

*Overall research theme:*

**Lipoprotein metabolism and atherosclerosis**

*Latest update:*

Monday, 16 August 2004

*Senior staff member(s): Position(s):*  
*addresses:*

*Degrees:*

*E-mail*

Lars Bo Nielsen

1. reserevelæge, Assoc. Prof.

MD,PhD,Dr.Med.Sci.

larsbo@rh.dk

*Department/institution/address/telephone/fax:*

Department Clinical Biochemsitry, KB3011, Rigshospitalet, blegdamsvej 9, DK2100 Copenhagen, Denamrk. Ph: +45 3545 2304. FAX: +45 35452524

*Characteristics of the research group:*

We seek to gain insights into new aspects of lipoprotein metabolism and atherosclerosis by combining molecular, pathological and physiological studies of normal and genetically modified mice, including new models that are generated by ourselves. In parallel we are studying human biopsy material to correlate our basic observations with human disease states. The group currently include three phd students, one postdoc, two technicians and one medical student – we are collaborating with several clinical departments at Rigshospitalet and other international research groups.

*Running projects: Titles and abstracts:*

### **1. Intestinal lipoprotein production and its effect on atherosclerosis**

Atherosclerosis is the major cause of heart disease. Plasma lipoproteins play essential roles in formation of atherosclerotic lesions. It is widely accepted that apolipoprotein B containing lipoproteins [VLDL, IDL, LDL, and lipoprotein(a)] promote atherosclerosis. However, it is likely that certain lipoprotein subfractions are particularly atherogenic.

It has often been proposed that postprandial lipoproteins are important initiators of atherosclerosis. Nevertheless, tests of this idea have been halted by a lack of experimental animal models and suitable methods for measuring postprandial lipoproteins in plasma. We are currently examining the effect of MTP overexpression in a cell model of intestinal absorptive enterocytes (CaCo-2 cells grown on filters) and will generate a mouse model of intestinal hyperlipidemia to assess the atherogenicity of intestinal lipoproteins *in vivo*. In addition, we are collecting human endarterectomy specimens (a collaboration with the Department of Vascular Surgery) and will use those to examine the accumulation of intestinally derived (apoB-48 containing) lipoproteins in atherosclerotic lesions. We have obtained a newly developed apoB48-specific monoclonal antibody that hopefully will allow us to do immunohistochemical studies of apoB48's localisation in plaques. Also we plan to wash out vascular lipoproteins and examine the size and density of vascular apoB48 versus apoB100 containing lipoproteins.

Combined the studies of transfected cells, an MTP transgenic mouse models, and human arterial specimens hopefully will be of value in a long-standing debate on the role of postprandial hyperlipoproteinemia. If intestinal lipoproteins appear to be particularly atherogenic in the mouse and/or if apoB48 containing lipoproteins accumulate in excess to apoB100 particles in plaques, the studies should encourage efforts to identify biochemical and genetic markers of intestinal hyperlipidemia.

### **2. Apolipoprotein M**

High plasma levels of HDL cholesterol decrease the risk of atherosclerosis. However, HDL particles constitute a heterogenous population with varying lipid and apolipoprotein composition. Many aspects of HDL metabolism and its effect on atherosclerosis are unknown. We have initiated studies of a novel HDL apolipoprotein, apoM. We wish to use mice with increased or decreased apoM production to define biological roles of apoM, particularly its effects on lipoprotein metabolism and atherogenesis. At this stage we have developed reagents and assays for characterization of apoM gene expression. We have

also generated polyclonal rabbit antibodies to human and mouse apoM which are useful for western blotting, ELISA quantification, and immunohistochemistry. We wish to determine the biological roles of apoM and its impact on atherogenesis by generating and studying mice with genetically altered apoM expression. Also we wish to use *in vivo* overexpression of mutated apoM to determine the importance of the preserved signal peptide. We have finalized the generation of mice that overexpress apoM in the liver and are currently breeding these mice in order to be able to study them. We are also making apoM deficient mice with embryonic stem cell technology. The apoM deficient mice are made in collaboration with Prof. Reinhard Fässler, Munich, Germany. We have recently cloned a targeting vector with arms of apoM homology and will use this vector in ES cell transfections in September 2004.

The studies of apoM will hopefully elucidate biological functions of a novel apolipoprotein involved in HDL metabolism. The clinical implications are impossible to predict at this stage, but several potentially important functions are implied by our preliminary results.

### **3. Formation of apolipoprotein B containing lipoproteins in unexpected tissues.**

It has been known for decades that the liver and the intestine produce apoB-containing lipoproteins. The principal biological function is the packaging and secretion of lipids. During Lars Bo Nielsen's post-doctoral fellowship, it was discovered that the heart also produces apoB-containing lipoproteins. Although the expression levels in the heart is rather low compared to liver and intestine, subsequent studies from our group have strongly supported the concept that the secretion of apoB containing lipoproteins serves as an effective means of removing excess triglycerides from the cardiac myocytes, and that it affects cardiac function.

Serendipitously, we recently found substantial MTP activity on extracts of human placenta biopsies. The only known biological function of MTP is participation in lipoprotein lipidation during their formation in liver and intestine. Interestingly, in mice, the yolk sac but not placenta produces lipoproteins, and no study has previously investigated lipoprotein secretion from the human placenta. Our preliminary studies support the hypothesis that human placenta indeed might produce lipoproteins because we can easily detect both apoB and MTP mRNA in human placenta biopsies and because pre-incubated of placental biopsies with <sup>35</sup>S-Methionine results in the secretion of <sup>35</sup>S- apoB. We are currently characterizing the placental lipoproteins in further detail since it may pose a novel pathway for lipid transfer from mother to fetus.

### **4. Inflammation in classical and uremic atherosclerosis.**

The view of atherogenesis has moved - from a slow passive deposition of amorphous material, mainly cholesterol, in arteries eventually leading to narrowing of the coronary lumen - to a highly dynamic process characterized by inflammation and multiple cellular interactions. We have recently developed novel techniques for quantitative and qualitative studies of the interaction of blood lymphocytes with the arterial intima *in vivo*. We have also developed an uremic apolipoprotein E-deficient mouse model which allows us to study the impact of renal failure on atherogenesis. The uremic mouse develops extremely severe vascular lesions compared with non-uremic mice. Biochemical, morphological and gene expression studies indicate that uremic atherosclerosis share key features of classical atherosclerosis (ie macrophage/foam cell infiltration of the intima, cholesterol and cholesterol ester accumulation, and increased ICAM-1 and VCAM-1 expression). We are currently examining the cause of uremic atherosclerosis by several different approaches including cDNA array analysis and by treating uremic mice with compounds that specifically target potential causes of the accelerated vascular disease.

### **5. Other projects**

In addition to the above mentioned main projects, we are also engaged in projects addressing the biology of natriuretic peptides in the heart and are currently collecting blood samples from 500 patients undergoing coronary arteriography to test putative novel markers (eg BNP, CD163, and PPAP-A) for atherosclerosis in a clinical setting.

*Recent publications related to the projects described above:*

Selected publications from the last 5 years:

1. Lars B. Nielsen, Murielle Veniant, Martin Raabe, Jan Boren, Laura Flynn, Carmen Tamm, Jenni Wong, Micheal D. Gunn, Ira Goldberg, Robert Hamilton, Stephen G. Young. The apolipoprotein B and microsomal triglyceride transfer protein genes are expressed in the heart. Indications that the heart has capacity for synthesis and secretion of lipoproteins. *Circulation*, 1998;98:13-16
2. Lars B. Nielsen, Debra Kahn, Thomas Duell, Heinz-Ulrich G. Weier, Stacy Taylor, and Stephen G. Young. Apolipoprotein B gene expression in a series of human apolipoprotein B transgenic mice generated with recA-assisted restriction endonuclease cleavage-modified bacterial artificial chromosomes. An intestine-specific enhancer element is located between 54 and 62 kilobases 5' to the structural gene. *J Biol Chem* 1998;273(34):21800-7
3. Martin Raabe, Murielle Veniant, Meghan Sullivan, Constance H. Zlot, Lars B. Nielsen, Jinny S. Wong, Robert L. Hamilton, Stephen G. Young. Analyzing the role of microsomal triglyceride transfer protein in the liver with tissue-specific knock-out mice. *J Clin Invest*, 1999;103:1287-98
4. Emil D. Bartels, Morten Lauritsen, Lars B. Nielsen. Expression of microsomal triglyceride transfer protein and triglyceride secretion are increased in livers of obese diabetic mice. *Diabetes*, 2002;51:1233-1239.
5. Lars B. Nielsen, Emil D. Bartels, Entela Bollano. Overexpression of apolipoprotein B in the heart impedes cardiac triglyceride accumulation and development of cardiac dysfunction in diabetic mice. *J Biol Chem*, 2002;277:27014-20
6. Christina Christoffersen, Jens P. Goetze, Emil D. Bartels, Marianne O. Larsen, Ulla Ribel, Jens F. Rehfeld, Bidida Rolin, Lars B. Nielsen. Chamber-dependent expression of BNP and its mRNA in normal and diabetic pig heart. *Hypertension*, 2002;40:54-60.
7. Lars B. Nielsen, Mario Perko, Henrik Arendrup, Claus B. Andersen. Microsomal triglyceride transfer protein gene expression and triglyceride accumulation in hypoxic human hearts. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2002;22:1489-94.
8. Flemming Moeller, Lars B. Nielsen. Aortic recruitment of blood lymphocytes is most pronounced in early stages of lesion formation in apolipoprotein-E-deficient mice. *Atherosclerosis*. 2003;168:49-56.
9. Goetze JP, Christoffersen C, Perko M, Arendrup H, Rehfeld JF, Kastrup J, Nielsen LB. Increased cardiac BNP expression associated with myocardial ischemia. *FASEB J*. 2003;17:1105-7
10. Flemming Møller, Finn C. Nielsen, Lars B. Nielsen. New tools for quantifying and visualizing adoptively transferred cells in recipient mice. *J Immunol Methods*. 2003;282:73-82.
11. Susanne Bro, Jacob F. Bentzon, Erling Falk, Claus B. Andersen, Klaus Olgaard, Lars B. Nielsen. Chronic renal failure accelerates atherogenesis in apolipoprotein e-deficient mice. *J Am Soc Nephrol*. 2003;14:2466-74.
12. Christina Christoffersen, Entela Bollano, Emil Bartels, Marie S. Lindegaard, Jens P. Goetze, Claus B. Andersen, Lars B. Nielsen. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology*. 2003;144:3483-90.
13. Helena Ledmyr, Alex D. McMahon, Ewa Ehrenborg, Lars B Nielsen, Hans Lithell, Peter W MacFarlane, Christopher J. Packard, Fredrik Karpe. The microsomal triglyceride transfer protein gene -493T variant lowers cholesterol but increases the risk of coronary heart disease. *Circulation* 2004;109:2279-84
14. Susanne Bro, Claus B. Andersen, Claus B. Andersen, Klaus Olgaard, Lars B. Nielsen. Increased expression of adhesion molecules in uremic atherosclerosis in apolipoprotein-E deficient mice. *J Am Soc Nephrol* 2004;15:1495-503
15. Kirsten Faber, Olof Axler, Bjorn Dahlback, Lars B. Nielsen. Characterization of apoM in normal and genetically modified mice. *J Lipid Research* 2004 45:1272-8
16. Jens Peter Goetze, Alicia Gore, Christian H. Møller, Daniel A. Steinbrüchel, Jens F. Rehfeld, Lars B. Nielsen. Myocardial Hypoxia Increases BNP Gene Expression, *FASEB J*, in press.

*Oversigtsartikler*

17. Lars Bo Nielsen. Lipoprotein production by the heart: a novel pathway of triglyceride export from cardiomyocytes. *Scand J Clin Lab Invest*, 2002;237:35-40

18. Sally P.A. McCormick, Catherine Y.Y. Liu, Stephen G. Young, Lars B. Nielsen. Manipulating large insert clones for transgenesis. *Methods Mol Biol.* 2002;209:105-23