JAMA. 290:2030 (2003)

- **Increasing the risk of CVD (11 studies):**

- Hirano K. ATVB. 17:1053 (1997)
- Zhong S, Tall AR.Japanese-Americans in Honolulu. J.Clin Invest. 97:2917 (1996).
- Bruce C, Tall A. Japanese-Americans in Honolulu associated with high TG.JLR. 39:1071(1998)
- Agerholm-Larsen B. Circulation. 101:1907 (2000)
- Kakko S. (in hyperlipidemics in Finland) Eur. J. Clin. Invest. 30:18 (2000)
- Kimura H. (in hemodialysis patients) Am J Kidney Dis. 38:70 (2001)
- Blankenberg S. J. Am. Coll. Cardiol. 41:1983 (2003)
- Marschang P. (patients treated with pravastatin). J. Intern. Med. 260:151 (2006)
- Ritsch A. (in patients undergoing coronary angiography). Circulation. 121:366(2010)
-Duwensee K. Karola study in CAD patients). Eur. J. Clin. Invest. 40:616 (2010)
- Yamashita S. in Japanese-Americans and in the Omagari area. BBA. 1529:257 (2000)
- Regieli JJ. In statin treated men. Eur. Heart J. 29:2792 (2008)

- **Neutral regarding CVD (one study and one meta-analysis):**

-de Grooth GJ. J. Am. Coll. Cardiol. 43:854 (2004)
-Thompson A. JAMA 299:2777(2008): Meta-analysis on CETP polymorphisms: 92 studies (113833 healthy participants) and 46 studies (27196 CHD cases and 55338 controls): along 18 years - January 1970 through January 2008 – diminished CHD has weak correlations with low CETP activity and is best explained by elevated plasma HDL-C

1) **diminished plasma CETP and CVD was related with the B2 polymorphsim of CETP:** Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, Lahoz C, Coltell O, Wilson PW, Schaefer EJ. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham Offspring Study. ATVB. 20:1323-1329 (2000) - **Framingham Offspring Study**

2) **Inverse correlation between CETP and CVD:** Vasan RS, Pencina MJ, Robins SJ, Zachariah JP, Kaur G, D'Agostino RB, Ordovas JM. Association of Circulating Cholesteryl Ester Transfer Protein Activity With Incidence of Cardiovascular Disease in the Community. Circulation. 120:2414-2420 (2009) - **Framingham Heart Study**

3) **Diminished CETP associated with increased CVD:** .Zhong R, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest. 97:2917-2923 (1996) - **Honolulu Heart Program**

4) **Diminished CETP associated with low CVD:** Curb JD, Abbott RD, Rodriguez BL, Masaki K, Chen R, Sharp DS, Tall AR. A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. J Lipid Res. 45:948-953 (2004): **Honolulu Heart Program**

CETP inhibitors:

Previous studies:

- Torcetrapib (Pfizer) RADIANCE 1 and 2, ILLUSTRATE and ILLUMINATE: off-target toxicity (elevated blood pressure); no benefit over statins; no halting of progression of atherosclerosis plaques.
- Dalcetrapib (Hoffmann-LaRoche): dal-VESSEL, dal-PLAQUE and Dal-OUTCOMES: failed to lower mortality.

Present investigations:

- Anacetrapib (Merck&Co)(DEFINE; REVEAL)
- Evacetrapib (Eli Lilly)/BAY 60-5521

In the ILLUMINATE study in 15067 subjects

(Torcetrapib + Atorvastatin *vs* Atorvastatin alone)

	Torcetrapib + Atorvastain	Atorvastatin alone
Total deaths	93	59
Cardiovascular	49	35
Cancer	24	14
Infection	9	0

Could these failures in the prevention of CVD be secondary to unknown adverse effects of the CETP inhibition, like inflammation?

Suggesting arterial inflammation due to CETP inhibition

In mice expressing huCETP: Torcetrapib and atorvastatin alone reduced atherosclerotic lesion size (-43% and -46%; P<0.05).
However, Torcetrapib enhanced monocyte recruitment and expression of monocyte chemoattractant protein-1 and resulted in lesions of a more inflammatory phenotype, as reflected by an increased macrophage content and reduced collagen content *(De Haan W. Circulation 117:2515, 2008)*

Against the role of inflammation by CETP inhibition

Plasma levels of hs-C-reactive protein, ICAM-1, VCAM-1, IL-6, Myeloperoxidase (MPO), t-PA, or PAI-1 did not differ at baseline or during treatment in both the placebo and Dalcetrapib groups: the dal-VESSEL randomized clinical trial *(Lüscher TF. Eur. Heart J. 33:857, 2012)*

Table 3 Distribution of plasma albumin and CETP levels among survivors and non-survivors in the sepsis case group

Variable	Non-survivors	Survivors	*P*-value
Albumin day 0	2·79 ± 0·73	2·60 ± 0·68	0·373†
Albumin day 3	1·67 ± 0·50	1·81 ± 0·56	0·424†
Δ* albumin	1·25 ± 1·00	0·79 ± 0·56	0·046‡
CETP day 0	1·63 ± 0·89	1·25 ± 0·49	0·162‡
CETP day 3	1·05 ± 0·65	1·23 ± 0·70	0·410†
Δ* CETP	0·78 ± 1·08	0·02 ± 0·58	0·010‡

Mean values at hospital admission (D0) and third day (D3) of severe sepsis. Albumin g dL^{-1}; CETP μg mL^{-1}.

CMC Grion, *LTQ Cardoso, TF Perazolo, AS Garcia, DS Barbosa, HK Morimoto, T Matsuo,* ***AJF Carrilho****. Lipoproteins and CETP levels as risk factors for severe sepsis in hospitalized patients. Eur J Clin Invest 40: 330–338 (2010).*

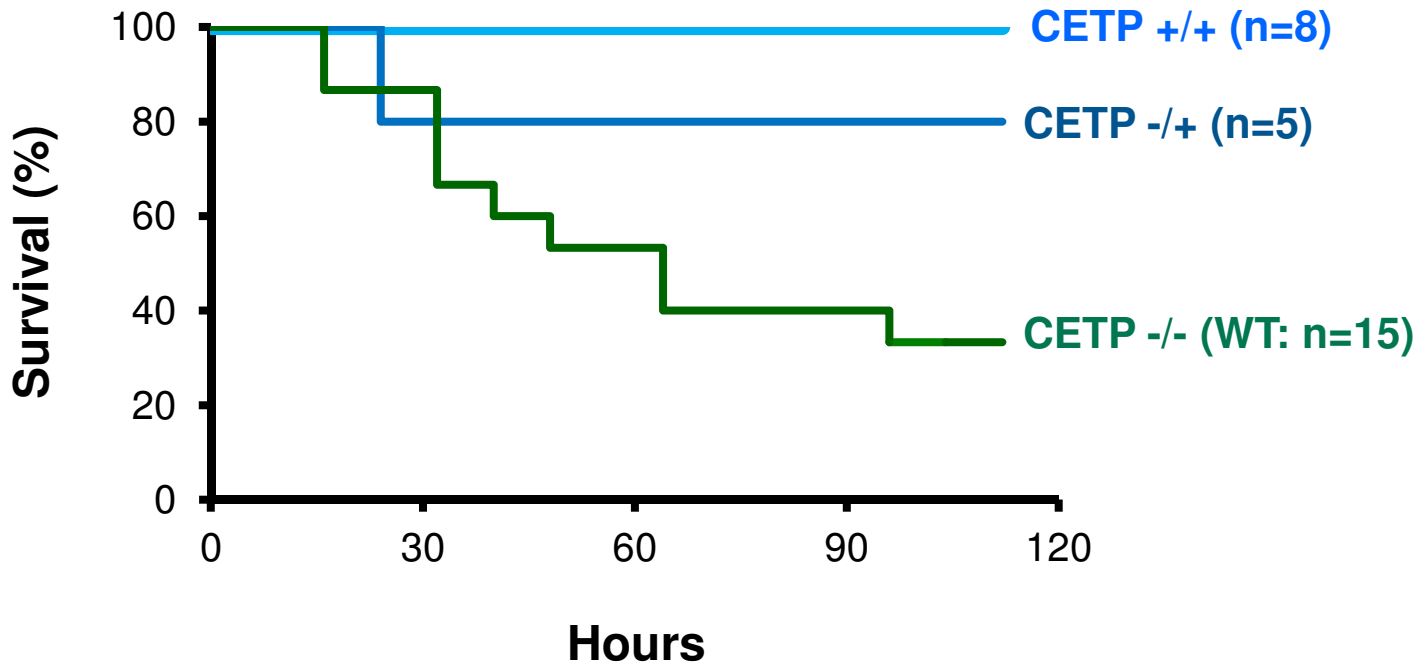
Infection influences plasma CETP concentration

- **Major point: administration of LPS to mice expressing huCETP resulted in a rapid marked decrease in hepatic CETP mRNA and plasma CETP concentration primarily as a result of adrenal corticosteroid release** *(Masucci-Magoulas L. JCI. 95:1587,1995)*

- **Minor point: LPS incubated with human blood had no effect on CETP activity** *(Clark RW. JLR 51:967, 2010)*

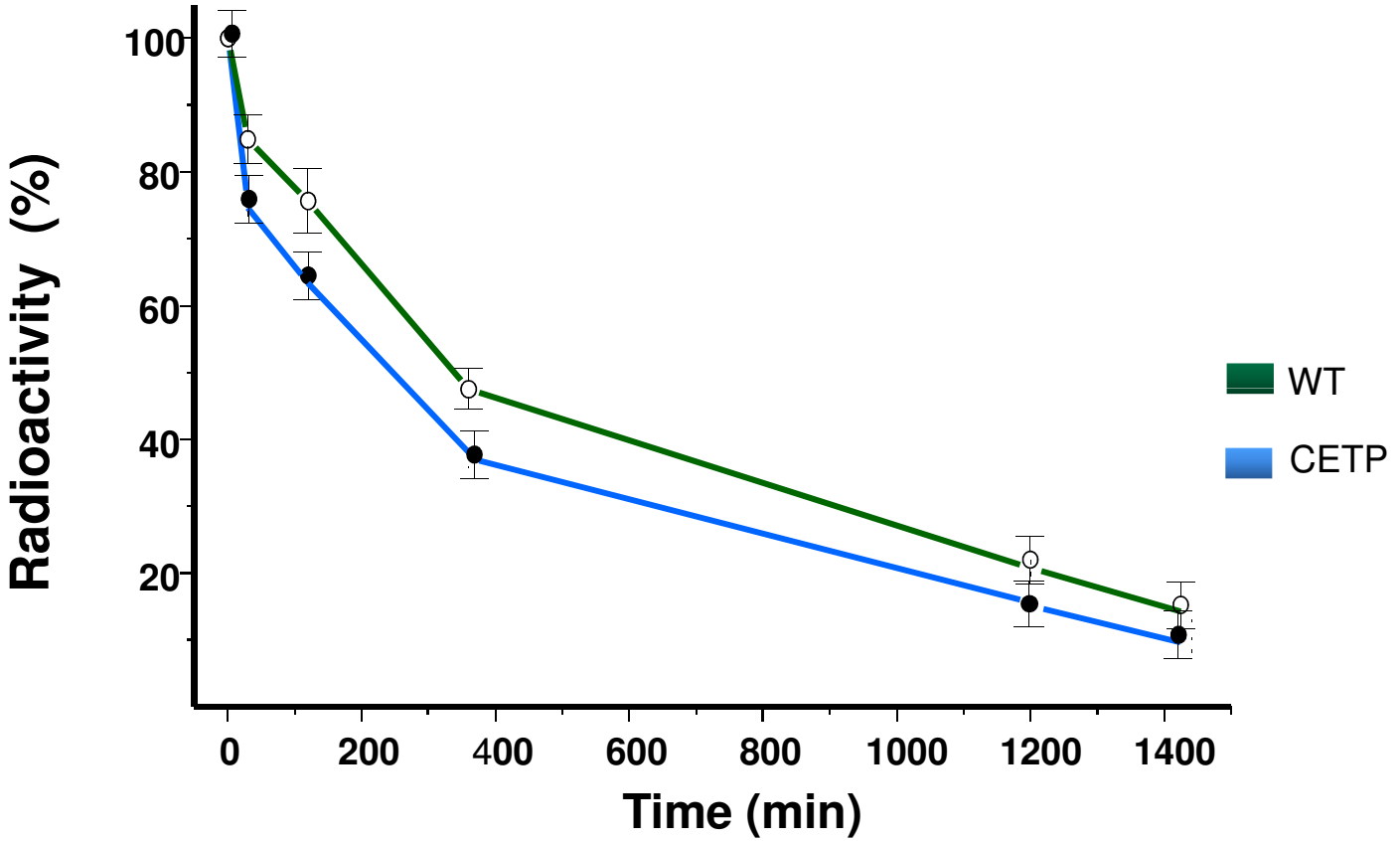
Animal models of infection: huCETP expressing mice

In huCETP expressing mice: after lipopolysaccharide (LPS) injection all CETP +/+ survived; CETP +/- survived longer than the wild type (CETP -/-) mice



***Cazita PM**, Barbeiro DF, Moretti AI, Quintão EC, Soriano FG. Shock. 30:590; 2008.*

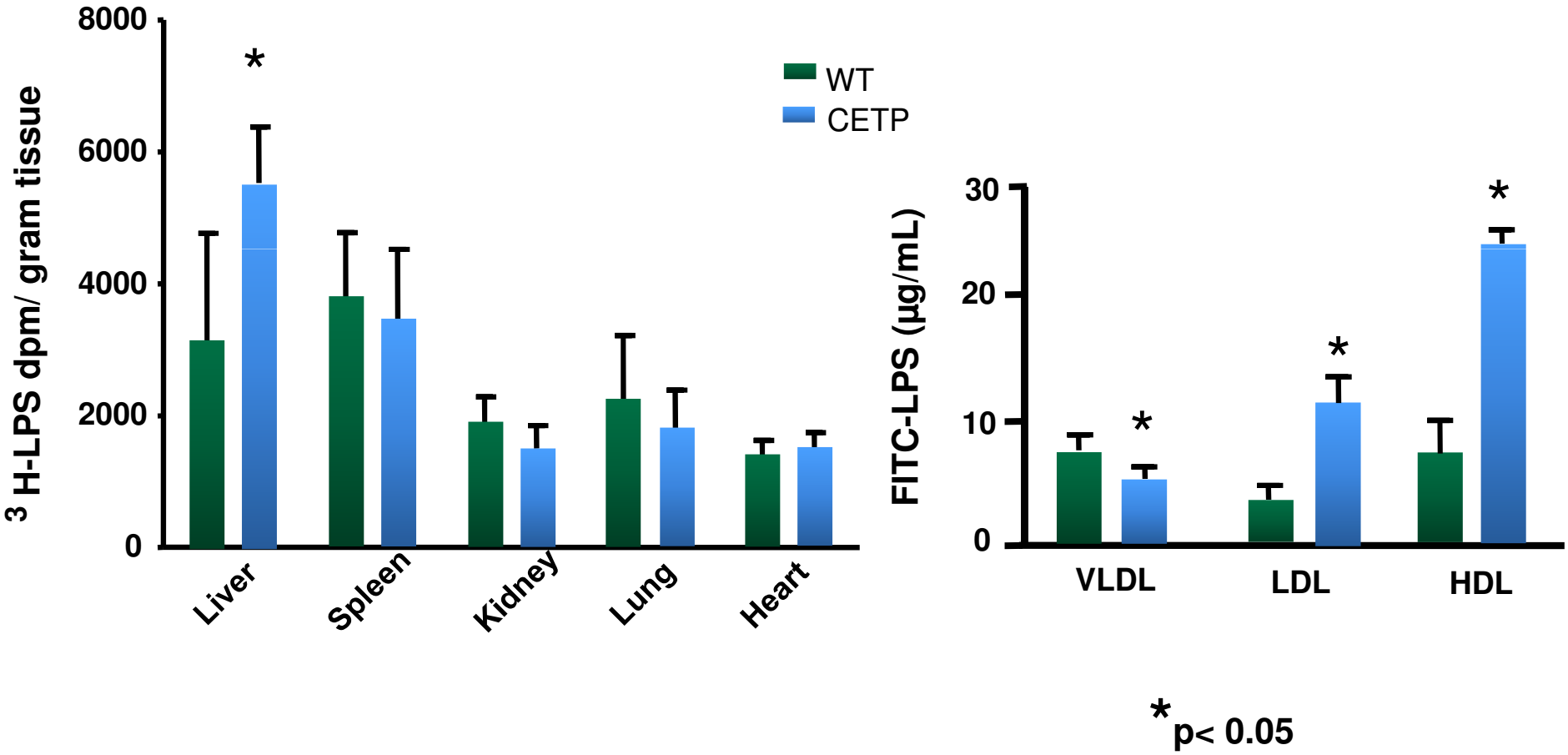
The clearance of ^{3}H-LPS from plasma was significantly faster in huCETP expressing than in wild type (WT) mice



*p< 0.05

Cazita PM *et al.: Circulation. 18:S_570; 2008*

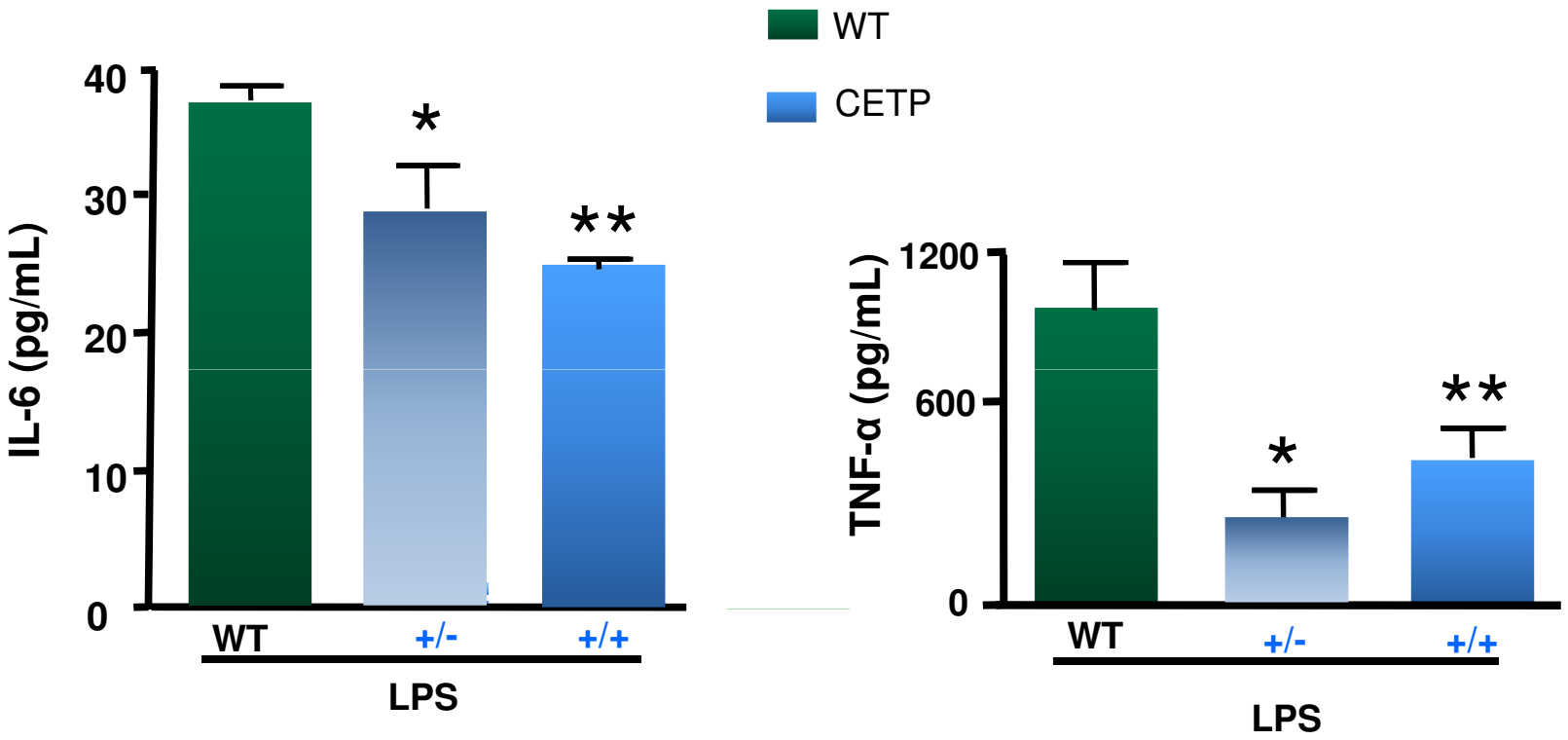
After LPS injection liver of huCETP mice took up more LPS than wild type mice; in huCETP mice LPS transport decreased in VLDL and increased in LDL and HDL; because plasma concentrations of LDL and HDL are greater than that of VLDL this may contribute to minimize LPS adverse effects



*p< 0.05

Cazita PM *et al.: Circulation. 18:S_570; 2008*

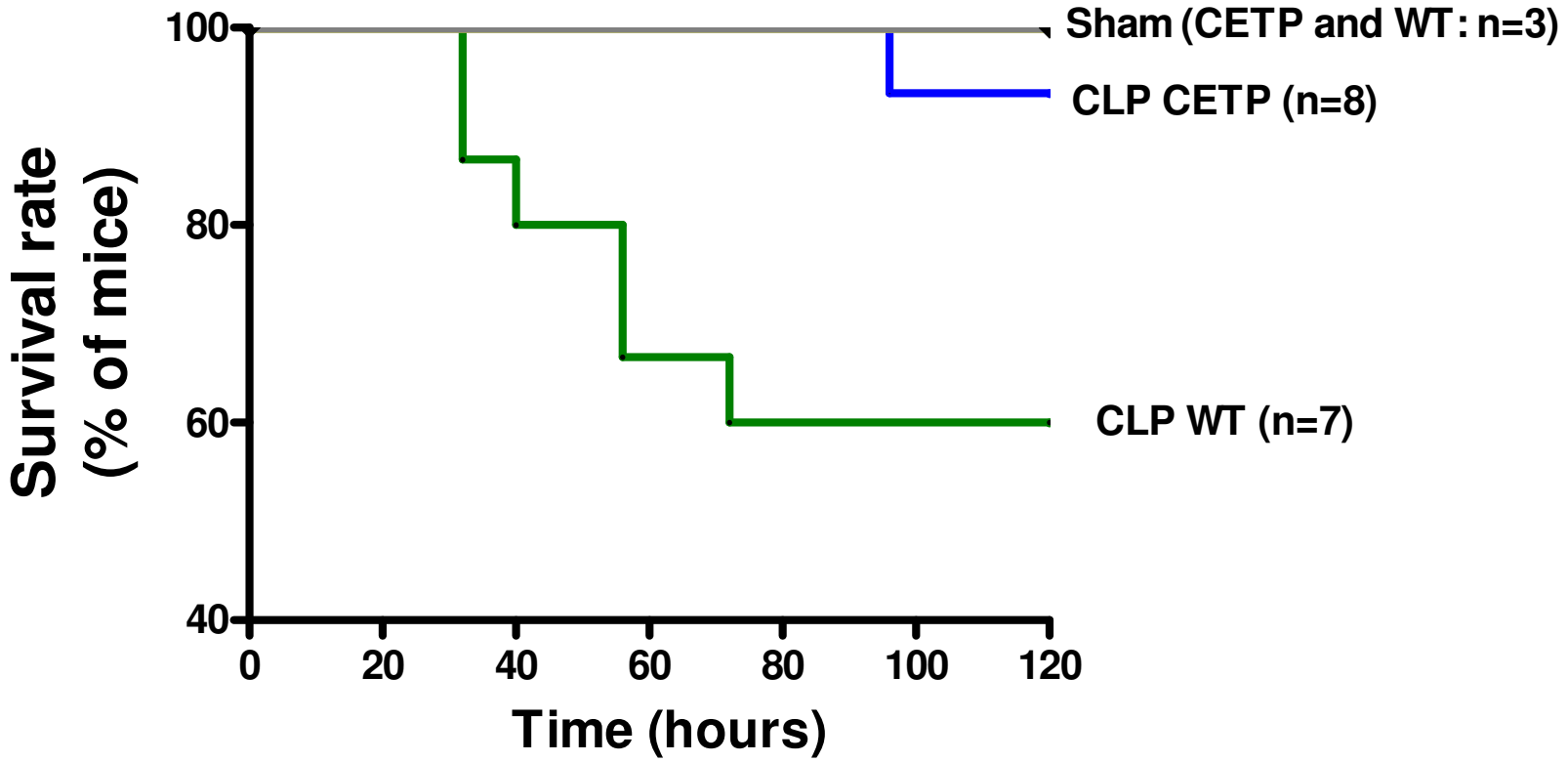
After LPS injection huCETP expressing mice presented lower plasma concentrations of IL-6 and TNF-α than wild type mice



*, ** p< 0.05 *vs* WT

Cazita PM *et al.: Circulation.18:S_570; 2008; Shock 30(5): 590-5, 2008.*

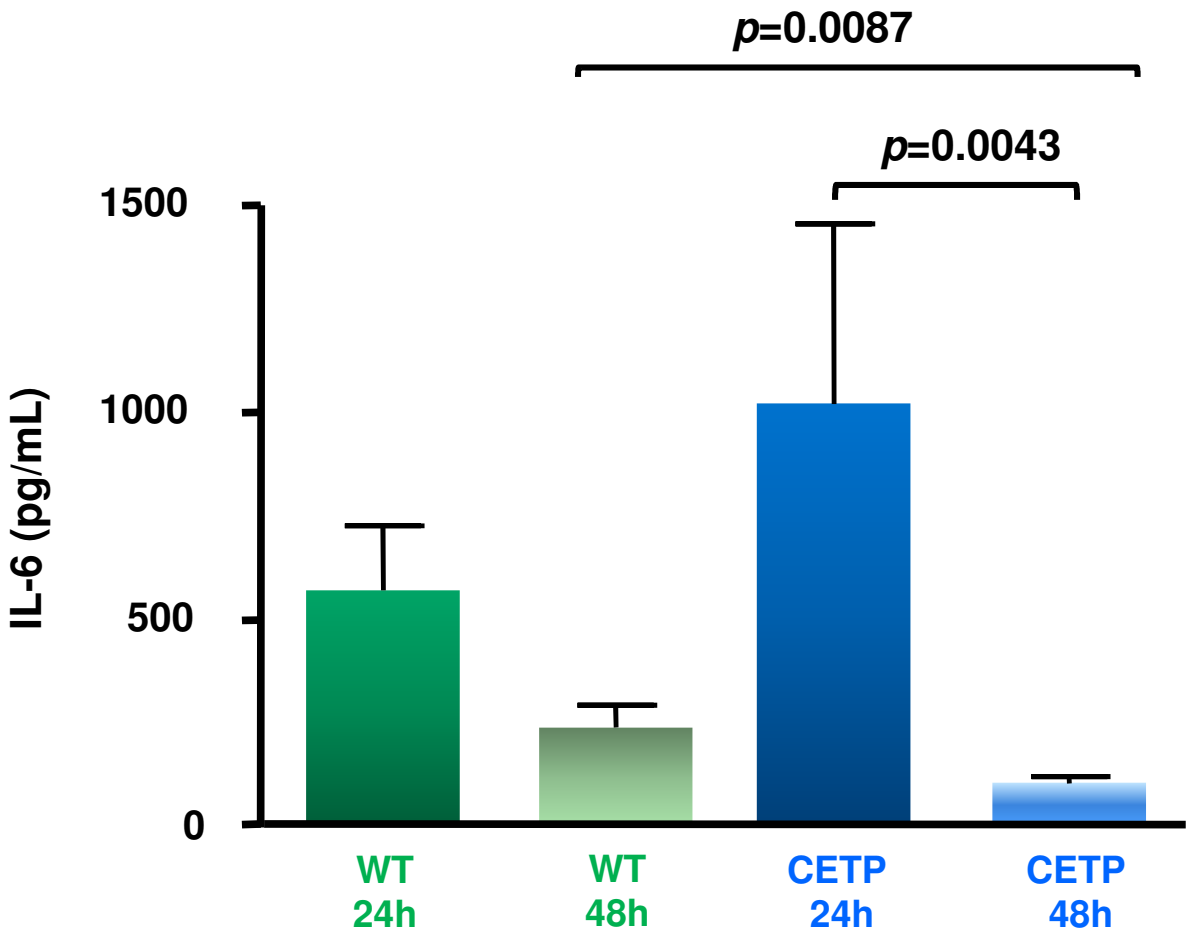
Survival rates were much greater after caecum ligation and puncture (CLP) in huCETP expressing mice than in wild type (WT) mice



***p*< 0.05 CLP: CETP *vs* WT**

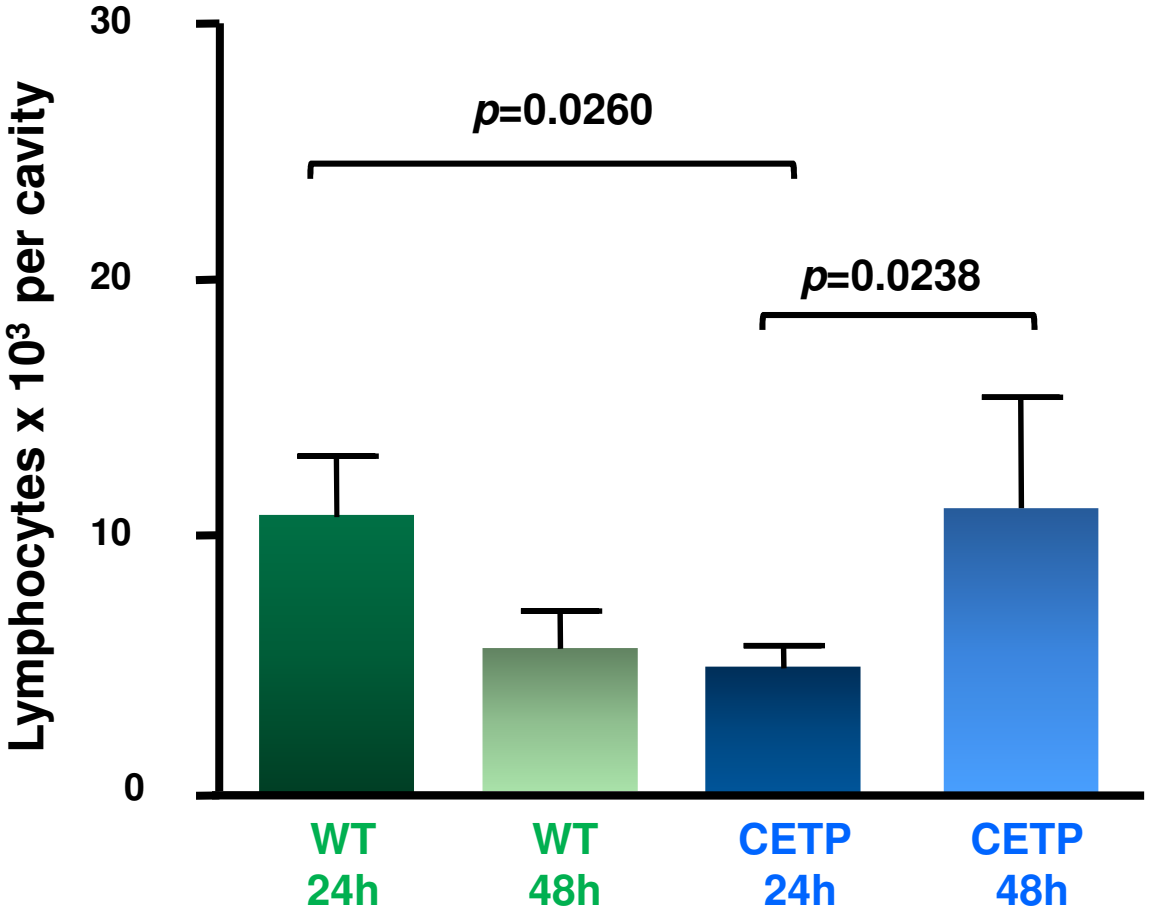
*Venancio, TM, **Cazita PM**. et al Circulation. 124: A1-A1; 2011*

48 hours after CLP plasma IL-6 concentration was reduced but reached much lower values in huCETP than in WT mice and at 48h than at 24h



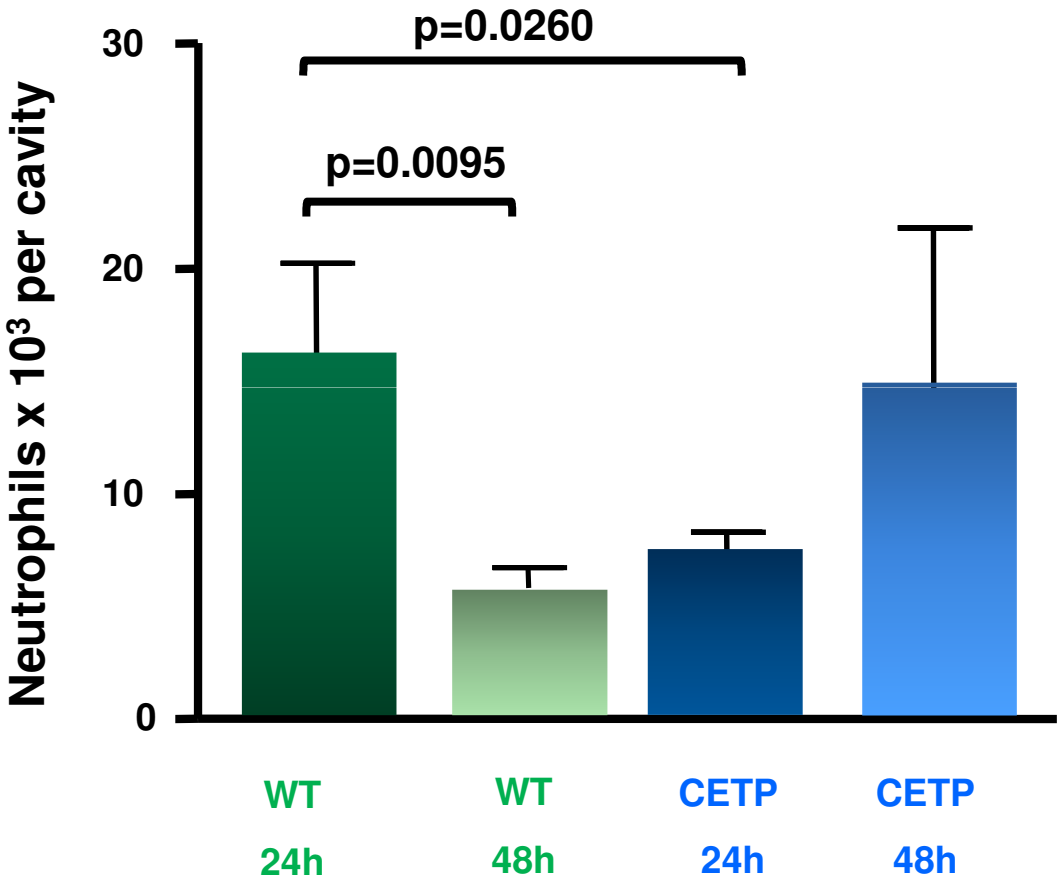
*Venancio, TM, **Cazita PM**. et al Circulation. 124: A1-A1; 2011*

48 hours after CLP: in huCETP mice, but not in WT mice, greater migration of lymphocytes into the peritoneal cavity



*Venancio, TM, **Cazita PM**. et al Circulation. 124: A1-A1; 2011*

48 hours after CLP: neutrophil migration into the peritoneal cavity was reduced in WT mice but not in huCETP expressing mice



*Venancio, TM, **Cazita PM**. et al Circulation. 124: A1-A1; 2011*

Our data support a study that showed *in vitro* failure of the chemotactic migration by neutrophils obtained from septic patients as compared to those from healthy individuals

Tavares-Murta BM et al. Failure of neutrophil chemotactic function in septic patients. Crit Care Med. 30(5):1056; 2002.

The largest role of the inflammatory response to blood born Gram-negative bacteria is played by the liver

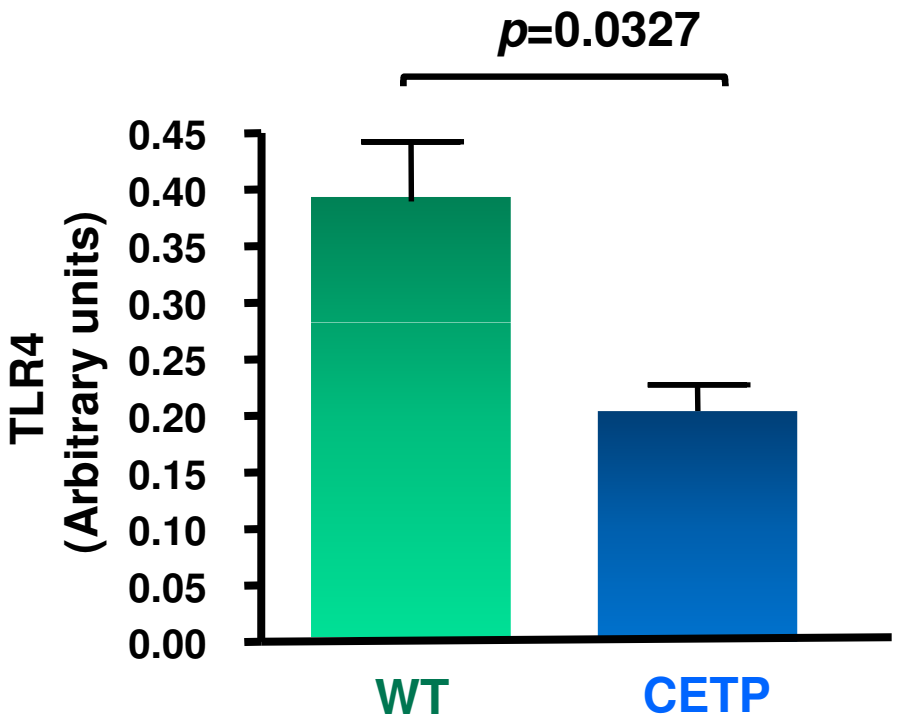
This organ removes bacteria and their lipopolysaccharide (LPS, endotoxin) from the bloodstream.

In the liver LPS undergoes deacylation by acyloxyacyl hydrolase (AOAH), which is an endogenous lipase that selectively removes fatty acid molecules.

The cellular activation by LPS is mediated by the MD-2-TLR4 (Toll-like receptor-4) complex.

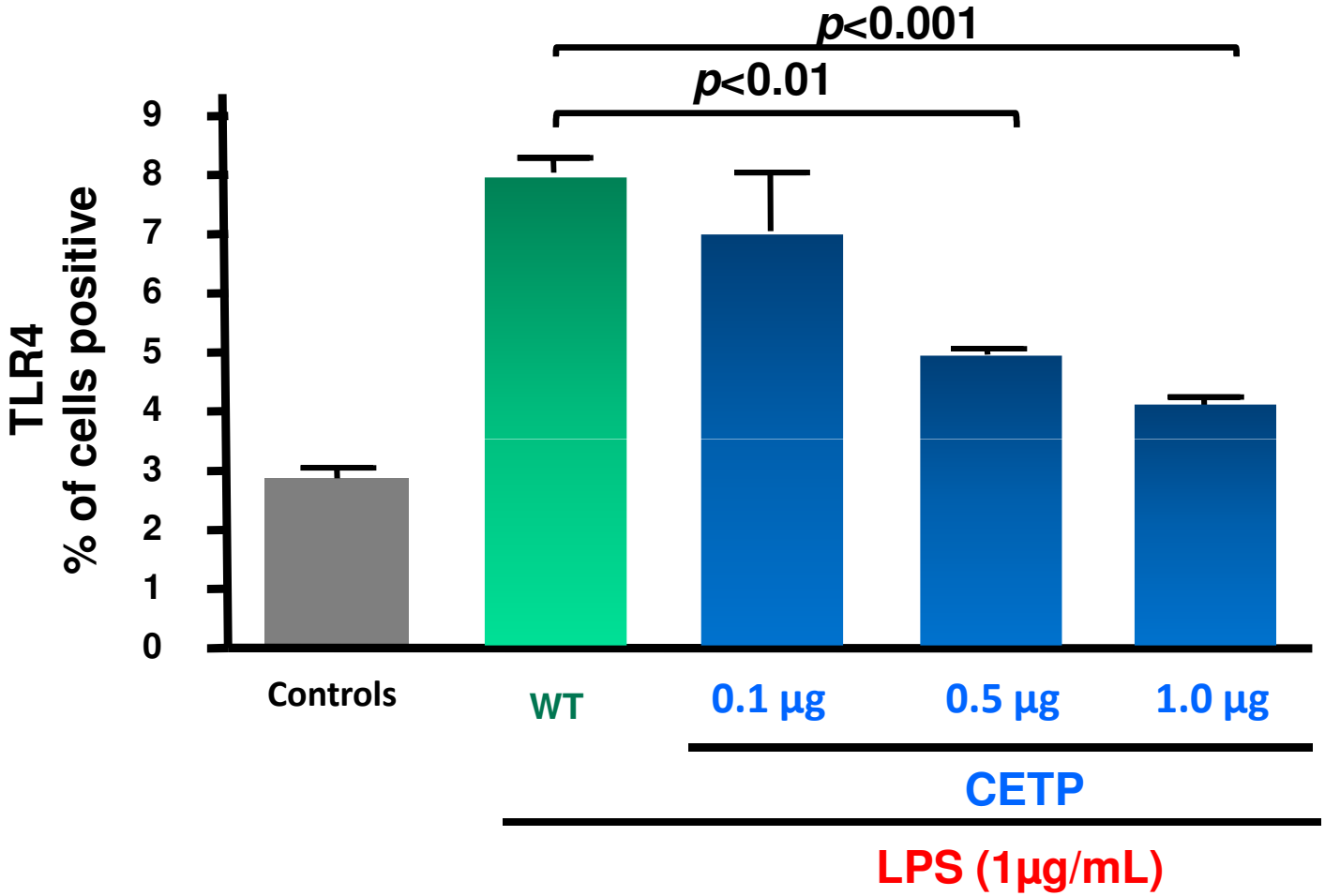
Thus, we investigated TLR4 hepatic expression after CLP in the presence of CETP as compared to its absence (wild type mice)

48 hours after CLP: liver TLR4 protein was lower in huCETP than in WT mice



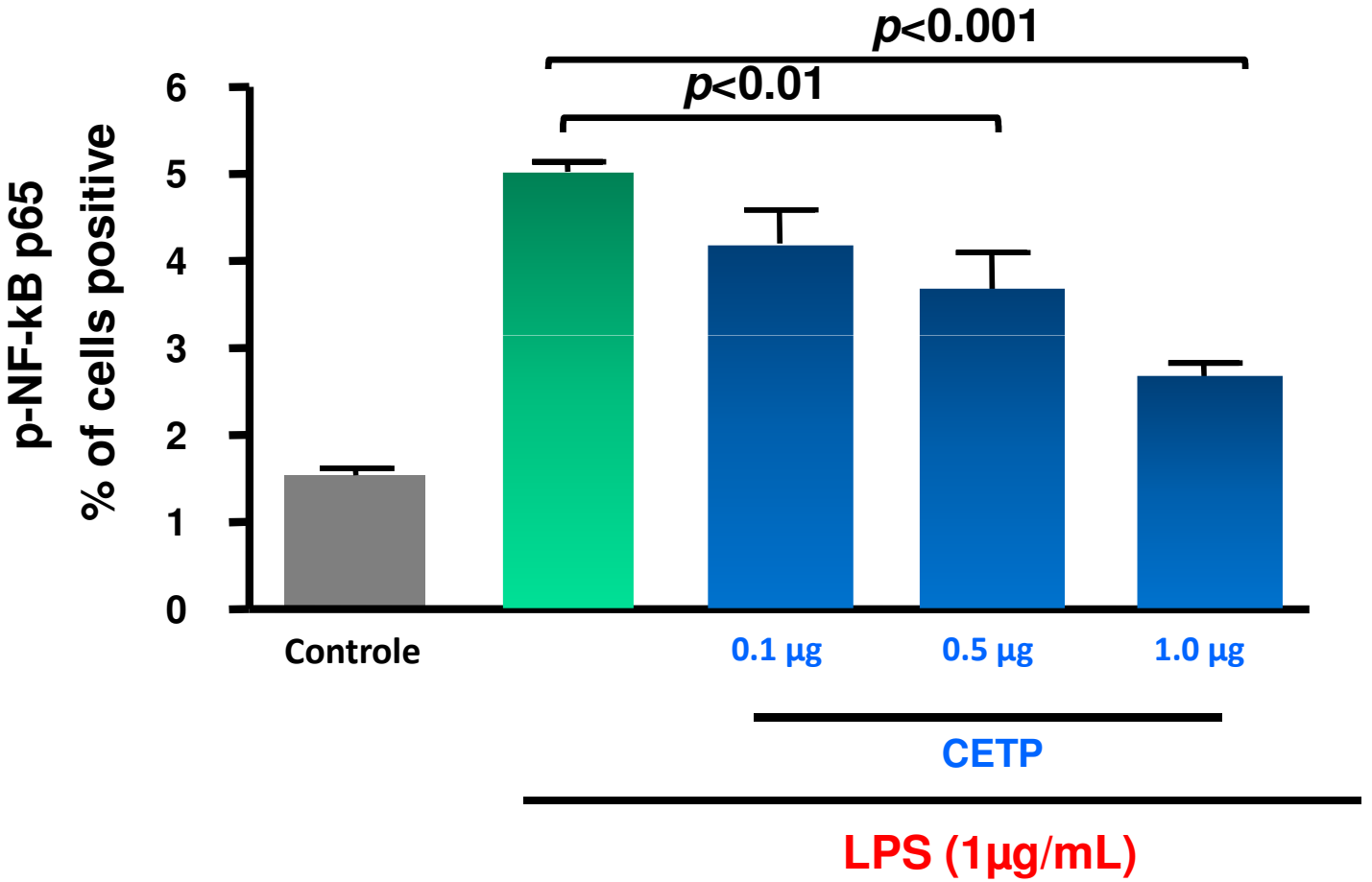
*Venancio, TM, **Cazita PM**. Lipids Lab, Univ. São Paulo*

***In vitro* LPS stimulated TLR4 expression in wild type mice macrophages. This effect was reduced after adding increasing amounts of recombinant huCETP to these macrophages**



*Venancio, TM, **Cazita PM**. Lipids Lab, Univ. São Paulo*

In vitro LPS stimulated p-NF-kB p65 expression in wild type mice macrophages. This effect was reduced after adding increasing amounts of recombinant huCETP to these macrophages



Venancio, TM, ***Cazita PM.*** *Lipids Lab, Univ. São Paulo*

Conclusions

- **CETP is an endogenous component that protects against the inflammatory responses to infection**

- **This may help explaining the CETP beneficial effects in sepsis and also the failure of CETP inhibition by drugs on the protection against the development of human atherosclerosis**

Acknowledgements

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Helena C. F. Oliveira (University of Campinas)



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Circulation. 2007 Sep 11;116(11):1267-73. Epub 2007 Aug 20.

Expression of cholesteryl ester transfer protein in mice promotes macrophage reverse cholesterol transport.

Tanigawa H[1], Billheimer JT, Tohyama J, Zhang Y, Rothblat G, Rader DJ.

METHOD AND RESULTS:

A vector based on adeno-associated virus serotype 8 (AAV8) with a liver-specific thyroglobulin promoter was used to stably express human CETP in livers of mice and was compared with an AAV8-lacZ control vector. The RCT assay was performed 4 weeks after vector injection and involved the intraperitoneal injection of acetylated low-density lipoprotein cholesterol-loaded and 3H-cholesterol-labeled J774 macrophages in mice with plasma sampling at several time points, liver and bile sampling at 48 hours, and continuous fecal collection to measure 3H-sterol as an integrated readout of macrophage RCT. In apobec-1-null mice,
CETP expression reduced plasma high-density lipoprotein cholesterol levels but significantly increased fecal 3H-sterol excretion. In low-density lipoprotein receptor/apobec-1 double-null mice, CETP expression reduced high-density lipoprotein cholesterol levels and had no effect on fecal 3H-sterol excretion. Finally, in scavenger receptor class B, type I-null mice, CETP expression reduced high-density lipoprotein cholesterol levels and significantly increased fecal 3H-sterol excretion.

CONCLUSION:

The present results demonstrate that CETP expression promotes macrophage RCT in mice, that this effect is dependent on the low-density lipoprotein receptor, and that CETP expression restores to normal the impaired RCT in mice deficient in scavenger receptor class B, type I.

Liver X receptor activation promotes macrophage-to-**feces** reverse **cholesterol** transport in a dyslipidemic hamster model.
Briand F, Tréguier M, André A, Grillot D, Issandou M, Ouguerram K, Sulpice T.
J Lipid Res. 2010 Apr;51(4):763-70.

The use of dyslipidemic hamsters to evaluate drug-induced alterations in reverse **cholesterol** transport. Briand F. Curr Opin Investig Drugs. 2010 Mar;11(3):289-97

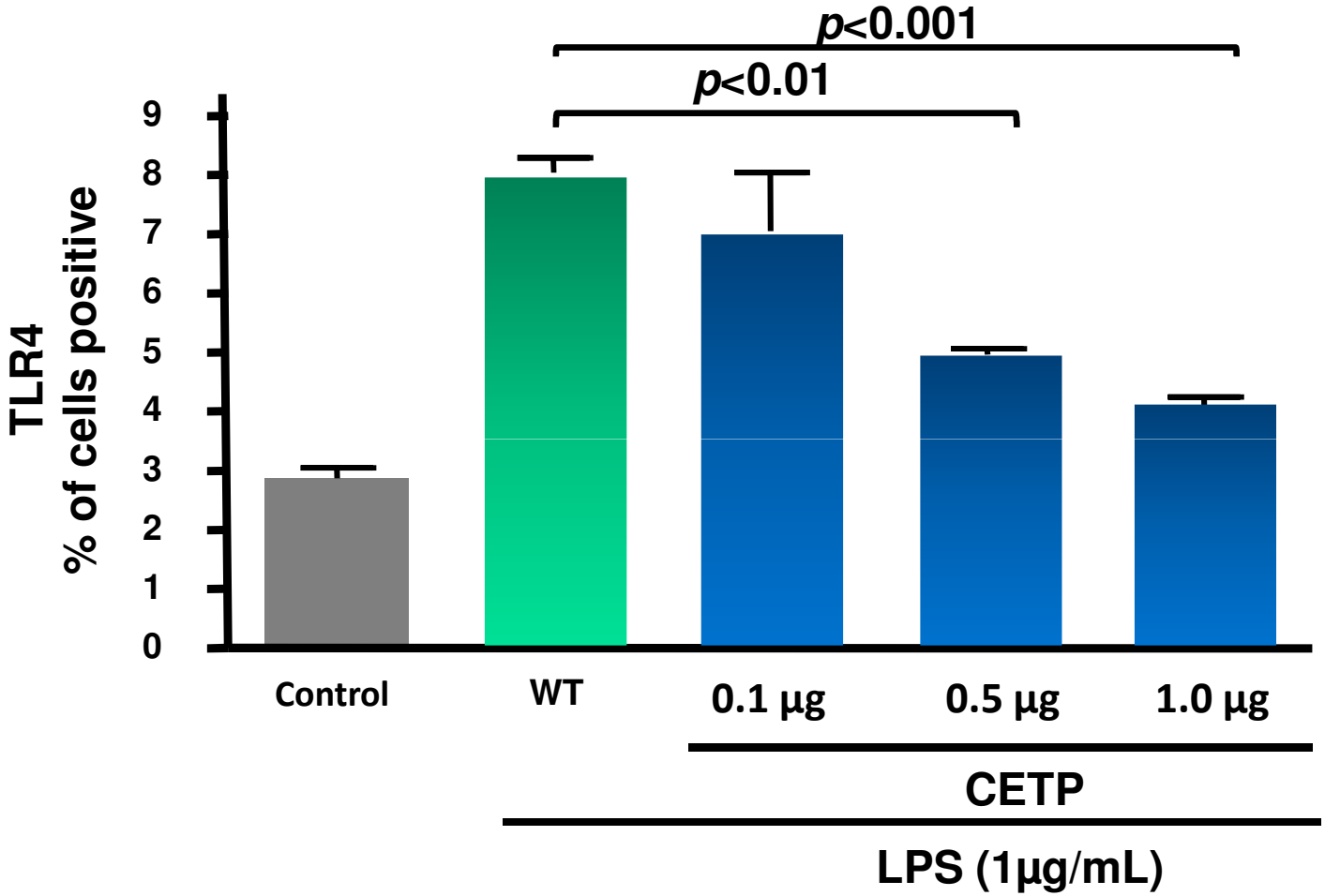
Clin Transl Sci. 2011 Dec;4(6):414-20.

CETP inhibitor torcetrapib promotes reverse cholesterol transport in obese insulin-resistant CETP-ApoB100 transgenic mice.

Briand F[1], Thieblemont Q, André A, Ouguerram K, Sulpice T.

Author information

We therefore evaluated the effects of CETP inhibitor torcetrapib in CETP-apolipoprotein (apo)B100 mice made obese and insulin resistant with a 60% high-fat diet. High-fat diet over 3 months increased body weight and homeostasis model of insulin resistance index by 30% and 846%, respectively ($p < 0.01$ for both vs. chow-fed mice). Total cholesterol (TC) increased by 46% and HDL-c/TC ratio decreased by 28% (both $p < 0.05$). Compared to vehicle, high-fat-fed mice treated with torcetrapib (30 mg/kg/day, 3 weeks) showed increased HDL-c levels and HDL-c/TC ratio by 41% and 37% (both $p < 0.05$). **Torcetrapib increased in vitro macrophage cholesterol efflux by 22% and in vivo RCT through a 118% increase in (3) H-bile acids fecal excretion after (3) H-cholesterol labeled macrophage injection ($p < 0.01$ for both). Fecal total bile acids mass was also increased by 158% ($p < 0.001$). In conclusion, CETP inhibition by torcetrapib improves RCT in CETP-apoB100 mice. These results emphasize the potential of CETP inhibition to prevent cardiovascular diseases.**


p<0.001
p<0.01
TLR4
% of cells positive
9
8
7
6
5
4
3
2
1
0
Control
WT
0.1 µg
0.5 µg
1.0 µg
CETP
LPS (1µg/mL)

CETP expression enhances liver HDL-cholesteryl ester uptake but does not alter VLDL and **biliary** lipid secretion.
Harada LM, Amigo L, Cazita PM, Salerno AG, Rigotti AA, Quintão EC, Oliveira HC.
Atherosclerosis. 2007 Apr;191(2):313-8.

Cholesteryl ester transfer protein modulates the effect of liver X receptor agonists on **cholesterol** transport and **excretion** in the mouse.
Masson D, Staels B, Gautier T, Desrumaux C, Athias A, Le Guern N, Schneider M, Zak Z, Dumont L, Deckert V, Tall A, Jiang XC, Lagrost L.
J Lipid Res. 2004 Mar;45(3):543-50. CETP trangenic increases hepatic cholesterol – CETP inhibitors increased fecal steroids (b. acids) in hamsters

J Lipid Res. 2011 Nov;52(11):1965-73.
Anacetrapib promotes reverse cholesterol transport and bulk cholesterol excretion in Syrian golden hamsters.
Castro-Perez J[1], Briand F, Gagen K, Wang SP, Chen Y, McLaren DG, Shah V, Vreeken RJ, Hankemeier T, Sulpice T, Roddy TP, Hubbard BK, Johns DG. – increased fecal 3H-sterois from macrophages

Circulation. 2007 Sep 11;116(11):1267-73.
Expression of cholesteryl ester transfer protein in mice promotes macrophage reverse cholesterol transport.
Tanigawa H1, Billheimer JT, Tohyama J, Zhang Y, Rothblat G, Rader DJ.
Adenovirus CETP expression. In the mouse liver: increases fecal 3H-cholesterol excretion from macrophages

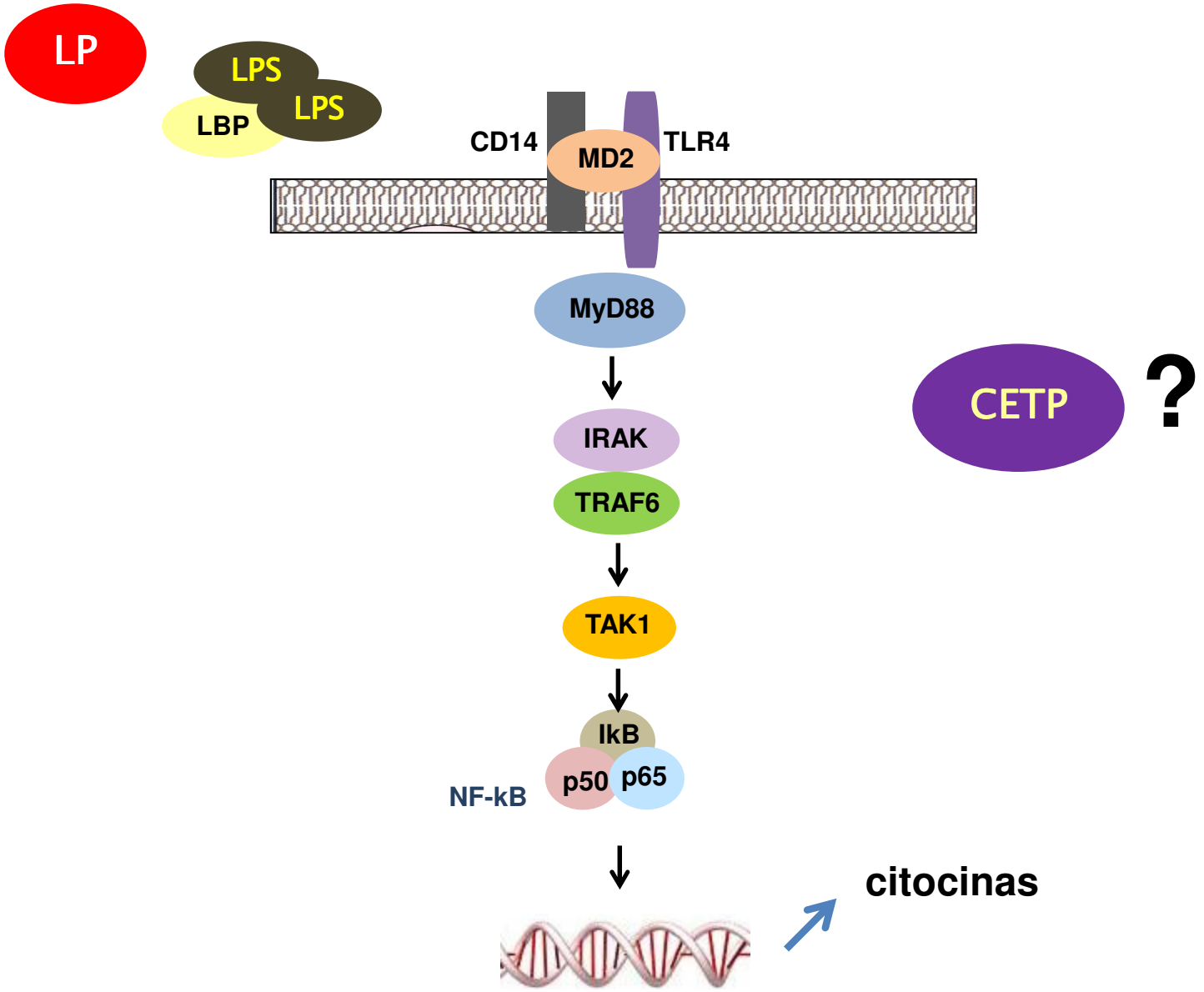
Anacetrapib and dalcetrapib differentially alters HDL metabolism and macrophage-to-**feces** reverse **cholesterol** transport at similar levels of **CETP** inhibition in hamsters.
Briand F, Thieblemont Q, Muzotte E, Burr N, Urbain I, Sulpice T, Johns DG. Eur J Pharmacol. 2014 Oct 5;740:135-43 but only anacetrapib increased HDL-derived (3)H-tracer fecal excretion significantly by 39% in hamsters

Effects of cholesteryl ester transfer protein inhibitors on human lipoprotein metabolism: why have they failed in lowering coronary heart disease risk?
Schaefer EJ.
Curr Opin Lipidol. 2013 Jun;24(3):259-64.

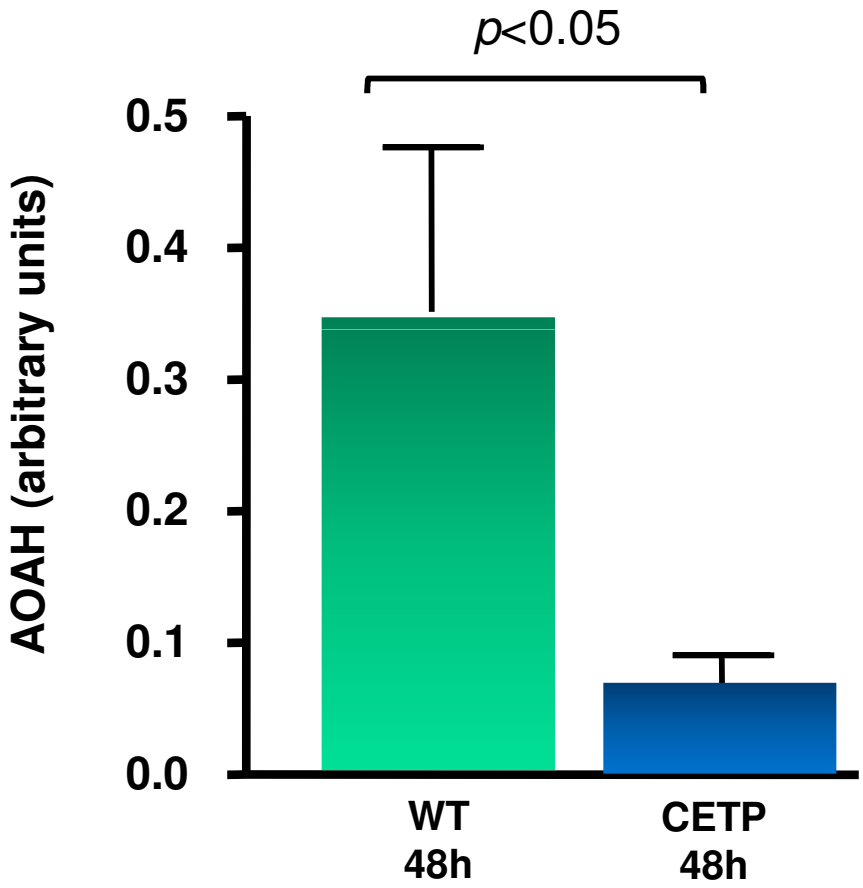
CETP inhibitor torcetrapib promotes reverse **cholesterol** transport in obese insulin-resistant **CETP**-ApoB100 transgenic mice.Briand F, Thieblemont Q, André A, Ouguerram K, Sulpice T.Clin Transl Sci. 2011 Dec;4(6):414-20. Torcetrapib increased in vitro macrophage cholesterol efflux by 22% and in vivo RCT through a 118% increase in 3H-bile acids fecal excretion after 3H-cholesterol labeled macrophage injection

Modulating cholesteryl ester transfer protein activity maintains efficient pre-β-HDL formation and increases reverse **cholesterol** transport. Niesor EJ, Magg C, Ogawa N, Okamoto H, von der Mark E, Matile H, Schmid G, Clerc RG, Chaput E, Blum-Kaelin D, Huber W, Thoma R, Pflieger P, Kakutani M, Takahashi D, Dernick G, Maugeais C.J Lipid Res. 2010 Dec;51(12):3443-54. In hamsters CETP inhibitors increased fecal steroids and radioactivity from macrophages

CETP NA RESPOSTA INFLAMATÓRIA

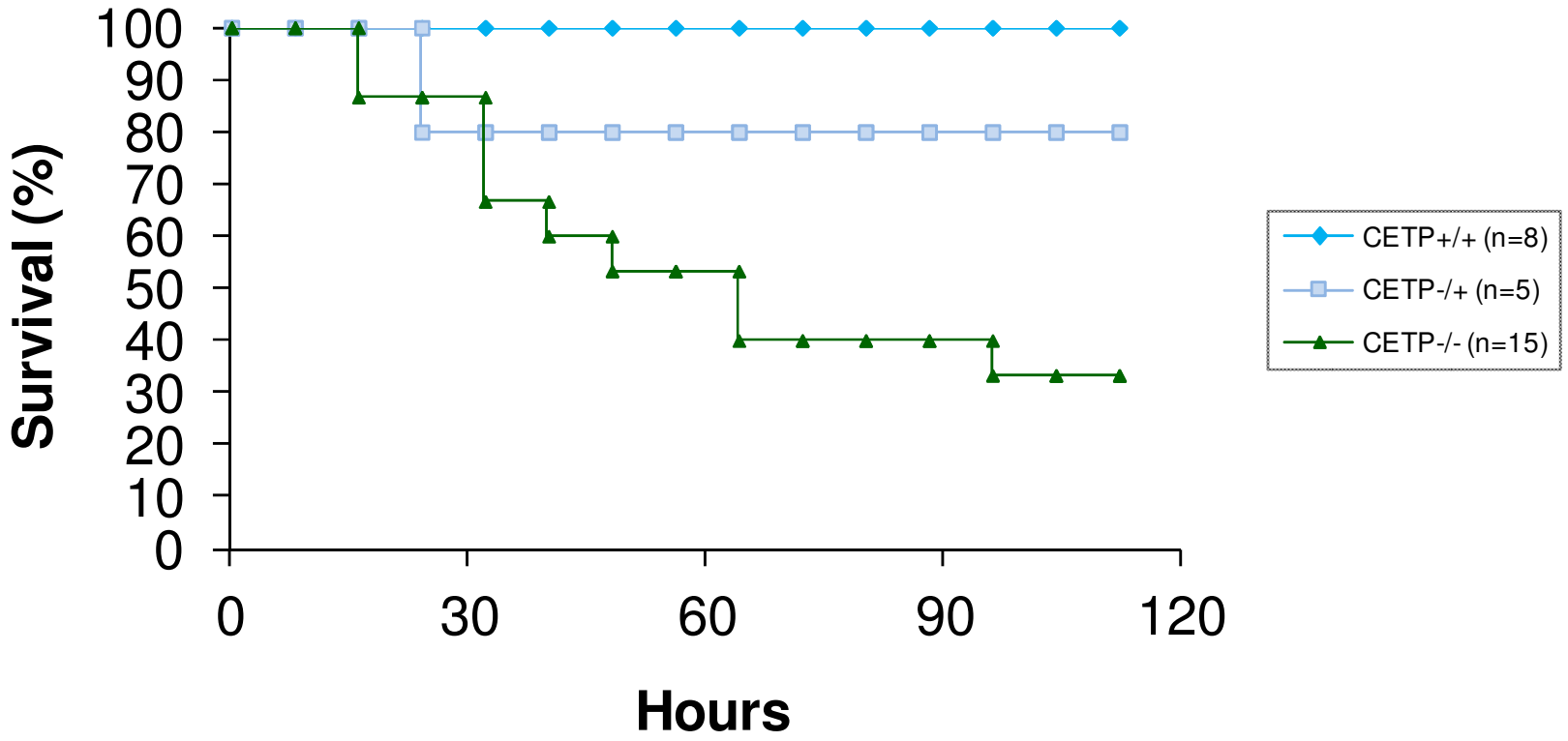


48 h after CLP liver lipopolysaccharide deacylating activity (Acycloacylhydrolase, AOAH) was lower in huCETP expressing than in WT mice



Teste *t* de *Student*; n=3

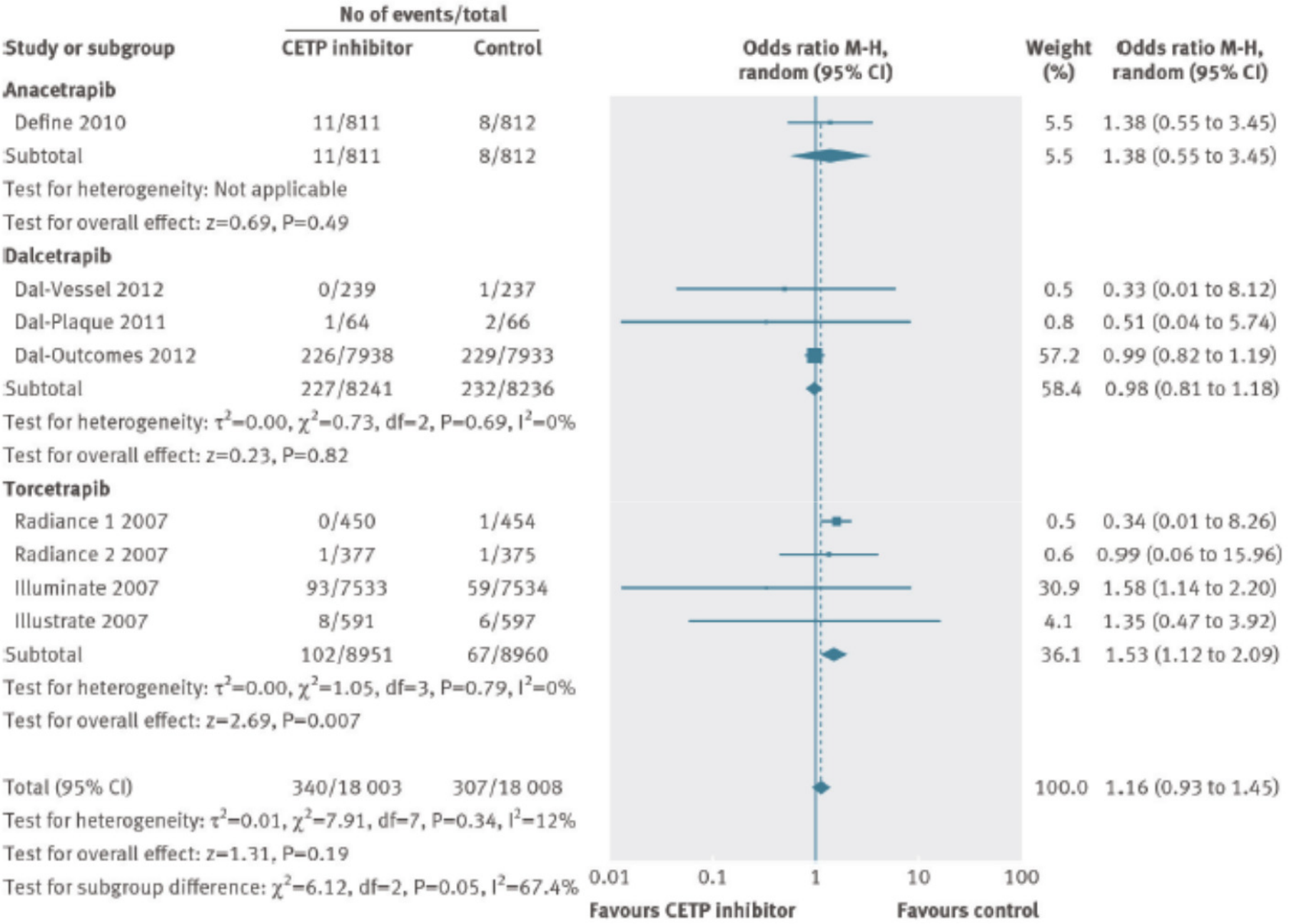
In huCETP expressing mice: after lipopolysaccharide (LPS) injection all CETP +/+ survived, and CETP +/- survived longer than the wild type (CETP -/-) mice



Cazita PM, Barbeiro DF, Moretti AI, Quintão EC, Soriano FG. Shock. 2008 Nov;30(5):590-5.

As compared to healthy individuals, in septic patients it was observed a failure of the *in vitro* neutrophil migration (chemotactic function)

Tavares-Murta BM et al. Failure of neutrophil chemotactic function in septic patients. Crit. Care Med. 2002 May;30(5):1056-61



Keene D et al. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments Niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117411 patients. BMJ. 349:g4379 (2014)

CETP expression prolongs mice survival rate in sepsis by increasing leukocyte migration and reducing the concentrations of plasma IL-6 and of hepatic TLR4

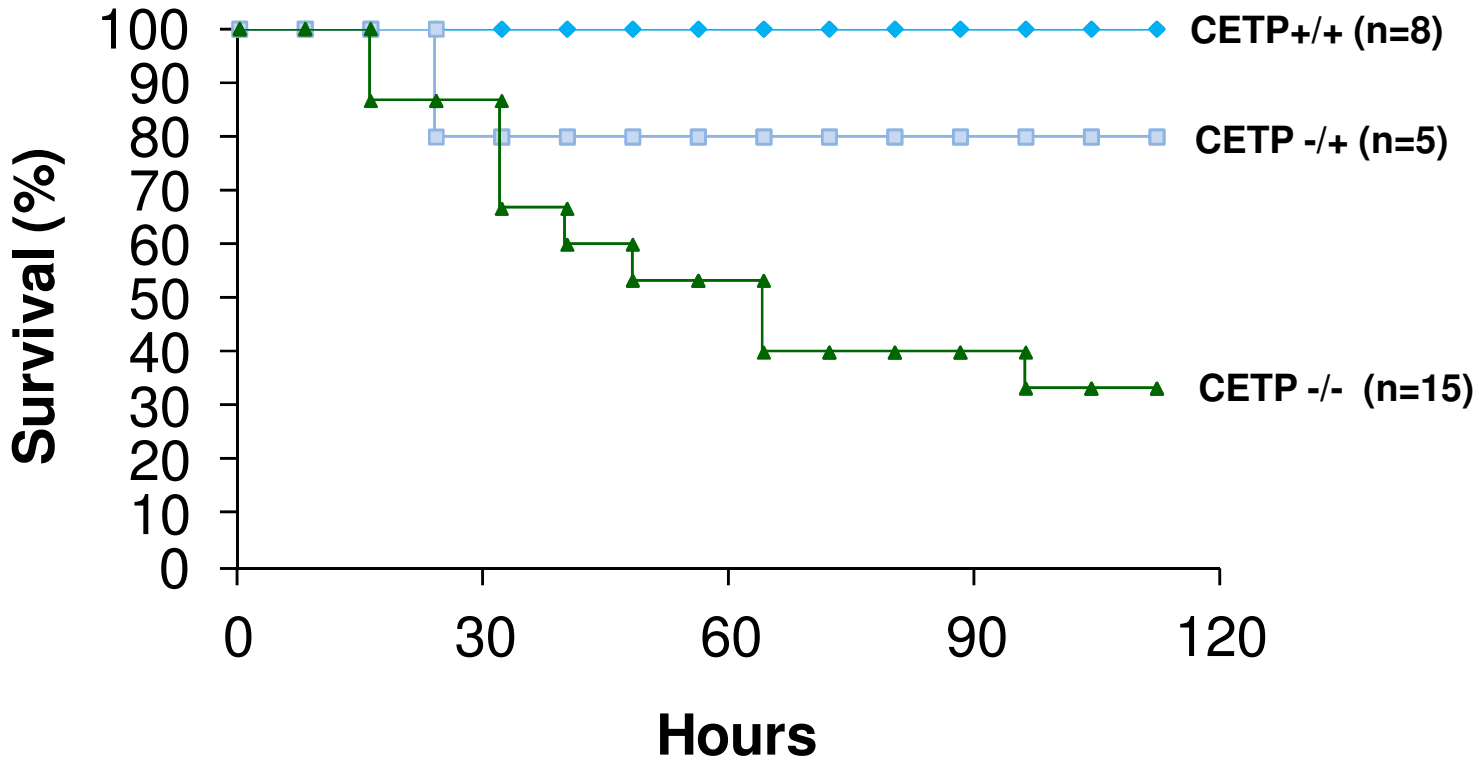
Eder C R Quintão[1], Tatiana Martins Venancio[1], Roberta Marcondes Machado[1],
Valéria Sutti Nunes[1], Alessandro Salerno[2], Francisco Garcia Soriano[3], Chin Jia Lin[4], Patrícia Miralda Cazita[1].
Lipids Lab, Faculty of Medical Sciences[1], Department of Physiology and Biophysics, State University of Campinas[2], Emergency Care Research Unit Laboratory [3], Hormones and Molecular Genetics Lab[4], University of São Paulo, Sao Paulo, Brazil

Cholesteryl ester transfer protein (CETP) transfers neutral lipids among plasma lipoproteins; its inhibition raises plasma HDL. There has been considerable debate on the role of CETP in human atherogenesis. This may in part be explained by the involvement of CETP on the protection against against microbial infection (Cazita PM et al. Shock. 30(5):590(2008) and human sepsis (Grion CM et al. Eur J Clin Invest. 40(4):330 (2010).

In order to evaluate the role of CETP in polymicrobial sepsis induced by caecum ligation and puncture (CLP), mice expressing human CETP, and wild-type mice (WT) underwent CLP sepsis. Sham-operated mice were utilized as controls. After CLP, mice survival rates were evaluated over five days. Also, mice were sacrificed at 24 or 48 hours after CLP, and blood, peritoneal cells and liver were collected.

After CLP, as compared to wild type mice, CETP mice survived longer, had increased leukocyte migration into the peritoneal cavity, lower plasma IL-6 and TLR4 and acyloxyacyl hydrolase (AOAH) expressions in their liver. CETP mice had reduced liver inflammation and plasma inflammatory factors, and increased leukocyte recruitment to the infectious focus. Thus, CETP is involved in the first line of defense against an exacerbated production of proinflammatory mediators. These results indicate that the regulation of TLR4 in the liver plays a role in the proinflammatory response and pathophysiology of polymicrobial sepsis that helps explaining why CETP pharmacological inhibition has consistently failed to provide protection against atherosclerosis in human investigations.

In huCETP expressing mice: after lipopolysaccharide (LPS) injection all CETP +/+ survived, and CETP +/- survived longer than the wild type (CETP -/-) mice



Cazita PM, Barbeiro DF, Moretti AI, Quintão EC, Soriano FG. Shock. 2008 Nov;30(5):590-5.

Arterioscler Thromb Vasc Biol. 2005 May;25(5):1057-64. Epub 2005 Mar 10.

Effects of cholesteryl ester transfer protein inhibition on high-density lipoprotein subspecies, apolipoprotein A-I metabolism, and fecal sterol excretion.

Brousseau ME[1], Diffenderfer MR, Millar JS, Nartsupha C, Asztalos BF, Welty FK, Wolfe ML, Rudling M, Björkhem I, Angelin B, Mancuso JP, Digenio AG, Rader DJ, Schaefer EJ.

CONCLUSIONS:

These data indicate that partial inhibition of CETP via torcetrapib in patients with low HDL-C: (1) normalizes apoA-I levels within alpha1-migrating HDL, (2) increases plasma concentrations of HDL apoA-I by delaying apoA-I catabolism, and (3) does not significantly influence fecal sterol excretion.

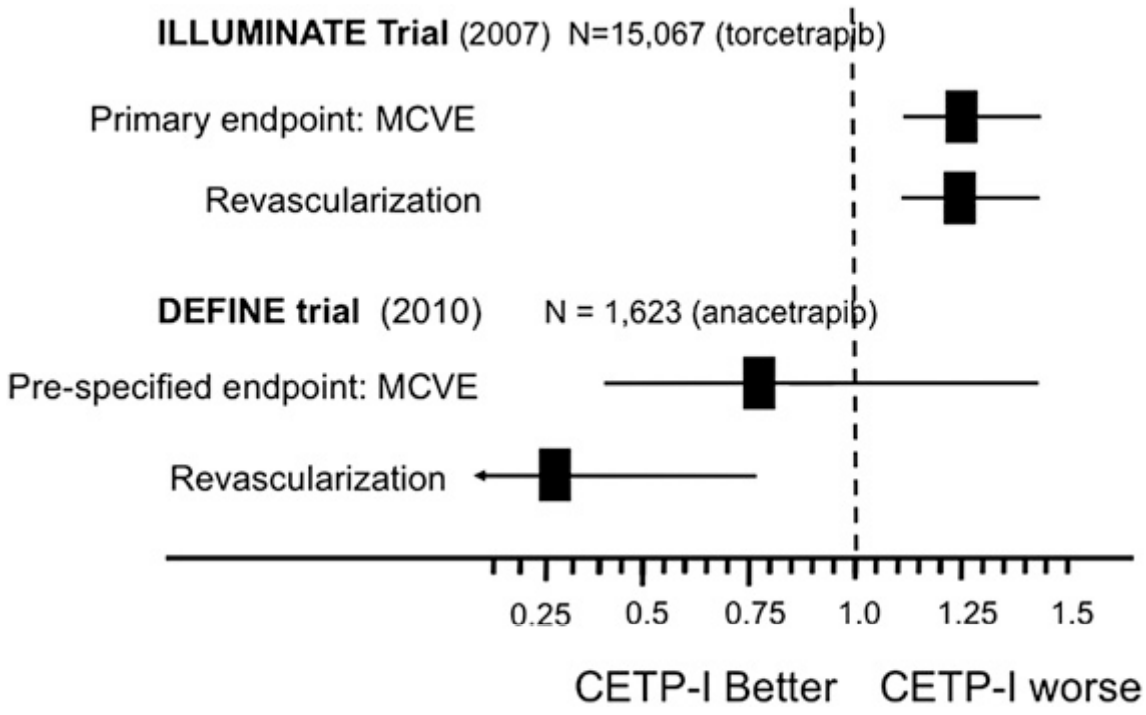
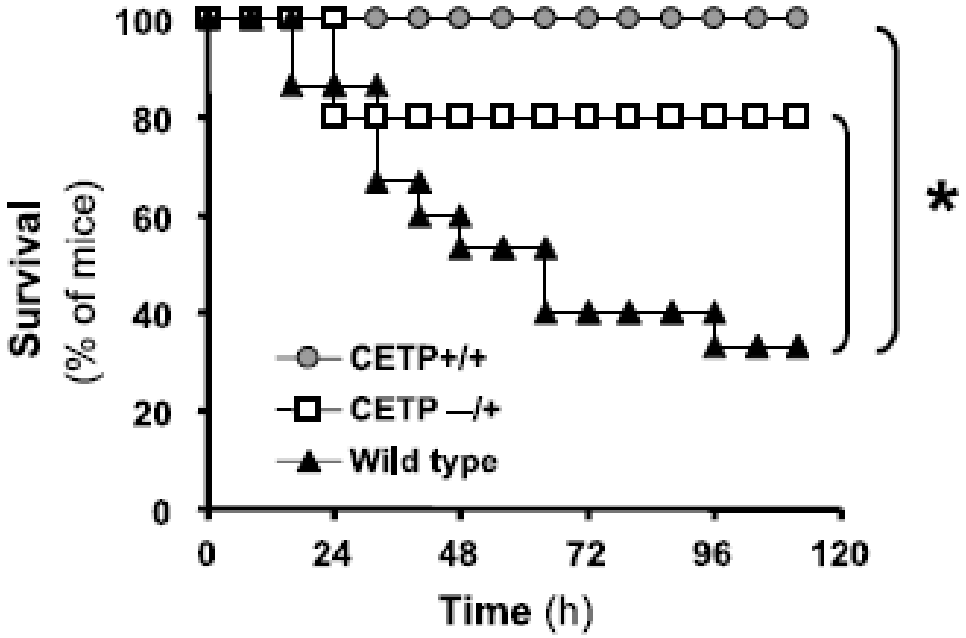
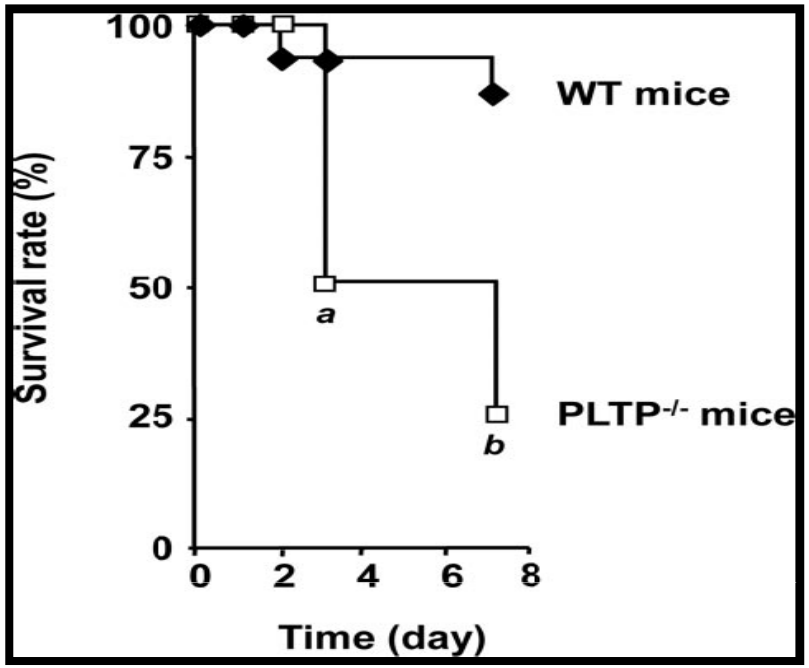


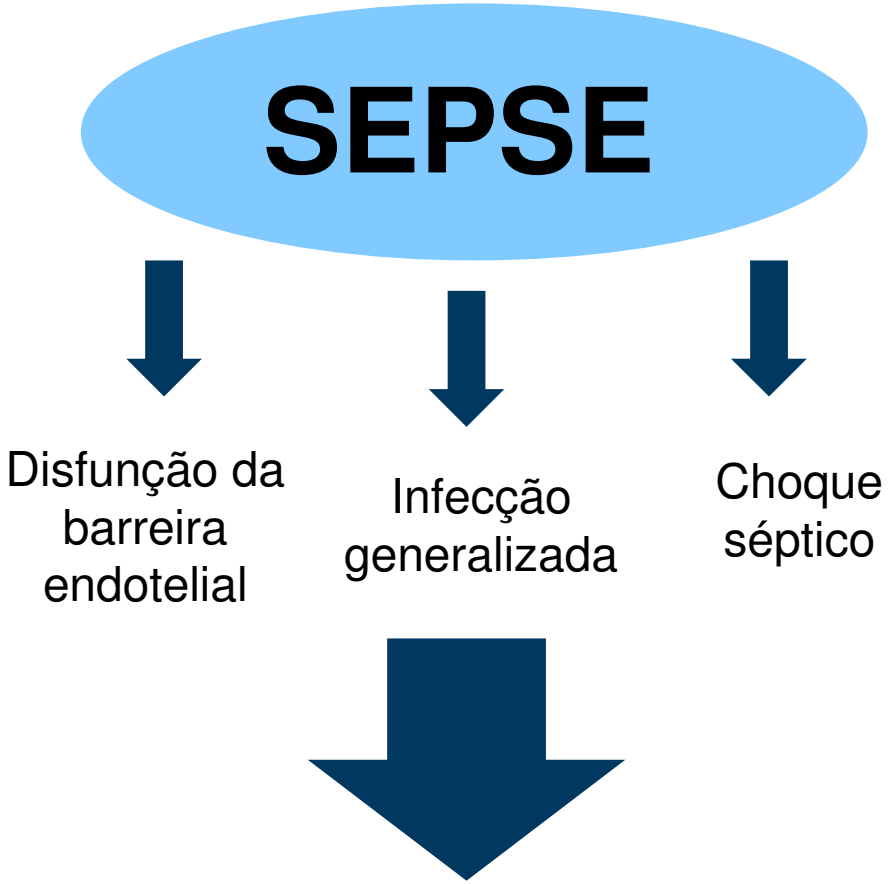
Fig. 6. Cardiovascular end points in the DEFINE (31) and ILLUMINATE (3) trials. The hazard ratios and 95% confidence intervals are shown. The primary end point in ILLUMINATE was time to first occurrence of a major cardiovascular event (MCVE), comprising a composite of death from coronary heart disease, nonfatal myocardial infarction, stroke, and hospitalization for unstable angina. A pre-specified end point in DEFINE was time to first occurrence of a MCVE, comprising death from cardiovascular causes, nonfatal myocardial infarction, hospitalization for unstable angina, and nonfatal stroke. CETP-I, CETP inhibitor.



Cazita PM, Barbeiro DF, Moretti AI, Quintão EC, Soriano FG. Shock. 2008 Nov;30(5):590-5.



Gautier T, Klein A, Lagrost L. et al. J Biol Chem. 283(27):18702-10. (2008).



Hotchkiss *et al.* N Engl J Med. (2003); 9;348(2): 138-50.

Skrupky *et al.* Anesthesiology. (2011); 115(6):1349-62.

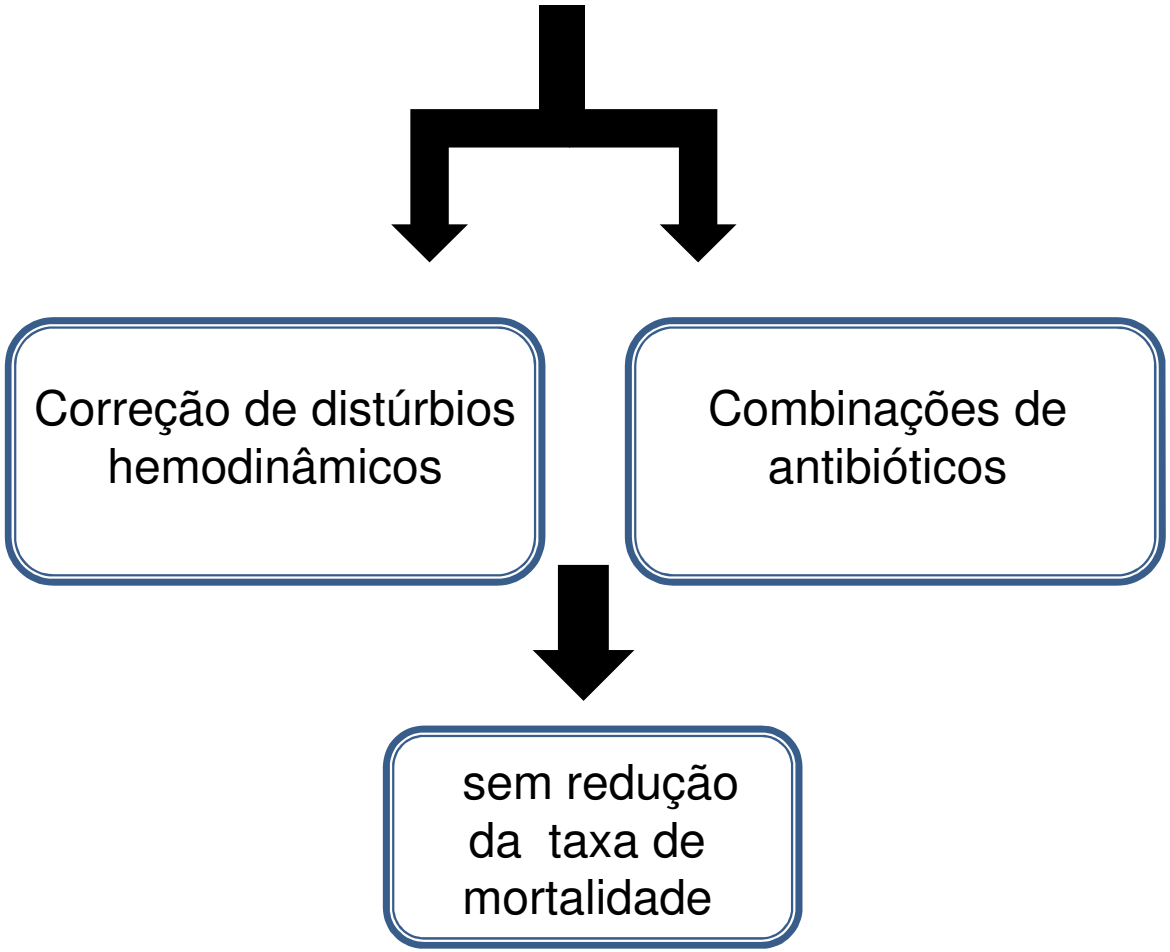
Leelahavanichkul *et al*, J Immunol. (2012);188(6):2749-58.

EPIDEMIOLOGIA DA SEPSE

- ✓ **Incidência é maior em crianças até 12 meses e em adultos acima dos 65 anos;**
- ✓ **Incidência: EUA: 751.000 casos/ano**
  Europa: 200.000 casos/ano
  Brasil: 250.000 casos/ano
- ✓ **Aumento de 1,5% ao ano até 2050;**
- ✓ **Mortalidade hospitalar no Brasil: 48,1% dos casos.**

Instituto Latino Americano da Sepse (ILAS) 2013

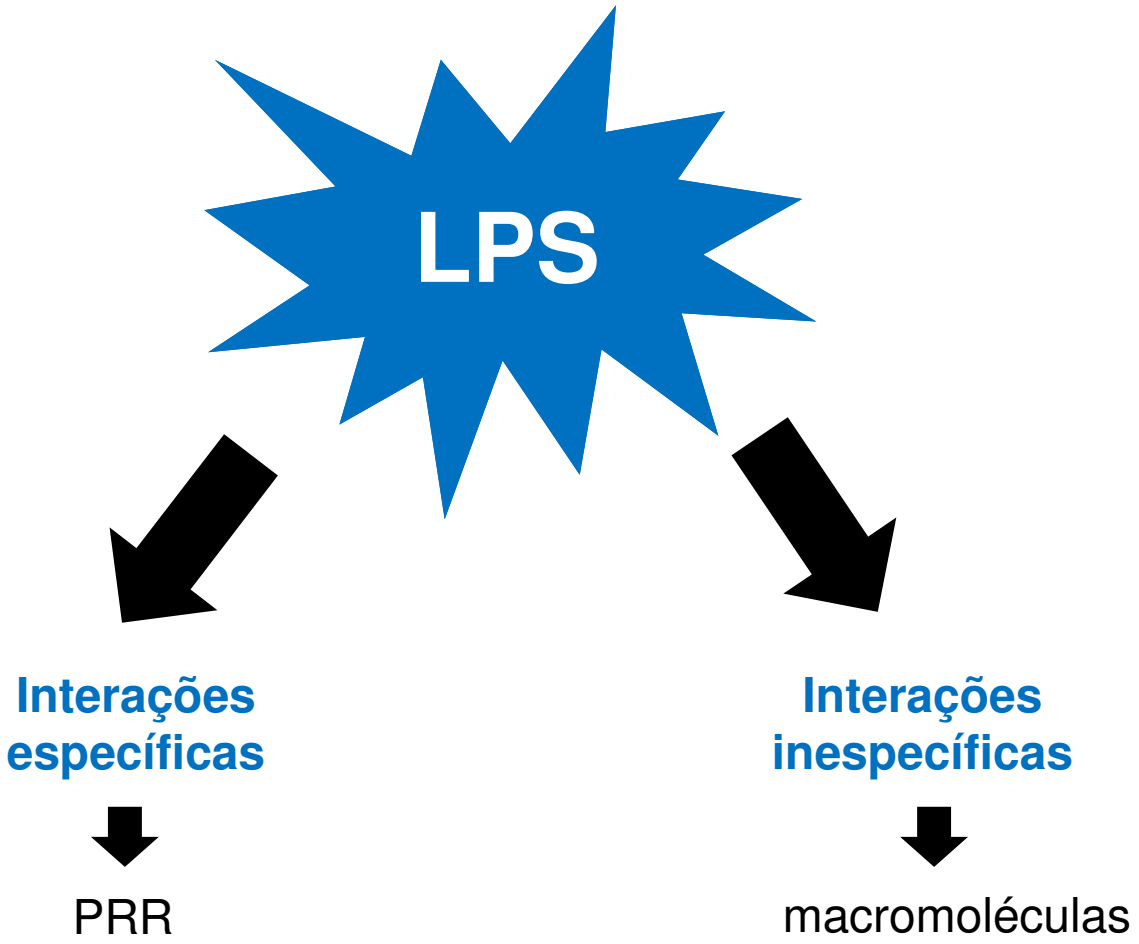
SEPSE: MEDIDAS TERAPÊUTICAS



Wynn. *et al,* Pediatrics (2010) 125 (5): 1031-41.

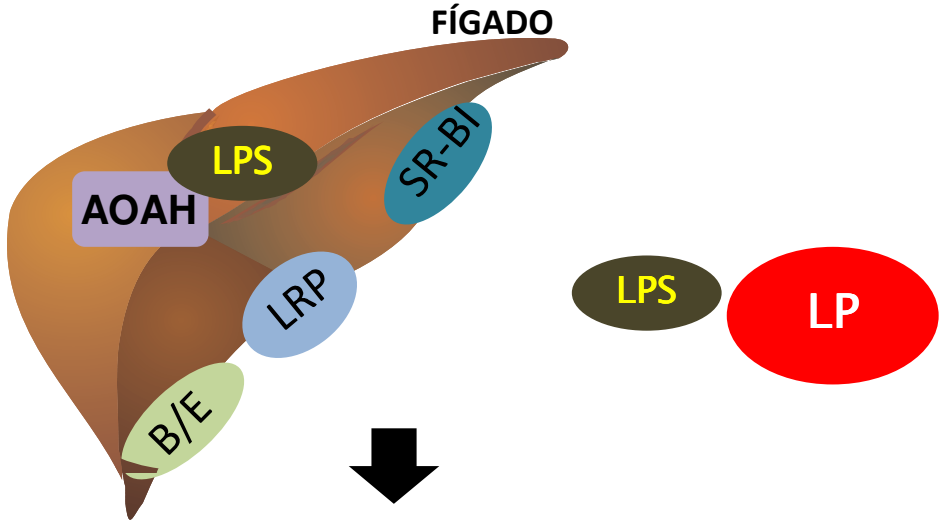
Daniels. J Antimicrob Chemother (2011) 66 Suppl 2: ii11–ii23.

LIPOPOLISSACARÍDEO (LPS)



Kang *et al.*, Electron Microsc. Rev. 1992; 5(2): 381-419.

LIPOPROTEÍNAS (LP): AGENTES ANTI-INFLAMATÓRIOS

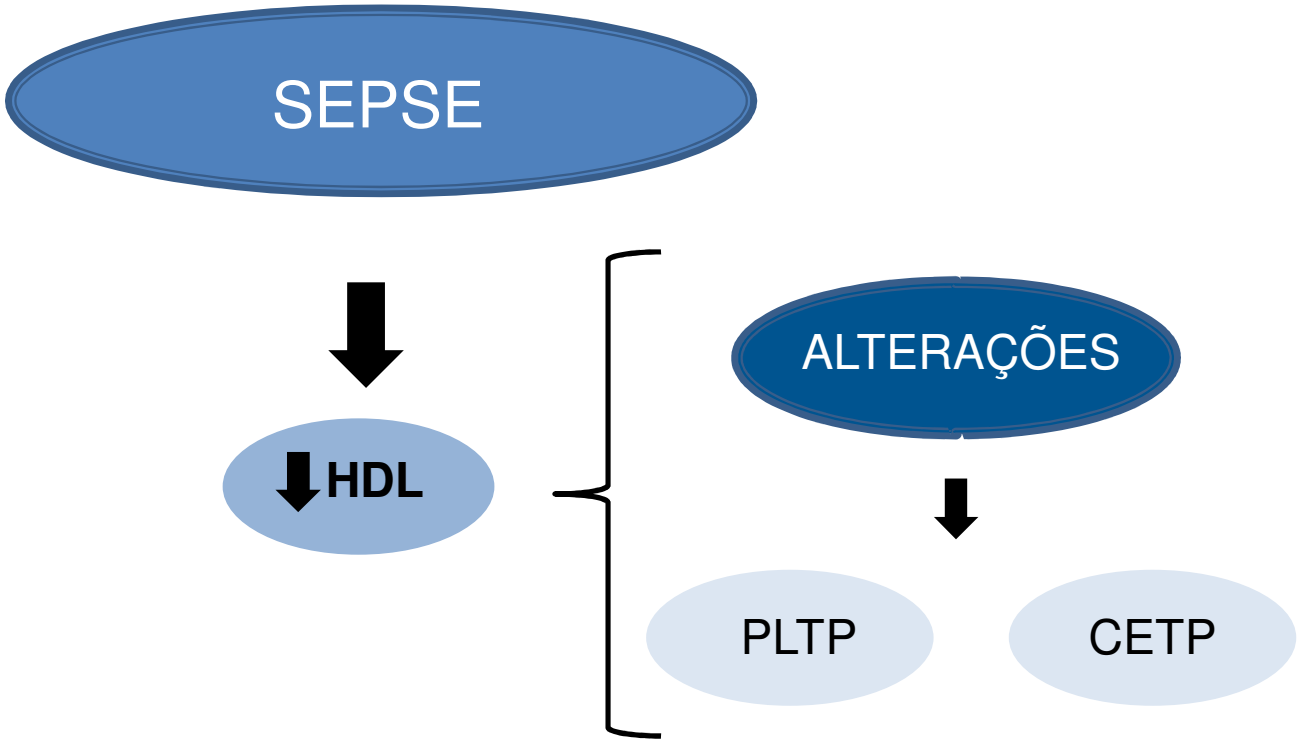


⬇Resposta inflamatória

⬇Taxa de mortalidade

van Leeuwen *et al*, Crit Care Med (2003) 31(5):1359-66.

Much, O. et al, Int. Care Med (2007) 33:13-24.

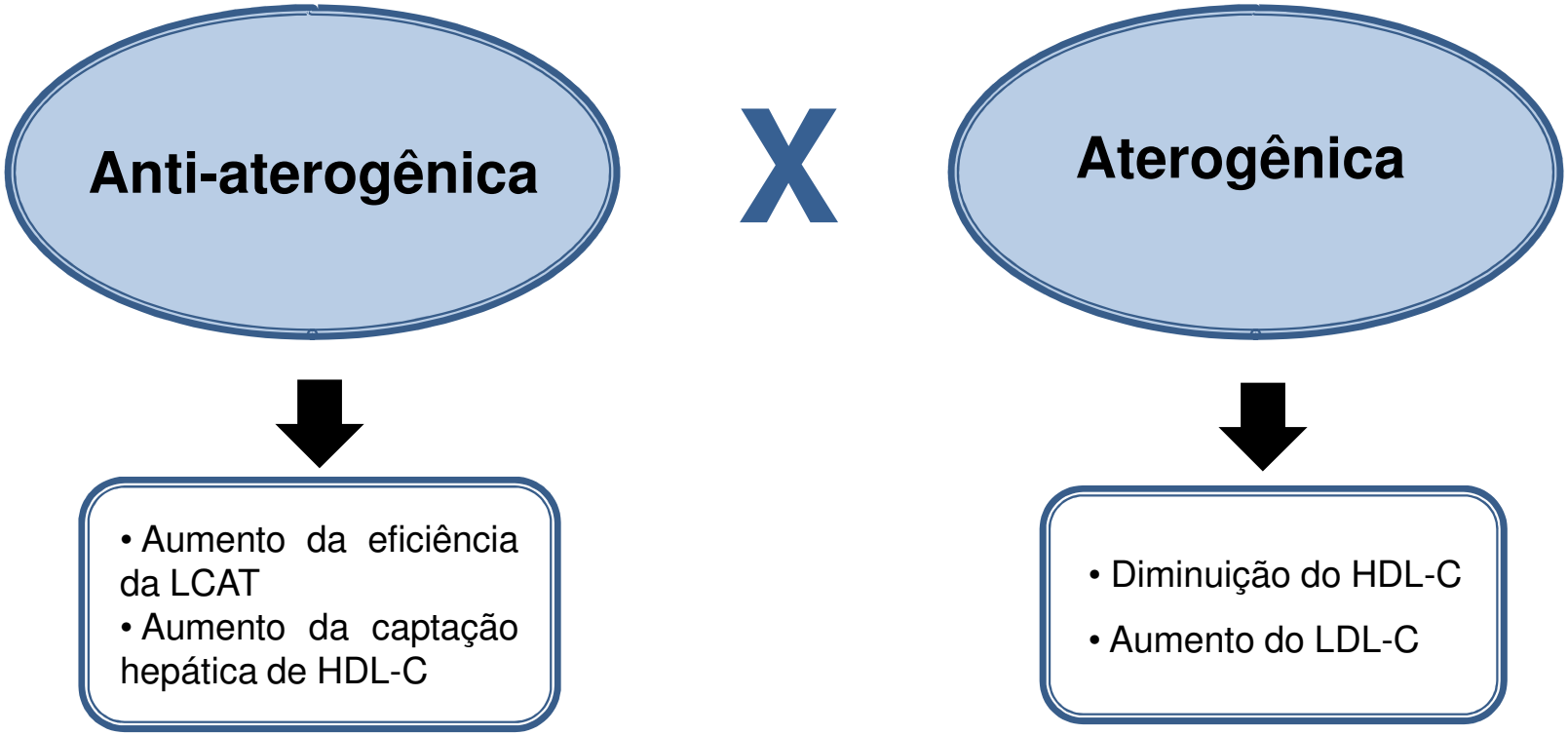


Levels *et al,* BBA. (2007); 1771(12):1429-38.

Quintão, E. C. & Cazita, P. M. Atherosclerosis . (2010); 209(1): 1-9.

Grion , C. M. C. *et al*, Eur J Clin Invest . (2010); 40 (4): 3030-338.

O PAPEL CONTROVERSO DA CETP NA ATEROSCLEROSE

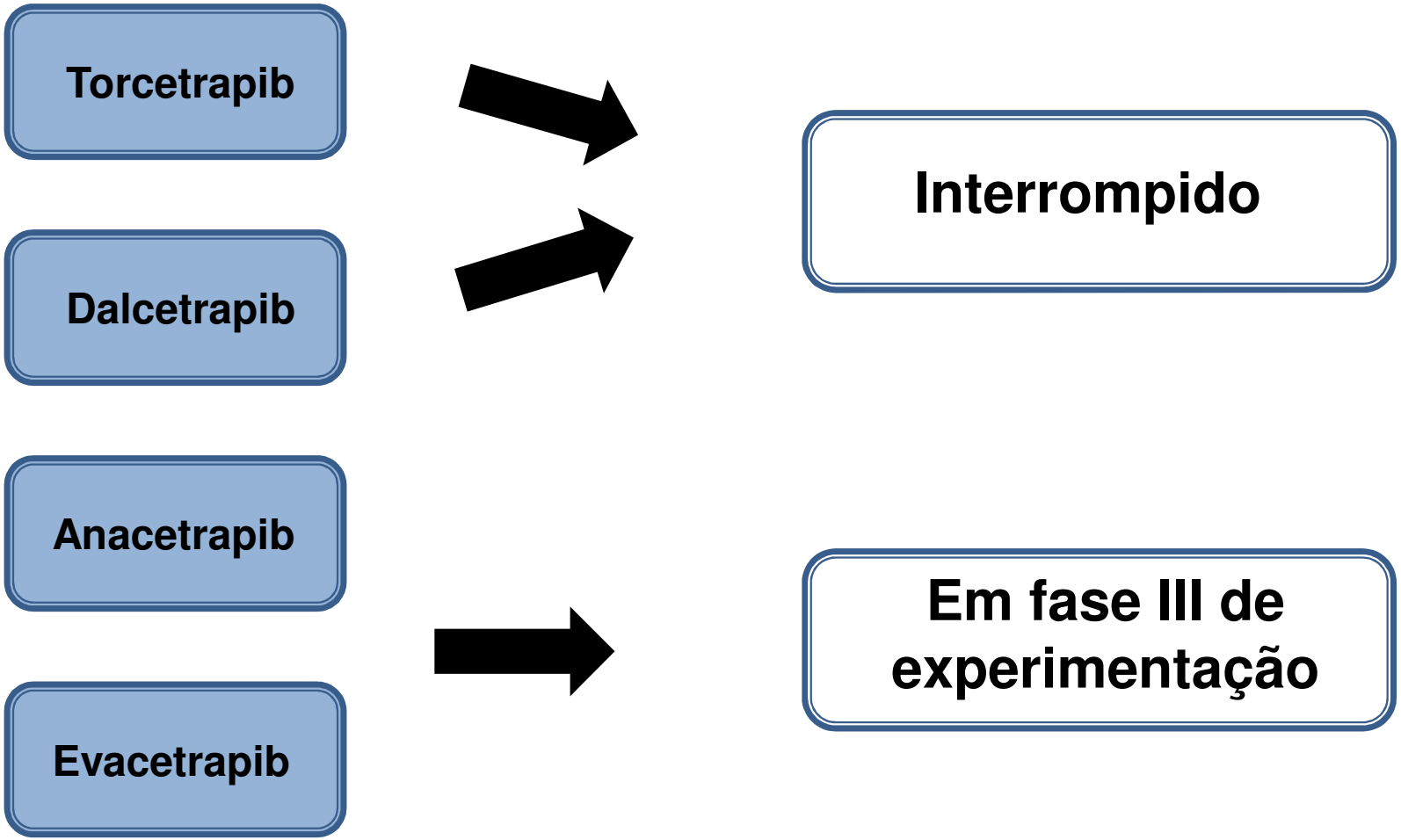


Inazu *et al.*, N. Engl. J. Med. (1990); 323(18): 1234–1238

Oliveira & de Faria, 2011. IUBMB Life. 2011; 63(4): 248-57.

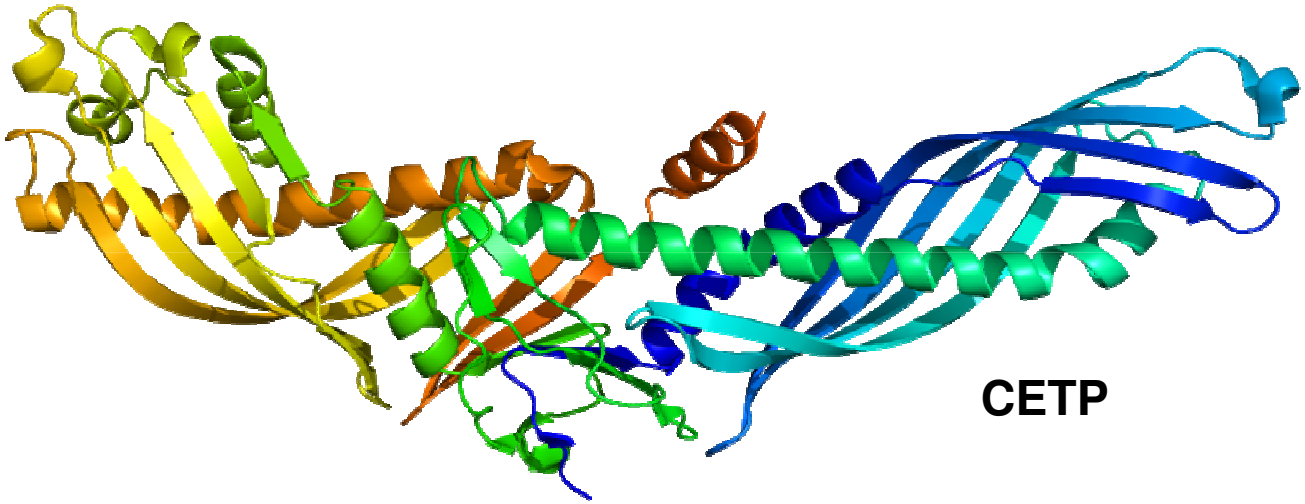
Zhong *et al.*, J Clin Invest. (1996); 97(12):2917-23.

INIBIDORES DE CETP



Sirtori . Exp. Opin Investig Drugs. (2011); 20(11):1543-54.
Barter P. & Rye K. J Lipid Res. (2012); 53(9):1755-66.

HOMOLOGIA ESTRUTURAL ENTRE A CETP E A PROTEÍNA DE LIGAÇÃO DO LPS (LBP)



CETP

Xiayang Qiu *et al*, Nature (2007) 14(2):106-113.

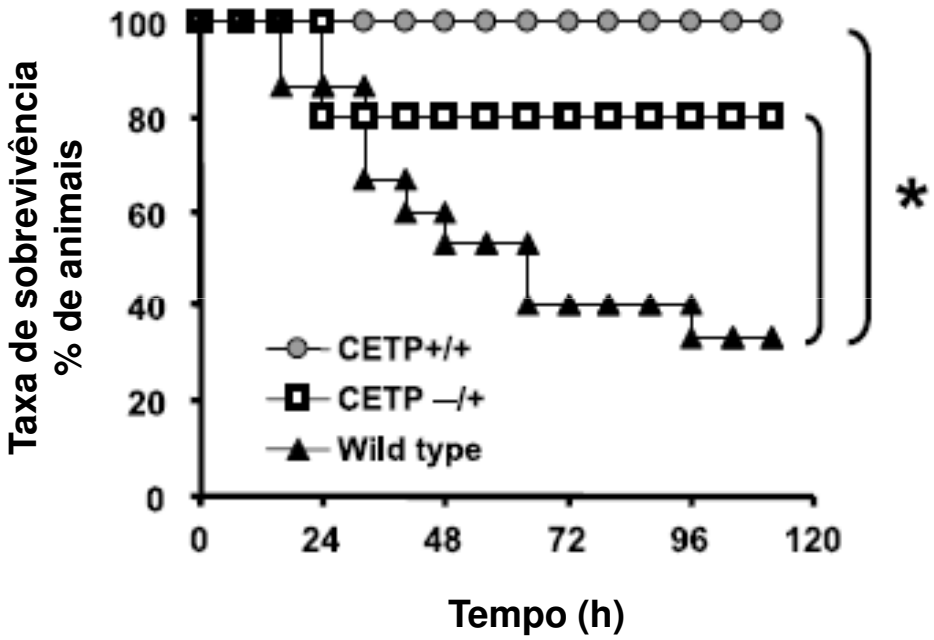
INFLUÊNCIA DA CETP NA RESPOSTA INFLAMATÓRIA EM MODELO EXPERIMENTAL DE ENDOTOXEMIA



CETP ***vs.*** **WT**



Injeção intraperitoneal de LPS (25 µg/kg de peso)



* p<0,05

Log-Rank test (n=15)

Cazita *et al*, Shock (2008) 30(5): 590-5.

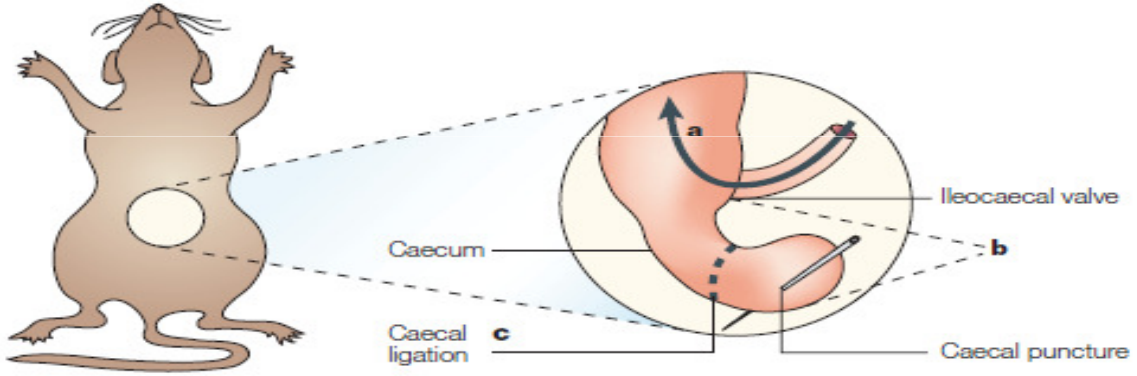
OBJETIVO

Avaliar a influência da CETP na resposta inflamatória em modelo experimental de sepse polimicrobiana.

MÉTODOS



CETP ***vs.*** **WT**



Curva de mortalidade 120 h

Sacrifício 24 h

Sacrifício 48 h

RESULTADOS

CONCLUSÃO

Os resultados indicam que a CETP é um componente endógeno que está envolvido na resposta inflamatória.

Esses achados devem ser considerados nas doenças inflamatórias e nos futuros estudos relacionados à inibição da CETP, além de estabelecer novas perspectivas para o tratamento da sepse.

AGRADECIMENTOS

LIM-10

Dra. Patrícia M. Cazita

Dra. Edna R. Nakandakare

Dr. Eder C.R. Quintão

Amigos do LIM-10

UNICAMP

Dra. Helena C. F. Oliveira



LIM- 51

Dr. Francisco G. Soriano

Table 2

Area under the plasma radioactivity vs. time curve (dpm h 10^6) of labeled HDL-cholesteryl ester obtained from CETP Tg (^{3}H-HDL$_{CETP}$) and non-transgenic mice (^{14}C-HDL$_{NTG}$) during 6 h after simultaneous injection of both HDL into non-transgenic (NTG) and CETP-Tg mice

	NTG mice	CETP Tg mice	p^*
Male			
^{14}C-HDL$_{NTG}$	0.98 ± 0.16	0.70 ± 0.09	<0.003
^{3}H-HDL$_{CETP}$	2.05 ± 0.22	1.44 ± 0.19	<0.002
$p^\#$	<0.001	<0.001	
Female			
^{14}C-HDL$_{NTG}$	0.89 ± 0.16	0.76 ± 0.15	n.s.
^{3}H-HDL$_{CETP}$	2.02 ± 0.18	1.47 ± 0.29	<0.001
$p^\#$	<0.001	<0.001	

Mean ± S.D. ($n = 10$). Mann–Whitney test: *NTG mice vs. CETP mice, $^\#$^{14}C-HDL$_{NTG}$ vs. ^{3}H-HDL$_{CETP}$.

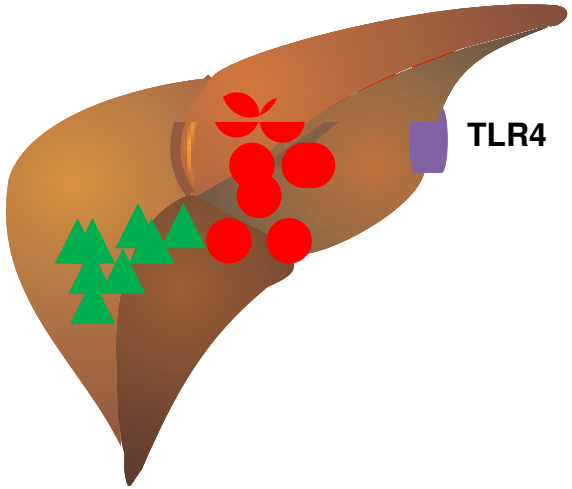
CETP expression enhances liver HDL-cholesteryl ester uptake but does not alter VLDL and biliary lipid secretion. Harada LM, Amigo L, Cazita PM, Salerno AG, Rigotti AA, Quintão EC, Oliveira HC. Atherosclerosis. 2007 Apr;191(2):313-8.

SUMMARY

CETP

↑ Survival rate

↓ plasma IL-6

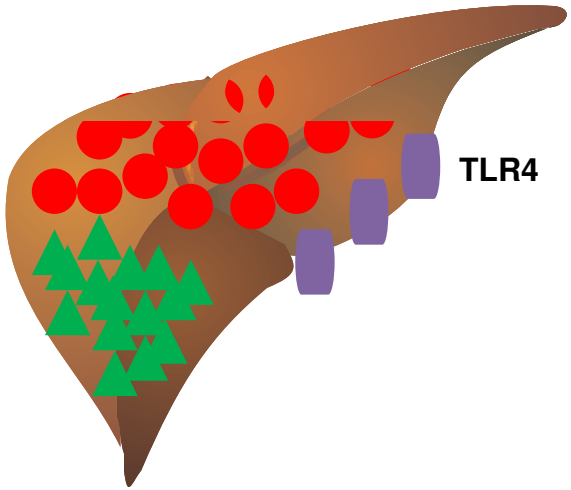


↑ Leukocyte migration

WT

↓ Survival rate

↑ plasma IL-6



↓ Leukocyte migration

● **LPS**

▲ **AOAH**