

# Regulation of metabolism and inflammation in liver and skeletal muscle



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Akademisk avhandling

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av

Annika Nerstedt

Fakultetsopponent: Professor Anna Krook,  
Institutionen för fysiologi och farmakologi, Karolinska Institutet, Stockholm

Avhandlingen baseras på följande arbeten:

- I. Vieira E, Nilsson EC, Nerstedt A, Ormestad M, Long YC, Garcia-Roves PM, Zierath JR, Mahlapuu M. **Relationship between AMPK and the transcriptional balance of clock-related genes in skeletal muscle.** *Am J Physiol Endocrinol Metab.* 2008 Nov;295(5):E1032-7.
- II. Nerstedt A, Johansson A, Andersson CX, Cansby E, Smith U, Mahlapuu M. **AMP-activated protein kinase inhibits IL-6-stimulated inflammatory response in human liver cells by suppressing phosphorylation of signal transducer and activator of transcription 3 (STAT3).** *Diabetologia.* 2010 Nov;53(11):2406-16.
- III. Nerstedt A, Cansby E, Amrutkar M, Smith U, Mahlapuu M. **Pharmacological activation of AMPK suppresses inflammatory response evoked by IL-6 signaling in mouse liver and human hepatocytes.** Manuscript.
- IV. Nerstedt A, Cansby E, Andersson CX, Laakso M, Stančáková A, Blüher M, Smith U, Mahlapuu M. **Serine/threonine protein kinase 25 (STK25): a novel negative regulator of lipid and glucose metabolism in rodent and human skeletal muscle.** *Diabetologia.* 2012 Jun;55(6):1797-807

## Regulation of metabolism and inflammation in liver and skeletal muscle

Annika Nerstedt

The Lundberg Laboratory for Diabetes Research  
The Sahlgrenska Academy at University of Gothenburg, Sweden

Type 2 diabetes (T2D) is a complex metabolic disorder characterised by hyperinsulinaemia, hyperglycaemia and dyslipidaemia. Obesity is the major risk factor for development of insulin resistance, a main predictor of T2D. Recent evidence indicates that nutrient excess and obesity lead to chronic low-grade inflammation in metabolic tissues, which further promotes insulin resistance.

AMP-activated protein kinase (AMPK), a central regulator of energy homeostasis, increases insulin sensitivity in liver and skeletal muscle and lowers the plasma glucose level, thus reverting the major metabolic disturbances in T2D. Serine/threonine protein kinase 25 (STK25) was found to be differentially expressed in skeletal muscle, comparing AMPK $\gamma$ 3 (*Prkag3*<sup>-/-</sup>) knockout mice to wild-type littermates, indicating a potential role for STK25 in regulation of energy homeostasis in skeletal muscle.

In *Paper I*, genes regulating the circadian rhythm (*Cry2*, *Nr1d1* and *Bhlhb2*) were shown to be differentially expressed in skeletal muscle from wild-type mice treated with the AMPK agonist 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), while they remained unaltered in AMPK $\gamma$ 3 knockout mice. Furthermore, the respiratory exchange ratio (RER) was elevated during the dark period of observation in wild-type mice reflecting a diurnal shift in substrate utilisation from lipid oxidation at daytime to carbohydrate utilisation during nighttime. However, no day/night shift in the RER profile was observed in *Prkag3*<sup>-/-</sup> littermates. Thus, this study suggests that AMPK, as a central energy sensor, could be one important node linking energy metabolism to the circadian clock function.

In *Papers II and III*, the AMPK agonists, AICAR and metformin, are shown to markedly decrease the expression of IL-6-induced serum amyloid A (*SAA*) cluster genes, haptoglobin and suppressor of cytokine signalling 3 (*SOCS3*) in the human hepatocyte cell line HepG2. By repressing AMPK activity with small interfering (si)RNA the inhibitory effect of AMPK on *SAA* expression by both AICAR and metformin was reversed (*Paper II*), indicating that the effect of the agonists is mediated by AMPK activation. Further, we show that AMPK interferes with IL-6 signalling by decreasing IL-6-induced phosphorylation of Janus kinase 1 (JAK1), src homology 2 domain containing protein tyrosine phosphatase 2 (SHP2) and signal transducer and activator of transcription 3 (STAT3) in HepG2 cells (*Papers II and III*). In addition, pharmacological activation of AMPK was shown to repress IL-6-induced inflammation *in vivo* by suppression of STAT3 activity in mouse liver (*Paper III*). This suggests that AMPK is an important intracellular link between metabolic and inflammatory pathways in liver.

In *Paper IV* we show that partial reduction of STK25 by siRNA increases uncoupling protein 3 (UCP3), glucose transporter 1 (GLUT1), GLUT4 and hexokinase 2 (HK2) in the rodent myoblast cell line L6, both at mRNA and protein level. Correspondingly, the rates of palmitate oxidation and insulin-stimulated glucose uptake were elevated after partial depletion of STK25. In conclusion, our studies suggest a role of STK25 as a negative regulator of glucose and lipid metabolism in skeletal muscle. Impaired glucose uptake and fatty acid metabolism by skeletal muscle is a *hallmark of insulin resistance*, and therefore, STK25 could be an important new mediator to be evaluated for therapeutic intervention in T2D and related complications.

Keywords: AMPK; IL-6; Inflammation; Liver; JAK1; STK25; Glucose metabolism; Lipid oxidation; Skeletal muscle; Circadian clock; Type 2 diabetes

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