

Overall research theme:

Molecular mechanisms of exercise on local and systemic functions in health and disease

Latest update:

November 30, 2002

<i>Senior staff member(s):</i>	<i>Position(s):</i>	<i>Degrees:</i>	<i>E-mail addresses:</i>
Thorkil Ploug	Associate professor	MD	tp@mfi.ku.dk

Department/institution/address/telephone/fax:

Department of Medical Physiology, University of Copenhagen
The Panum Institute, bldg. 12/4, Blegdamsvej 3, DK-2200 Copenhagen
Tel.: 3532 7435 Fax: 3532 7555

Characteristics of the research group:

Using experimental systems ranging from the intact organism to perfused or incubated organs and cultured cells we study how acute exercise or repeated bouts of exercise (training) affects the function of skeletal muscle as well as the whole organism. In particular, at the local level we are interested in elucidating the molecular mechanisms behind insulin resistance of glucose metabolism in skeletal muscle, a cardinal feature of the metabolic syndrome. At the systemic level it is well recognized that exercise training has a number of beneficial effects on various systems, e.g. the cardio-vascular system, body weight, glucose homeostasis and mood. We are exploring to what extent these effects may be mediated by "hormones" liberated from the contracting muscle.

Running projects: Titles and abstracts:

Signal transduction mechanisms for GLUT4 translocation in skeletal muscle

Skeletal muscle glucose metabolism serves a dual purpose. It provides fuel during muscle contractions and stores blood glucose as glycogen in the postprandial phase. The failure of normal insulin levels to stimulate muscle glucose uptake, i.e., insulin resistance, is a major contributor to type 2 diabetes mellitus. GLUT4 is the major glucose transporter protein in skeletal muscle and in response to stimulation with insulin or muscle contractions (exercise) it translocates from intracellular storage sites to the plasma membrane. This exocytotic process is dramatically decreased in type 2 diabetes. Whereas a number of signaling intermediates in the insulin transduction cascade have been identified much less is known about how contractions initiate the translocation of intracellular GLUT4 vesicles. The aim of the research project is to identify targets for therapeutic rectification of reduced GLUT4 translocation as seen in insulin resistant states as type 2 diabetes. It is worth noting that the smooth muscle cells of the cardio-vascular system also contain GLUT4 transporters.

Morphometric analysis of human muscle biopsies

In rat muscle we have identified a number of morphological parameters that correlates with insulin sensitivity of glucose metabolism. By confocal immunofluorescence microscopy and electron microscopy we are investigating if any of these parameters are affected in muscle biopsies obtained from different groups of human subjects, e.g. young and old, male and females, trained and untrained, type 2 diabetics and McArdel patients (deficient in glycogen phosphorylase). We are also exploring to what extent the status of the blood vessels associated with the muscle fibers can be characterized by these morphological techniques.

In vivo use of the "green fluorescent protein" tagging technique and other fluorescent probes to study protein dynamics and metabolism in skeletal muscle

One problem with using fixed tissue for morphological observations is that it is difficult (impossible) to obtain a good dynamic resolution, e.g. to follow minute by minute the itinerary of GLUT4 vesicles after stimulation with insulin. To overcome this problem we are transfecting (by gene gun bombardment or electroporation) skeletal muscle of mice with various genes (e.g. GLUT4, hormone sensitive lipase (HSL)) tagged with the gene encoding the "green fluorescent protein" (GFP). Several days after transfection the mice are again anesthetized and mounted in vivo on the stage of a confocal microscope, the transfected muscle exposed and observed by time-lapse microscopy. The possibility of studying skeletal muscle and blood vessel metabolism in vivo in intact animals by using fluorescent labelled glucose will be explored.

Skeletal muscle as an endocrine organ?

Our studies of GLUT4 trafficking in skeletal muscle have led us to speculate that the intracellular GLUT4 vesicles may contain substances that are liberated to the extracellular space and which may serve autocrine, paracrine or endocrine functions. We have now identified a number of peptides and proteins that are secreted from skeletal muscle and some of which are dramatically affected by exercise. These potential “hormones” are currently being characterized by protein chemistry methods (MALDI-TOF and MS-MS) and their potential influence on the cardiovascular system (e.g. atherosclerotic plaques, blood pressure and angiogenesis), body weight (obesity), glucose homeostasis (liver glucose production) and mood (depression) will be investigated.

Recent publications related to the projects described above:

- Ploug, T. and Ralston, E.: Exploring the whereabouts of GLUT4 in skeletal muscle (*review*). *Mol. Membr. Biol.*, 19: 39-49, 2002.
- Lauritzen, H.P.M.M., Reynet, C., Ralston, E., Thomas, S.W., Galbo, H. and Ploug, T.: Gene gun bombardement mediated expression and translocation of GFP-tagged GLUT4 in skeletal muscle fibers in vivo. *Pflugers Arch.* 444: 710 – 721, 2002.
- Ai, H., Ihlemann, J., Hellsten, Y., Lauritzen, H.P.M.M., Hardie, D.G., Galbo, H. and Ploug, T.: Effect of fiber type and nutritional state on AICAR and contraction stimulated glucose transport in rat skeletal muscle. *Am. J. Physiol.*, 282: 1291-1300, 2002.
- Ralston, E., Ploug, T., Kalhovde, J. and Lømo, T.: Golgi complex, endoplasmic reticulum exit sites, and microtubules in skeletal muscle fibers are organized by patterned activity. *J. Neurosci.* 21: 875-883, 2001.
- Derave, W., Ai, H., Ihlemann, J., Withers, L.A., Kristiansen, S., Richter, E.A. and Ploug, T.: Dissociation of AMP-activated protein kinase activation and glucose transport in contracting slow-twitch muscle. *Diabetes* 49: 1281-1287, 2000.
- Ralston, E., Lu, Z. and Ploug, T.: The organization of the Golgi complex and microtubules in skeletal muscle is fiber type dependent. *J. Neurosci.* 19: 10694-10705, 1999.
- Ihlemann, J., Galbo, H. and Ploug, T.: Calphostin C is an inhibitor of contraction, but not insulin stimulated glucose transport, in skeletal muscle. *Acta Physiol. Scand.* 167: 69-75, 1999.
- Ralston, E. and Ploug, T.: Caveolin-3 is associated with the T-tubules of mature skeletal muscle fibers. *Exp. Cell Res.* 246: 510-515, 1999.
- Ploug, T., van Deurs, B., Ai, H., Cushman, S.W., Ralston, E.: Analysis of GLUT4 distribution in whole skeletal muscle fibers: Identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J. Cell Biol.* 142: 1429-1446, 1998.
- Ploug, T., Han, X.X., Petersen, L.N. and Galbo, H.: Effect of in vivo injection of cholera and pertussis toxin on glucose transport in rat skeletal muscle. *Am. J. Physiol.* 272: E7-E17, 1997.