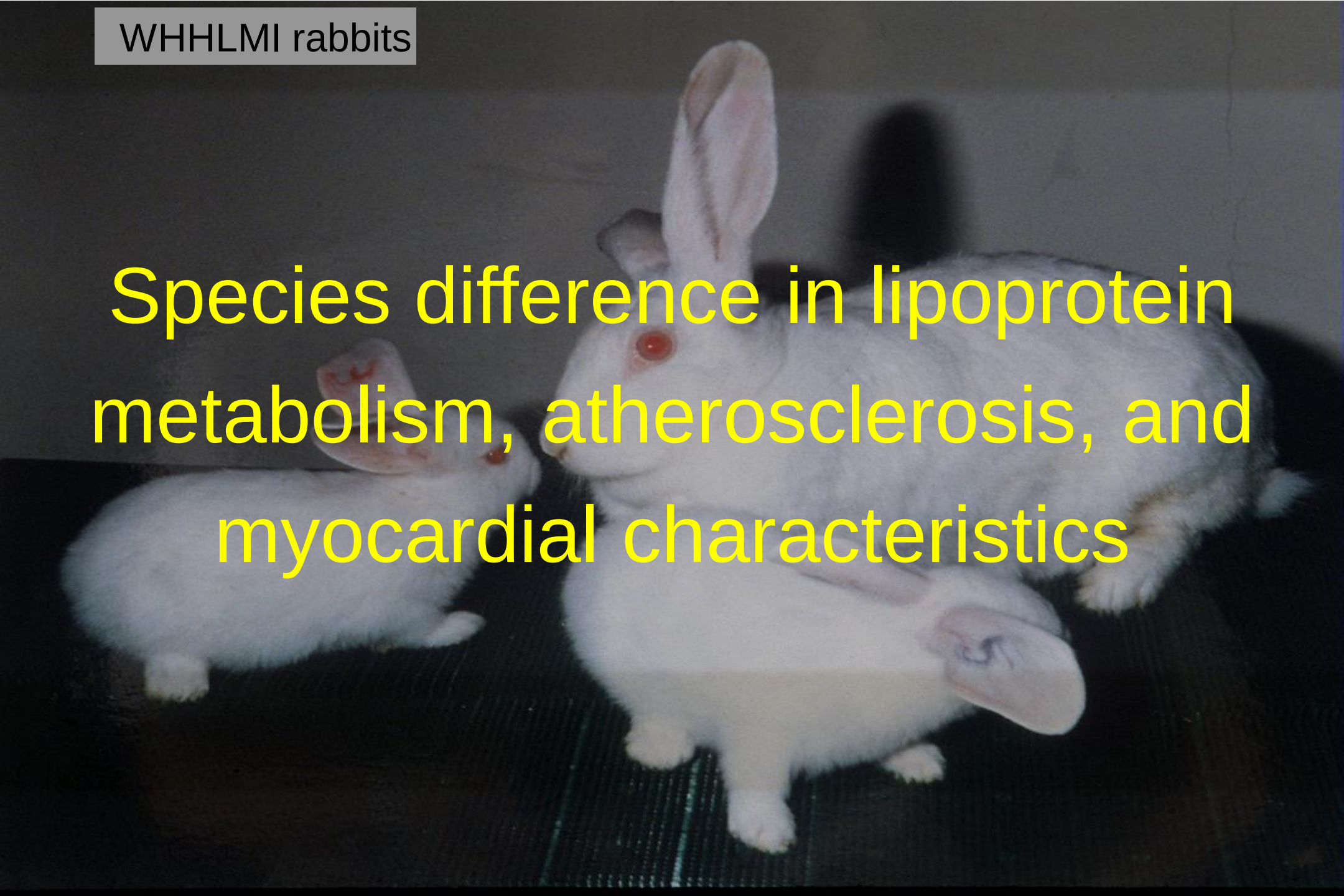
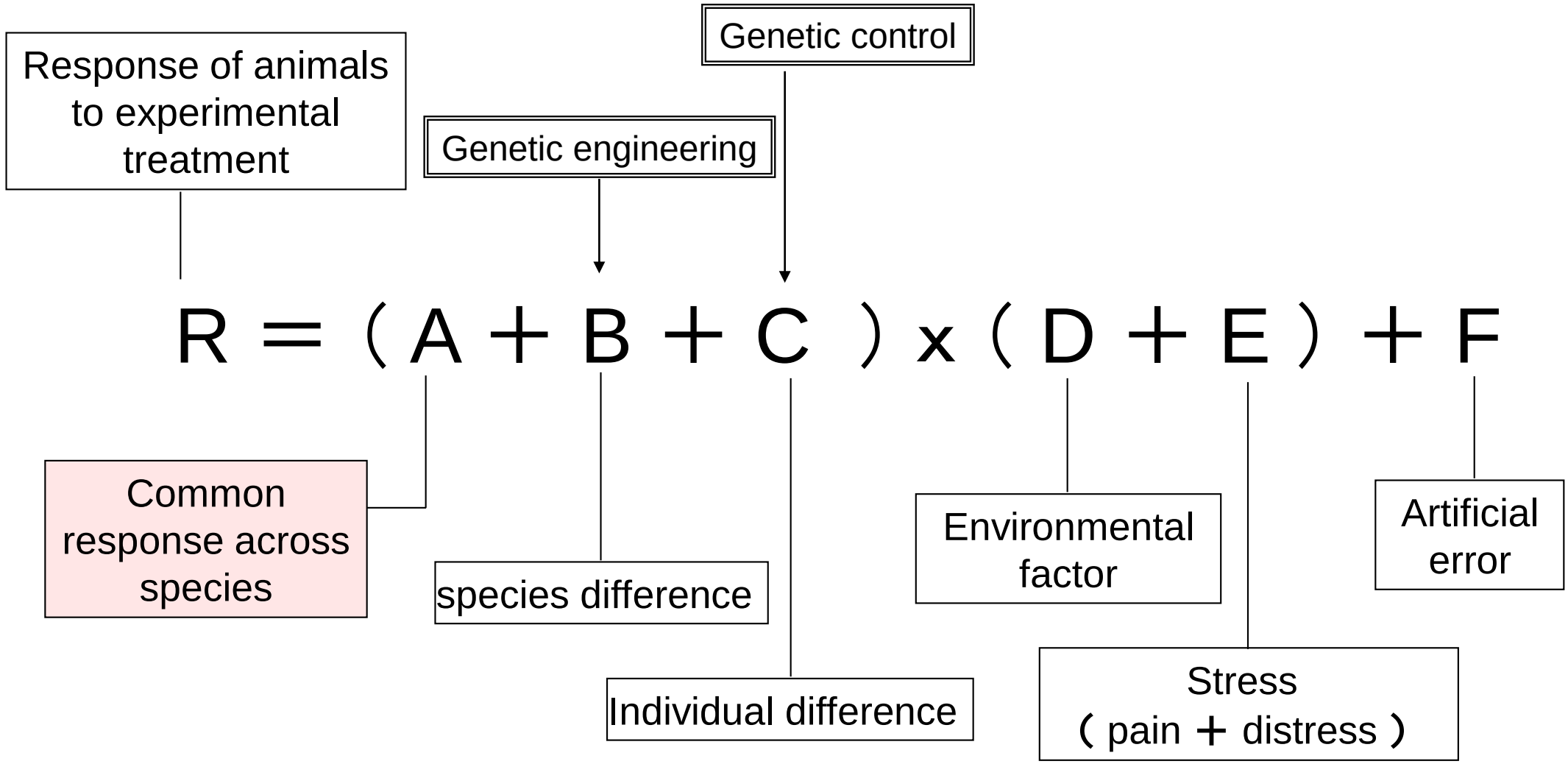

WHHLMI rabbits
Species difference in lipoprotein metabolism, atherosclerosis, and myocardial characteristics

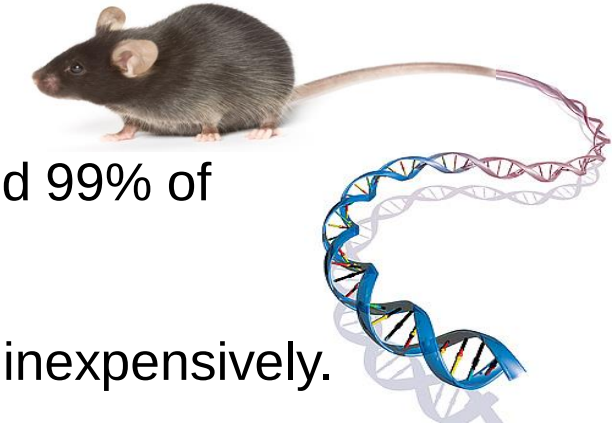
Composition of animal's response to experimental treatment



(modified after Dr. Yoshio Tajima)

Mice are major model animals in biomedical research.

Why are mice the most used for biomedical research?

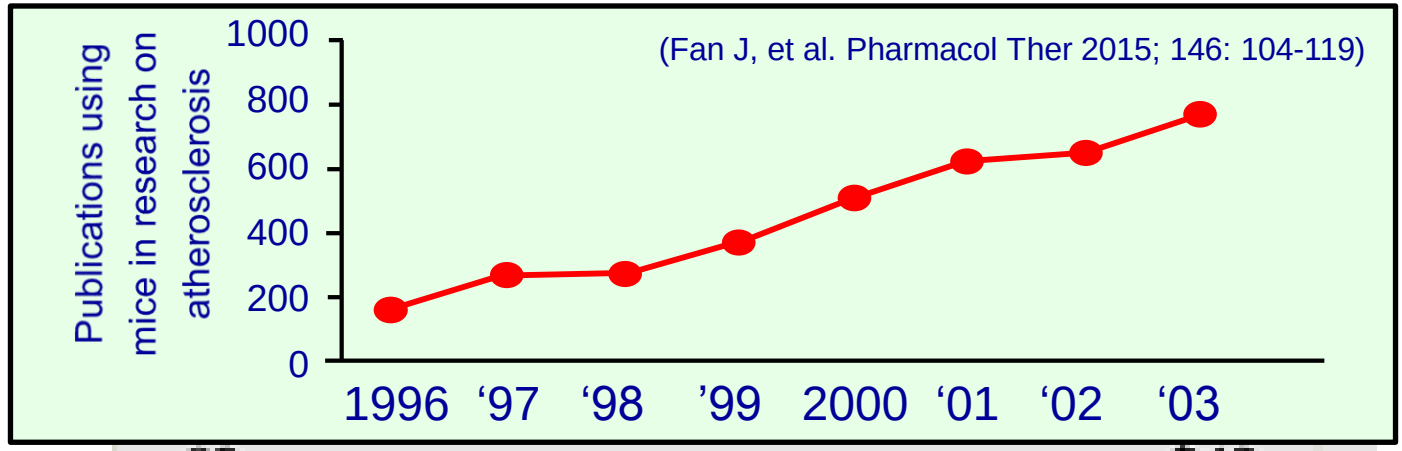


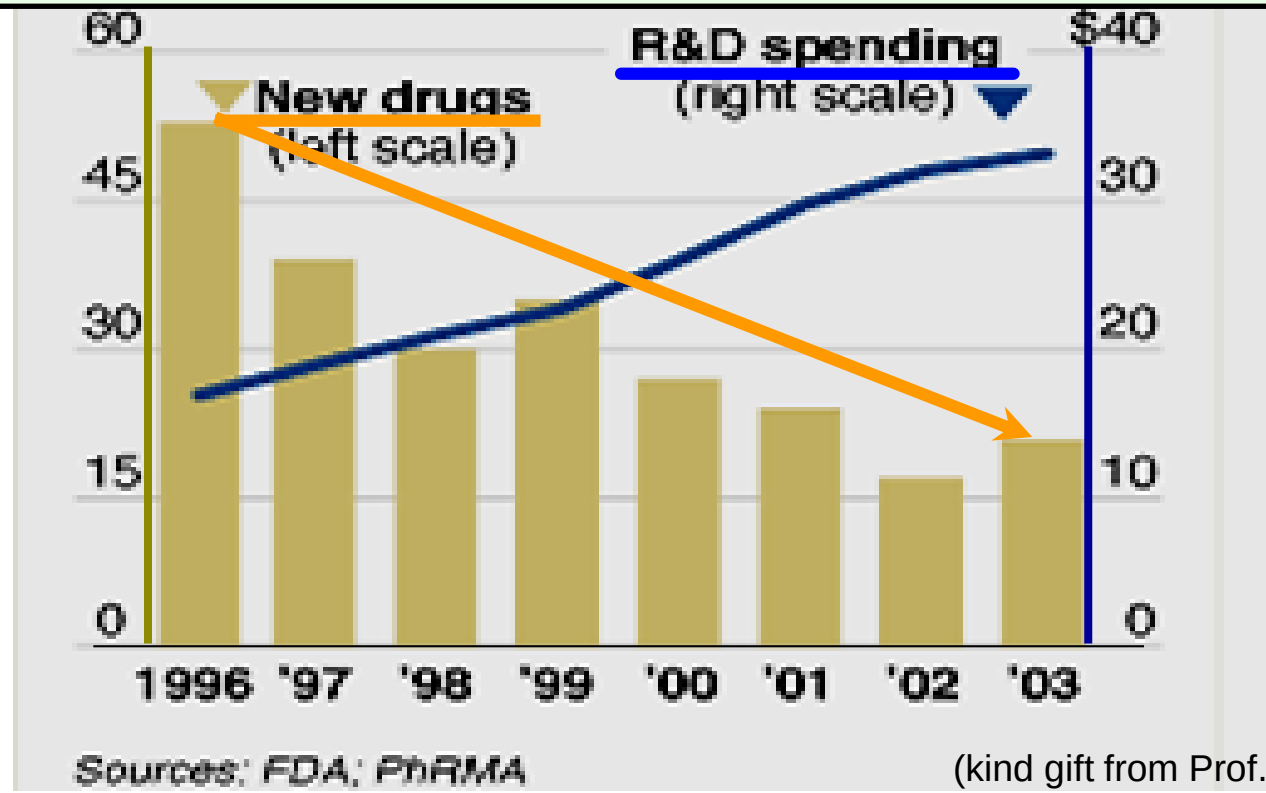
- The DNA sequence of the mouse has been determined and 99% of the mouse gene corresponds to human.
- Gene mutation mice can be prepared easily and relatively inexpensively.
- An inbred strain whose gene sequence has been determined can be used for experiments.
- Compared to other animal species, research costs are low.
  （small breeding space, small amounts of administration reagents）
- Experimental results can be obtained in a short time compared to other animal species.

▼

- Elucidation of disease onset mechanism and life phenomenon
- Identification of related genes

WSJ.com THE WALL STREET JOURNAL. ONLINE **Feb. 24, 2004**





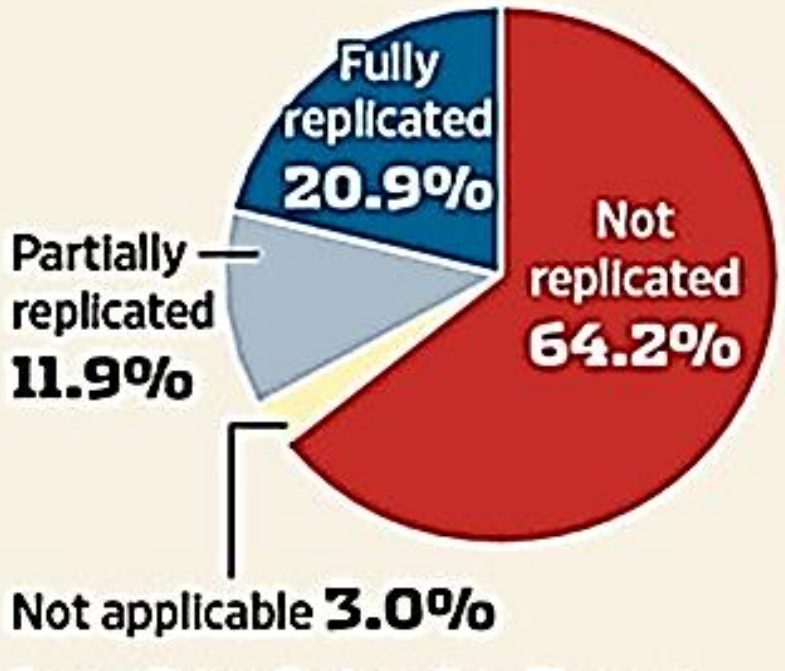
(kind gift from Prof. Chen, Michigan Univ)

WSJ.com THE WALL STREET JOURNAL. ONLINE

(Dec. 2, 2011)

No Cure

When Bayer tried to replicate results of 67 studies published in academic journals, nearly two-thirds failed.



(kind gift from Prof. Chen, Michigan Univ)

NIH mulls rules for validating key results

US biomedical agency could enlist independent labs for verification.

BY MEREDITH WADMAN

In biomedical science, at least one thing is apparently reproducible: a steady stream of studies that show the irreproducibility of many important experiments.

In a 2011 internal survey, pharmaceutical firm Bayer HealthCare of Leverkusen, Germany, was unable to validate the relevant preclinical research for almost two-thirds of 67 in-house projects. Then, in 2012, scientists at Amgen, a drug company based in Thousand Oaks, California, reported their failure to replicate 89% of the findings from 53 landmark cancer papers. And in a study published in May, more than half of the respondents to a survey at the MD Anderson Cancer Center in Houston, Texas, reported failing at least once in attempts at reproducing published data (see 'Make believe').

The growing problem is threatening the reputation of the US National Institutes of Health (NIH) based in Bethesda, Maryland, which funds many of the studies in question. Senior NIH officials are now considering adding requirements to grant applications to make experimental validations routine for certain types of science, such as the foundational work that leads to costly clinical trials. As the NIH pursues such top-down changes, one company is taking a bottom-up approach, targeting scientists directly to see if they are willing to verify their experiments.

There is the looming

NATURE.COM
For more on the challenges of reproducibility:
go.nature.com/zqtnp

- Compounds that were reproducible in clinical trials in drug development by major pharmaceutical companies are less than 21% of animal experiments.

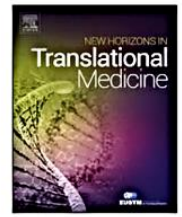
(Nature 2013; 500:14-16)

New Horizons in Translational Medicine 2 (2014) 5–11

Contents lists available at ScienceDirect

New Horizons in Translational Medicine

journal homepage: www.elsevier.com/locate/nhtm



Research Articles

Animal models in translational medicine: Validation and prediction

Tinneke Denayer, Thomas Stöhr*, Maarten Van Roy

Ablynx NV, Dept. Pharmacology, Zwijnaarde, Belgium

Situation of development of drug candidate that falls out in clinical trials

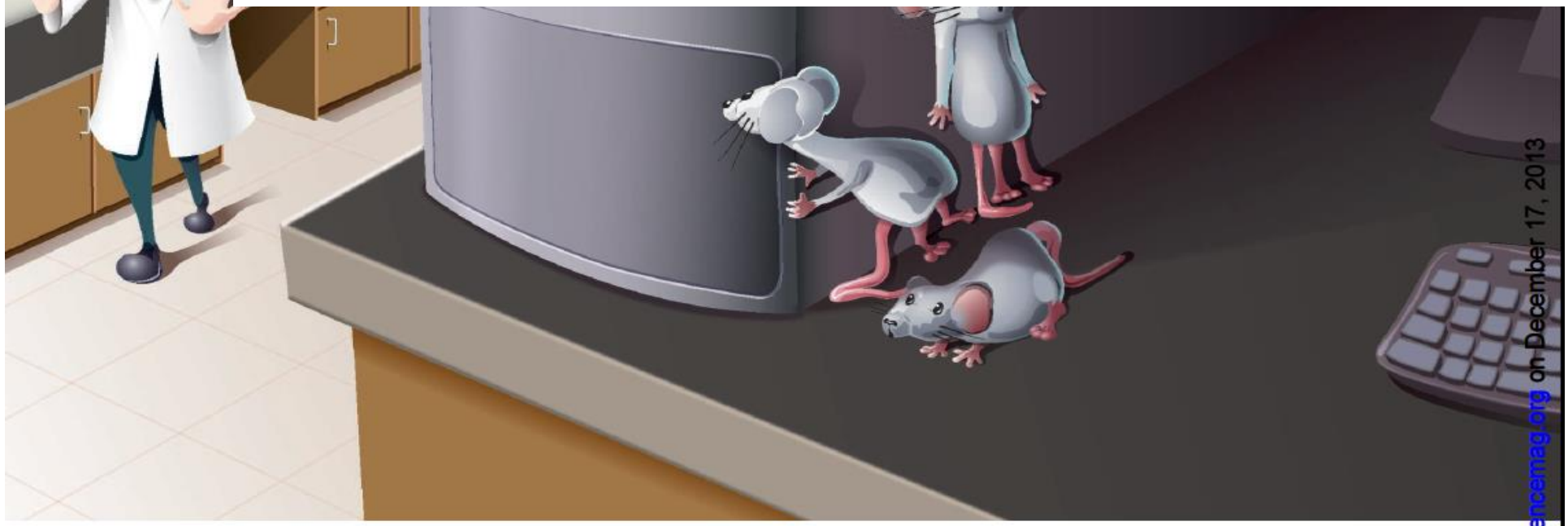
- Failure of drug development is due to the fact that there is no effect in clinical trial phases II and III.
- Many new drug candidate compounds dropped out were derived from novel compounds based on the human genome project focusing on potential new drug discovery targets, molecular biological approaches, compound development by computer simulation, and nano-bodies.

As a result, in the last decade, many compounds developed as research on targets that are not very effective in humans advanced to clinical trials.

(New Horizons in Translational Medicine 2014; 2: 5-11)

Science 2013; 342:922-925

1. Researchers should be more cautious whether studies with mice showing different diseases can accurately reflect the disease condition occurring in patients.
2. Many animal experiments are carried out without sufficient planning.
3. Compared to clinical trials, there are few experimental standards in animal experiments, and they are not managed.



When Mice Mislead

Tackling a long-standing disconnect between animal and human studies, some charge that animal researchers need stricter safeguards and better statistics to ensure their science is solid

OPEN ACCESS Freely available online PLoS MEDICINE

Research in Translation

Can Animal Models of Disease Reliably Inform Human Studies?

PLoS Med.
2010 Mar 30;7(3):e1000245

H. Bart van der Worp[1]*, David W. Howells[2], Emily S. Sena[2,3], Michelle J. Porritt[2], Sarah Rewell[2], Victoria
C

The design of animal experiments and the condition of human patients are very different.

Box 2. General reasons that the effectiveness of animal studies has declined: Inadequate research design

	Animal experiments	Human
Condition of disease	Young with single disease Homogeneous strain	Seniors with multiple diseases Individual difference
Gender	Either males or females	Both males and females
Onset of disease	Induction	Spontaneous onset
Timing of treatment	Induced or early stage	After the symptoms progressed
Dose of drug	Extremely high doses that are toxic in humans	

Numbers matter

Researchers need help in making the statistical power of animal experiments clear.

Albert Einstein is said to have noted that theories should be as simple as possible, but no simpler. By the same token, biomedical researchers doing *in vivo* experiments should use as few animals as possible, but no fewer. On page 271, *Nature* reports a move by UK government funding agencies to require grant applicants to show how they calculated the number of animals needed to make the results of an experiment statistically robust. In recent years there have been concerns that sample sizes in individual experiments can be too low, especially in preclinical research that attempts to determine whether a drug is worth pursuing in human studies.

Too-small sample sizes can lead to promising drugs being discarded when their effectiveness is missed, or to false positives, as well as to ethical issues if animals are being used in studies that are too small to provide reliable results.

The UK research councils' move is to be applauded. And Britain is

Journals are also responsible for ensuring that the research they publish is reported in sufficient detail for readers to fully appreciate key details of experimental and analytical design. Many publications — including *Nature* — have endorsed the ARRIVE guidelines for reporting animal research (C. Kilkenny *et al. PLoS Biol.* **8,** e1000412; 2010). These are, however, hugely detailed, and compliance at this level is difficult for early, exploratory research.

Journals published by Nature Publishing Group nevertheless encourage the use of ARRIVE. In 2013, we implemented a reporting checklist that demands that authors supply key details of study design. For animal studies, these include the methods of sample-size determination, randomization and study blinding, as well as exclusion criteria (see *Nature* **496,** 398; 2013). An impact analysis on the effectiveness of the changes introduced in 2013 is currently under way.

Sample size is just one of a suite of issues that need to be addressed if poor reproducibility is to be tackled. Journals have a key part to play in dealing with this problem, but so do others. Credit to

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

The ARRIVE guidelines:

Animal Research: Reporting of In Vivo Experiments

ARRIVE guidelines contains sample size determination methods, randomization, blindness, exclusion criteria in animal-based studies.

Pioneering Better Science

What makes an ideal animal model?

- **Represents the human condition**
- **Cost-effective**
- **Large enough for imaging/repeated sampling**
- **Reproducible pathology**
- **Therapeutic target is present naturally**

Kavanagh (Winston-salem, US)
In which animal models of atherosclerosis can interventions be translated to human therapy?
17th International Symposium on Atherosclerosis. (Amsterdam, May 23-26, 2015)

Research design is extremely important for reproducing the condition of human patients in animal experiments.

Integrative Physiology

OPEN

Methodological Rigor in Preclinical Cardiovascular Studies

Targets to Enhance Reproducibility and Promote Research Translation

F. Daniel Ramirez, Pouya Motazedian, Richard G. Jung, Pietro Di Santo, Zachary D. MacDonald, Robert Moreland, Trevor Simard, Aisling A. Clancy, Juan J. Russo, Vivian A. Welch, George A. Wells, Benjamin Hibbert

Rationale: **Methodological sources of bias and suboptimal reporting contribute to irreproducibility in preclinical science and may negatively affect research translation. Randomization, blinding, sample size estimation, and considering sex as a biological variable are deemed crucial study design elements to maximize the quality and predictive value of preclinical experiments.**

Objective: **To examine the prevalence and temporal patterns of recommended study design element implementation in preclinical cardiovascular research.**

Methods and Results: **All articles published over a 10-year period in 5 leading cardiovascular journals were reviewed. Reports of in vivo experiments in nonhuman mammals describing pathophysiology, genetics, or therapeutic interventions relevant to specific cardiovascular disorders were identified. Data on study design and animal model use were collected. Citations at 60 months were additionally examined as a surrogate measure of**

substantially improved over the past 10 years, and may be overlooked when basing subsequent studies. Resultant risks of bias and threats to study validity have the potential to hinder progress in cardiovascular medicine as preclinical research often precedes and informs clinical trials. Stroke research quality has uniquely improved in recent years, warranting a closer examination for interventions to model in other cardiovascular fields. (*Circ Res*. 2017;120:1916-1926. DOI: 10.1161/CIRCRESAHA.117.310628.)

Review

Critical Issues for the Translation of Cardioprotection

Gerd Heusch

Abstract: **The translation from numerous successful animal experiments on cardioprotection beyond that by reperfusion to clinical practice has to date been disappointing. Animal experiments often use reductionist approaches and are mostly performed in young and healthy animals which lack the risk factors, comorbidities, and comedications which are characteristics of patients suffering an acute myocardial infarction or undergoing cardiovascular surgery. Conceptually, it is still unclear by how much the time window for successful reperfusion is extended by preconditioning, and how long the duration of ischemia can be so that adjunct cardioprotection by postconditioning at reperfusion still protects. Experimental studies addressing long-term effects of adjunct cardioprotection beyond infarct size reduction, that is, on repair, remodeling, and mortality, are lacking. Technically, reproducibility and robustness of experimental studies are often limited. Grave faults in design and conduct of clinical trials have also substantially contributed to the failure of translation of cardioprotection to clinical practice. Cardiovascular surgery with ischemic cardioplegic arrest is only a surrogate of acute myocardial infarction and confounded by the choice of anesthesia, hypothermia, cardioplegia, and traumatic myocardial injury. Trials in patients with acute myocardial infarction have been performed on agents/interventions with no or inconsistent previous animal data and in patients who had either some reperfusion already at admission or were reperfused too late to expect any myocardial salvage. Of greatest concern is the lack of adequate phase II dosing and timing studies when rushing from promising proof-of-concept trials with surrogate end points such as infarct size to larger clinical outcome trials. Future trials must focus on interventions/agents with robust preclinical evidence, have solid phase II dosing and timing data, and recruit patients who have truly a chance to benefit from adjunct cardioprotection. (*Circ Res*. 2017;120:1477-1486. DOI: 10.1161/CIRCRESAHA.117.310820.)**

ILAR Journal, 2017, Vol. 58, No. 1, 1–3

doi: 10.1093/ilar/ilx017
Article

Bridging the Gap between Reproducibility and Translation: Data Resources and Approaches

Caroline J. Zeiss and Linda K. Johnson

Caroline J. Zeiss, BVSc, PhD, is Professor of Comparative Medicine and Director of the Phenotyping Core at the Yale University School of Medicine in New Haven, Connecticut. Linda K. Johnson, DVM, MS, MPH, is Professor of Pathology and Director of the Comparative Pathology Shared Resource at the University of Colorado, Anschutz Medical Campus in Aurora, Colorado.

Address correspondence and reprint requests to Caroline J. Zeiss, Section of Comparative Medicine, Yale University School of Medicine, 375 Congress

design, reproducibility and operational norms within the biomedical research enterprise. In this issue, we explore the range of information resources available for the comparative study of disease, as well as challenges to the ultimate translation of preclinical findings. Genomics resources in support of translational research are described for zebrafish, mice, rats and non-human primates. The utility of transcriptomics to explore the temporal basis of lesion development in toxicologic pathology is reviewed. Integration of the ever-increasing volume of text-based and bioinformatics data is a significant challenge, and in this issue, informatics resources and general text mining methodologies to explore and aggregate text data are described. Finally, factors contributing to both reproducibility and translatability are examined. Guidelines designed to address reproducibility are essential to improving individual studies. To this end, a viewpoint from the National Institutes of Health on measures needed to enhance rigor and reproducibility is given, as well as an overview of the role of the Institutional Animal Care and Use Committee in this regard. The challenge of improving generalizability of animal experiments so that their findings can be more frequently extended to the intended human population remains. Reasons why models that replicate key aspects of human disease fail to be predictive in humans are explored in two fields in which translation has been a challenge: sepsis and neurodegeneration.

PERSPECTIVE

JBMR®

The Road to Reproducibility in Animal Research

Robert L Jilka

Center for Osteoporosis and Metabolic Bone Diseases, Division of Endocrinology and Metabolism, University of Arkansas for Medical Sciences, Little Rock AR, USA

ABSTRACT

Reproducibility of research findings is the hallmark of scientific advance. However, the recently noted lack of reproducibility and transparency of published research using animal models of human biology and disease has alarmed funders, scientists, and the public. Improved reporting of methodology and better use of statistical tools are needed to enhance the quality and utility of published research. Reporting guidelines like Animal Research: Reporting In Vivo Experiments (ARRIVE) have been devised to achieve these goals, but most biomedical research journals, including the *JBMR*, have not been able to obtain high compliance. Cooperative efforts among authors, reviewers and editors—empowered by increased awareness of their responsibilities, and enabled by user-friendly guidelines—are needed to solve this problem. © 2016 American Society for Bone and Mineral Research.

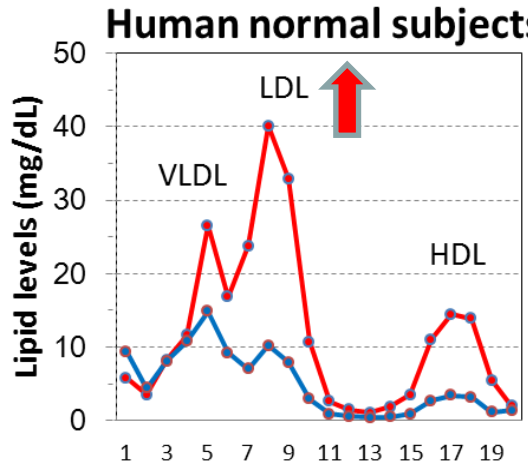
Journal of Bone and Mineral Research, Vol. 31, No. 7, July 2016, pp 1317–1319

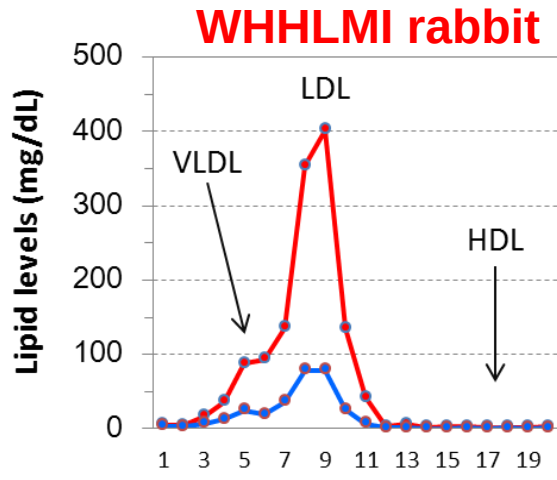
It is still important to increase the reproducibility of the results of animal experiments in clinical trials

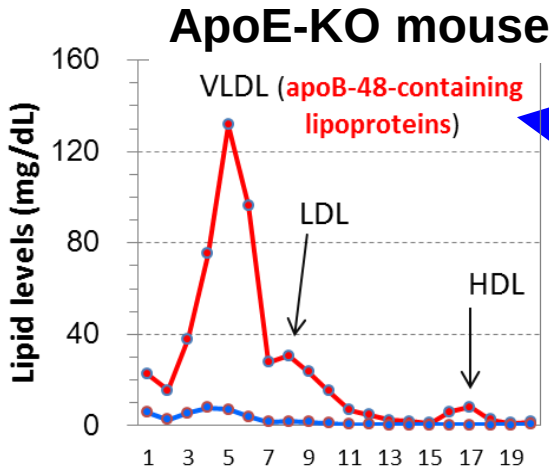
Species differences in lipoprotein metabolism

Species difference in plasma lipoprotein profile

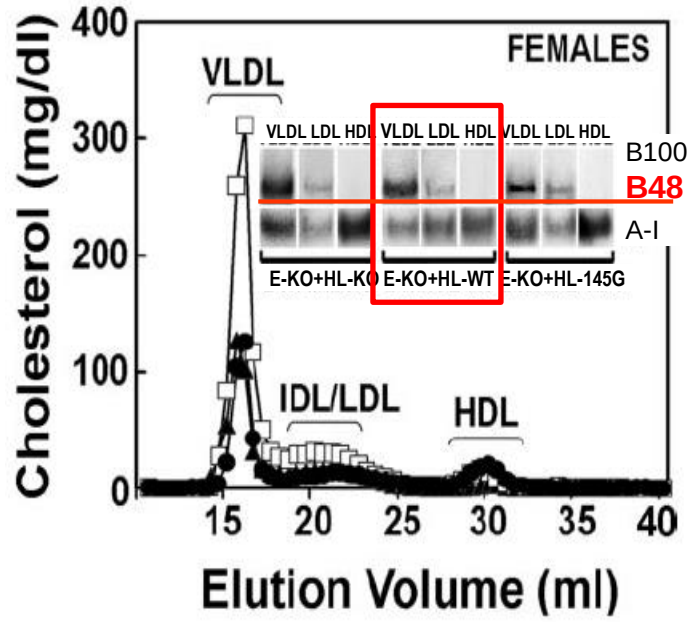
Hyper-cholesterolemia of apoE-KO mice is essentially different from human hypercholesterolemia.







Total cholesterol
Triglyceride



Plasma cholesterol levels (mg/dl)			
	Human	WHHLMI	apoE-KO mice
Normal chow	188	1336	513 ± 33

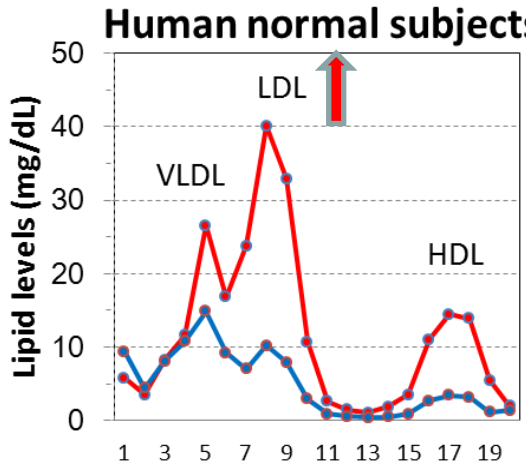
Herminia Gonzalez-Navarro, et al.

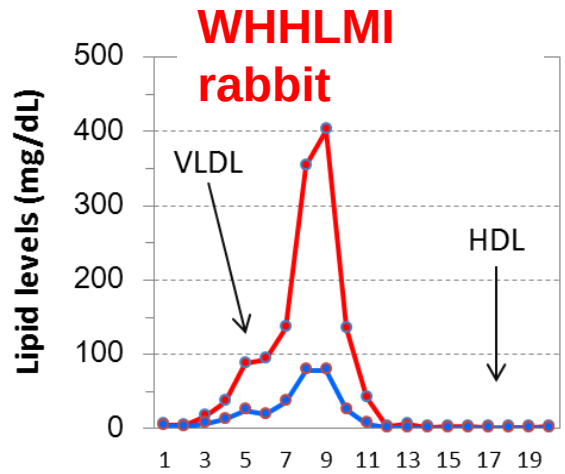
J Biol Chem 2004; 279: 45312-21

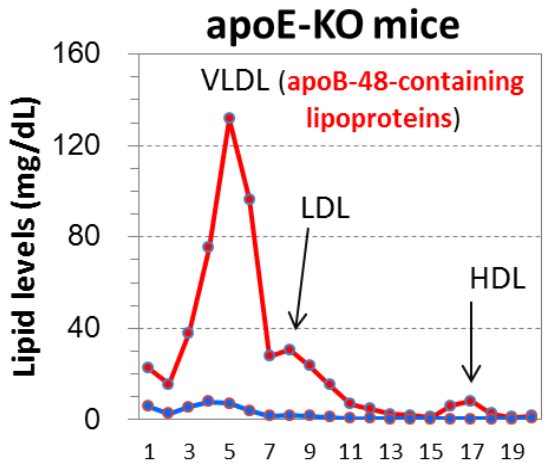
Species differences in plasma lipoproteins

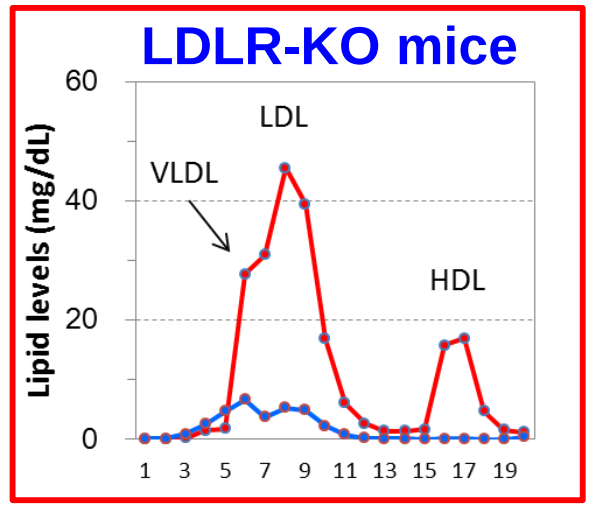
Total cholesterol
Triglyceride

Despite the absence of LDL receptor expression in LDLR-KO mice, the serum cholesterol level is not so high in feeding a standard chow, which is very different from human familial hypercholesterolemia and WHHL / WHHLMI rabbits.









Plasma cholesterol levels (mg/dl)				
	Human	WHHLMI	**LDLR-KO mice**	apoE-KO mice
Normal chow	188	1336	**225 ± 27**	513 ± 33
Animals fed 1.25% cholesterol diets.			**1583 ±120**	

ASBMB

Method to measure apolipoprotein B-48 and B-100 secretion rates in an individual mouse: evidence for a very rapid turnover of VLDL and preferential removal of B-48- relative to B-100-containing lipoproteins

Xiaohua Li,* Fernando Catalina,†† Scott M. Grundy,*,†,§,** and Shailesh Patel[1,*,†]

In mice, apoB-48-containing VLDL particles are secreted from the liver.

Molar Ratio of ApoB-48 to ApoB-100 in mouse VLDL

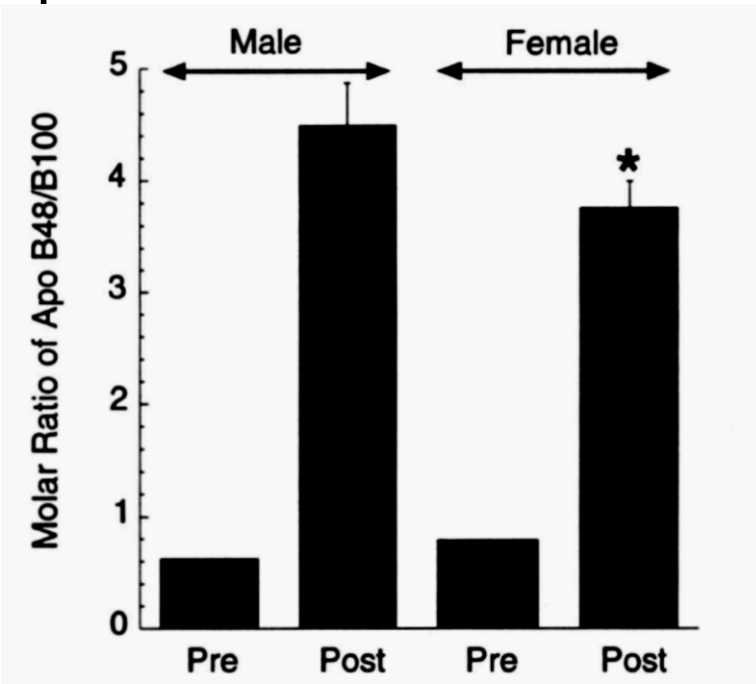


Fig. 6. Pre- and post-injection ratios of apoB-48 to B-100 in VLDL. The molar ratio of B-48 to B-100 was determined at baseline or after 5 h post-injection of tyloxapol in male and female FVB/N mice. Compared to a pre-injection ratio of 0.63, the ratio increased to 4.50 ± 0.37 (SD) in the males, compared with a baseline value of 0.79 in the females that rose to 3.75 ± 0.24 in the females. Analysis of the post-injection ratios between the males and females showed a statistically significant difference ($P = 0.0098$). See text for discussion.

Apolipoprotein turnover rate in mice

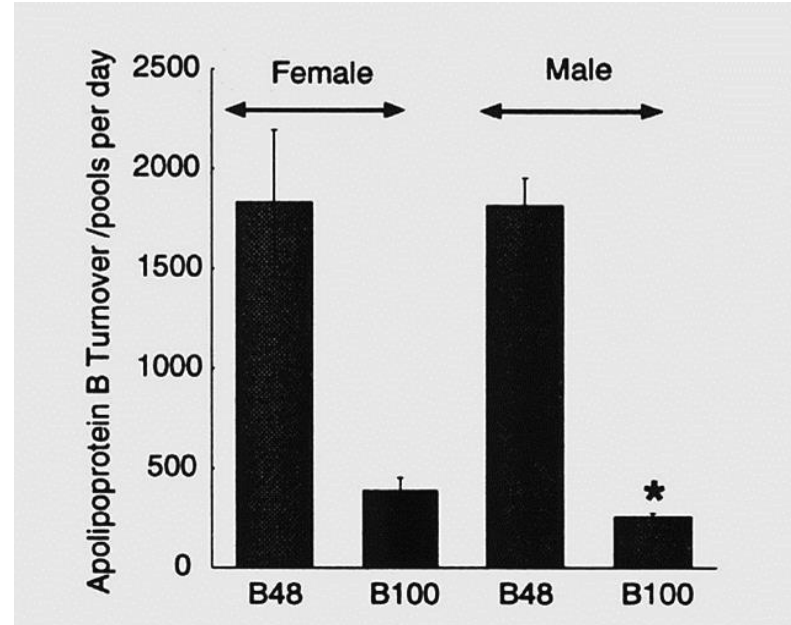
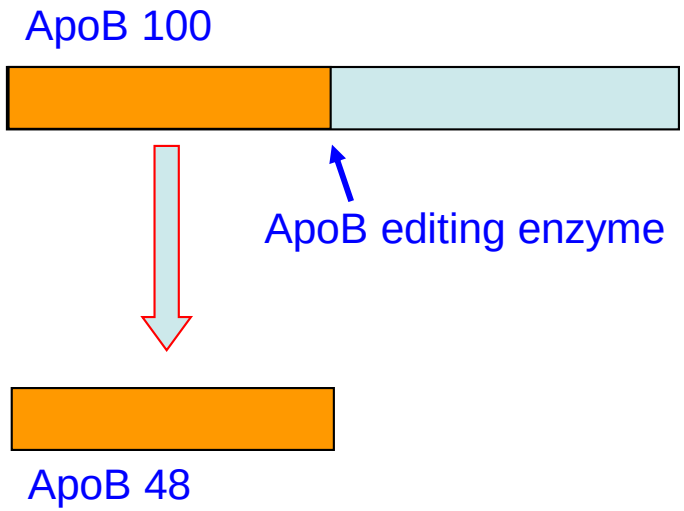


Fig. 8. VLDL apoB turnover rates in male and female FVB/N mice. Turnover rates for apoB-48 and B-100 were computed from the steady state baseline values and from the rates of secretion of apoB after tyloxapol injection. A pool is defined as the total amount of apoB in the plasma at baseline. The error bars indicate SD. Hence, for apoB-48-VLDL, males have a turnover of 1814 ± 139 pools per day compared with 1831 ± 365 pools per day in the female ($P = 0.92$). For B-100-VLDL, males have a turnover of 255 ± 19 pools per day compared with 386 ± 66 pools per day for the female ($P = 0.0055$).

The catabolic rate of apoB-48-containing VLDL is extremely fast, which affects lipoprotein metabolism markedly .

The gene of apoB-48 editing enzyme, *apobec-1*, is not expressed in the liver of wild-type WHHL rabbits.

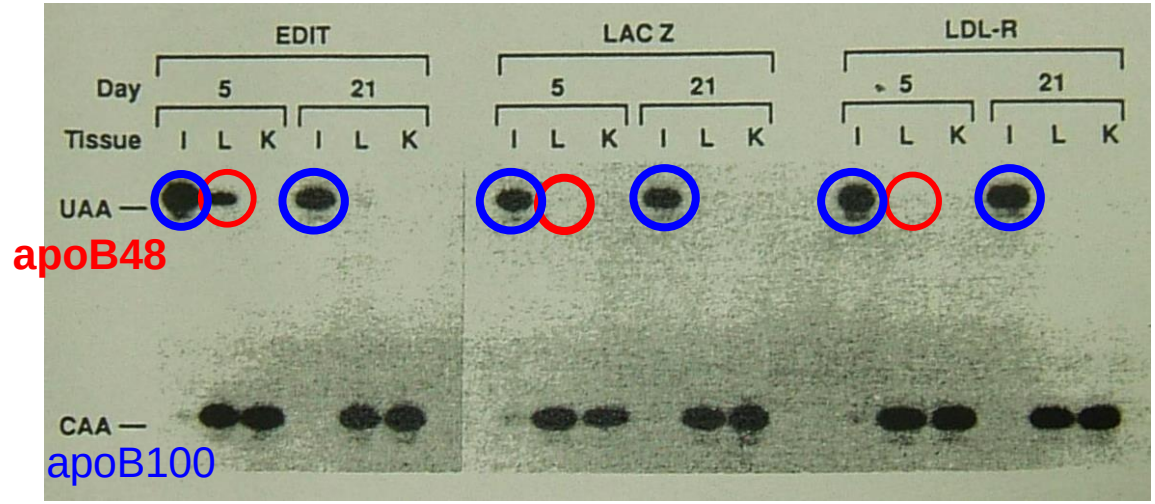


Primer expression assay of mRNA of apoB editing enzyme in WHHL rabbits infused various recombinant adenoviruses

Rat apobec-1 cDNA

lacZ gene

Human LDL receptor cDNA

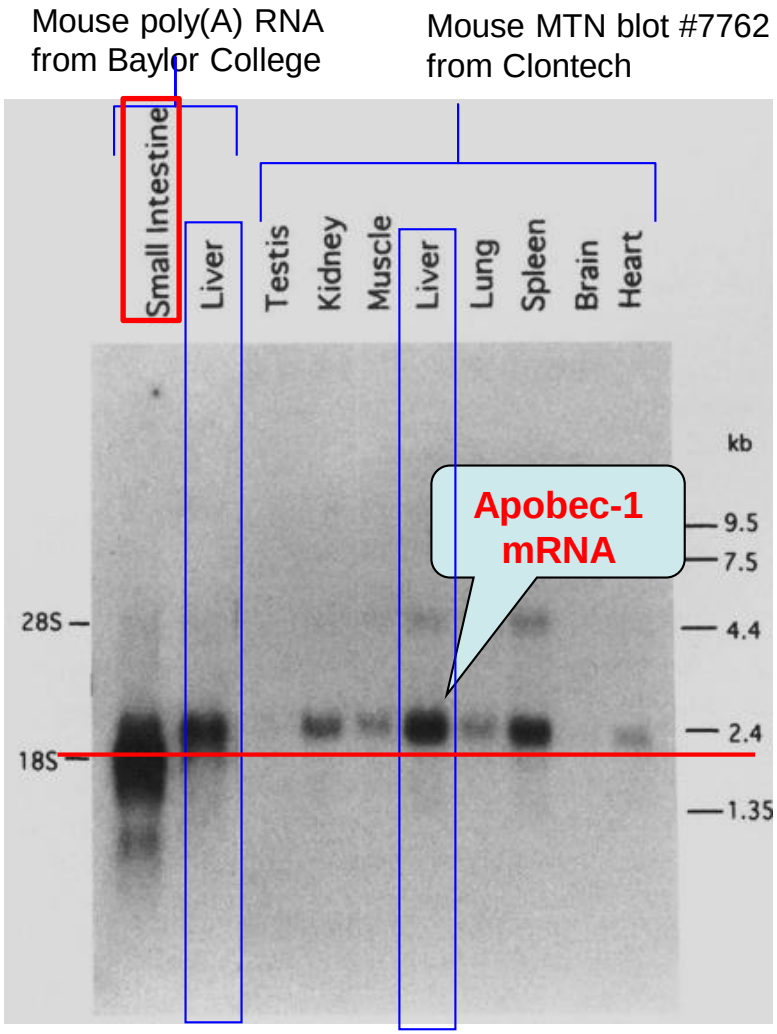


Detection of apoB48 and apoB100 mRNA in intestine (I), liver (L), and kidney (K) tissues of **WHHL rabbits** infused with recombinant adenoviruses.

UAA, stop codon in apoB48 mRNA

CAA, glutamine codon in apoB100 mRNA

(Kozarsky KF, et al. Human Gene Therapy 1996; 7:943-57)



Northern blot analysis of apobec-1 mRNA expression in various **mouse** tissues

(Nakamuta M, et al. J Biol Chem 1995; 270:13042-56)

Apo B editing enzyme is expressed in the liver of mice and rats.

The species differences between mice or rats and humans or rabbits on the expression of apoB-editing enzyme greatly affect lipoprotein metabolism.

Plasma CETP activity in WHHL rabbits

Rabbits	Gender	Plasma cholesterol (mg/dl)	Transfer activity (% kt/ml plasma) TG	CE
Control New Zealand white	male (n=5)	48.7 ± 11.5	563 ±124[A]	594 ± 99[E]
	female (n=5)	52.2 ± 8.2	862 ±206[B]	901 ± 177[F]
WHHL	male (n=5)	798 ± 209	1068 ± 341[C]	1562 ± 579[G]
	female (n=5)	640 ± 134	1000 ± 268[D]	1430 ± 360[H]

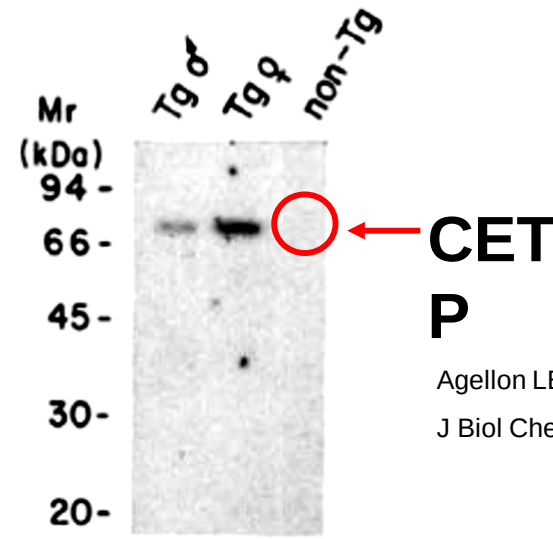
Mean ± SD . A vs B (P<0.025); A vs C (P<0.025); B vs D (P<0.025); E vs F (P<0.01) E vs G (P<0.01); F vs H (P<0.02)

Son YC, et al. (Arteriosclerosis 1986; 6: 345-351)

CETP (cholesteryl ester transfer protein) transfers cholesterol from HDL to VLDL, IDL, and LDL. In humans, CETP is thought to be a key player that transports cholesterol from the peripheral macrophages to the liver.

Since CETP does not exist in the blood in mice, the reverse cholesterol transport pathway of mice is greatly different from humans and rabbits.

Immunoblot analysis for CETP

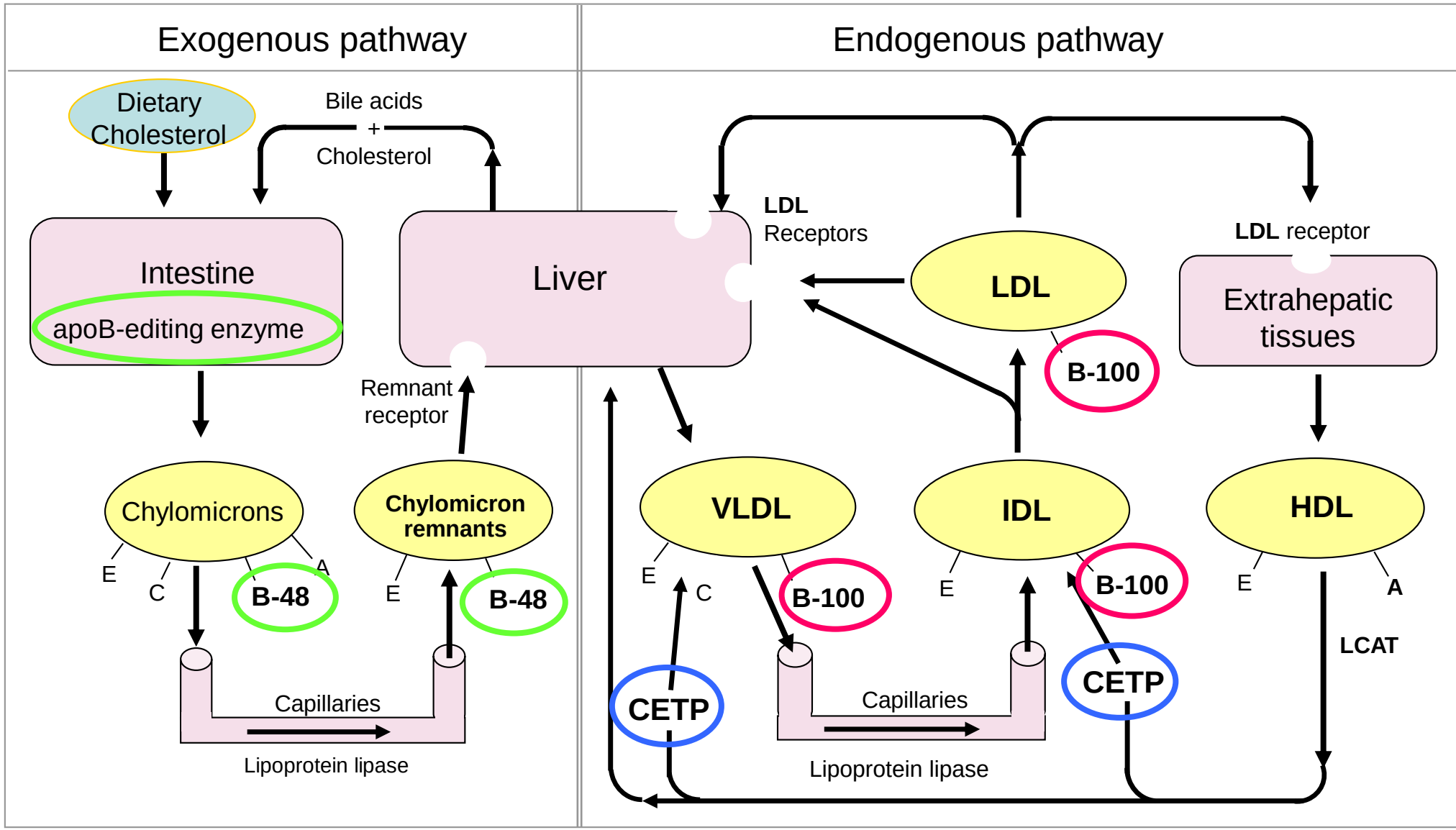


Agellon LB, et al.

J Biol Chem 1991; 266 (17): 10796-10801

FIG. 2. **Detection of human CETP in transgenic mouse plasma.** Mouse plasma was passed through a CETP immunoaffinity column constructed with a monoclonal antibody (mAb TP2) that recognizes an epitope at the carboxyl terminus of human CETP (16). The retained fraction was eluted, blotted, and then probed with ^{125}I-TP2. An immunoreactive protein is clearly visible in the plasma of CETP transgenic mice (*lane 1*, pooled female plasma; *lane 2*, pooled female plasma). Pooled plasma from nontransgenic littermates contains no detectable TP2 immunoreactive protein (*lane 3*). Mobility of molecular weight standards are indicated on the *left side* of the figure. *Tg*, transgenic.

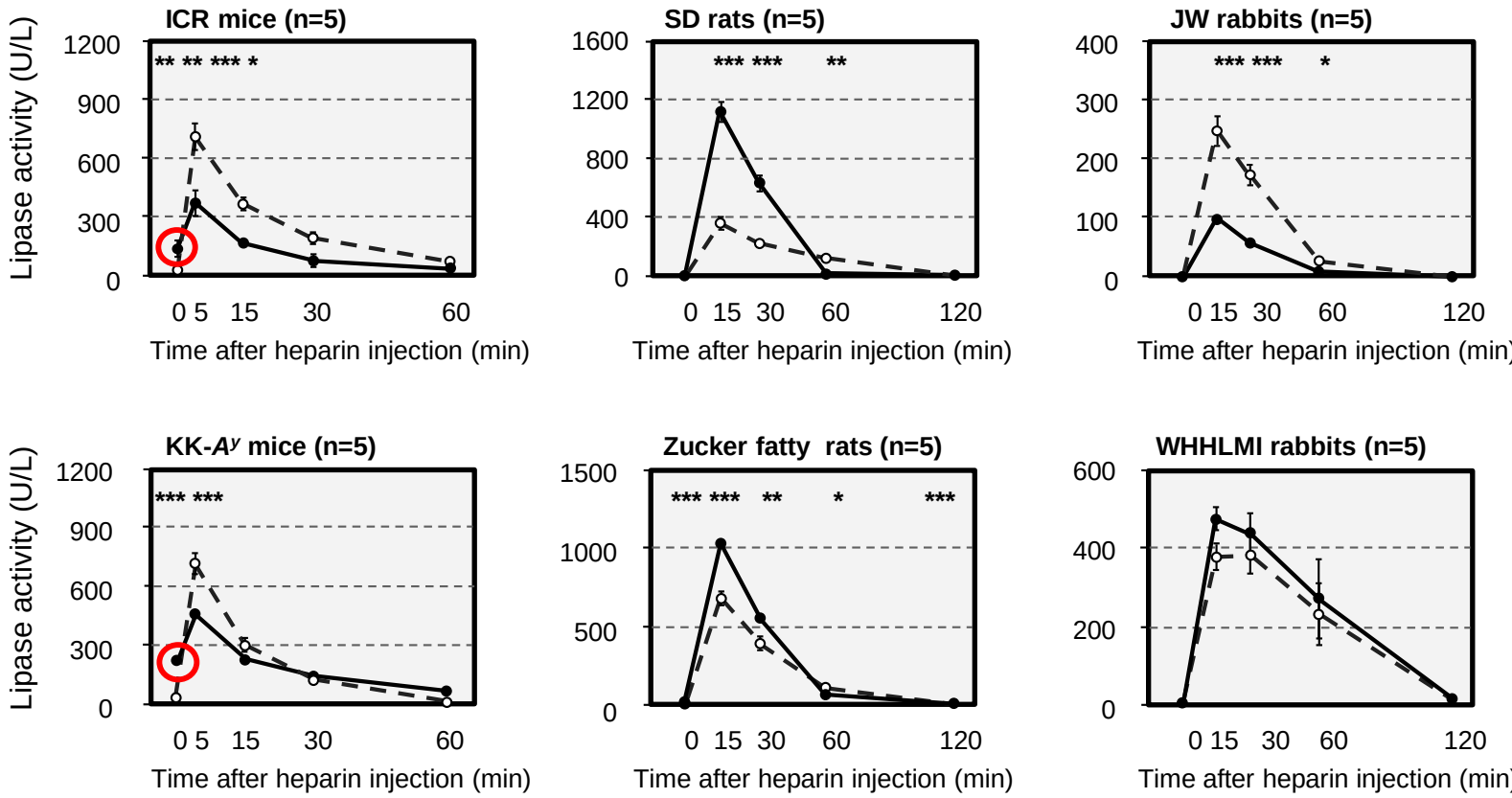
Lipoprotein metabolism in humans and rabbits



(Modified after Goldstein et al. N Engl J Med 1983; 309:288-286)

Activities of LPL and HTGL of mice, rats, and rabbits.

Mice have HTGL activity in pre-heparin plasma



Changes in activity of LPL (dotted lines) and HTGL (solid lines) after heparin intravenous injection in various animals.
Animals used in this examination were females in mice and rats, and males in rabbits.
Data are presented as the mean +/- SEM. Statistical analyses between LPL activity and HTGL activity were performed with the Student t-test (*, P<0.05; **, P<0.01; ***, P<0.001).

(Kimura et al. (Exp Anim 2019, in press)

Lipid-lowering effects of pravastatin

Dose (mg/kg per day)	Percent of initial value (mean ± S.D.)		
	total cholesterol	phospholipid	triacylglycerol
A. Beagle dog (18 days, $n = 6$)			
Control	96 ± 5	96 ± 4	103 ± 10
スタチンの用量 0.625	88 ± 5 [b]	88 ± 6 [b]	83 ± 8 [b]
スタチンの用量 1.25	82 ± 6 [c]	64 ± 6 [b]	89 ± 14
B. Cynomolgus monkey (18 days, $n = 4$)			
Control	96 ± 4	85 ± 8	110 ± 37
スタチンの用量 20	85 ± 6 [a]	87 ± 9	96 ± 7
スタチンの用量 50	69 ± 11 [b]	84 ± 11	94 ± 15
C. Japanese white rabbit (18 days, $n = 6$)			
Control	96 ± 9	96 ± 3	94 ± 35
スタチンの用量 6.25	78 ± 10 [b]	84 ± 7 [b]	79 ± 18
スタチンの用量 12.5	68 ± 11 [b]	73 ± 7 [b]	77 ± 33
D. WHHL rabbit (12 days, $n = 4$)			
Control	100 ± 20	93 ± 4	108 ± 13
スタチンの用量 12.5	82 ± 5 [c]	88 ± 5	92 ± 11
スタチンの用量 50	72 ± 9 [c]	84 ± 5 [b]	107 ± 10
E. Wistar-Imamichi rat (14 days, $n = 8$)			
500	118 *	101 *	69 *

Significantly different from control value: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

* The values represent percent of control.

Pravastatin decreased serum cholesterol levels in dogs, monkeys, HW rabbits and WHHL rabbits.

However, in rats, pravastatin did not decrease the serum lipid levels.

(Tsujita Y, et al. BBA 1986; 877: 50-60)

Statins do not reduce serum lipid levels in mice.

http://www.kidney-international.org original article

Pravastatin improves renal ischemia–reperfusion injury by inhibiting the mevalonate pathway

Satoru Sharyo[1,3,6], Naoko Yokota-Ikeda[2,6], Miyuki Mori[1,6], Kazuyoshi Kumagai[3], Kazuyuki Uchida[4], Katsuaki Ito[1], Melissa J Burne-Taney[5], Hamid Rabb[5] and Masahiro Ikeda[1]

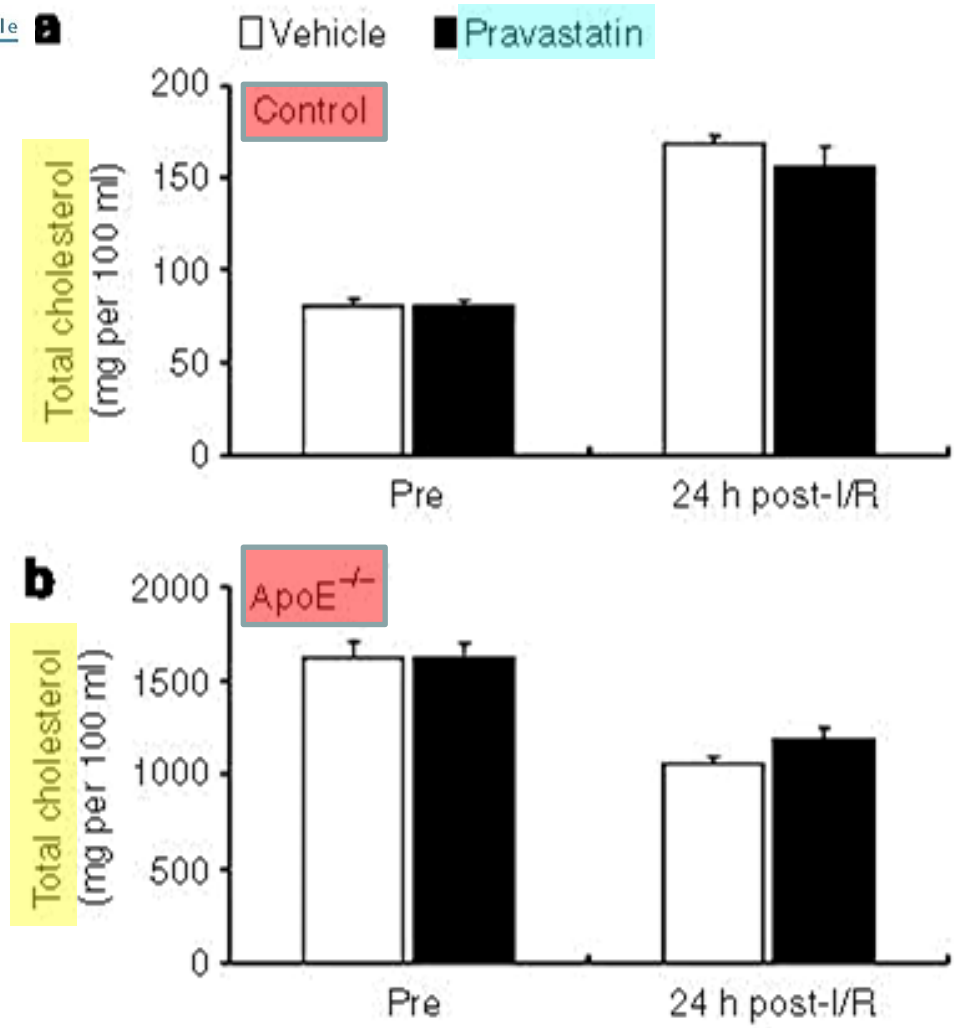


Figure 5 | Effect of pravastatin on plasma total cholesterol and creatinine concentrations after renal I/R in the genetic control mice and ApoE−/−. Pravastatin (100 mg/kg) or vehicle was administered to the (**a** and **c**) genetic control mice and (**b** and **d**) ApoE−/− for 5 consecutive days before I/R. Plasma for measurements of (**a** and **b**) total cholesterol and (**c** and **d**) creatinine concentrations was collected before I/R (Pre) and 24 h after I/R. Values are mean ± s.e. ($N = 5$ per group). *$P < 0.01$ vs vehicle with I/R.

Renal ischemia–reperfusion (I/R) injury

Species differences in lipoprotein metabolism

	Humans	WHHLMI rabbits		Mice	
Main lipoprotein	**LDL**	**LDL**	◎	**HDL, VLDL**	✕
ApoB in VLDL particles	**apoB-100**	**apoB-100**	◎	apo**B-48**	△
Expression of apob-editing enzyme	Intestine	Intestine	◎	Intestine, **liver**	△
CETP	**Yes**	**Yes**	○	**none**	✕
HTGL activity in pre-heparin plasma	none	none	◎	High	✕
Sensitivity to dietary fat	sensitive	sensitive	○	resistance	✕
Endothelial lipase	No effects on LDL	No effects on LDL	◎	LDL-lowering	✕
Acute inflammatory marker	**CRP**	**CRP**	◎	Serum Amyloid P component **SAP**	✕
Effects of statin	Effective	Effective		Ineffective	✕

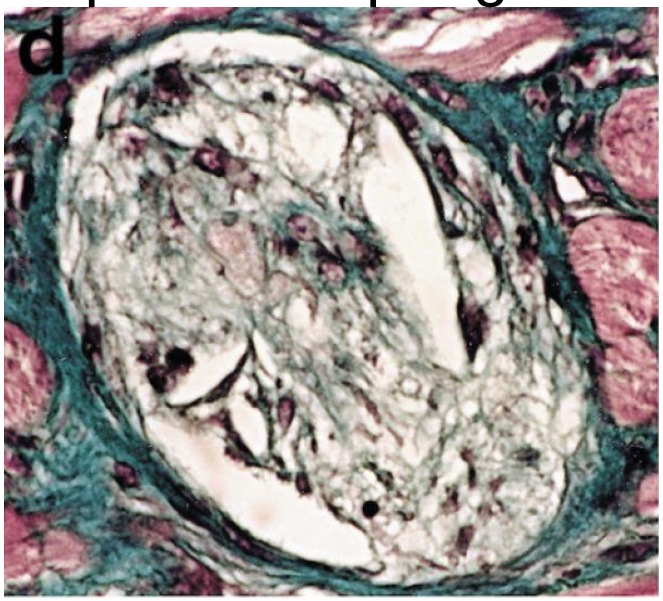
Species differences in lipoprotein metabolism-2

	Humans	WHHL/WHHLMI rabbits		Mice	
Apolipoprotein(a)	Bound to LDL	Bound to LDL	◎	Not bound to LDL	✕
HDL	heterogeneous	heterogeneous	◎	homogeneous	X
Apolipoprotein A-II	Dimmer	Absent	X	Monomer	△
Hepatic LDL receptor activity	Down regulated	Down regulated	◎	Usually high	✕
Cholesterol pool	Mainly from hepatic synthesis	Mainly from hepatic synthesis	◎	Mainly from dietary origin	✕
Excretion of bile acid	Low	Low	◎	High	✕
Response to cholesterol diet	Sensitive	Sensitive	◎	Resistant	✕

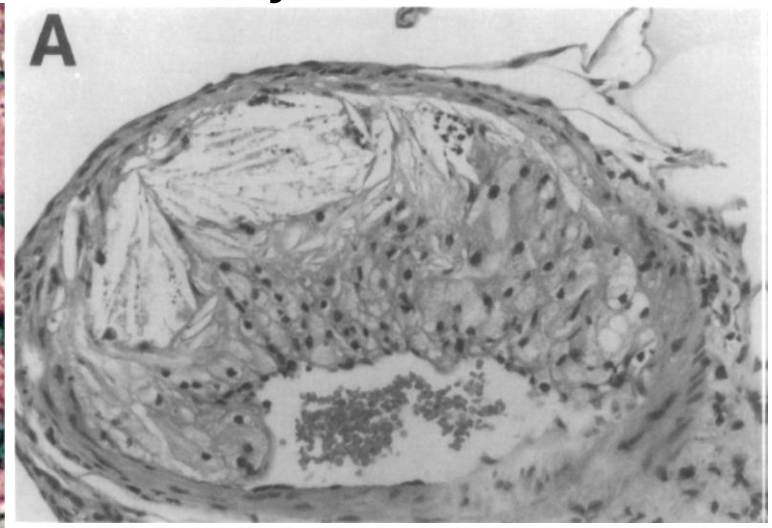
(Modified after Fan et al. Pharmacol Ther 2015; 146: 104-119)

Species differences in atherosclerotic lesions

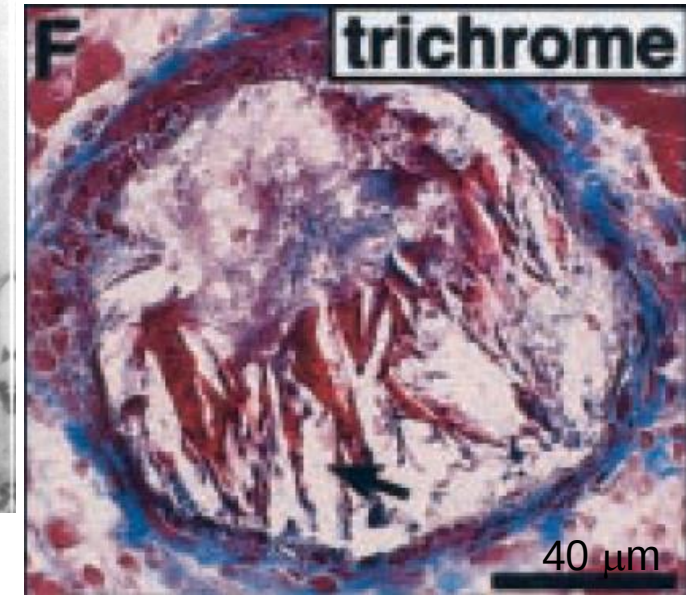
Lipid/macrophage- rich coronary lesions in mice



apoE-KO/LDLR-KO mouse fed high fat diet （Masson's Trichrome stain）
Caligiuri G, et al. PNAS 1999; 96: 6920-6924

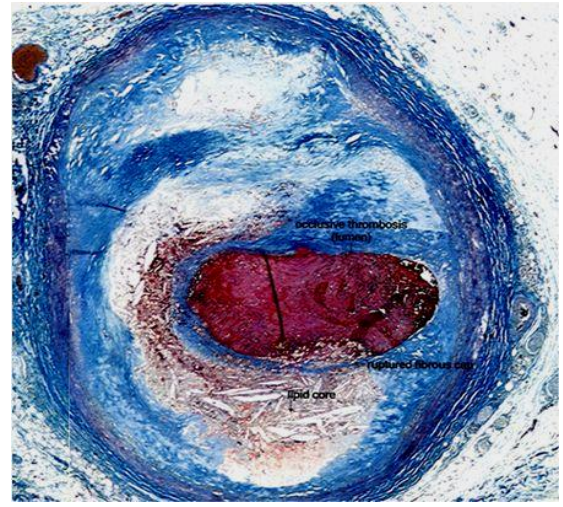


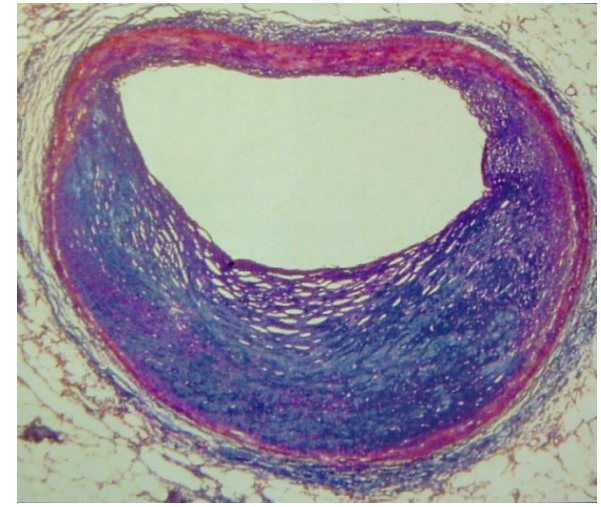
LDLR-KO mouse fed high fat diet （H&E stain）
Ishibashi S, et al. JCI 1994; 93: 1885-1893



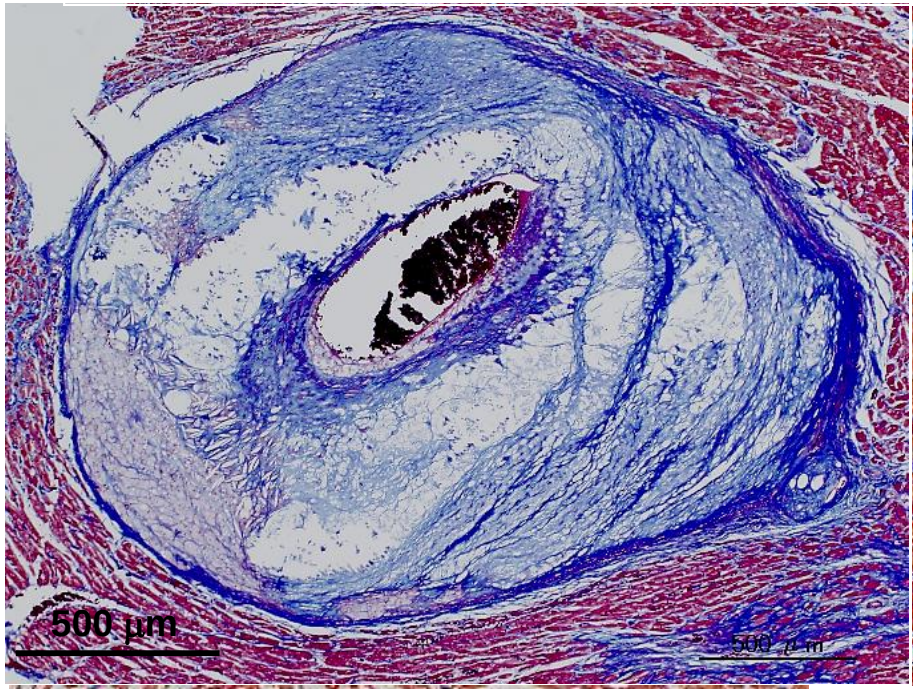
ApoE-KO/SRBI-KO mouse fed standard chow （Masson's Trichrome stain）
Braun A, et al. Cir Res 2002; 90: 270-276

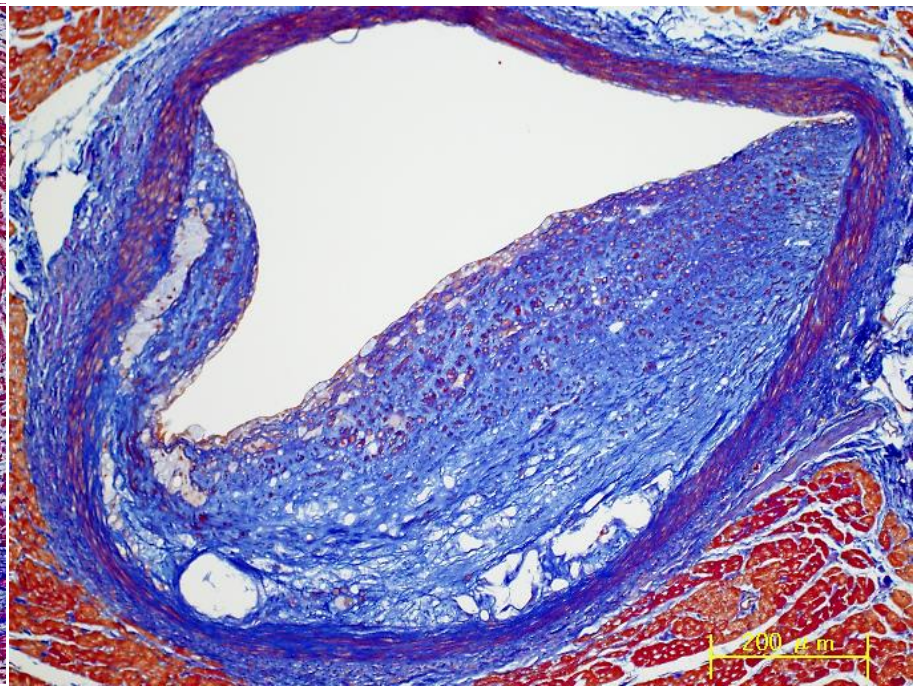
Stable and instable coronary lesions of humans

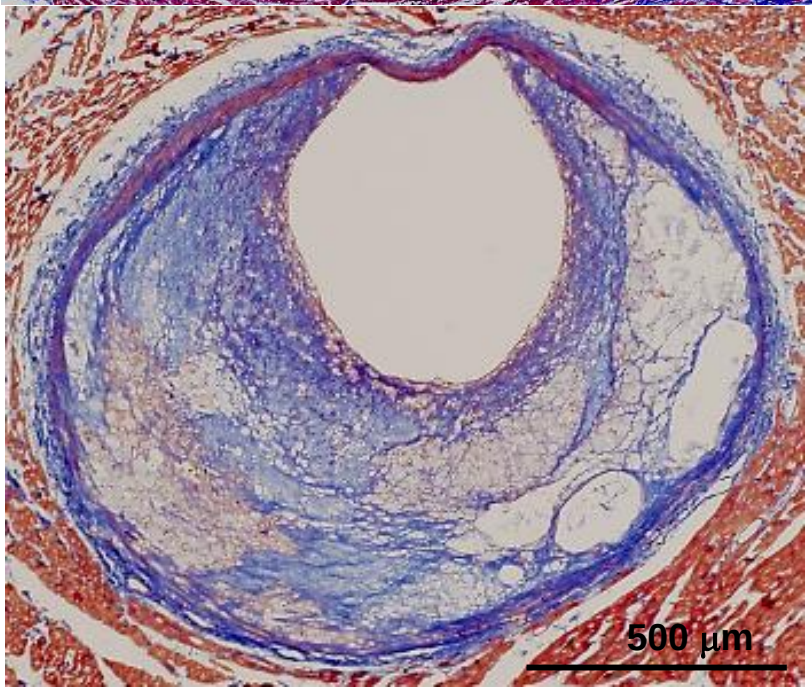




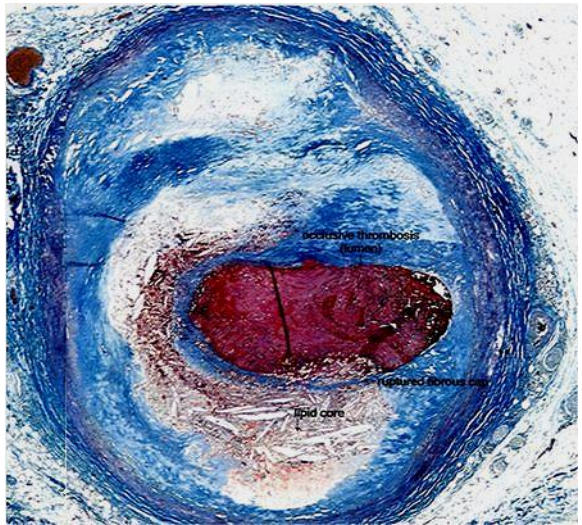
Stable and instable coronary lesions of WHHLMI rabbits

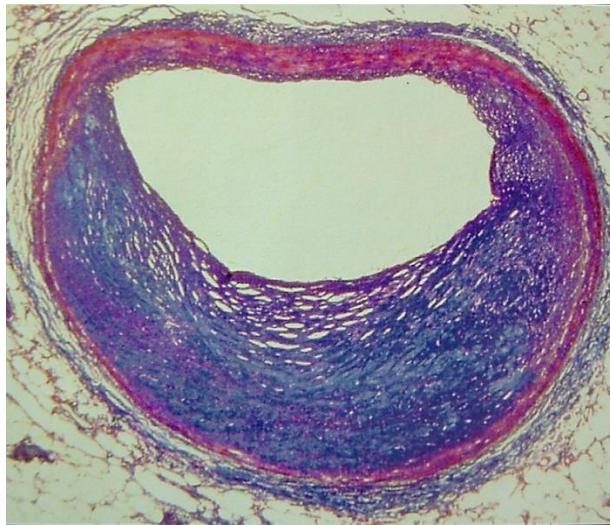






Stable and instable coronary lesions of humans





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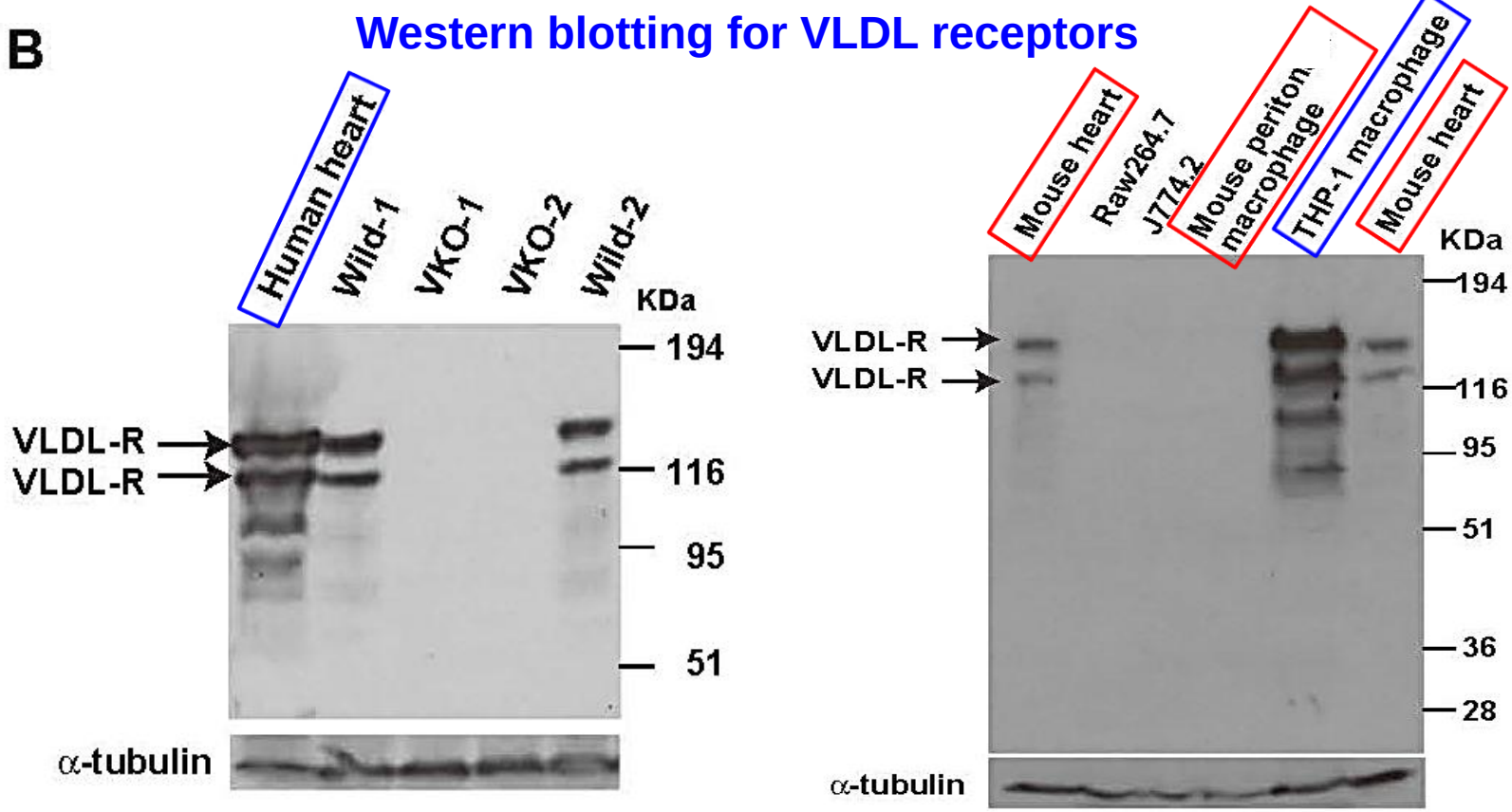
Species differences of macrophage very low-density-lipoprotein (VLDL) receptor protein expression

Sadao Takahashi [a,b,*], Takashi Ito [c], Yasuo Zenimaru [a], Jinya Suzuki [a], Isamu Miyamori [a], Masao Takahashi [d], Masafumi Takahashi [e], Takafumi Ishida [f], Tatsuro Ishida [g], Ken-ichi Hirata [g], Tokuo T. Yamamoto [h], Tadao Iwasaki [i], Hiroaki Hattori [i], Masashi Shiomi [c,j]



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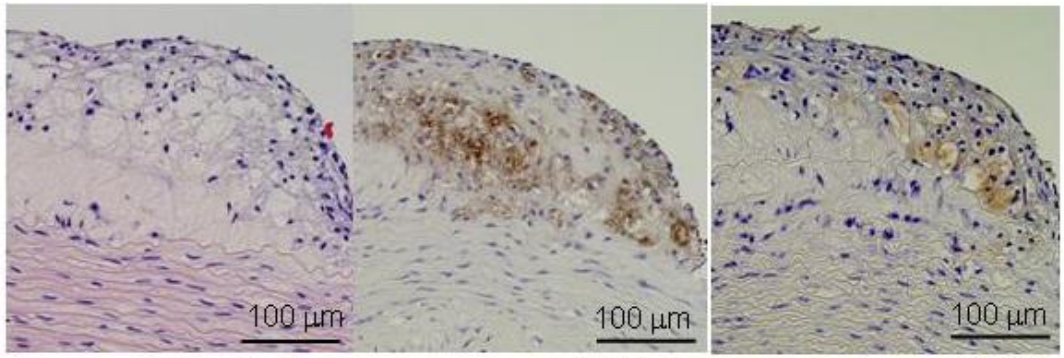
- The VLDL receptor is expressed in macrophages in arterial lesions of rabbits and humans, but not in mice.
- The development and development process of atherosclerotic lesions in mice may be different from humans and WHHL rabbits.

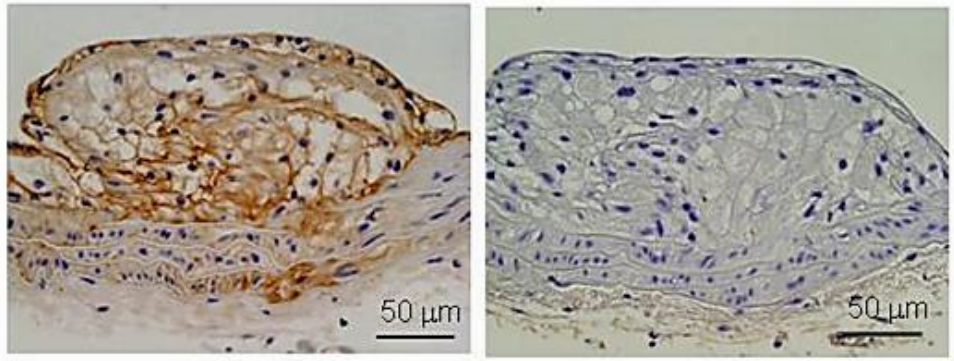
Species differences of macrophage very low-density-lipoprotein (VLDL) receptor protein expression

Sadao Takahashi [a,b,*], Takashi Ito [c], Yasuo Zenimaru [a], Jinya Suzuki [a], Isamu Miyamori [a], Masao Takahashi [d], Masafumi Takahashi [e], Takafumi Ishida [f], Tatsuro Ishida [g], Ken-ichi Hirata [g], Tokuo T. Yamamoto [h], Tadao Iwasaki [i], Hiroaki Hattori [i], Masashi Shiomi [c,j]

Aortic plaque from a WHHLMI rabbit



Plaques developed on aortic sinus in an **apoE-KO mouse**



Species differences in atherosclerotic lesions

	Humans	WHHLMI		Mice	
Atherogenesis	Sensitive	Spontaneous	△	Resistance	X
Coronary lesions	Frequent	Frequent	◎	Rare	X
Property of coronary lesions	Various types	Various types	◎	Excessive lipid deposits	X
Expression of VLDL receptors in lesions	Macrophages	Macrophages	◎	No expression	X
Destabilization of plaques by MMP	Yes	Yes	◎	Inconsistent results	X
Inflammatory marker	CRP	CRP	◎	SAP (Serum Amyloid-P Compound)	X

Species differences in the property of myocardium

	Humans	WHHLMI		Mice	
Cardiac myosin heavy chain	β-type	β-type	◎	α-type	X
Ion channel of myocardial myosin	I_{kr} and I_{ks}	I_{kr} and I_{Ks}	◎	I_{to} and $I_{k,slow}$	X
Electrocardiogram	12-lead ECG	12-lead ECG	◎	Single lead ECG	X
Wave form of ECG	T-wave (diastolic phase)	Similar to human (T-wave)	◎	Different from Human (J-wave)	X

Differences between mouse and rabbit at overexpressing the same human gene

	Mice	Rabbits
Lecitin:cholesterol acyltransferase	**Pro**-atherogenic	**Anti**-atherogenic
Hepatic TG lipase	**Pro**-atherogenic	**Anti**-atherogenic
apoE3	**Anti**-atherogenic	**Pro**-atherogenic
15-lipoxygenase	**Pro**-atherogenic	**Anti**-atherogenic
Apolipoprotein (a)	**No** Lp(a) formation	**Lp(a) formation**
Lipoprotein lipase	**No** effects on visceral fat accumulation	**Decrease** in
CRP	**No** function	**Functional**

(modify. after Koike T & Fan J, Laboratory Animal Technology and Science 2005; 17: 91-96)

Lessons from species differences in hypercholesterolemia and coronary heart disease

1) For translational research, we have to select appropriate model animals

2) Not all genetically modified animals correspond to human diseases.