Regulation of metabolism and inflammation in liver and skeletal muscle

UNIVERSITY OF GOTHENBURG

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg

Fredagen den 8 mars 2013, kl 9.00

av

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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*

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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 AMPKγ3 (*Prkag3*$^{-/-}$) 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 AMPKγ3 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*$^{-/-}$ littermates. Thus, this study suggests that APMK, 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, S*TK25 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

ISBN 978-91-628-8562-5
http://hdl.handle.net/2077/31713 Gothenburg 2013