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Abstract

Skeletal muscle is the largest organ and critical for whole-body glycemic control. Furthermore, skeletal muscles are crucial for maintaining an independent lifestyle, and muscle strength is inversely associated with death from all causes. Since skeletal muscle wasting is common in several metabolic pathological conditions associated with muscle insulin resistance, understanding the molecular mechanisms underlying this phenomenon is vital.

In response to insulin but also muscle contraction, glucose transporter (GLUT)-4 is translocated to the plasma membrane of the muscle fibre resulting in increased glucose uptake. Although the intracellular signalling mechanisms in response to insulin and muscle contraction has been extensively studied, the signalling pathways are still incompletely understood. The Rho family GTPase Rac1 has been implicated in both insulin- and contraction-stimulated GLUT4 translocation. However, the upstream regulators involved in the increased Rac1 activity and downstream mechanisms are incompletely understood.

The present PhD thesis aimed to identify the Rac1 interactome and reveal potential candidates involved in the regulation of Rac1 activity and downstream mechanisms. In continuation, the effect of RhoGDIα overexpression or knockdown on insulin-stimulated Rac1-activity, GLUT4 translocation and glucose uptake were investigated in muscle cells and mouse. Using a pharmacological inhibitor and transgenic mice, the role of group I PAKs, downstream of Rac1, in the regulation of glucose uptake in response to insulin and muscle contraction was explored.

In the present PhD thesis, RhoGDIα was identified as an endogenous inhibitor of Rac1 in skeletal muscle cells and additionally a negative regulator of insulin sensitivity *in vivo*. Muscle-specific RhoGDIα overexpression impaired whole-body glucose tolerance in mice. Moreover, RhoGDIα protein content was increased in skeletal muscle from insulin-resistant patients with type 2 diabetes potentially explaining the previously reported dysfunctional insulin-stimulated Rho GTPase signalling in these subjects. Interestingly, RhoGDIα was also identified as a negative regulator of muscle mass. Lastly, downstream of Rac1, glucose uptake in response to insulin and electrically-induced muscle contraction was found to partly depend on PAK2, but not PAK1, in glycolytic mouse skeletal muscle. In conclusion, RhoGDIα is identified as a novel regulator of muscle mass and insulin sensitivity and evidence is provided that Rac1-mediated glucose uptake partly depends on PAK2, but not PAK1.

Resumé

Skeletmuskulaturen er kroppens største organ og er kritisk for den glykæmiske kontrol. Kroppens muskler er også nødvendige for at vi kan bevæge os, og nedsat muskelstyrke er associeret med højere dødelighed. Muskelsvind er ikke unormalt i mange metaboliske sygdomme associeret med nedsat insulin sensitivitet. Derfor er det vigtigt at udrede de molekylære mekanismer, der er ansvarlige.

Både insulin og kontraktion af musklerne stimulerer til translokation af glukose transporter (GLUT)-4 ud til plasma membranen af muskelfibrene, hvilket fører til øget glukoseoptagelse. Selvom de intracellulære mekanismer, der regulerer insulin- og kontraktions-stimuleret GLUT4 translokation er blevet undersøgt dybdegående, så er der meget, som vi stadig ikke ved. Rho GTPasen Rac1 er tidligere vist både at regulerer insulin- og kontraktions-stimuleret GLUT4 translokation. Endnu vides det dog ikke præcist, hvordan Rac1 bliver aktiveret, og hvilken signalering Rac1 efterfølgende sætter i gang.

Formålet med denne ph.d. afhandling var at identificere Rac1's bindingspartnere og afsløre potentielle kandidater involveret i reguleringen af Rac1 aktivitet og nedstrøms aktioner. I forlængelse af dette blev effekten af RhoGDIα overudtryk eller knockdown for insulin-stimuleret Rac1 aktivitet, GLUT4 translokation og glukoseoptagelse undersøgt i muskelceller og mus. Ved brug af en farmakologisk hæmmer og transgene mus blev betydningen af gruppe I PAKs, nedstrøms for Rac1, i reguleringen af insulin- og kontraktionsstimuleret glukoseoptagelse undersøgt.

I denne ph.d. afhandling blev RhoGDIα identificeret som en endogen hæmmer af Rac1 i muskelceller og en negativ regulator af insulin-sensitivitet *in vivo*. Muskel-specifik overudtryk af RhoGDIα nedsatte glukosetolerancen på helkropsplan i mus. Derudover blev det fundet, at RhoGDIα proteinekspressionen var højere i patienter med type 2 diabetes, hvilket potentielt kan forklare den nedsatte insulin-stimulerede Rho GTPase signalering tidligere rapporteret i disse patienter. Undersøgelserne viste også, at RhoGDIα var vigtig i reguleringen af muskelmasse. Afslutningsvis blev det fundet nedstrøms for Rac1, at glukoseoptagelse i respons til insulin eller elektrisk-stimuleret muskelkontraktioner delvist var afhængig af PAK2, men ikke PAK1. Disse resultater identificerer RhoGDIα som en hidtil ukendt regulator af muskelmasse og insulin

sensitivitet, samt fastslår at Rac1-medieret glukoseoptagelse i muskler delvist er afhængig af PAK2, men ikke PAK1.

Enclosed manuscripts

The present thesis is based on three manuscripts and some unpublished observations not included in the enclosed manuscripts. Throughout the thesis, the manuscripts are referred to as study 1-3. All manuscripts are located at the back of the thesis.

Study 1

Møller, L.L.V, Watt, K.I., Davey, J, Jedrychowski, M.P., Ali, M., Andersen, N.R., Long, J.Z., Qian, H., Jeppesen, J.F., Jensen, T.E., Abrigo, J., Cabello-Verrugio C., Goodman, C.A., Højlund, K., Wojtaszewski J.F.P., Nielsen, J., Klip, A., Gregorevic, P., Richter, E.A., & Sylow, L. Rho GDP-dissociation inhibitor α inhibits Rac1 and is a novel negative regulator of skeletal muscle mass and insulin sensitivity. *In preparation*.

Study 2

Møller, L.L.V, Jaurji, M., Kjøbsted, R., Joseph, G.A., Madsen, A.B., Knudsen, J.R., Lundsgaard, A.-M., Andersen, N.R., Schjerling, P., Jensen, T.E., Krauss, R.S., Richter, E.A., & Sylow, L. Insulin-stimulated glucose uptake partly relies on p21-activated kinase (PAK)-2, but not PAK1, in mouse skeletal muscle. *bioRxiv*; doi: https://doi.org/10.1101/543736. *To be submitted to Journal of Physiology.*

Study 3

Møller, L.L.V, Nielsen, I.L., Knudsen, J.R., Andersen, N.R., Jensen, T.E., Sylow, L., & Richter, E.A. The role of group I p21-activated kinases in contraction-stimulated skeletal muscle glucose transport. *bioRxiv*, doi: https://doi.org/10.1101/2020.01.29.925024. *In review Physiological Reports, February 2020*.

1. Introduction

Skeletal muscle accounts for approximately 40% of the total body mass in healthy, lean men and 30% in women (Kim et al., 2002; Lee et al., 2000). The preservation of skeletal muscle mass and strength is crucial for maintaining an independent lifestyle, and muscle strength is inversely associated with death from all causes in men (Landi et al., 2013, 2015; Leong et al., 2015; Ruiz et al., 2008). Skeletal muscle wasting is common in several metabolic pathological conditions associated with muscle insulin resistance (Kalyani et al., 2014), including type 2 diabetes (Bassil and Gougeon, 2013; DeFronzo et al., 1985; Pereira et al., 2008), several types of cancer (Dev et al., 2018; Esposito et al., 2012; Han et al., 2020; Wagner and Petruzzelli, 2015), and ageing (Batsis and Villareal, 2018; Cleasby et al., 2016; Rasmussen et al., 2006). Understanding the molecular interactions underlying this phenomenon is vital given the increasing prevalence of type 2 diabetes in the adult population (International Diabetes Federation, 2019), the number of new cancer incidence every year (National Cancer Institute, 2018), and the demographic evolution of an increasing proportion of elderly citizens (World Health Organization, 2018), constituting a remarkable socioeconomic burden. Hence, the overarching aim of the present PhD thesis was to delineate novel molecular mechanisms regulating skeletal muscle mass and insulin sensitivity.

During a euglycemic hyperinsulinemic clamp or an oral glucose load, skeletal muscle accounts for the majority of whole-body glucose disposal (Baron et al., 1988; DeFronzo et al., 1985; Ferrannini et al., 1985), making skeletal muscle critical for whole-body glycemic control. Additionally, skeletal muscle insulin resistance is an early defect in the pathophysiology of whole-body insulin resistance and type 2 diabetes mellitus (DeFronzo et al., 1985; Kahn, 2003). The glucoregulatory role of insulin is generally exerted via inhibition of hepatic glucose production and stimulation of glucose uptake in peripheral tissues (Saltiel and Kahn, 2001).

Glucose transport across the muscle membrane occurs via specific transmembrane glucose transporters (GLUTs). The main muscle-expressed GLUT, GLUT4 is not exclusively located in the plasma membrane. Instead, trafficking of GLUT4 from intracellular stores and insertion into the plasma membrane increases membrane permeability (Klip et al., 2019). Distinct signalling mechanisms increase glucose uptake in response to insulin stimulation and muscle contraction in skeletal muscle (Douen et al., 1990; Hansen et al., 1994; Lee et al., 1995; Lund et al., 1995;

Ploug et al., 1998; Sakamoto et al., 2006). Importantly, muscle contraction increases glucose uptake similarly in both insulin-sensitive and insulin-resistant skeletal muscle (Kennedy et al., 1999; Martin et al., 1995; Richter and Hargreaves, 2013; Wallberg-Henriksson and Holloszy, 1985), making muscle contraction an important alternative pathway to lower blood glucose in insulin-resistant states.

Although the intracellular signalling mechanisms responsible for increased glucose uptake in response to insulin and muscle contraction have been extensively studied, the signalling pathways are still incompletely understood. Elucidating these mechanisms is not only of relevance to type 2 diabetes mellitus, but also other pathological conditions characterized by insulin resistance, such as cancer and ageing.

1.1. Aim, hypotheses and objectives

To gain deeper insight into the molecular mechanisms involved in the regulation of glucose uptake and metabolism in skeletal muscle, the present PhD thesis aimed to investigate the upstream regulators and downstream mechanisms involved in GLUT4 translocation and glucose uptake mediated by the Rho family GTPase Rac1 in response to insulin stimulation and muscle contraction. Specific hypotheses and objectives for Study 1-3 are presented in Table 1. During the investigation of RhoGDIα in **study 1**, we observed changes in muscle mass upon manipulation of RhoGDIα protein expression. Consequently, the role of RhoGDIα in the regulation of muscle mass was investigated in addition to the role in the regulation of muscle glucose uptake.