Original Article

MOLECULAR METABOLISM

# Skeletal muscle O-GlcNAc transferase is important for muscle energy homeostasis and whole-body insulin sensitivity

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## ABSTRACT

**Objective:** Given that cellular O-GlcNAcylation levels are thought to be real-time measures of cellular nutrient status and dysregulated O-GlcNAc signaling is associated with insulin resistance, we evaluated the role of O-GlcNAc transferase (OGT), the enzyme that mediates O-GlcNAcylation, in skeletal muscle.

**Methods:** We assessed O-GlcNAcylation levels in skeletal muscle from obese, type 2 diabetic people, and we characterized muscle-specific OGT knockout (mKO) mice in metabolic cages and measured energy expenditure and substrate utilization pattern using indirect calorimetry. Whole body insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp technique and tissue-specific glucose uptake was subsequently evaluated. Tissues were used for histology, qPCR, Western blot, co-immunoprecipitation, and chromatin immunoprecipitation analyses.

**Results:** We found elevated levels of O-GlcNAc-modified proteins in obese, type 2 diabetic people compared with well-matched obese and lean controls. Muscle-specific OGT knockout mice were lean, and whole body energy expenditure and insulin sensitivity were increased in these mice, consistent with enhanced glucose uptake and elevated glycolytic enzyme activities in skeletal muscle. Moreover, enhanced glucose uptake was also observed in white adipose tissue that was browner than that of WT mice. Interestingly, mKO mice had elevated mRNA levels of *Il15* in skeletal muscle and increased circulating IL-15 levels. We found that OGT in muscle mediates transcriptional repression of *Il15* by O-GlcNAcylating Enhancer of Zeste Homolog 2 (EZH2).

**Conclusions:** Elevated muscle O-GlcNAc levels paralleled insulin resistance and type 2 diabetes in humans. Moreover, OGT-mediated signaling is necessary for proper skeletal muscle metabolism and whole-body energy homeostasis, and our data highlight O-GlcNAcylation as a potential target for ameliorating metabolic disorders.



**Keywords** O-GlcNAc signaling; Type 2 diabetes; N-acetyl-D-glucosamine; Tissue cross talk; Epigenetic regulation of *Il15* transcription; Insulin sensitivity

## 1. INTRODUCTION

Protein modification by O-linked β-D-N-acetylglucosamine (O-GlcNAc) is a post-translational event that results in the transfer of a sugar derivative emanating from the hexosamine biosynthetic pathway to a variety of cellular proteins [1]. Several lines of evidence support the notion that the addition and removal of an O-GlcNAc moiety from serine and threonine hydroxyls by O-GlcNAc transferase (OGT) and O-

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GlcNAcase (OGA), respectively, serve as an integrated cellular nutrient sensor. First, the amount of O-GlcNAc-modified cellular proteins increases in response to elevated nutrient and energy availabilities [2]. Second, the activity of key signaling molecules in the canonical insulin signaling pathway, such as the β subunit of the insulin receptor, IRS1, IRS2, p85 and p110 subunits of PI3K, PDK1, and Akt is generally inhibited by OGT-dependent O-GlcNAcylation [3–7]. Third, transcription co-factors such as FOXO1 and PGC-1α, which are master regulators of gluconeogenesis and lipid metabolism, respectively, are targeted by OGT which results in the stabilization of the proteins [8]. Fourth, OGT and the energy sensor AMP-activated protein kinase (AMPK) physically interact and mutually modify each other's function and/or localization [6,9]. Accordingly, dysregulation of cellular O-GlcNAcylation may be involved in the pathogenesis of a number of metabolic diseases, including obesity and type 2 diabetes [10,11]. This is evidenced by findings showing critical roles of O-GlcNAcylation status in AgRP and α-CaMKII positive neurons for maintaining whole body energy balance in mice [12,13].

Skeletal muscle is an important metabolic tissue and plays essential roles in locomotion and thermogenesis, both having significant impacts on global glucose metabolism and insulin sensitivity [14–17]. Skeletal muscle utilizes a highly complex set of intercalated and coordinated signaling mechanisms to respond to altered environmental nutritional cues [18–20]. Previous studies have suggested that dysregulated O-GlcNAc signaling in skeletal muscle is associated with the development of several metabolic diseases. For example, over-expression of OGT in muscle and adipose tissue causes insulin resistance and impairs glucose transport, respectively [21,22]. In addition, over-expression of OGA, which eliminates O-GlcNAcylation, impairs myogenesis and induces muscle atrophy [23,24]. These findings suggest that O-GlcNAcylation may be part of a complex signaling mechanism within skeletal muscle that is responsible for monitoring cellular nutrient availability and conveying this information to downstream targets to induce metabolic adaptation. Apart from the studies mentioned above, however, the precise role of O-GlcNAcylation in skeletal muscle remains largely unexplored. Herein, we show that skeletal muscle from type 2 diabetic humans has elevated O-GlcNAcylation levels compared with matched controls. Moreover, we show that knockout of OGT in skeletal muscle in mice causes alterations of muscle metabolism that improves whole-body insulin sensitivity and increases energy expenditure. We propose a mechanism whereby lack of OGT results in perturbed regulation of *Il15* expression, which subsequently drives the observed phenotype.

## 2. MATERIALS AND METHODS

### 2.1. Human samples

Human skeletal muscle samples were obtained from a previous study [25]. In short, muscle samples were obtained from the vastus lateralis muscle (Bergström needle biopsy) before and after a 4-hour euglycemic hyperinsulinemic clamp (40 mU * $m^{-2}$ * $min^{-1}$) under local anesthesia. Muscle biopsy samples were quickly blotted free of blood and frozen in liquid nitrogen. Muscle lysates were prepared from freeze-dried muscle dissected free of visible blood, fat, and connective tissue and homogenized as previously described [26]. Lysates were collected from the supernatant of homogenates centrifuged for 20 min at 16,000 *g* and 4 °C. Total protein content in lysates was analyzed by the bicinchoninic acid method (ThermoFisher Scientific). Muscle lysates were prepared in Laemmli buffer and heated for 10 min at 96 °C after which equal amounts of protein were separated by SDS-PAGE. Proteins were transferred to PVDF membranes and incubated with the indicated antibodies.

### 2.2. Animals

Mice were generated and experiments performed at either the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, or Virginia Polytechnic Institute and State University. Muscle specific *Ogt* deletion was achieved by breeding $HSA^{Cre/+}$;$Ogt^{LoxP/Y}$ (JaxMice, strain: B6.Cg-Tg(ACTA1-cre)79Jme/J) males with $Ogt^{LoxP/LoxP}$ females to generate $HSA^{+/+}$;$Ogt^{LoxP/Y}$ (WT) and $HSA^{Cre/+}$;$Ogt^{LoxP/Y}$ (mKO) mice. Inducible muscle-specific OGT knockout mice were generated by breeding HSA-rtTA/TRE-Cre (JaxMice, strain B6; C3-Tg(ACTA1-rtTA,tetO-Cre)102Monk/J) male mice with $Ogt^{LoxP/LoxP}$ females. All procedures were approved by the Danish Animal Experiments Inspectorate and complied with the European convention for protection of vertebrate animals used for scientific purposes (EU Directive 2010/63/EU for animal experiments), or they were approved by Institutional Animal Care and Use Committee of the Virginia Polytechnic Institute and State University and complied with the National Institutes of Health guidelines (NIH Publications No. 8023). Mice were group-housed in an enriched environment with a 12 h light:12 h dark cycle with *ad libitum* feed (#1310, Altromin for mice at University of Copenhagen, and #2918, Envigo for mice at Virginia Polytechnic Institute and State University) and water in a temperature-controlled (22 °C ± 1 °C) room. Mice used for HFD study were fed Teklad rodent diets (#TD.06414, Envigo) with 60% calories coming from fat. The fatty acid profile (% of total fat) is as follows: 37% saturated, 47% monounsaturated, 16% polyunsaturated. The control diet was the standard chow (#2918, Envigo). Mice were fed HFD or normal chow from 4 weeks of age for the indicated weeks.

### 2.3. Whole body metabolic assessment

Whole body metabolic phenotyping was performed at Virginia Tech Metabolic Phenotyping Core using the TSE LabMaster Indirect Calorimetry System. For acclimation purpose, mice were housed in a mock LabMaster cage for one week before they were transferred to recording cages to measure locomotor activity and indirect calorimetry. Mice were fed *ad libitum* during the indicated experimental period. Cage activity, energy expenditure normalized to lean body mass, and respiratory exchange rates were recorded for a 48-h period at the interval of 20 min. Whole body composition was measured by MRI (Minispec Whole Body Composition Analyzer, Bruker). Rectal temperature was recorded at 4PM using an ETI Microtherma 2 Type T thermometer (ThermoWorks). The probe was inserted into the anal ducts of the mice (around 2 cm) when animal was awake in the absence of anesthesia.

### 2.4. Histology and immunohistochemistry

Muscle, WAT, liver, and pancreas were collected from WT and mKO mice and fixed in 10% neutral buffered formalin (0.4% sodium phosphate monobasic, 0.65% sodium phosphate dibasic, 10% formalin, pH 6.8) overnight. The tissues were then washed twice in 70% ethanol, and paraffin-embedded. For H&E staining, tissue sections were de-paraffinized with two exchanges of xylene, rehydrated in 100%, 95%, and 70% ethanol respectively. After a brief wash in water, sections were stained in Harris hematoxylin for 8 min, washed in running water for 1 min, soaked in 0.2% ammonia water for 1 min. After washing in running water for 5 min, the tissue sections were rinsed in 95% ethanol for 10 s. Sections were then counterstained with eosin solution for 30 s, dehydrated in 95% ethanol, 100% ethanol, and

 