



**Karolinska
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Institutionen för molekylär medicin och kirurgi

Insulin Signalling and TBC1D1 in Skeletal Muscle Metabolism

AKADEMISK AVHANDLING

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av

Ferenc L.M. Szekeres

Huvudhandledare:

Professor Juleen R. Zierath
Karolinska Institutet
Institutionen för Molekylär medicin och kirurgi
Sektionen för Integrativ fysiologi

Bihandledare:

Professor Anna Krook
Karolinska Institutet
Institutionen för Fysiologi och farmakologi
Sektionen för Integrativ fysiologi

Fakultetsopponent:

Professor Arild Rustan
Oslos Universitet
Institutionen för Farmaceutisk bioteknologi
Enheten för Farmakologi

Betygsnämnd:

Docent Abram Katz
Karolinska Institutet
Institutionen för fysiologi och farmakologi

Docent Ingo Leibiger
Karolinska Institutet
Institutionen för Molekylär medicin och
kirurgi

Docent Elisabet Stener-Victorin
Göteborgs Universitet
Institutionen för Neurovetenskap och
fysiologi

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Abstract

Type 2 diabetes mellitus (T2DM) has taken the form of a pandemic disease globally as people adopt a more western lifestyle. High circulating glucose levels indicates that an individual is in the risk zone for developing T2DM. One of the hallmarks for T2DM is insulin resistance in skeletal muscle. Skeletal muscle is the primary target for insulin-stimulated glucose uptake. Thus, it is of clinical importance to understand of how the key hormone, insulin, affects the sensitivity of the tissues to maintain the rate of glucose uptake, and also how glucose uptake could be altered on a cellular level through metabolic switches. The aim for thesis is to investigate whether functional insulin signalling, and hence glucose uptake, occurs in incubated skeletal muscle specimens. Another aim of this thesis is to understand how the molecular switches TBC1D1 and NT5C1A are involved in the regulation of glucose uptake in skeletal muscle

In paper I, the canonical insulin signalling cascade is investigated in homogenates and cross-sections of skeletal muscle. Muscle specimens were incubated *in vitro*, which is a commonly used experimental technique, to measure glucose uptake and utilisation in skeletal muscle. We show that the canonical pathway of insulin signalling is activated throughout the muscle specimen due to insulin diffusion. We also validate the preparation for the study of insulin signalling. We provide evidence that the experimental approach is valid to assess insulin signalling.

In paper II the effect of silencing the NT5C1A enzyme in skeletal muscle and NT5C2 in cultured myotubes and intact muscle was investigated. We hypothesised that AMPK could be increased if less AMP was hydrolysed as a result of a reduction in the expression of the NT5-enzyme. We demonstrate that in myotubes grown from human biopsies, as well as in mouse tibialis anterior muscle, NT5C silencing increases phosphorylation of AMPK and ACC through changes in the AMP:ATP ratio to thereby increase glucose uptake and lipid oxidation. This means that there is alternative approach to activate AMPK and increase glucose uptake, rather than exercise or chemical stimulation, which is a common way to activate metabolism.

In paper III we investigate the role of TBC1D1 in insulin signalling and metabolism. We provide evidence that the TBC1D1-deficient congenic B6.SJL-Nob1.10 (SJL/SJL) mice have enhanced suppression of hepatic glucose production during the euglycemic-hyperinsulinemic clamp. Moreover, glucose uptake in extensor digitorum longus (EDL) and tibialis anterior muscle was increased during an insulin-stimulated 2-deoxyglucose clamp. Conversely, *in vitro* glucose uptake in response to insulin, contraction or AICAR was impaired in EDL muscle, but not in soleus muscle from the SJL/SJL mouse. These data provide evidence that TBC1D1 is a regulator of glucose transport and metabolism in skeletal muscle.

In conclusion, insulin signalling is functional in incubated skeletal muscle specimens. Moreover, the molecular switches TBC1D1 and NT5C1A have high impact on glucose uptake in skeletal muscle, which is of great importance for the investigation and understanding of T2DM.