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Review

Proteomic study of skeletal muscle in obesity and type 2 diabetes: progress and potential

Rikke Kruse\textsuperscript{1,2}, Kurt Højlund\textsuperscript{1,2}

\textsuperscript{1}The Section of Molecular Diabetes and Metabolism, Department of Clinical Research and Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark

\textsuperscript{2}Steno Diabetes Center Odense, Odense University Hospital, Odense, Denmark

Correspondence:

Kurt Højlund

Steno Diabetes Center Odense, Odense University Hospital, Kløvervænget 10, DK-5000 Odense, Denmark.

Email: kurt.hoejlund@rsyd.dk

Phone: +45 25320648
Abstract

Introduction: Skeletal muscle is the major site of insulin-stimulated glucose uptake and imparts the beneficial effects of exercise, and hence is an important site of insulin resistance in obesity and type 2 diabetes (T2D). Despite extensive molecular biology-oriented research the molecular mechanisms underlying insulin resistance in skeletal muscle remain to be established.

Areas covered: The proteomic capabilities have greatly improved over the last decades. This review summarizes the technical challenges in skeletal muscle proteomics studies as well as the results of quantitative proteomic studies of skeletal muscle in relation to obesity, T2D, and exercise.

Expert commentary: Current available proteomic studies contribute to the view that insulin resistance in obesity and T2D is associated with increased glycolysis and reduced mitochondrial oxidative metabolism in skeletal muscle, and that the latter can be improved by exercise. Future proteomics studies should be designed to markedly intensify the identification of abnormalities in metabolic and signaling pathways in skeletal muscle of insulin-resistant individuals to increase the understanding of the pathogenesis of T2D, but more importantly to identify multiple novel targets of treatment of which at least some can be safely targeted by novel drugs to treat and prevent T2D and reduce risk of cardiovascular disease.

Keywords: Skeletal muscle, type 2 diabetes, subcellular fractionation, quantitative proteomics, mitochondria
1.0 Introduction

Skeletal muscle is a highly-specialized tissue that accounts for ~40% of the total body mass in healthy humans [1]. Skeletal muscle plays a prominent role in the metabolic homeostasis and directly imparts the beneficial effects of exercise [2]. In both healthy individuals and patients with type 2 diabetes (T2D), exercise training improves insulin sensitivity [3-6] and increases mitochondrial oxidative capacity in skeletal muscle by enhancing mitochondrial biogenesis [3,4,7], mainly through activation of peroxisome proliferator-activated receptor-γ co-activator (PGC)-1α [8,9]. Skeletal muscle is the major site of insulin-stimulated glucose uptake [10]. As such, skeletal muscle is quantitatively the predominant site of peripheral insulin resistance in obesity and T2D [11], which are associated with an increased risk of cardiovascular disease (CVD). Insulin resistance in human skeletal muscle is characterized by a reduced insulin-stimulated glucose uptake and metabolism, increased accumulation of lipids, as well as reduced content and functional capacity of mitochondria [11-15]. Molecular biology-oriented research has generated a large amount of knowledge in this field. However, we do still not fully understand the cellular or molecular mechanisms underlying insulin resistance in skeletal muscle in obesity or T2D.

While hypothesis-driven investigations targeting a few genes or proteins of interest have indeed elucidated important details of the complex mechanisms leading to insulin resistance and T2D, hypothesis-free global approaches, such as microarray-based transcriptional profiling and quantitative proteomic analyses, have the advantage of visualizing patterns in the molecular signatures of complex diseases like T2D, which may be used to generate new hypotheses. Unbiased and large-scale tandem mass spectrometry (MS/MS)-based analyses of the total protein content, the proteome, and post-translational modifications (PTMs) at a given time and state are thus potent tools in the characterization of a metabolic state or a disease. Despite continuous improvements in the technical and analytical capabilities of mass spectrometry (MS), a comprehensive characterization and quantification of mammalian proteomes is still a challenging task [16], with the skeletal muscle proteome and PTMs being particularly challenging [17,18]. The skeletal muscle proteome has a large dynamic range due to the presence of both highly abundant contractile proteins and low abundant regulatory proteins [19] and combined with the heterogeneous nature of skeletal muscle, this makes it challenging, if not impossible to detect and quantify all proteins in the skeletal muscle proteome, even with the currently available techniques. Nevertheless, over the past decade, the technical advances in MS [20] and the improved tools for bioinformatic analysis [21] have driven the remarkable progress of proteomic science. With the present review, we aim to address the challenges faced when carrying out large-scale MS-based proteomics in human skeletal muscle. We furthermore aim to summarize the existing knowledge on the proteomic signature of human skeletal muscle in insulin-resistant conditions such as obesity.
and T2D, and to present suggestions to novel studies within this field. Unless otherwise mentioned, we will focus on large-scale quantitative proteomic studies on human skeletal muscle biopsies in vivo from individuals with insulin resistance due to either obesity or T2D.

2.0 Strategies for quantitative proteomic studies of human skeletal muscle

The development of quantitative proteomic approaches has shifted protein research from studies targeting a few proteins of interest into a global approach making it possible to identify and quantify a large number of proteins and PTMs in a given sample in an unbiased manner.

Some of the quantitative proteomic studies of skeletal muscle from individuals with obesity and T2D have applied gel-based techniques such as two-dimensional gel electrophoresis (2-DE) and two-dimensional difference gel electrophoresis (2D-DIGE), and we will therefore briefly introduce these techniques. 2-DE separates highly complex protein samples according to their pl and molecular weight. Gel-based proteomics in general gives an excellent coverage of soluble proteins, while being biased against hydrophobic proteins, such as membrane and nuclear proteins, as well as highly acidic or basic proteins [22,23]. On the negative side, 2-DE separation is limited by its inherent resolving power, and the presence of highly abundant proteins, such as structural proteins in skeletal muscle, may distort certain areas of the gel and thus disturb proper identification and quantification of other proteins in the same area [22,23]. 2-DE may be used to quantify changes in the abundance of the detected proteins when combining e.g. Coomassie blue or silver staining and software-based analysis of gel images with MS for protein identification. However, coomasie blue staining has a quite low sensitivity in protein detection, while silver staining displays improved sensitivity but may interfere with MS-based protein identification [23,24].

The development of 2D-DIGE, which involves labeling of proteins with multiplexed fluorescent dyes, greatly increased the sensitivity and reproducibility in 2-DE-based proteomics [23,24]. Each sample is labeled with a distinct fluorescent cyanine dye and can easily be differentiated by imaging after protein separation by 2-DE, which allows running up to three samples on the same gel, thus avoiding gel-to-gel variability.

Despite the advances in protein quantification using gel-based strategies, the extensive improvements in MS instrumentation, including improved sensitivity, resolution, and a higher sequencing speed [16] as well as the development of advanced gel-free techniques, have made MS-based proteomics the method of choice for both protein identification and quantification. Various methods have been developed for protein quantification, both label-based and label-free approaches. Chemical labeling using isobaric tags, such as tandem mass tag (TMT) and isobaric tags for relative and absolute quantitation (iTRAQ), was developed to enable relative quantification of protein abundances between samples. Isobaric labeling with TMT and iTRAQ is similar in
concept. The isobaric tags are added to the samples after proteolytic digestion after which the samples are mixed and subjected to fractionation and MS/MS analysis for quantification [20]. Due to the isobaric nature of the tags, an identical peptide from each sample will appear as a single peak in the MS spectra, whereas the individual tags produce a distinct reporter ion in the MS/MS spectra, thus enabling a relative comparison between samples [20]. Relative quantification using isobaric tags is accurate, however, it is an expensive approach and it only enables comparison of a limited number of samples (4- and 8-plex with iTRAQ and 10-plex with TMT) [20,25]. When compared to chemical labeling using isobaric tags, relative quantification using a label-free approach is cost-efficient and easy to handle. It either takes advantage of spectral counting, that is counting the number of peptide-to-spectrum matches obtained for each protein, or by measuring the average MS/MS signal intensities [20,25]. Label-free quantification enables comparison of an unlimited number of samples, however, it is associated with a higher variation (determined as the coefficient of variation) since the samples are prepared individually and do not have a labeled internal standard [20,25]. The higher variation may cause small differences between groups to be missed when using this approach for quantification.

The proteomic studies of human skeletal muscle biopsies that we will address in the following sections of this review take advantage of a bottom-up proteomic approach with data-dependent acquisition, in which the most abundant precursor ions are selected for fragmentation. This reduces reproducibility and additionally, leads to loss of a large proportion of the information, especially in complex samples [26]. Targeted proteomic approaches, such as selected reaction monitoring (SRM) or parallel reaction monitoring (PRM), allows for consistent high-sensitive identification and quantification of multiple proteins that are preselected prior to data acquisition [27,28]. These approaches may be particularly useful in hypothesis-driven investigations, including clinical and translational studies, which often focuses on biomarkers and requires large sample cohorts because of the biological variability [29]. Contrary to strategies based on data-dependent acquisition, data-independent acquisition-based strategies, such as sequential window acquisition of all theoretical spectra (SWATH), involves fragmentation of all detected precursor ions and therefore does not involve any loss of information [30,31]. In SWATH, peptide identification relies on the use of ion libraries created from verified MS/MS spectra that specifically identify the peptides of interest and since all precursor ions are fragmented, SWATH support both discovery-mode and hypothesis-driven investigations [29]. While discovery-mode proteomics is still essential in the characterization of e.g. the skeletal muscle proteome but also the plasma proteome of patients with T2D, targeted proteomic approaches on e.g. plasma samples could in the future detect biomarkers that may be useful in the prevention and/or early detection of the metabolic syndrome and insulin resistance prior to the development of T2D.
2.1 Strategies for phosphopeptide enrichment and phosphoproteomic studies

It is a challenging task to detect PTMs in a complex sample but the development of techniques for selective enrichment of PTMs has greatly improved their coverage in proteomic studies. Based on the number of publications, protein phosphorylation is by far the most studied PTM [32]. It is one of the most abundant PTMs and is known to be involved in a variety of cellular functions, including cell signaling, metabolism, protein degradation, and cell differentiation [33,34]. In the present review, we will focus on the phosphoproteome as this has been characterized in human skeletal muscle [35-37].

The low stoichiometry of most phosphoproteins (ratio of phosphorylated to non-phosphorylated peptides) represents a huge analytical challenge in proteomic research since complex biological samples consist of thousands of proteins differing in abundance over four or more orders of magnitude [38]. Enrichment of phosphopeptides prior to MS-based analysis will therefore greatly improve the outcome. There are numerous approaches available for phosphopeptide enrichment, including strong cation exchange chromatography (SCX), immobilized metal affinity chromatography (IMAC), metal oxide affinity chromatography using titanium dioxide (TiO$_2$), and calcium phosphate precipitation (CPP) [38,39]. In this review, we will only briefly describe phosphopeptide enrichment using TiO$_2$ as this has been the enrichment method of choice in the human skeletal muscle proteomic studies presented in a later section of this review. We refer to some of the many excellent reviews on this topic for additional information [38,39].

TiO$_2$-mediated chromatography has evolved as a commonly used strategy for phosphopeptide enrichment due to its high recovery and selectivity [40-42]. It is generally assumed that TiO$_2$ has a preference for mono-phosphorylated peptides and the level of multi-phosphorylated peptides enriched by this technique is therefore quite low. It has been suggested that TiO$_2$ binds multi-phosphorylated peptides but that their high binding affinity makes them difficult to elute [43]. Contrary to TiO$_2$, IMAC has a preference for multi-phosphorylated peptides [41,43] and Thingholm et al. therefore introduced sequential IMAC (SIMAC) in which the two modes of phosphopeptide enrichment are combined to obtain a complete coverage of both mono- and multi-phosphorylated peptides from complex samples [43]. No enrichment procedure ensures a complete coverage of the phosphoproteome, in particular not in samples of high complexity. Most large-scale phosphoproteomics studies therefore take advantage of other peptide fractionation strategies, such as SCX or hydrophilic interaction chromatography (HILIC), in combination with TiO$_2$ for selective phosphopeptide enrichment [35-37,44,45]. In a study by Zhao et al., four different phosphopeptide enrichment strategies (TiO$_2$ batch mode, TiO$_2$ micro column, CPP + TiO$_2$ batch mode, and HILIC + TiO$_2$ batch mode) showed distinct phosphopeptide recognition patterns and identified different phosphorylated peptides from the same proteins (encoded by the genes VDAC1, VDAC2, IMMT,
and CKMT2) in mitochondria isolated from human skeletal muscle [36]. This illustrates the need for complementary phosphopeptide enrichment methods in discovery-mode phosphoproteomics. In a more recent study, we took advantage of TiO$_2$-based phosphopeptide enrichment followed by fractionation of the enriched phosphorylated peptides using capillary-HILIC prior to liquid chromatography (LC)-MS/MS analysis [37]. This appeared to give a higher throughput and specificity compared to HILIC fractionation followed by TiO$_2$ enrichment [36,37]. Especially in small and complex biological samples, the combination of different enrichment approaches will maximize the coverage of phosphopeptides from individual proteins.

2.2 Subcellular fractionation
Subcellular fractionation is a useful approach to enhance the number of proteins and/or PTMs identified and quantified in a proteomic study as it greatly reduces sample complexity. As stated in the introduction, skeletal muscle insulin resistance is associated with a reduced content and functional capacity of mitochondria [13-15] and the present paragraph will therefore focus on the isolation of mitochondria.

Global proteomic studies of whole-cell and tissue extracts have successfully identified thousands of proteins and phosphorylation sites. However, work in our and other groups has shown that use of isolated mitochondrial fractions significantly improves the identification of mitochondrial peptides and proteins by MS/MS in human skeletal muscle when compared to studies on whole-cell and whole-tissue extracts [35,46,47]. In two studies using isolated, functional mitochondria from human skeletal muscle, Lefort et al. and Zhao et al. could assign 59-75% of the peptide MS/MS spectra to mitochondrial proteins [36,46], while a proteomic characterization of whole-cell lysates from human skeletal muscle only assigned 8% of the MS/MS peptide spectra to mitochondrial proteins [47].

One of the challenges when performing proteomic studies on isolated organelles, such as mitochondria, is to obtain reliable and reproducible data. The methods used for e.g. preparation of pure and functionally viable mitochondria are obviously crucial as any mitochondrial preparation with intact matrix space and outer membranes will be exposed to extra-mitochondrial contamination. The mitochondrial purity and functionality may be evaluated in various ways, including western blotting of markers of various subcellular compartments for contamination with non-mitochondrial proteins and enrichment of mitochondrial proteins [46,48], assessment of respiratory measurements [36,46,49,50], membrane potential measurements [50], cytochrome c oxidase assay [49], and/or electron microscopy for morphological examination [48].

Another matter to consider when isolating mitochondria from skeletal muscle is the fact that muscle contains two distinct mitochondrial subpopulations, the subsarcolemmal and the
intermyofibrillar mitochondria, which are present just beneath the sarcolemma and between the myofibrillar, respectively. Several studies have suggested that the two mitochondrial subpopulations differ functionally but also in their morphology [51,52]. Of particular interest to the present review is the fact that Ritov et al. found a subsarcolemmal-specific reduction in the mitochondrial electron transport chain activity in skeletal muscle from patients with T2D and in obese non-diabetic individuals when compared with lean healthy individuals [53].

As stated in the beginning of this paragraph, proteomic characterization of isolated mitochondria could be a useful approach to enhance the number of proteins and/or PTMs identified and quantified in a proteomic study on whole-tissue extracts. Nevertheless, an important matter to consider is the fact that when using isolated mitochondria for proteomic studies it is important to keep in mind that the isolation procedure requires a relatively large sample size and that it may cause some disruption of the mitochondrial structure and morphology, thereby altering the physiological state of the mitochondria [49,54]. This may influence the mitochondrial proteome identified.

2.3 Challenges in skeletal muscle proteomic analysis

As stated in the introduction, skeletal muscle is a highly-specialized tissue [1] and it is responsible for voluntary body movements and is indispensable in the regulation of whole-body energy metabolism. Aside from the technical challenges in large-scale proteomic experiments, also physiological and biological parameters contribute to the challenges faced when carrying out proteomic investigations of human skeletal muscle biopsies.

Human skeletal muscle biopsies are highly heterogenous and are often contaminated with other cell types such as adipocytes, neurons, and proteins originating from connective tissue, blood, and blood vessels. Besides contamination arising from other cells types and tissues, human skeletal muscle is usually composed of different fiber types, which contribute to the heterogeneity of the samples. Generally, adult human skeletal muscle fibers can be classified as either slow oxidative (type I), fast oxidative (type 2A), or fast glycolytic (type 2X) fibers [55]. The various muscle fiber types are metabolically different, which should be considered when performing studies of human skeletal muscle biopsies. The fiber type composition differs from one type of muscle to another [56] and it may be remodeled by hormonal and metabolic changes as well as by muscle activity [57]. Despite the metabolic differences between muscle fiber types and the different muscle fiber type compositions in various human muscles, most proteomic studies performed so far have utilized muscle biopsies from the vastus lateralis muscle [35-37,46,47,58-66], which is a mixed fiber muscle. However, since MS-based studies have revealed differences in the proteome of
human vastus lateralis, trapezius, and deltoideus muscle [67,68], findings should be extended from one group of muscle to another with caution.

The heterogeneity may be circumvented by studying isolated single muscle fibers. Recently it was found that an MS-based proteomics approach can be used for fiber-type assignment in skeletal muscle biopsies from mice [68]. Interestingly, this study by Murgia et al. revealed fiber-type-specific characteristics showing that the abundance of proteins involved in oxidative metabolism, β-oxidation, and the tricarboxylic acid (TCA) cycle was fiber-type-dependent in mice skeletal muscle [68]. This is consistent with findings in biceps muscle biopsies from healthy humans where experiments in isolated single fibers have revealed metabolic heterogeneity among muscle fibers as there appears to be a higher prevalence of glycolytic enzymes in type 2 fibers and an increased content of oxidative enzymes in type I fibers [69]. Recently, the fiber-type-specific protein content and phosphorylation-dependent regulation of components in the insulin signaling cascade and/or metabolic enzymes were investigated in skeletal muscle biopsies from healthy lean and obese individuals and from patients with T2D [70]. This study indicated that human type I muscle fibers have a higher capacity for glucose handling, but a similar insulin-mediated regulation of protein phosphorylation. Thus, Albers et al. found impaired insulin-stimulated phosphorylation of Akt at Ser473 and Thr308 in muscle from patients with T2D, but with a similar reduction in type I and II fibers [70]. This study only characterized the protein abundance and the phosphorylation of relatively few proteins of interest, whereas an unbiased quantitative MS-based approach could have revealed multiple novel fiber-type-specific differences in protein abundance and phosphorylation in skeletal muscle of patients with T2D and weight-matched healthy controls. Nevertheless, Albers et al. propose a model in which human type I fibers appears to have a higher abundance of protein related to glucose transport, the phosphorylation of glucose, and glucose oxidation compared to type II fibers [70]. This molecular fiber-type specific differences may explain the positive correlation between proportions of type I fibers in muscle and whole-body insulin sensitivity that has been described in humans [71-73]. Furthermore, a decreased proportion of type I fibers has been found in various insulin-resistant states, including obesity [74,75] and T2D in some [73-75] but not all studies [71,76]. This insulin resistance-associated change in fiber-type composition was not evident on the proteins measured by Albers et al. [70]. It should be mentioned that although proteomic studies on isolated single fibers may provide us with a more homogenous sample and thus allow a more accurate characterization of the skeletal muscle proteome, the small protein amount available in these experiments will make it a challenge to characterize PTMs on single fiber level.

The very high amount of structural proteins and the wide dynamic range of proteins and enzymes found in skeletal muscle possess major challenges in skeletal muscle proteomics. This
became evident in a study by Deshmukh et al. in which the dynamic range of the proteomes of mice skeletal muscle and cultured C2C12 myotubes was found to be spread over eight orders of magnitude [19]. In mice muscle, the top 10 most abundant proteins made up 50% of the total protein mass, with proteins annotated to the contractile machinery accounting for 53.6% of the total protein mass. In the other end of the scale, the lower half of the proteome accounted for less than 0.1% of the total protein mass. The presence of such high-abundance proteins widens the dynamic range of the skeletal muscle proteome and has contributed to the limited coverage of the human skeletal muscle proteome so far. The technical developments in MS-based proteomics presented previously in this manuscript will greatly improve the proteome coverage, and thus the knowledge gained from future MS-based characterizations of human skeletal muscle.

3.0 Quantitative proteomic studies of skeletal muscle in obesity and T2D

Insulin resistance in skeletal muscle of patients with T2D greatly contributes to the overall disease manifestation. Over the last decade, both gel- and MS-based proteomics has been used to characterize the proteome of healthy human skeletal muscle [17,35,47,59-61,77]. Recently, The Human Skeletal Muscle Proteome Project was initiated, which aims to characterize proteins in skeletal muscle and how they change with ageing and disease. As a first initiative, a systematic review of peer-reviewed studies was performed, and we refer to this review for an extensive overview of the published skeletal muscle proteomic research in general [17]. In the present section, we will elaborate on the quantitative proteomic studies published on human skeletal muscle biopsies from individuals with insulin resistance such as obesity or T2D. The studies are summarized in Table I. The identity of the proteins mentioned below will be given by either the name of protein and/or the gene name encoding them.

More than a decade ago, we combined 2-DE with silver staining, computerized image analysis and matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS to identify changes in the skeletal muscle proteome in T2D. We identified 11 proteins that showed altered abundance in skeletal muscle of patients with T2D compared with age- and gender-matched control individuals [58]. The increased abundance of the stress-inducible heat shock proteins 78 kDa glucose-regulated protein (GRP78) and heat shock protein 90 (HSP90) β as well as an increased content of the major extracellular matrix protein collagen type VI, α1-chain (COL6A1) pointed toward increased muscle stress in skeletal muscle of patients with T2D. Interestingly, we also identified reduced abundance of a slow muscle protein isoform (MYL2), whereas a fast muscle protein isoform (MYLPF) showed increased abundance in skeletal muscle of patients with T2D, thus suggesting an altered fiber type distribution in these. Furthermore, an increased content of phosphoglucomutase 1, the enzyme facilitating the interconversion between glucose-1-
phosphate and glucose-6-phosphate, suggested an altered balance between glycogen metabolism and glycolysis and could indicate a more glycolytic phenotype in skeletal muscle of patients with T2D [58]. Consistent with these findings suggesting an altered metabolism, we found a reduced protein content and altered phosphorylation at Thr213 in the nucleotide-binding region of the catalytic β-subunit in the ATP synthase complex (ATP5B) in skeletal muscle of patients with T2D [58]. Using a targeted proteomic approach including immunoprecipitation of the ATP synthase β-subunit and high-performance liquid chromatography (HPLC) coupled to MS/MS, we subsequently discovered seven potential phosphorylation sites [78]. Two of these sites (Thr213 and Tyr361) showed reduced phosphorylation in the basal state in skeletal muscle in obesity and T2D [78]. These changes occurred concurrently with a reduction in protein content of subunits of complex I (ND6), II (SDHA), III (UQCRC1), and V (ATP5B and ATP5A1) in the mitochondrial electron transport chain, thus indicating an abnormal mitochondrial function and reduced content in obesity and T2D [78].

Consistent with our findings suggesting a shift toward a glycolytic metabolism phenotype in skeletal muscle of individuals with insulin resistance and T2D [58], Hittel et al. demonstrated increased abundance and activity of adenylate kinase 1 (AK1) as well as a higher abundance of the glycolytic enzymes aldolase A (ALDOA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in obese and morbidly obese insulin-resistant women compared with lean women when using 2-DE, coomassie blue staining, image analysis, and MALDI-TOF MS to quantify differences in the cytosolic proteome of rectus abdominus muscle [79]. The subcellular fractionation used in the study by Hittel et al. focused on extraction of cytosolic proteins, which may explain the lack of differences in the abundance of mitochondrial proteins between the groups in this study. Nevertheless, Hittel et al. concludes that their findings may represent a compensatory glycolytic drift to counteract a reduced mitochondrial function, which is consistent with data from enzyme studies of skeletal muscle from obese individuals [80] and patients with T2D [81].

In a study from 2010, Hwang et al. investigated the proteome in skeletal muscle biopsies from patients with T2D and lean and obese non-diabetic individuals. Muscle lysate proteins were separated using one-dimensional gel electrophoresis (1-DE), with protein assignment using HPLC-electrospray ionization (ESI)-MS/MS and label-free quantification based on the normalized spectral abundance factor (NSAF) method [62]. In total, 1218 proteins were assigned in at least one of the 24 participants. Of these, 92 proteins were increased or decreased by 2-fold in at least one of the two insulin-resistant groups (obesity or T2D) versus lean controls, and 15 of these proteins were significantly (p<0.05) changed. The study demonstrated an increased abundance of chaperone proteins (HSP90 and T complex 1 isoforms) and proteins involved in proteasomal degradation (Cullin 5 and proteasomal subunits). This is consistent with the findings from in our initial proteomic
characterization of the skeletal muscle proteome in individuals with T2D [58]. Importantly, Hwang et al. [62] also identified a decreased abundance of several mitochondrial proteins (UQCRCCQ, COX6C, and CHCHD3), which is consistent with several previous studies that have reported abnormalities in mitochondrial oxidative metabolism, also termed mitochondrial dysfunction, in skeletal muscle in obesity and T2D [13-15,62,82-86].

To further characterize the abnormalities in skeletal muscle mitochondria associated with insulin resistance, Lefort et al. assessed mitochondrial bioenergetics and reactive oxygen species (ROS) production rates combined with identification of the mitochondrial proteome in insulin-resistant skeletal muscle by combining mitochondrial isolation, 1-DE, HPLC-ESI MS/MS, and quantification using the NSAIFs method [63]. As stated in a previous paragraph, use of purified mitochondrial fractions significantly increases the identification of mitochondrial peptides and proteins by MS/MS in human skeletal muscle compared to studies on whole-cell and whole-tissue extracts [35,46,47,63]. While no intrinsic abnormalities in fuel oxidation were identified between groups, the production of ROS was increased and the total mitochondrial protein and citrate synthase activity per muscle wet weight suggested a reduced mitochondrial content in skeletal muscle of obese insulin-resistant individuals compared with lean controls [63]. The quantitative MS/MS-based study of the muscle mitochondrial proteome identified altered abundance of 21 proteins per mitochondrial mass in insulin-resistant obese individuals, including a lower abundance of several complex I subunits, which may explain the increased ROS production identified in these individuals. Muscle mitochondria from insulin-resistant, obese individuals were furthermore found to have a lower abundance of carnitine palmitoyltransferase 1β, which may explain the intramuscular accumulation of lipids associated with insulin resistance [87]. Additionally, isolated mitochondria from insulin-resistant skeletal muscle showed a lower abundance of enzymes involved in branched-chain amino acid metabolism (ALDH6A1 and PCCB). Consistently, other metabolomic studies in humans have suggested that insulin resistance and T2D are associated with alterations in branched-chain amino acid metabolism [88,89].

In the latest attempt to characterize T2D-related alterations in the skeletal muscle proteome, Giebelstein et al. took advantage of protein separation and quantification by 2D-DIGE and protein identification using nano-HPLC/ESI-MS/MS to compare the skeletal muscle proteome of patients with T2D with healthy lean and obese non-diabetic individuals [64]. In this study, 25 proteins were found to be at least 1.5-fold and significantly (p<0.05) altered in T2D and/or obesity versus lean individuals in either the basal or insulin-stimulated state. The abundance of multiple glycolytic proteins (GAPDH, PGAM2, ENO3, PKM2) and fast muscle protein isoforms (MYL1, MYLPF, TNNT3) was higher in obese non-diabetic individuals and patients with T2D when compared with the lean non-diabetic individuals. Consistent with these findings, the abundance of most protein
spots containing glycolytic enzymes and fast muscle protein isoforms correlated negatively with the insulin-stimulated glucose disposal rate and glucose oxidation, while correlating positively with insulin-stimulated lipid oxidation. In contrast to the glycolytic proteins, proteins involved in mitochondrial functions (ECH1, GBAS, HADHB, HES1) and slow muscle protein isoforms (MYL2, MYL3, TNNT1) showed reduced abundance in the insulin-resistant groups. The abundance of these proteins and other mitochondrial proteins involved in the TCA cycle and mitochondrial respiration correlated positively with the insulin-stimulated glucose disposal rate and/or the non-oxidative glucose metabolism.

Combined, the data presented by Giebelstein et al., in line with the other proteomic studies presented in the section above, suggest that obesity and T2D are associated an increased abundance of glycolytic proteins, fast muscle protein isoforms, muscle stress proteins, and proteins involved in proteasomal degradation, while there appears to be a reduced abundance of mitochondrial proteins and slow muscle protein isoforms (Figure 1). These findings are consistent with microarray-based transcriptomic studies of skeletal muscle from insulin-resistant individuals indicating a switch toward a glycolytic phenotype as well as a reduced mitochondrial content with respect to both the TCA cycle and oxidative phosphorylation in skeletal muscle [82-84]. This supports the hypothesis that altered glycolytic and oxidative capacities are important molecular signatures of insulin resistance in skeletal muscle in obesity and T2D.

3.1 Exercise-mediated regulation of the muscular proteome
Insulin sensitivity is directly related to the degree of physical activity and regular physical exercise is an effective physiological intervention to improve insulin sensitivity as well as mitochondrial content and function in skeletal muscle of insulin-resistant individuals and to prevent/postpone the manifestation of T2D in healthy individuals [90,91]. A single bout of exercise can enhance insulin sensitivity and glucose tolerance for up to 12-48 hours after completion of an exercise session in both healthy individuals [92,93], obese individuals [94,95], and in patients with T2D [96], although not all studies demonstrate this [97,98]. A few proteomic studies have been carried out to increase our understanding of the molecular mechanisms underlying the beneficial effects of exercise on skeletal muscle metabolism in healthy individuals [59-61]. Holloway et al. were the forerunners in the characterization of the exercise training-mediated changes in the human skeletal muscle proteome. In 2009, they did a protein expression profiling using either 2-DE and MALDI-TOF/TOF MS or 1-DE and LC-MALDI-TOF/TOF MS with iTRAQ labelling for quantification. They found that 6-weeks high-intensity interval exercise training led to an increased abundance of metabolic enzymes (KCRM, ENOB, ATPA, ATPB, DHSA, and ECHA), myofibrillar proteins (TNNT1 and TNNT3), and the heat shock protein CH60 (HSPD1), while the intervention reduced the abundance
of the heat shock protein β-1 (HSPB1) [61]. Egan et al. used 2D-DIGE for quantification and LC-MS/MS for protein identification to determine the effect of 14 days of endurance exercise training on stationary bike on isolated mitochondrial proteins of skeletal muscle biopsies from young, healthy, sedentary males [59]. The 2D-DIGE analysis identified 31 protein spots showing a change in protein abundance after either 7 or 14 days of training. Among the proteins that increased in abundance in response to 14 days of endurance exercise training were components of the respiratory complex I (NDUFA5, NDUFA8, and NDUFA13) and complex V (ATP5A1 and ATP5B), the TCA cycle (MDH, FH, DLD, and DLST), creatine kinase S-type, mitochondrial (CKMT2), and adenylate kinase 3 (AK3). Additionally, endurance exercise training reduced the abundance of proteins involved in muscle contraction (ATP2A1, MYLPF, and TRIM72) and respiratory complex IV (COX5A). The findings from Egan et al. suggest that exercise training induces mitochondrial remodeling. Combined, the studies by Holloway et al. and Egan et al. suggest that an exercise training improves mitochondrial metabolism and oxidative phosphorylation, thus underlining its importance in the prevention and treatment of T2D. In the most comprehensive characterization of the exercise-mediated effects on the skeletal muscle proteome Schild et al. subjected five endurance-trained athletes and five untrained individuals to a 1-h cycle ergometer intervention at 80% VO2 max and analyzed the skeletal muscle lysates using HPLC-ESI-MS/MS for protein identification and the MaxQuant software for label-free quantification [60]. The abundance of 71 and 42 proteins were significantly affected by the exercise intervention in endurance-trained and untrained individuals, respectively. Only three proteins were found significantly regulated by exercise in both groups, thus reflecting the diversity of the molecular response to exercise and possibly also the complexity of carrying out in vivo proteomic experiments on human muscle biopsies. The abundance of 92 proteins differed between endurance-trained and untrained individuals in the basal resting state of which almost half of them could be assigned to the skeletal muscle mitochondrial proteome. More specifically, Schild et al. found an increased abundance of proteins involved in oxidative phosphorylation, the TCA cycle, as well as fatty acid transport, utilization, and β-oxidation in endurance trained individuals when compared with untrained individuals at rest. This study, therefore, reflects the fact that training status is associated with differences in abundance of key energy metabolism proteins. A recently published systematic review and meta-analysis found that only three proteins involved in mitochondrial oxidative phosphorylation (NDUFAB, NDUFA13, and ATPB) have been found significantly regulated by exercise in more than one study identifying exercise-mediated changes in the skeletal muscle proteome of healthy individuals [99]. This once again underlines the complexity of carrying out proteomic studies in human skeletal muscle and the fact that specific findings are extremely
difficult to reproduce due to the large genetic variability in humans and an apparent lack of reproducible methods for sample preparation.

In 2013, Hussey et al. described the effect of exercise on the skeletal muscle proteome in patients with T2D using 1-DE, HPLC-ESI MS/MS, and the NSAFs method for quantification. In these patients with T2D, only the abundance of 17 muscle proteins were significantly changed in response to exercise, of which 12 proteins showed increased and five decreased abundance [66]. The muscle proteins that showed exercise-mediated changes were related to energy metabolism (LDHB, CKMT2, GOT2, ACAT1, UQRC2, SUCLA2, FH, HADHA, IDH2, PFKM, ALDOA, and GLO1), the cytoskeleton (ANXA2 and PDLIM3), or had a currently unknown function in skeletal muscle. To our knowledge, the study by Hussey et al. is the only study reporting the effect of exercise on the proteome in skeletal muscle of individuals with T2D. Future studies in this area therefore holds the possibility of elucidating whether exercise, also on the proteome level, induces similar adaptions in skeletal muscle in patients with T2D as in healthy individuals.

3.2 PTMs in skeletal muscle of patients with T2D
Several molecular defects have been identified in insulin signalling to both glucose transport and glycogen synthesis in skeletal muscle of insulin-resistant conditions such as obesity, polycystic ovarian syndrome, and T2D using targeted analysis of a few enzymes by classical protein chemistry [11]. As reviewed recently [11], impaired insulin-mediated activation of the intrinsic tyrosine kinase of the insulin receptor, tyrosine phosphorylation of insulin receptor substrate (IRS)-1, IRS-1 associated phosphoinositide 3-kinase (PI3K) activity, Akt phosphorylation and activity, as well as glycogen synthase dephosphorylation and activity have been documented in skeletal muscle of patients with T2D in some but not all studies [11]. However, in addition to these defects in insulin signalling, it is highly likely that several other metabolic and signalling pathways are abnormally regulated in insulin-resistant skeletal muscle and these may be identified in large-scale proteomic studies designed to characterize and quantify PTMs.

We have previously characterized the phosphoproteome of healthy human skeletal muscle [35] and of isolated muscle mitochondria in the resting, basal state [36,37] and in the insulin-stimulated state [37]. In 2009, we carried out the first large-scale in vivo phosphoproteomic study of skeletal muscle from lean, non-diabetic volunteers. We took advantage of phosphopeptide enrichment using SCX and TiO₂ followed by protein identification using HPLC-ESI-MS/MS [35]. In two additional studies on isolated muscle mitochondria from healthy individuals, we used TiO₂ alone or in combination with HILIC or CPP for phosphopeptide enrichment and HPLC-ESI-MS/MS for protein identification [36,37]. These studies identified several phosphorylation sites in proteins involved in mitochondrial metabolism [36,37], sarcomeric function [35], glycolysis [35], and
glycogen metabolism [35]. Although these studies increased the understanding of skeletal muscle metabolism, they had a limited proteome coverage compared to what is possible today. The functionality and importance of all of these phosphorylation sites remains to be elucidated as do their occurrence in e.g. conditions of insulin resistance. To our knowledge, there has been no studies quantifying changes in the skeletal muscle phosphoproteome, or any other PTM for that matter, in response to physiological insulin concentrations nor in individuals with obesity or T2D compared with lean individuals. In future studies, it will be of great interest to quantify and relate the changes in e.g. mitochondrial phosphorylation sites with measures of ATP synthesis, substrate oxidation, and insulin sensitivity. Such studies may expand our understanding of the molecular pathogenesis of insulin resistance in obesity and T2D.

Recently, Hoffman et al. carried out a global analysis of the phosphoproteome in skeletal muscle biopsies from untrained healthy males before and after a single bout of high-intensive exercise using quantitative multiplexed isobaric labeling and phosphopeptide enrichment coupled to MS/MS. In this extensive study, Hoffman et al. isobarically labelled peptides with iTRAQ or TMT tags for quantification and enriched phosphopeptides using TiO2 chromatography and SIMAC. HILIC was used to separate the unbound non-phosphorylated and phosphorylated fractions even further after which the samples were analyzed using nano-ultra HPLC-MS/MS [65]. This study revealed an extensive exercise-mediated regulation of the human skeletal muscle phosphoproteome as 1004 unique phosphopeptides were found regulated on 562 proteins. Although these sites included substrates of known exercise-regulated kinases, such as 5’-AMP activated protein kinase (AMPK), protein kinase A (PKA), calcium/calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinase (MAPK), and mechanistic (previously referred to as mammalian) target of rapamycin (mTOR), many of the identified phosphorylation sites and their predicted upstream kinases have not previously been implicated in exercise signaling. The impressive number of identified and regulated phosphopeptides identified by Hoffman et al. is indeed promising for future proteomic studies in human skeletal muscle as it provides an insight into the possibilities and insight gain from the use of the protocols, machinery, and tools for data analysis available today.

4.0 Conclusion
In conclusion, current available quantitative proteomic studies suggest that obesity and T2D are associated with an increased abundance of glycolytic proteins, fast muscle protein isoforms, muscle stress proteins, and proteins involved in proteasomal degradation as well as a reduced abundance of mitochondrial proteins and slow muscle proteins. These changes in the skeletal muscle proteome indicate a switch toward a glycolytic phenotype and impaired mitochondrial
oxidative metabolism in skeletal muscle of insulin-resistant individuals. This supports the hypothesis that altered glycolytic and oxidative capacities are important molecular signatures of insulin resistance in skeletal muscle in obesity and T2D, whereas a causal role for these changes, if any, in the pathogenesis of T2D remains to be established. Interestingly, quantitative proteomic studies of human skeletal muscle also indicate that exercise interventions improve the mitochondrial oxidative metabolism, thus underlining the importance of exercise in the prevention and treatment of T2D.

Although the quantitative proteomic studies summarized above have described several differences in protein abundance between insulin-sensitive and insulin-resistant human skeletal muscle and in response to exercise, the findings reflect the proteomic technologies available at that time. These studies are therefore likely to have a limited proteome coverage and less robust quantifications. Characterization of the skeletal muscle proteome of individuals with obesity and T2D using the mass spectrometers, fractionation strategies, and computational tools available today will strengthen the findings presented above and most likely identify novel cellular pathways and functions altered in these individuals.

5.0 Expert commentary
Insulin resistance plays a major role for the increased risk of T2D and CVD. Targeted treatment of insulin resistance is, however, non-existing, except for weight-loss and physical activity. This is a key weakness in the current treatment options for T2D and may explain the inability of current antidiabetic drugs to robustly reduce the risk of CVD morbidity and mortality in patients with T2D despite clear reductions in blood glucose levels. So far, hypothesis-driven, targeted studies of proteins/enzymes known to be involved in insulin action on glucose metabolism have increased our understanding of some of the molecular defects underlying insulin resistance in skeletal muscle. However, it has not helped us to develop an effective and safe treatment of insulin resistance. A much deeper understanding of the complex cellular and molecular mechanisms underlying insulin resistance in skeletal muscle is therefore strongly needed. The current wide gaps of knowledge within this field can only be filled out by an increased and improved application of the most recent and forthcoming developments in quantitative MS/MS-based proteomic technologies and equipment, possibly in combination with other novel global approaches such as deep RNA sequencing, metabolomics, genetics, and epigenetics. A challenge when working particularly with skeletal muscle is the complex nature of this organ, which will influence the proteomic data gained from studies on human skeletal muscle biopsies. Since mitochondrial dysfunction is often associated to insulin resistance and T2D, proteomic characterization of isolated functional mitochondrial may be a strategy for reducing sample complexity, thus providing
knowledge that is otherwise missed. Another approach that may be used to reduce sample complexity was suggested by Albers et al., who characterized the skeletal muscle proteome of patients with T2D in isolated fibers. This is a strategy that should be considered in future studies as it substantially reduces sample complexity. It may not be a feasible strategy in all studies as it requires a larger amount of biopsy material but given the fiber-type switch often identified in skeletal muscle of patients with obesity and/or T2D, it may be of particular importance in these studies to obtain a more informed proteomic analysis. Finally, the use of data-independent acquisition-based strategies, such as SWATH, will also extend the depth of proteome coverage and thus, the information gained from proteomic studies. It is foreseen that the combination of several improved strategies for tissue sample preparation, subcellular fractionation and enrichment of PTMs together with enhanced MS/MS based targeted and global quantification of changes in protein abundance and PTMs will lead to the identification of multiple novel proteins and PTMs associated with insulin resistance in skeletal muscle. At the same time, the development of targeted proteomic strategies, such as PRM and SRM, will enable validation of potential interesting findings from both targeted molecular characterizations of specific proteins and/or PTMs but also from discovery-mode proteomic studies on larger populations to confirm their importance in relation to obesity and T2D. Hopefully many of these can be safely targeted by novel drugs to reduce insulin resistance and hence prevent the development or treat T2D and reduce the increased risk of CVD.

6.0 Five-year view: A speculative viewpoint on how the field will evolve in 5 years

As evidenced in the study by Hoffman et al. [65], the improvements in the mass spectrometers, the development of strategies for more accurate quantification of protein abundances, PTMs, and differences between groups as well as the improved strategies for enrichment of PTMs and the rapid development of tools and databases used for data analysis have greatly expanded the amount of information that may be gained from future proteomic experiments.

We believe that the translational research combining biopsy material and metabolic characteristics from patients, e.g. with T2D, and the novel capabilities within the field of proteomic research will allow us to make substantial progress in the understanding of the dysfunctional molecular mechanisms in skeletal muscle of patients with T2D. The combination of proteomic data on e.g. the regulation of proteins involved in mitochondrial oxidative metabolism and measurements of mitochondrial respiration, substrate oxidation, and insulin sensitivity will substantially increase our understanding of the metabolic consequences of the molecular dysfunction. Characterization of the abovementioned parameters in e.g. pre-diabetic individuals could provide information as to whether the molecular alterations identified in skeletal muscle of
patients with T2D are in fact the cause or consequence of insulin resistance, and eventually type 2 diabetes. Furthermore, the use of single fibers for proteomic analysis may yield relevant and important information that is otherwise blurred by the use of a muscle biopsy with a mixed fiber-type composition.

So far, no studies have reported the skeletal muscle phosphoproteome of patients with T2D nor how insulin or exercise alters the phosphoproteome in skeletal muscle of patients with T2D when compared to healthy individuals. Such studies will provide novel information and understanding on the molecular mechanisms underlying T2D. Importantly, it is increasingly recognized that other PTMs may play an important role in the regulation of cellular metabolism. An organ-wide map of lysine acetylation in rats have demonstrated that lysine acetylation has a cellular prevalence comparable to protein phosphorylation [100]. How protein acetylation is acutely regulated by e.g. physiological insulin concentrations or how it is influenced in skeletal muscle of patients with T2D remains to be determined. The advancement in all aspects of proteomic research will hopefully also allow us to consider how the various PTMs interact since this crosstalk may reveal a novel level of regulation that are not recognized today.

Finally, the rapid development of metabolomics will allow us to understand the connection between molecular dysfunctions, the metabolite pattern, and/or the whole-body metabolic measurements such as substrate oxidation and insulin sensitivity. Combined, these studies will further enhance our understanding of the molecular causes and consequences of T2D and will potentially reveal novel targets for drug development and/or early disease detection and prevention.

**Key issues**

- The heterogeneous composition, the presence of various muscle fiber types, and the wide dynamic range of protein abundance in skeletal muscle possess a major challenge in skeletal muscle proteomics
- Gel- and mass spectrometry-based quantitative proteomic studies suggest that individuals with obesity and T2D have an increased abundance of glycolytic proteins, fast muscle protein isoforms, muscle stress proteins, and proteins involved in proteasomal degradation in their skeletal muscle, while there appears to be a reduced abundance of mitochondrial proteins and slow muscle protein isoforms
- Skeletal muscle proteomics suggest that an exercise intervention improves mitochondrial oxidative metabolism, thus underlining its importance in the prevention and treatment of T2D
• Characterization of the mitochondrial phosphoproteome in the basal- and insulin-stimulated state in healthy individuals revealed a large number of phosphorylation sites in proteins involved in mitochondrial metabolism, sarcomeric function, glycolysis, and glycogen metabolism. To our knowledge, there are no studies characterizing the phosphoproteome, or any other PTM, in skeletal muscle of patients with T2D

• An acute exercise intervention was found to induce changes in 1004 unique phosphopeptides on 562 proteins in human skeletal muscle. Although these sites included substrates of known exercise-regulated kinases, such as AMPK, PKA, CaMK, MAPK, and mTOR, many of the identified phosphorylation sites and their predicted upstream kinases have not previously been implicated in exercise signaling. Whether a similar effect can be seen in skeletal muscle from patients with T2D remains to be determined

• The use of single fibers should be considered for future mass spectrometry-based characterizations of human skeletal muscle as it may reveal novel information regarding the molecular abnormalities associated with obesity and insulin resistance

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References

Papers of special note have been highlighted as:

* of interest

** of considerable interest


** An extensive review of studies of the skeletal muscle proteome in humans as a first initiative in the Human Proteome Project


* This paper describes the technical limitations of skeletal muscle proteomics


26. Michalski A, Cox J, Mann M. More than 100,000 detectable peptide species elute in single shotgun proteomics runs but the majority is inaccessible to data-dependent LC-MS/MS. J Proteome Res. 2011 Apr;10(4):1785-1793.


44. McNulty DE, Annan RS. Hydrophilic interaction chromatography reduces the complexity of the phosphoproteome and improves global phosphopeptide isolation and detection. Mol Cell Proteomics. 2008 May;7(5):971-980.


** A comprehensive characterization of the skeletal muscle proteome in patients with T2D versus obese and lean non-diabetic individuals


* An study that characterizes how exercise training alters the skeletal muscle proteome in patients with T2D


** This paper reports muscle fiber type specific differences in insulin signaling and metabolic enzymes in lean and obese non-diabetic individuals and patients with T2D


* A comprehensive systematic review and meta-analysis of changes in the human skeletal muscle proteome in obesity and T2D and in response to exercise training

**Figure 1: Proteomic signature of skeletal muscle in obesity and type 2 diabetes**

A summary of the proteins and related pathways showing altered (increased ↑ or reduced ↓) abundance in skeletal muscle in obesity and T2D in the gel- or MS-based quantitative proteomic studies by Højlund et al. [58] (blue), Hittel et al. [79] (yellow), Hwang et al. [62] (orange), Lefort et al. [63] (red), and Giebelstein et al. [64] (green) with colors indicating the study or studies in which the abundance of a protein was altered.
Table 1: Quantitative proteomic studies of skeletal muscle in obesity and type 2 diabetes

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Muscle type</th>
<th>Participants/intervention</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-DE Silver staining Image analysis MALDI-TOF MS</td>
<td>m. vastus lateralis</td>
<td>Patients with T2D: n=9 Matched controls: n=6</td>
<td>Abundance of 8 proteins (in 11 protein spots) was significantly altered in T2D.</td>
<td>Hojlund et al. 2003 [58]</td>
</tr>
<tr>
<td>2-DE Coomassie blue staining Image analysis MALDI-TOF MS</td>
<td>m. rectus abdominis (cytosolic extracts)</td>
<td>Lean women: n=6 Obese women: n=6 Morbidly obese women: n=6</td>
<td>Abundance of 3 protein spots was significantly increased in obese and morbidly obese</td>
<td>Hittel et al. 2005 [79]</td>
</tr>
<tr>
<td>1-DE HPLC-ESI MS/MS NSAF</td>
<td>m. vastus lateralis</td>
<td>Lean individuals: n=8 Obese individuals: n=8 Patients with T2D: n=8</td>
<td>Abundance of 15 proteins was 2-fold and significantly altered either in obesity and/or T2D.</td>
<td>Hwang et al. 2010 [62]</td>
</tr>
<tr>
<td>1-DE HPLC-ESI MS/MS NSAF</td>
<td>m. vastus lateralis (isolated mitochondria)</td>
<td>Lean individuals: n=20 Obese individuals: n=14</td>
<td>Abundance of 21 proteins was significantly altered in obesity</td>
<td>Lefort et al. 2010 [63]</td>
</tr>
<tr>
<td>2D-DIGE Fluorescence staining Image analysis nano-HPLC/ESI-MS/MS</td>
<td>m. vastus lateralis</td>
<td>Lean individuals: n=10 Obese individuals: n=11 Patients with T2D: n=10</td>
<td>Abundance of 25 proteins (in 44 protein spots) was 1.5-fold and significantly altered in either obesity and/or T2D before or after insulin.</td>
<td>Giebelstein et al. 2012 [64]</td>
</tr>
<tr>
<td>1-DE HPLC-ESI MS/MS NSAF</td>
<td>m. vastus lateralis</td>
<td>Patients with T2D: n=6 4 weeks exercise training</td>
<td>Abundance of 17 proteins was altered in response to exercise training in T2D</td>
<td>Hussey et al. [66]</td>
</tr>
</tbody>
</table>

ESI Electrospray ionization, HPLC High performance liquid chromatography, MALDI Matrix-assisted laser desorption/ionization, MS Mass spectrometry, MS/MS Tandem mass spectrometry, NSAF Normalized spectral, abundance factor, TOF Time-of-flight, T2D Type 2 diabetes, 2D-DIGE Two-dimensional difference gel electrophoresis, 1-DE One-dimensional gel electrophoresis, and 2-DE Two-dimensional gel electrophoresis
Fig. 1. Proteomic signature of skeletal muscle in obesity and type 2 diabetes

Glycolysis
PGM1
PGAM2
ALDOA
ENO3
GAPDH
PKM2

Muscle stress
HSP90AB1
CDC37
CCT4
HSPA5
PDIA3
CCT8
COL6A1
HSPA2
TRIM72

Fast muscle isoforms
MYLPF
MYH1
MYL1
MYH2
TNNT3

Proteasomal degradation
PSMB3
CUL5

Mitochondrial function
ATP5B
ECH1
NDUFC2
COX5B
UQCRQ
HES1
NDUFS2
ALDH6A1
COX6C
GBAS
NDUFA8
CPT1B
CHCHD3
HADHB
NDUFA8
PCCB
NDUFA11

Slow muscle isoforms
MYL2
MYL3
TNNT1