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Profiling of human myotubes reveals an intrinsic proteomic signature associated with type 2 diabetes

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ABSTRACT

The development of insulin resistance and type 2 diabetes (T2D) involves a complex array of metabolic defects in skeletal muscle. An in vitro cell culture system excludes the acute effects of external systemic factors existing in vivo. Thus, we aimed to determine whether intrinsic differences in the protein profile exist in cultured myotubes derived from T2D versus normal glucose tolerant (NGT) healthy people. Applying two dimensional difference gel electrophoresis technology (2-D DIGE), the abundance of 47 proteins differed in myotubes derived from T2D patients versus NGT donors. Proteins involved in fatty acid and amino acid metabolism, TCA cycle, mitochondrial function, mRNA processing, DNA repair and cell survival showed higher abundance, while proteins associated with redox signaling (PARK7; Parkinson disease 7), glutathione metabolism (glutathione S-transferase, GST, isoforms T1, P1 and M2), and protein dynamics (heat shock protein, HSP, isoform B1 and 90A) showed reduced abundance in myotubes derived from T2D versus NGT donors. Consistent with our proteome analysis results, the level of total glutathione was reduced in myotubes obtained from T2D versus NGT donors. Taken together, our data provide evidence for intrinsic differences in the profile of proteins involved in energy metabolism, cellular oxidative stress, protein dynamics and gene regulation in myotubes derived from T2D patients. These differences thereby suggest a genetic or epigenetic influence on protein content level, which can be further investigated to understand the molecular underpinnings of T2D progression and lead to new therapeutic approaches.

1. Introduction

Type 2 diabetes (T2D) is a metabolic disease characterized by derangements in glucose and lipid homeostasis in insulin-sensitive organs such as liver [1], adipose tissue [2] and skeletal muscle [3]. Skeletal muscle accounts for over 80% of insulin-stimulated glucose uptake, and impairments in insulin action on non-oxidative glucose metabolism in this tissue are among the earliest metabolic defects in T2D [4].

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Substantial evidence from proteomic and genomic studies suggests that metabolic defects exist in skeletal muscle from people with T2D versus normal glucose tolerance (NGT) [5–10]. A broad spectrum of cellular defects, including mitochondrial function, fatty acid metabolism and inflammation have been observed in skeletal muscle from T2D patients [11,12]. Due to the complexity of T2D, greater insight into mechanisms underlying the development of skeletal muscle insulin resistance is warranted, due to the important role of this tissue in the maintenance of whole body glucose, amino acid and lipid homeostasis [13–15].

T2D and related metabolic diseases impart a coordinated, progressive dysfunction in skeletal muscle that is manifested through alterations in both local gene transcription [16] and circulating metabolites and hormones [17,18]. Thus, the inter-individual variation, and the influence of external systemic factors such as hormones, cytokines and metabolites, which may influence the identification of inherent T2D-related differences, must be taken into consideration when performing a global profiling of proteins in skeletal muscle to detect T2D-specific signatures. Primary differentiated myotubes display many features of mature skeletal muscle [19]. Thus culturing satellite cells has become a useful research model to study molecular mechanisms underlying cellular and physiological processes such as cell growth, differentiation, apoptosis and the regulation of specific gene expression in skeletal muscle. In spite of the non-similarity to a whole mature muscle phenotype, differentiated human myotubes may also maintain the diabetic phenotype, as evidenced by impaired glucose metabolism and insulin action [7,20,21]. Another advantage of primary differentiated myotube cultures is the higher protein extraction yield acquired from cells verses the amount typically obtained from small muscle biopsies. Therefore, in vitro studies of cultured primary differentiated human myotubes may reveal intrinsic differences in metabolism and protein abundance in T2D [20].

The genome-to-protein system functions in a coordinated manner to maintain metabolism and cell protein homeostasis. Quantitative proteomics has served as a helpful tool in the characterization of cellular processes or diseases, such as T2D in skeletal muscle [22–24]. However, the use of primary tissue is a major limitation in clinical OMICS studies due to inter-individual variability since low technical variability is essential when clinical material is studied [25,26]. Few studies have investigated the proteome of primary cultured myotubes derived from people with T2D [27]. For cell culture-based comparative proteomic studies, different methods have been used, such as the isobaric peptide tags for relative and absolute quantification (iTRAQ), the metabolic labeling technique, stable isotope labeling of amino acids in cell culture (SILAC), as well as the quantitative 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE). Quantitative data from SILAC has shown to be consistent with data obtained by 2-D DIGE [28]. However, due to the restriction on serum and amino acid content in the SILAC technology, 2-D DIGE can be used as a platform for accurate quantification of large number of cellular proteins through normalization at the individual protein level. Thus, we used 2-D DIGE, followed by the liquid chromatography–mass spectrometry (LC–MS) to identify intrinsic proteome differences in cultured myotubes derived from skeletal muscle biopsies obtained from T2D patients.

Table 1 – Clinical characteristics of the study participants.

	NGT (10)	T2D (10)
Age (year)	60.4 ± 1.8	60.7 ± 1.5
BMI (kg/m^2)	27.7 ± 0.7	29.6 ± 1.2
Waist (cm)	99.6 ± 2.2	101.2 ± 2.5
Systolic blood pressure (mmHg)	135.0 ± 4.3	142.5 ± 4.3
Diastolic blood pressure (mmHg)	86.0 ± 3.3	82.3 ± 1.6
Plasma glucose (mmol/L)	5.3 ± 0.1	7.5* ± 0.2
OGTT 2 h glucose (mmol/L)	6.9 ± 0.2	13.5* ± 1.0
HbA1c (%)	4.7 ± 0.0	5.8* ± 0.3
Insulin (pmol/L)	52.3 ± 7.2	50.2 ± 8.6
Cholesterol (mmol/L)	5.4 ± 0.4	4.4* ± 0.2
HDL (mmol/L)	1.7 ± 0.2	1.3 ± 0.1
LDL (mmol/L)	3.4 ± 0.4	2.5* ± 0.2
Triglycerides (mmol/L)	1.3 ± 0.2	1.3 ± 0.1
Hemoglobin (g/L)	148.4 ± 3.1	146.2 ± 2.7

Results are mean ± SEM. BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin, A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
* $p < 0.05$, T2D versus NGT.

2. Materials and methods

2.1. *Human subjects*

A cohort of age- and BMI-matched normal glucose tolerant NGT (10) and T2D (10) male volunteers were selected for study. Clinical characteristics, including morphometric measurements, urine analysis, blood chemistry and measurements of blood pressure, were assessed at Karolinska University Hospital, Stockholm, Sweden (Table 1). Biopsies were obtained from the *vastus lateralis* portion of the *quadriceps femoris* muscle. All protocols were approved by the ethical committee at Karolinska Institutet and informed consent was received by all participants.

2.2. *Human skeletal muscle cell culture*

Satellite cells were isolated from skeletal muscle biopsies derived from NGT and T2D individuals by trypsin-EDTA digestion and cultured as described previously [29]. Myoblasts were propagated in growth medium (F12/DMEM, 20% FBS, 1% PeSt and 1% fungizone) (Gibco, Invitrogen, Sweden), and differentiated at >80% confluence in medium (DMEM-1 g/L glucose, 2% FBS, 1% PeSt and 1% Fungizone). Experiments presented in this study were performed on cultured myoblasts (passages 2–5 of cell cultures derived from either T2D patients or NGT individuals, with no skewed distribution between the groups on number of passages), that were differentiated at >80% confluence in a 150 mm Petri dish for 6 days and serum-starved for 24 h prior to harvest. For the metabolic assays, myoblasts were seeded in 6 well plates, and differentiated at >80% confluence. The serum-free media was refreshed after 18 h and all experiments were terminated after 6 h in fresh medium. All chemical reagents were purchased from Sigma–Aldrich Sweden AB, if not otherwise indicated.