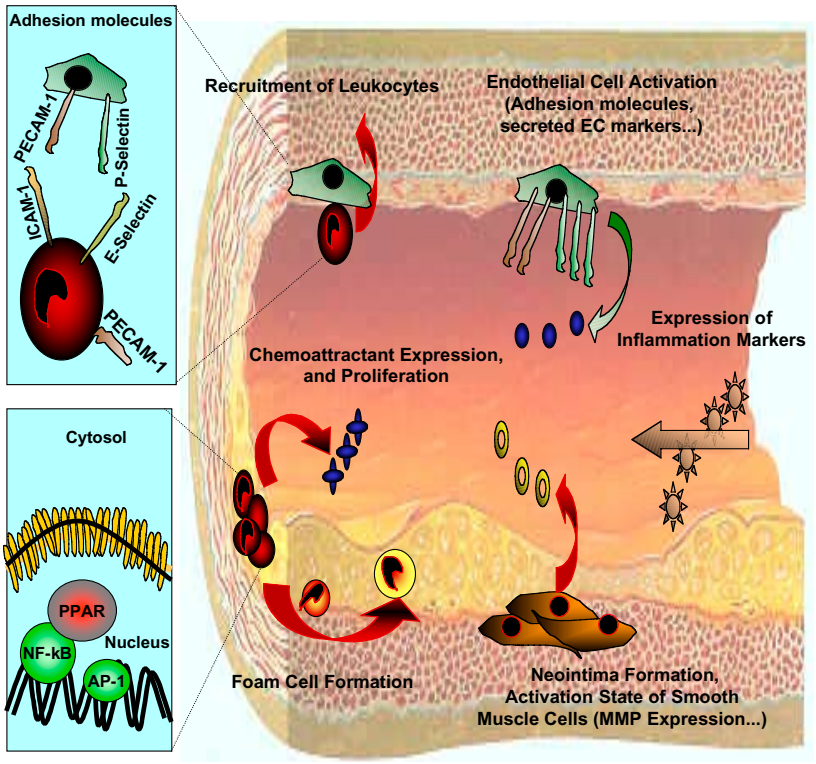
TNO | Knowledge for business

Chronic inflammation in Vascular and Metabolic Disease

Atherosclerosis, insulin resistance/diabetes and obesity are very complex multifactorial diseases. Chronic inflammation has been identified as a common denominator. Inflammatory processes in the vessel wall, adipose tissue, intestine and the liver drive the diseases. Our extensive knowledge of the dynamics of the disease processes will help you to discover, develop and test new drugs and treatments and to identify novel targets for therapy. TNO offers:

- humanized models of inflammation, atherosclerosis, IR/DM2, obesity, non-alcoholic fatty liver disease, liver steatosis, adipose quality.
- experienced pathological & morphometrical analysis of vessel wall, adipose tissue, liver, gut etc. combined with state-of-the-art clinical chemistry.
- advanced immunohistochemistry of macrophages, SMC, adipocytes, adhesion molecules, transcription factors etc.
- functional transcriptomic pathway analysis combined with metabolomics in microvolumes of plasma (>350 identified metabolites).



Chronic inflammation is an important risk factor and is associated with obesity, insulin resistance/diabetes and atherosclerosis. TNO's expertise in the field of chronic inflammation will help you to develop and test new strategies for disease prevention and treatment and to identify new targets for therapy.

Typical test compounds include:

Nutrients	Pharmaceuticals
Probiotics	Statins
Fish oil	PPAR (α,δ,γ) activators
Specific fatty acids (Ω-3, Ω-6)	Fibrates
Fruits & Vegetables (tomatoes, grapes, garlic...)	LXR activators
Anti-oxidants (vitamines, green tea, ...)	Anti-inflammatory drugs (e.g. salicylate)
Flavonoids (quercetin, lycopeen, ...)	Hypotensive drugs Cytokine-inhibiting antibodies

Chronic Inflammation Models

- ApoE*3-Leiden transgenic mice (E3L) [ref 1-5]: a humanized atherosclerosis model leading to atherosclerotic lesions which resemble human lesions. Unique for the ApoE*3-Leiden mice is their human-like response to drugs (e.g. statins, fibrates) and nutrients (e.g. fish oil, flavonoids). Low-grade inflammation can be evoked in this model with specific diets. Also regression studies are possible.
- LDLR-/- mice [ref 6]: A rapid atherosclerosis model; simultaneous development of insulin resistance and atherosclerosis.
- C-reactive protein (CRP) transgenic mice [ref 3, 7, 8] Transgenic mice which express human CRP, a predictor of future metabolic and cardiovascular disease. The mice are

sensitive to typical anti-inflammatory and anti-atherosclerotic drugs.
- Cellular assays using primary human cultures and human cell lines allow rapid screens (e.g. potency of a test compound to quench adhesion molecule expression).

Supporting technologies

Analysis of monocyte adhesion and immune cell infiltration [ref 2-4, 6]

Mononuclear cell adhesion to the endothelium and infiltration into the vasculature can be quantified by immunohistochemistry. T cells and SMCs can also be quantified by IHC.

Plaque stability [ref 2-4]

- Quantification of the cellular composition of lesions (monocytes/macrophages, T cells and SMCs).
- Quantification and intracellular localization of relevant transcription factors, e.g. the activated forms of NFkB and c-jun in cytosol or nucleus (by IHC and photometry).
- Analysis of (de)stabilizing molecular processes by functional microarrays and bioinformatical analysis of the biological process affected.

Functional assays for inflammatory key processes [ref 5, 9-11]

The activity and expression of transcription factors can be analyzed in the above mentioned *in vivo* models in the vessel wall and other tissues both descriptive (by IHC photometry) and functionally (by rapid binding assays and the TransAM system).

Comprehensive analysis of processes across pathways [ref 5, 9, 12]

Functional microrray analysis in combination with metabolomics allows us to assess all biological processes in virtually all tissues (mouse and human).
Relevant active signaling pathways and transcription factors are identified by state-of-the art Systems Biology analysis [ref 6, 10].

Anti-inflammatory and anti-oxidative potential of compounds [ref 7, 8, 9-11]

Anti-inflammatory potency of test compounds can be tested in human CRP transgenic mice and in designated *in vitro* assays.

Obesity and adipose tissue biology [ref 13]

Testing of compounds on obesity and risk factors of the metabolic syndrome (e.g. Macrophage infiltration into adipose tissue).

Selected references (full list available on request)

[1] Zadelaar *et al.* ***Arterioscler.Thromb.Vasc. Biol.*** 2007, 27:1706-1721 (review).
[2] Kleemann *et al.* ***Circulation*** 2003, 108:1368-1374.
[3] Verschuren *et al.* ***Arterioscler.Thromb. Vasc.Biol.*** 2005, 25:161-167.
[4] Kooistra *et al.* ***Arterioscler.Thromb.Vasc. Biol.*** 2006, 26:2322-2330.
[5] Kleemann *et al.* ***Genome Biology*** 2007, 8(9):R200.
[6] Bernhagen *et al.* ***Nat.Med.*** 2007, 170:809-817.
[7] Kleemann *et al.* ***Blood*** 2004, 103:4188-4194.
[8] Rein *et al.* ***J.Nutr.*** 2006,136:2331-2337.
[9] Lindeman *et al.* ***Clin.Sci.(Lond)*** 2008, 114:687-697.
[10] Kleemann *et al.* ***Blood*** 2003, 101:545-551.
[11] Kleemann *et al.* ***Nature*** 2000, 408:211-216.
[12] Bobeldijk *et al.* ***J.Chromatogr.B.*** 2008 (in press).
[13] Madsen *et al.* ***J.Biol.Chem.*** 2008, 283:7196-7205.

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. As a research partner TNO works 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.
info-pharma@tno.nl

The Netherlands, Zeist
P +31 30 694 48 44
F +31 30 694 48 45

The Netherlands, Leiden
P +31 71 518 14 99
F +31 71 518 19 01

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