

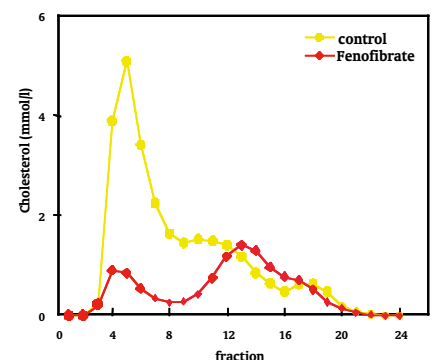
Models, Science and Technologies for Cardiovascular and Metabolic Diseases

Cardiovascular disease is a major cause of mortality and morbidity, affecting millions of individuals in the world. TNO has a range of services to promote the understanding of the mechanisms of cardiovascular disease. These services involve efficacy testing of potentially new and registered therapeutic compounds or interventions and risk factor analysis. We offer state-of-the-art animal models to evaluate the effects of drugs and nutritional factors in the fields of atherosclerosis, diabetes and obesity, restenosis, therapeutic angiogenesis, myocardial infarction and heart failure. Multiple cardiovascular risk factors can be combined in one model to simulate the human situation. Our models are well characterized and evaluated in close collaboration with pharmaceutical and clinical partners. For each model we can offer drug and dietary intervention studies, involving mechanism-oriented sub-studies as needed and employing tailor-made protocols within a well-defined timeframe.



ApoE*3-Leiden transgenic mice offer the unique opportunity to investigate the effect of compounds to treat cardiovascular and metabolic disease. This single animal model simulates conditions, such as: hyperlipidaemia, insulin resistance, atherosclerosis, hyperhomocysteinaemia together with myocardial infarction, restenosis and angiogenesis.

Effects of Fenofibrate on Lipoprotein Profiles in ApoE*3-Leiden Transgenic Mice



The ApoE*3-Leiden transgenic mouse

Because of its unique characteristics, the ApoE*3-Leiden transgenic mouse plays a pivotal role in most of our cardiovascular disease models. These mice display human-like lipoprotein profiles and lipid levels. They also develop atherosclerosis with all the characteristics of the human lesions. As one of the few animal models, the ApoE*3-Leiden mice respond to all hypolipidaemic drugs currently used in the clinic, such as statins, fibrates and ezetimibe, at similar dosages and in a similar way to humans. This offers the opportunity to investigate the effect of new drugs on top of standard treatment and combination therapy. Important risk factors that contribute to vascular disease in man, such as hyperlipidaemia, obesity and insulin resistance, can be induced in a controlled manner in this model.

The model allows us to titrate cholesterol and triglycerides to any desired level and to conduct atherosclerosis studies. You can use a progression (prevention) design or a regression (therapeutic) design to investigate the effect of compounds on lipid metabolism or atherosclerosis under conditions relevant to the clinical situation in humans.

The ApoE*3-Leiden.CETP mouse

ApoE*3-Leiden.CETP mice are derived from the ApoE*3-Leiden transgenic mice by cross-breeding with huCETP transgenic mice, expressing cholesterol ester transfer protein under control of its natural flanking regulatory regions. This results in an even more human-like lipoprotein profile with transfer of cholesterol ester from HDL to the apoB-containing lipoproteins in exchange for triglycerides. This leads to higher levels of the apoB-containing lipoproteins and lower HDL levels. Consequently, ApoE*3-Leiden.CETP mice are more susceptible to develop atherosclerosis than ApoE*3-Leiden mice. The model has the same favorable characteristics as the ApoE*3-Leiden mouse, such as (i) responsiveness to all hypolipidaemic drugs currently used in the clinic, such as statins, fibrates, ezetimibe and niacin, at similar dosages and in a similar way to humans, (ii) the ability to titrate cholesterol and triglycerides to any desired level and (iii) to conduct atherosclerosis studies in a progression (prevention) design or a regression (therapeutic) design. Moreover, the ApoE*3-Leiden.CETP mouse is very well suited to testing the effect of drugs that modulate HDL levels. The mice demonstrate reduced apoB-containing lipoproteins and increased HDL levels upon treatment with the registered drugs atorvastatin, fenofibrate and niacin and with CETP-inhibitors. As in clinical studies the CETP-inhibitor torcetrapib did not show an additional anti-atherosclerotic effect on top of standard treatment with atorvastatin.

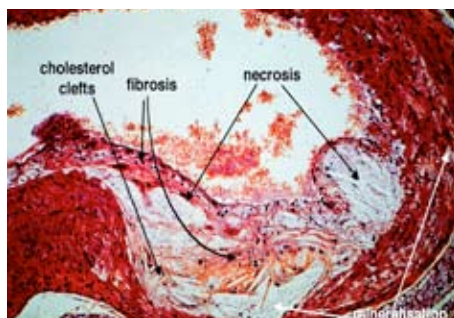
Models in the field of atherosclerosis

– Hyperlipidemia

Since ApoE*3-Leiden- and ApoE*3-Leiden.CETP mice have a human-like plasma lipoprotein profile and lipid levels, they are ideally suited for drug and dietary intervention studies in the field of hyperlipidaemia. The model's plasma lipid levels respond to pharmacological interventions similarly to how humans would respond.

– Atherosclerosis

On a Western Type Diet, ApoE*3-Leiden mice develop atherosclerotic lesions with all the characteristics of human lesions. The various stages of atherosclerosis (from type I to type V) develop within 3 to 6 months, depending upon the magnitude of cholesterol exposure (which can be chosen at any desired level to suit your specific problem). The ApoE*3-Leiden.CETP transgenic mice on a Western-type diet develop increased atherosclerosis as compared to ApoE*3-Leiden mice. This is a result of the adverse lipoprotein distribution and the higher amount of atherogenic apoB-containing lipoproteins.



Severe atherosclerotic plaque (type V) in the aorta of an ApoE*3-Leiden transgenic mouse fed 3 months a high cholesterol diet.

Tailor-made drug and dietary intervention trials include quantification of atherosclerosis development in progression and regression studies and (immuno-) histological characterisation of lesion composition in aortic root and arch. Cholesterol-independent effects, combination therapy and mechanistic studies on atherosclerosis development.

– Plaque rupture

In ApoE*3-Leiden mice plaque rupture can be mimicked. This unique model offers the possibility to study the effects of drugs or interventions on plaque rupture or stability.

– Atherosclerosis and inflammation

We can offer several models of studies on the role of inflammatory factors in vascular diseases in general and in atherosclerosis in particular. An example is the human CRP transgenic mouse, which carries the complete human CRP gene including its regulatory promoter elements. These mice allow us to determine the effect of compounds on general inflammation and on the cardiovascular risk factor CRP.

Models in the field of diabetes and obesity

– ApoE*3-Leiden(.CETP) transgenic mouse on a high-fat diet

Most conventional models (ob/ob, db/db or Zucker diabetic rat) develop rather exaggerated forms of obesity, hyperglycaemia or insulin resistance. ApoE*3-Leiden mice on a high-fat diet develop obesity with mild and reversible insulin resistance comparable to the clinical diagnosis of metabolic syndrome. In addition to insulin resistance, the mice display other components relevant to the metabolic syndrome, leading to type 2 diabetes, such as hyperlipidaemia and the development of atherosclerosis. Major favorable characteristics of the ApoE*3-Leiden mouse are (i) responsiveness to all hypolipidaemic drugs currently used in the clinic, such as statins, fibrates, ezetimibe and niacin, at similar dosages and in a similar way to humans, (ii) the ability to titrate cholesterol and triglycerides to any desired level and (iii) to conduct atherosclerosis studies in a progression (prevention) design or a regression (therapeutic) design.

Moreover, the ApoE*3-Leiden.CETP mouse is very well suited to testing the effect of drugs that modulate HDL levels. The mice

demonstrate reduced apoB-containing lipoproteins and increased HDL levels upon treatment with the registered drugs atorvastatin, fenofibrate and niacin and with CETP-inhibitors.

– **Insulin resistance/Obesity**

On a high-fat, high-caloric diet, wild type mice become obese and insulin-resistant. In these mice we can measure insulin sensitivity under highly standardized conditions using the hyperinsulinaemic euglycemic clamp analysis. Application of this “golden standard” technique allows you to evaluate the effect of your compound regarding its effect on insulin sensitivity in general, as well as on glucose or fatty acid metabolism specifically. It offers the possibility to focus on separate tissues of interest, like liver, adipose tissue and (heart) muscle.

Conventional models:

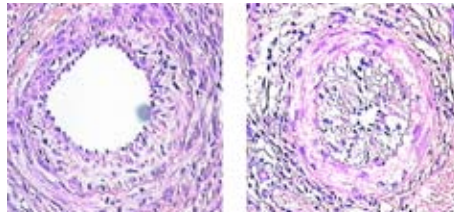
e.g. diet induced obese (DIO)-mouse, ob / ob mouse, db / db mouse, KKya mouse, Zucker rat and Zucker diabetic rat.

Models in the field of restenosis

Cuff-induced intimal hyperplasia in the femoral artery of (transgenic) mice

Placement of a stiff polyethylene cuff around the femora artery induces within 3 weeks the formation of a smooth muscle cell-rich neointima. In the ApoE*3-Leiden mice a hypercholesterolaemic diet greatly increases the size of the neointima, at the same time inducing foam cell accumulation in the neointima. This model is eminently

suitable for studying the effect of drugs on the early events of (post-interventional) atherosclerosis, including the role of inflammation.



Cross section of cuffed femoral arteries in ApoE*3-Leiden mice on normal chow diet (left) or hypercholesterolemic diet (right).

Drug eluting cuff model

The model described above for neointima formation can be activated using a drug eluting polymer cuff. This provides a rapid response model that mimics drug-eluting stent approaches. The model also allows you to access the local anti-inflammatory effect of your compound. It has been validated with rapamycine and paclitaxel.

Models in the field of myocardial infarction

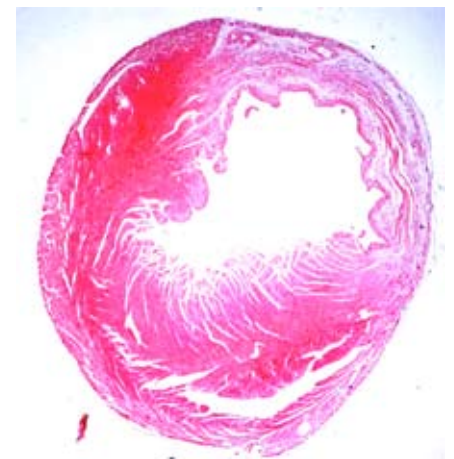
TNO offers a myocardial infarction model in mice, based on ligation of the left anterior descending coronary artery (LAD). This model can be used for studying the effects of drugs or therapeutic interventions on ischemic reperfusion damage or heart failure.

– **Ischaemia reperfusion**

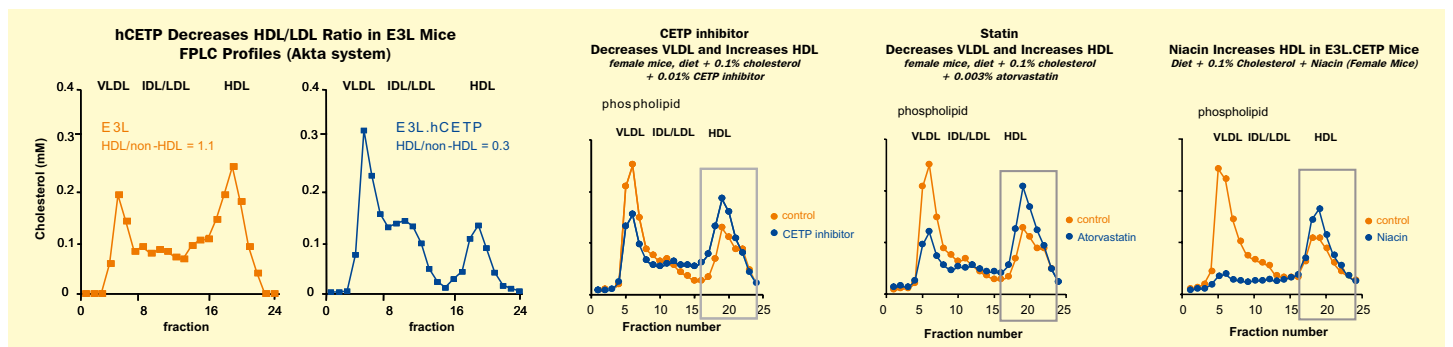
After removal of the ligation the heart can be reperfused for the desired time (from hours to months). In this model, the size of the myocardial infarction, the magnitude of reperfusion damage and functional parameters such as intraventricular pressure-volume relationships can be measured.

– **Heart failure**

When the mice are kept alive for longer periods of time, they develop a condition which is comparable to heart failure in man. Using this myocardial infarction/ heart failure mouse model, the effects of drugs can be investigated in combination with other cardiovascular risk factors such as hyperlipidaemia, insulin resistance, hyperhomocysteinaemia and atherosclerosis, if required on a different genetic background.



Ventricular wall thinning at four weeks after coronary artery ligation.



Selected references

1. Zadelaar S, *et al.* Mouse models for atherosclerosis and pharmaceutical modifiers. **Arterioscler Thromb Vasc Biol.** 2007 Aug;27(8):1706-21.
2. van der Hoogt CC, *et al.* Fenofibrate increases HDL-cholesterol by reducing cholesteryl ester transfer protein expression. **J Lipid Res.** 2007 Aug;48(8):1763-71.
3. Westerterp M, *et al.* Cholesteryl ester transfer protein decreases HDL and severely aggravates atherosclerosis in ApoE*3-Leiden mice. **Arterioscler Thromb Vasc Biol.** 2006 26;2552-2559.
4. Delsing DJM, *et al.* Rosuvastatin reduces plasma lipids by inhibiting VLDL production and enhancing hepatobiliary lipid excretion in ApoE*3-Leiden mice. **J Cardiovasc Pharmacol** 2005 45;53-60.
5. Van der Hoorn JW, *et al.* Olmesartan and pravastatin additively reduce development of atherosclerosis in ApoE*3-Leiden transgenic mice. **J Hypertens.** 2007 Dec;25(12):2454-62.
6. Kleemann R, *et al.* Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis. **Genome Biol.** 2007 Sep;24;8(9):R200 [Epub].
7. Dual PPAR α/γ agonist tesaglitazar reduces atherosclerosis in insulin-resistant and hypercholesterolemic ApoE*3-Leiden mice. **Arterioscler Thromb Vasc Biol.** 2006 26;2560-2566.
8. Post SM *et al.* Fibrates suppress bile acid synthesis via PPAR α -mediated down-regulation of cholesterol 7 α -hydroxylase gene expression. **Arterioscler Thromb Vasc Biol.** 2001 21;1840-1845.

TNO Quality of life

TNO Quality of Life is a part of TNO; Europe's largest independent research institute for technological and strategic research and consultancy. By translating scientific knowledge into practice we optimise the innovative abilities of the industry and government. TNO is a research partner for various industries worldwide.

Pharma office**Visiting address**

Zernikedreef 9
2333 CK Leiden
The Netherlands

Postal address

P.O. Box 2215
2301 CE Leiden
The Netherlands

For more information, please contact us.

E info-pharma@tno.nl

The Netherlands, Leiden

P +31 71 518 14 99
F +31 71 518 19 01

The Netherlands, Zeist

P +31 30 694 48 44
F +31 30 694 48 45

North America

P +1 416 837 75 00
F +31 71 518 19 01

Japan

P +81 45 478 51 30
F +81 45 473 79 59

www.tno.nl/pharma

TNO | Knowledge for business

