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Protein Phosphatase 2a Function And Regulation In Insulin Resistant Human Skeletal Muscle Cells

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PROTEIN PHOSPHATASE 2A FUNCTION AND REGULATION IN INSULIN RESISTANT HUMAN SKELETAL MUSCLE CELLS

by

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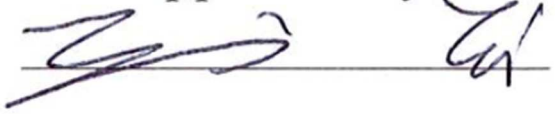

DEDICATION

To my family who supported me throughout my educational career

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CHAPTER 1: INTRODUCTION

1.1 Introduction to Diabetes and Insulin Signaling Pathway

1.1.1 Introduction to Diabetes

Diabetes is a metabolic disorder characterized by hyperglycemia, high blood glucose levels. In 2014, 29.1 million people in the United States had diabetes, out of which 21 million were diagnosed and 8.1 million were undiagnosed [1]. Prediabetes is a condition in which the blood sugar is high but not high enough to be considered diabetic and an estimated 86 million people in the U.S has prediabetes [1]. In 2013, diabetes was ranked as the seventh leading cause of death in the United States [1]. Other than elevated blood glucose levels, there are many other complications associated with diabetes that affect a number of organs/tissues in the body. About half of all people with diabetes have some form of diabetic neuropathy, nerve damage caused by diabetes [2]. The chances of experiencing a stroke or developing heart disease are higher than average for people with diabetes [3]. High levels of blood sugar, over time, can also lead to diabetic nephropathy, a kidney disease [4]. Diabetic retinopathy occurs when there is a retina disorder caused by diabetes [5].

There are two major classifications of diabetes, type 1 and type 2. Type 1 diabetes accounts for about 5% of all diagnosed cases and Type 2 diabetes accounts for about 90% to 95% of all cases [1]. Type 1 diabetes, commonly known as juvenile diabetes, is when the body produces little or no insulin due to the loss of pancreatic beta cells. This type of diabetes is usually diagnosed in young adults and children. Type 2 diabetes is caused by relative insulin

deficiency and insulin resistance, which is a condition when the body does not use insulin properly. This type of diabetes is usually diagnosed in older adults.

Insulin is a hormone produced by the islet cells in the pancreas that regulates a wide variety of biological processes like metabolism, growth and cell survival [6]. The majority of insulin-stimulated glucose uptake occurs in the skeletal muscle [7]. Insulin resistance in the skeletal muscle is one of the major defects in type 2 diabetes [8].

1.1.2 Insulin Signaling Pathway

Insulin increases glucose uptake in the muscle and fat and thus decreases blood sugar levels [9]. It also increases the storage of energy reserves in fat, liver, and muscle through the stimulation of lipogenesis, glycogen synthesis and protein synthesis [10]. These cellular responses are a result of the activation of a number of different pathways that occur in the liver, muscle and fat cells. Insulin binds to the insulin receptor (IR), a tyrosine kinase receptor, inducing a structural change enabling autophosphorylation of the receptor to take place. Once phosphorylated, the receptor phosphorylates various tyrosine residues on docking proteins [e.g., insulin receptor substrate 1 (IRS1), Shc and adaptor protein with PH and SH2 domain (APS)] [11]. Tyrosine phosphorylation of these substrates initiates the signaling of the phosphoinositide-3 kinase/Akt (PI 3K) and Ras/MAPK pathways [12]. In the PI 3K pathway, activated Akt/PKB increases insulin-stimulated translocation of a glucose transporter (GLUT4) in the muscle and adipose tissues [13]. Cell growth-related gene expression is regulated by the MAPK pathway through a signaling cascade that is also activated by insulin [14].

1.2 Protein Phosphatase 2A (PP2A) Regulation and Effects on Insulin

Reversible phosphorylation of proteins is a major mechanism that regulates a large number of intracellular events (like metabolism, transport and secretion, transcription and translation of genes) in eukaryotic cells [15]. Phosphatases carry out dephosphorylation by removing a phosphate group from phosphorylated serine, threonine and tyrosine residues, leading to activation or deactivation of substrate proteins.

PP2A is one of the most abundant serine/threonine protein phosphatases, and is responsible for regulating the activities of a large number of signal transduction proteins [16]. PP2A makes up as much as 1% of total cellular proteins and together with protein phosphatase 1 (PP1), accounts for >90% of all Ser/Thr phosphatase activities in most tissues and cells [17]. PP2A can exist in two different forms: a trimeric form [18] and a dimeric form [19]. The core enzyme, dimeric form, consists of a 36kDa catalytic subunit C (PP2AC) and a 65 kDa scaffold subunit A (PP2AA), while the trimeric form consists of the core enzyme and a 55kDa regulatory subunit B (PP2AB) [20] (Fig 2). There are 4 types of regulatory B subunits: B (B55/PR55), B' (B56/PR61), B'' (PR48/PR72/PR130) and B''' (PR93/PR110), while the A and C subunits exist in the α and β isoforms [17-20].

1.2.1 Regulation of PP2A

PP2A is regulated by subunit diversity, post-translational modifications, substrate protein interactions and auto-regulation [21]. Due to a large number of potential associations of the A and B regulatory subunits, a total of 75 dimeric and trimeric PP2A holoenzymes can be generated

[22]. The combination of the A, B and C subunit isoforms affects the activity and specificity of the PP2A complex against a particular substrate.

Experiments done in vivo and in vitro have shown that phosphorylation of tyrosine^{307} inhibited the activity of PP2Ac by preventing its interaction with the regulatory subunit [21-23]. In addition, methylation of Leu^{309} by leucine carboxylmethyltransferase 1 (LCMT1) has been shown to enhance the binding affinity of the A and C subunits to distinct regulatory subunits [21, 22].

Various cellular proteins such as I_1^{PP2A} and I_2^{PP2A} regulate the activity of PP2A by directly interacting with the free C subunit or with the AC core dimer [21-24]. Aside from cellular proteins, some small compounds found naturally such as okadaic acid can inhibit the enzymatic activity of PP2A [25].

1.2.2 PP2A in Insulin Signaling

The effect of insulin on PP2A abundance in the muscle of type 2 diabetic subjects was shown by Højlund et al. They showed that upon insulin stimulation in vivo, PP2A levels in control subjects were reduced (when compared to basal levels), but not in type 2 diabetic subjects [16].

Saturated fatty acids like palmitate have been shown to negatively regulate insulin signaling by activating PP2A [26-28]. Wu et al. observed a decrease in Thr^{172} phosphorylation of AMPK, a substrate of PP2A, when bovine aortic endothelial cells (BAECs) were incubated with palmitate [26]. In rat hepatocytes [27] and human skeletal muscle cells [28], treated with fatty acids, PP2A was seen to have been upregulated and Akt phosphorylation and activation was decreased.

PP2A activation was observed in INS-1 832/13, an insulin-secreting pancreatic β-cell line [29] and C2C12 skeletal myotubes [30] treated with ceramide. Cazzolli and associates observed a reduction in glycogen synthesis through the inhibition of phosphorylation on Akt/PKB upon insulin stimulation mediated through activated PP2A [30].

In the context of insulin signaling, PP2A interacts with various proteins involved in the signaling cascade (e.g. AKT, GSK3, etc.). In recent studies, 514 proteins were identified as PP2Ac interaction partners in pancreatic β-cells [31]. Of those 514 proteins, 89 proteins showed a significant difference in response to glucotoxic treatment [31]. Some of those interaction partners have been known to be involved in insulin secretion (Rab5c, RhoA), gluconeogenesis (WDR5) and glucose flux control (GFPT1) [31].

1.3 Insulin Sensitivity and Protein-Protein Interactions

1.3.1 Measuring Insulin Sensitivity

Insulin sensitivity can be measured directly using hyperinsulinemic euglycemic clamp and the insulin suppression test, and can be measured indirectly using minimal model analysis of frequently sampled intravenous glucose tolerance test (FSIVGTT) and oral glucose tolerance test (OGTT) [32].

Where the maintenance of steady-state conditions is crucial, the hyperinsulinemic euglycemic clamp is considered the “gold standard” for measuring insulin sensitivity because it directly measures the effects of insulin *in vivo* [33].

1.3.2 Protein-protein interactions (PPIs)

Protein-protein complexes are an important component of various biological functions such as cell cycle regulation, gene transcription, signal transduction [34], etc. Correct formation of these complexes is necessary for normal body function. Abnormality in the formation of these complexes can lead to irregular cell signals and ultimately cause diseases. Currently, scientists are targeting protein complexes to treat diseases like Huntington's disease [35]. Studying PPIs can help us understand the function of a particular target protein and analyze the signaling pathways.

PPIs can be classified as homo-oligomeric and hetero-oligomeric based on the type of protein units involved; non-obligate and obligate based on the stability of the structure, and transient and permanent based on the lifetime of the complex [36].

These interactions can be detected using biochemical methods (like co-immunoprecipitation and chemical cross-linking) and biophysical methods (like NMR Spectroscopy and X-ray crystallography). The most commonly used high-throughput methods are yeast two-hybrid screening and affinity purification coupled to mass spectrometry [37-39].

1.4 Mass Spectrometry

Mass spectrometry is a powerful approach for the identification, characterization and quantification of proteins [40]. This is a very sensitive technique that can be used to identify compounds as low as 1 part per trillion in chemically complex mixtures and as small as 10^{-12} g for a compound of mass 1000Da [40]. The main components of a mass spectrometer include an ion source, a mass analyzer and a detector.

1. Ion Source: This device generates charged particles and the two commonly used ion sources are Matrix-Assisted Laser Desorption Ionization (MALDI) and Electrospray Ionization (ESI) [40].
2. Mass Analyzer: This is used to separate ions based on their mass-to-charge ratio. A few commonly used mass analyzers include quadrupole, ion trap, orbitrap analyzers, etc. [40].
3. Detector: This device is used to record the charge induced when the ion hits a surface (for example the electron multiplier) or record the current produced when the ion passes by (for example the image current detector) [40].

CHAPTER 2: RESEARCH DESIGN AND METHODS

Clinical and proteomics data acquisition and data analysis used for this project were illustrated in Fig. 3. First, we recruited subjects and performed comprehensive screening tests (Visit 1) to ensure patient eligibility. This was then followed by in-patient clinical tests (Visit 2) which included the hyperinsulinemic-euglycemic clamp and muscle biopsies. The skeletal muscle cells collected were analyzed in the following order: homogenization of the biopsy sample; immunoprecipitation of PP2Ac; the eluate were subjected to in-solution trypsin digestion, peptide extraction and UPLC-nanoESI-MS/MS analysis to identify co-immunoprecipitating proteins. Immunoprecipitation of NIgG (non-specific control) was carried out to minimize the possibility of obtaining false positive results.

2.1 Materials

2.1.1 Reagents

Reagents were acquired from these suppliers: sequencing-grade modified trypsin (Promega, Madison, WI); protein A sepharose, protein G-agarose and iodoacetamide (Sigma, St Louis, MO); C18 ziptip (Millipore, Billerica, MA); Normal mouse IgG antibody, anti-PP2A C-subunit antibody and PP2A Immunoprecipitation phosphatase assay kit (Millipore, Billerica, MA).

2.1.2 Subjects

A total of 8 volunteers –glucose tolerant lean controls (LC)—were recruited and took part in the study at the Clinical Research Center at Wayne State University. The study was explained

in detail, including potential risks, to all participants and written consent was obtained before participation. None of the participants engaged in heavy exercise, nor did they have any significant medical problems. All forms of exercise were stopped at least 2 days before the study. The protocol was approved by the Institutional Review Board of Wayne State University.

2.1.3 Hyperinsulinemic-Euglycemic Clamp with muscle biopsies

To determine insulin sensitivity and expose the skeletal muscle to insulin *in vivo*, a hyperinsulinemic-euglycemic clamp was used, as previously described [41]. Having the participant fast for a minimum of ten hours overnight, the study began around 08:30 hours (time -60 mins). One catheter was placed in each arm, one to maintain the infusion of insulin and glucose throughout the study; the second was used to collect blood to observe the participants blood glucose levels. At 09:00 hours (time -30mins), a biopsy of the vastus lateralis muscle was performed. As soon as possible, the biopsy samples were cleaned of blood, connective tissue and fat, and then transferred to ice cold PBS for primary cell culture. At 09:30 hours (time 0 mins), a second muscle biopsy was taken and then continuous human insulin (Humulin R; Eli Lilly, Indianapolis, IN) infusion began and continued for 120 mins. The plasma glucose was measured every five 5 minutes throughout the clamp. Throughout the insulin infusion, euglycemia was maintained at ~90 mg/dl. At 11:30 hours (time 120), the third muscle biopsy was taken and the insulin infusion was shut off.

2.2 Sample Preparation and Analysis

2.2.1 Sample Preparation

Cultured cells from the skeletal muscle biopsies were generated from the 8 human muscle biopsies, and myoblasts were differentiated into myotubes in normal or hyperinsulinemic hyperglycemic condition; Each condition was stimulated with or without insulin for 15 minutes before harvesting the cells (cells were treated with the HGHI media to induce insulin resistance [41-43]). Therefore, there were four sets of samples: low glucose no insulin without acute 15-min insulin treatment (LGNI BAS), low glucose no insulin with acute 15-min insulin treatment (LGNI INS), high glucose high insulin without acute 15-min insulin stimulation (HGHI BAS) and high glucose high insulin with acute 15-min insulin stimulation (HGHI INS). Cells were then collected, homogenized and processed as described [44-46]. The lysate proteins were precleared with NIgG and then followed by a PP2Ac immunoprecipitation. The immunoprecipitates were washed three times in 700µL TBS. Once all the TBS solution was removed, the immunoprecipitates were boiled in 30µL SDS buffer and transferred to a 0.5mL centrifugal filter unit in a collection tube. The samples were then washed three times with 200µL 8M Urea, followed by three washes with 200µL 40mM ammonium bicarbonate (ABC). In solution trypsin digestion was then carried out by adding 50µL trypsin in ABC and incubated overnight at 37°C on a shaker. More ABC was added to the sample, and supernatant containing tryptic peptides were transferred to a collection tube, and placed in a vacuum centrifuge until the peptides were completely dried. The peptides were reconstituted in 0.1% TFA in water and purified using a C18 ziptip. UPLC-nanoESI analysis using an Orbitrap Fusion Lumos, as previously described [44], was performed.

2.2.2 Sample Data Analysis

Using the MaxQuant software [47-50], peptide/protein identification and quantification were performed. Using label-free quantification (LFQ), peak areas for each protein was obtained and only proteins with a minimum of 2 unique peptides and a false discovery rate (FDR) at 0.01 were considered. In total, 1933 proteins were identified in the 32 samples in addition to the two isoforms of PP2Ac, PP2Acα and PP2Acβ. To be considered as a PP2A interaction partner, the following criteria needed to be satisfied: i) an enrichment ratio >10 and 1063 out of the 1933 proteins satisfy this criterion; ii) identified with LFQ peak area in more than half of the PP2Ac IP (i.e. >16 out of 32 samples used), and 202 out of the 1063 proteins satisfy this criterion, and were classified as PP2Ac interaction partner. The enrichment ratio was calculated as follows:

First, peak area for a protein identified in a sample was normalized against the sum of the peak areas for all the proteins identified in that sample to obtain a normalized ratio for the individual protein, *Norm: i*.

$$Norm{:}\ i = \frac{PA_i}{\sum_1^n PA_i}$$

Then, the average of normalized ratio for each protein in the PP2Ac IP, *Average_Norm: i_PP2Ac*, as well as the average of normalized ratio for the same protein in the NIgG IP, *Average_Norm: i_NIgG*, were obtained. Finally, Average_Norm: i_PP2Ac was divided by Average_Norm: i_NIgG, which gives the enrichment ratio for each protein.

$$Enrichment\ Ratio{:}\ i = \frac{\text{Average\_Norm: i\_PP2Ac}}{\text{Average\_Norm: i\_NIgG}}$$

Since NIgG was employed as a negative control, proteins exclusively detected in the PP2Ac immunoprecipitates were classified as PP2Ac interaction partners. However, this would lead to false negatives because the high sensitivity of our approach would identify a trace amount

of a protein non-specifically absorbed on the NIgG beads. However, if this protein is a true PP2Ac interaction partner, higher peak area will be assigned to this protein in the PP2Ac sample than in the NIgG sample.

To determine the relative quantities of PP2Ac interaction partners in the samples, the peak area for each protein identified in a specific sample was normalized against the peak area for PP2Ac identified in the same sample, *Norm: j*.

$$Norm: j = \frac{PA_j}{PA\_PP2Ac}$$

The normalization strategy is widely used in proteomics studies involving protein-protein interactions [49]. The normalized peak area for each PP2Ac interaction partner, Norm: j, was compared among the 4 sets of samples to assess the effects of acute insulin stimulation and /or chronicle hyperinsulinemia and hyperglycemia on protein-protein interactions involving PP2Ac.

2.2.3 Statistical Analysis

Although a large number of proteins were assigned in at least one of the 32 samples that were studied, a number of filters were applied to narrow down the total number of proteins that were used to compare the effects of acute insulin stimulation and /or chronicle hyperinsulinemia and hyperglycemia among the 4 sets of samples (Figure 4). Statistical calculations were performed using the paired t-test and changes were considered significant at $p<0.05$.

CHAPTER 3: RESULTS

Clinical characteristics of the eight glucose tolerant lean control participants are listed in Table 1. The participants' average BMI is below 25kg/m^2, HbA1c below 5.7 %, fasting plasma glucose below 100 mg/dl, 2h OGTT below 100 mg/dl, indicating there are indeed lean healthy non-diabetic controls. In addition, the mean M-value is at 11.0 mg/kg·min, suggesting they are insulin sensitive.

3.1 PP2Ac interaction partners in primary skeletal muscle cells from glucose tolerant lean human participants

PP2Acα and PP2Acβ were detected in PP2Ac immunoprecipitates from all 32 samples, but were not detected in all the NIgG immunoprecipitates. In total, 202 proteins met the criteria for classification as PP2Ac interaction partners (Figure 4 and Table 2).

Among the 202 interaction partners, 18 proteins were previously reported as PP2Ac interaction partners in islet beta cells. The known interaction partners include ACP1, CCDC6, DLST, GMPPB, IGBP1, NAMPT, PCBP2, PCBP3, PDHX, PPP2R1A, PPP2R2A, PPP4C, PPP4R2, PSMA6, SSBP1, TUBB6, VPS25 and VTA1.

DAVID pathway analysis on the 202 interaction partners and PP2Ac showed a number of pathways significantly enriched when compared to the whole genome background, such as pyruvate metabolism, cytoskeleton organization and FGF signaling pathway (Figure 5 and Table 3).

3.2 Nineteen interaction partners of PP2Ac with a significant difference among 4 treatment groups.

A total of 19 interaction partners showed a significant change in PP2Ac interaction among the 4 sets of samples (Table 4 and Figure 6). Under basal conditions (i.e., without acute 15-minute insulin stimulation), 6 proteins showed a significant change in PP2Ac interaction between the low glucose no insulin and chronicle hyperinsulinemia and hyperglycemia conditions (LGNI BAS vs HGHI BAS, $p<0.05$, Table 4 and Figure 6). Among these 19 partners, 2 proteins showed significant difference in PP2Ac interaction in LGNI condition in response to insulin stimulation (LGNI BAS vs LGNI INS, $p<0.05$, Table 4 and Figure 6). In HGHI conditions, 4 proteins showed significant difference in PP2Ac interaction in response to insulin stimulation (HGHI Bas vs HGHI INS, $p<0.05$, Table 4 and Figure 6). Upon acute insulin stimulation, 11 proteins showed significant difference in PP2Ac interaction in HGHI condition when compared to the LGNI condition (LGNI INS vs HGHI INS, $p<0.05$, Table 4 and Figure 6).

CHAPTER 4: DISCUSSION

4.1 PP2Ac interaction partners in primary skeletal muscle cells from glucose tolerant lean human participants

We have identified 202 PP2Ac interaction partners in the primary skeletal muscle cells of 8 glucose tolerant lean participants, using a proteomics approach developed in our laboratory [44]. Among them, 18 proteins are known PP2Ac interaction partners in islet beta cells recently reported by our group (Table 2).

From these 18 PP2Ac interaction partners, PPP2R1A and PPP2R2A are A subunit alpha isoform and B" subunit alpha isoform of the PP2A complex, respectively. DLST, dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex), is a part of the TCA cycle and is responsible for catalyzing the overall conversion of 2-oxoglutarate to succinyl-CoA and CO_2 [50]. PDHX, pyruvate dehydrogenase protein X component, is a part of the PDH complex which provides the primary link between glycolysis and the TCA cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA [51]. GMPPB, GDP-mannose pyrophosphorylase B, is an enzyme that catalyzes the reaction which converts mannose-1-phophatase and GTP to GDP-mannose [52]. GDP-mannose is necessary for some glycosylation pathways to occur. Glycosylation is a post translational modification of proteins that is important for proper protein folding [53]. IGBP1, immunoglobulin (CD79A) binding protein 1 and also known as alpha4, is involved in the regulation of the catalytic activity of PP2A by protecting its partially folded catalytic subunits from degradative polyubiquitination [54, 55]. VPS25, vacuolar protein-sorting-associated protein 25, is a protein that is part of the endosomal sorting complex required for transporting II (ESCRT-II), which functions in the sorting of ubiquitinated

membrane proteins during endocytosis [56]. VTA1, vacuolar protein sorting-associated protein VTA1 homolog, is a protein in the ESCRT-IV complex that works in trafficking multivesicular bodies [57, 58]. PSMA6, proteasome subunit alpha type-6, is a component of the 20S core proteasome complex, which is involved in the proteolytic degradation of most intracellular proteins [59]. NAMPT, nicotinamide phosphoribosyltransferase, belongs to the nicotinic acid phosphoribosyl transferase (NAPRTase) family and is involved in many biological processes, including metabolism, stress response and aging [60]. This protein catalyzes the condensation of nicotinamide with 5-phosphoribosyl-1-pyrophosphate to yield nicotinamide mononucleotide (an intermediate in the biosynthesis of NAD) [61]. SSBP1, single-stranded DNA-binding protein, is a protein that is involved in mitochondrial biogenesis and a decrease in mitochondrial function can lead to the aging, neurodegeneration and type 2 diabetes [62-64]. TUBB6, tubulin beta-6 chain, is a constituent of microtubules, which helps to control insulin secretion and a disturbance in this control can lead to beta cell dysfunction and type 2 diabetes [65]. PPP4C, serine/threonine-protein phosphatase 4 catalytic subunit, is shown to play a role in microtubule organization at centrosomes and PPP4R2, a regulatory subunit of PPP4C, helps regulate the activity of PPP4C [66]. PCBP2 and PCBP3, poly(rC)-binding protein 2 and poly(rC)-binding protein 3 respectively, are major poly(rC)-binding proteins that negatively regulates cellular antiviral responses mediated by the mitochondrial antiviral signaling pathway (MAVS) [67]. In connection to diabetes, studies have shown an association between viral infections, like hepatitis C, with diabetes [68].

4.2 Nineteen interaction partners of PP2Ac with a significant difference among 4 treatment groups.

Under basal conditions, 6 proteins showed a significant fold change in PP2Ac interaction among the low glucose no insulin and high glucose high insulin conditions (LGNI BAS vs HGHI BAS, $p<0.05$, Table 4 and Figure 6). Of these 6 proteins, 5 of them (ASTML, ESD, GLRX3, HINT1 and QKI) showed increased association while only GPX1 showed decreased association. QKI, protein quaking, is an RNA-binding protein that plays a central role in myelination, the formation of myelin sheath around a nerve fiber [69]. Abnormalities in myelin formation can lead to diabetic peripheral neuropathy [70]. GLRX3, glutaredoxin-3, is a part of the glutaredoxin family and binds to and modulates the function of PKC-theta [71]. PKC theta has been shown to induce insulin-mediated tightening of the muscles by inhibiting Akt [72]. GPX1, glutathione peroxidase 1, protects cells against oxidative damage and has been shown to be upregulated by IGF-1 in vascular endothelial cells [73]. ROS level elevation in diabetes may be due to decrease in destruction or increase in the production by glutathione peroxidase antioxidant [74]. HINT1 (histidine triad nucleotide-binding protein 1), a tumor suppressor gene, inhibits the Wnt/β-catenin pathway [75] and has been shown to interact with IRS1, insulin receptor substrate 1, along with PP2Ac in human skeletal muscle [44]. ESD, S-formylglutathione hydrolase, is an enzyme that is involved in the detoxification of formaldehyde [76].

Among these 19 partners, 2 proteins showed significant difference in PP2Ac interaction in the low glucose no insulin condition in response to insulin stimulation (LGNI BAS vs LGNI INS, $p<0.05$, Table 4). Both CCDC6 and GLRX3 showed increased association with PP2Ac. CCDC6 (coiled-coil domain containing 6), a tumor suppressor gene, is involved in apoptosis and

DNA damage response [77]. GLRX3, as mentioned previously, is a part of the glutaredoxin family and binds to and modulates the function of PKC-theta [71].

In hyperinsulinemia and hyperglycemia conditions, 4 proteins showed significant difference in PP2Ac interaction in response to insulin stimulation (HGHI BAS vs HGHI INS, $p<0.05$, Table 4). ACP1 and HINT1 both showed decreased association with PP2Ac, while CYCS and HSPB6 showed increased association with PP2Ac. HSPB6, heat shock protein beta-6, plays a role in regulating muscle function such as smooth muscle vasorelaxation and cardiac myocyte contractility [78]. CYCS (cytochrome c), a small heme protein, is known to be involved in electron transport chain in the mitochondria and involved in the initiation of apoptosis [79]. ACP1, low molecular weight phosphotyrosine protein phosphatase, is an acid phosphatase and protein tyrosine phosphatase that plays an important role in the control of insulin receptor activity [80].

Upon insulin stimulation, 11 proteins showed significant difference in PP2Ac interaction in the hyperinsulinemia and hyperglycemia condition when compared to the LGNI condition (LGNI INS vs HGHI INS, $p<0.05$, Table 4). All 11 proteins (TAK1L, DCAMKL1, ESD, GSS, HINT1, IGBP1, SEP11, TPPP3, UCK2, GRIPAP1 and S100A13) showed decreased association with PP2Ac. IGBP1, as previously mentioned, binds to PP2Ac to stabilize it [54, 55]. DCAMKL1, doublecortin Like Kinase 1, is a kinase involved in the Ca2+ signaling pathway controlling neuronal migration [81]. GRIPAP1, GRIP1-associated protein 1, functions as a JNK pathway scaffold protein, binding both JNK and the upstream kinase MEKK1 in neurons [82]. SEP11, septin-11, is a filament-forming cytoskeletal GTPase that plays a role in cytokinesis and vesicle trafficking [83]. TPPP3, tubulin polymerization-promoting protein family member 3, has microtubule binding activity and plays a role in cell proliferation and mitosis [84]. UCK2,

uridine-cytidine kinase 2, catalyzes uridine and cytidine phosphorylation to uridine monophosphate (UMP) and cytidine monophosphate (CMP), respectively [85]. GSS, Glutathione synthetase, is involved in the glutathione biosynthesis pathway [86]. Glutathione synthesis has been seen to be diminished in diabetic patients [87].

CHAPTER 5: SUMMARY

In the present work, PP2Ac was found to interact either directly or indirectly with 202 interaction partners in primary human skeletal muscle cells and 19 PP2Ac interaction partners were identified to show a significant change among the four treatment conditions (LGNI BAS, LGNI INS, HGHI BAS and HGHI INS). Validating the important partners that may affect the insulin signaling pathway (directly or indirectly) would be the next step. In addition, comparing PP2Ac interaction partners in primary human skeletal muscle cells with/out HGHI treatment with those from obese and type 2 diabetic participants will help to uncover the differences in the PP2Ac interaction partners among the 3 groups. With this information, we may have a better understanding of the role of PP2Ac in the insulin signaling pathway in primary human skeletal muscle cells and ultimately better knowledge on the development of insulin resistance and type 2 diabetes.

FIGURES

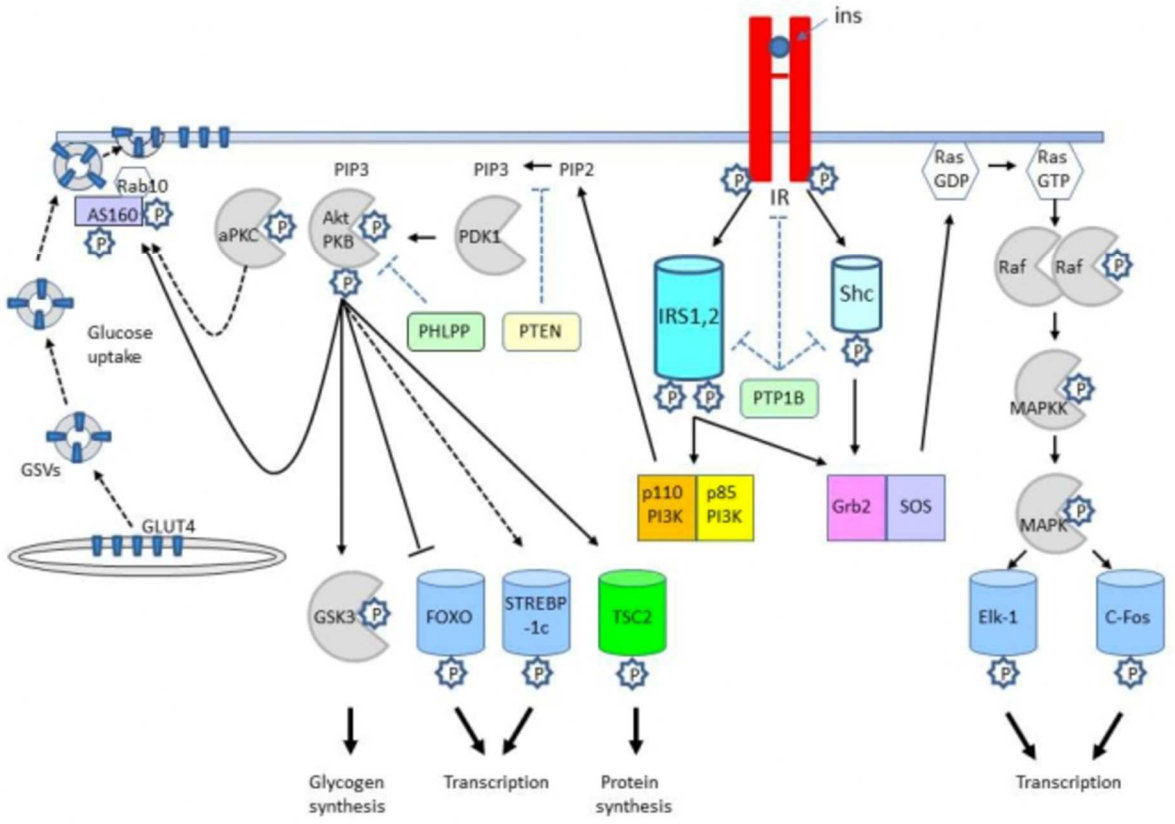


Figure 1. Insulin signaling pathway showing some of the signaling molecules involved and the effects seen [10].

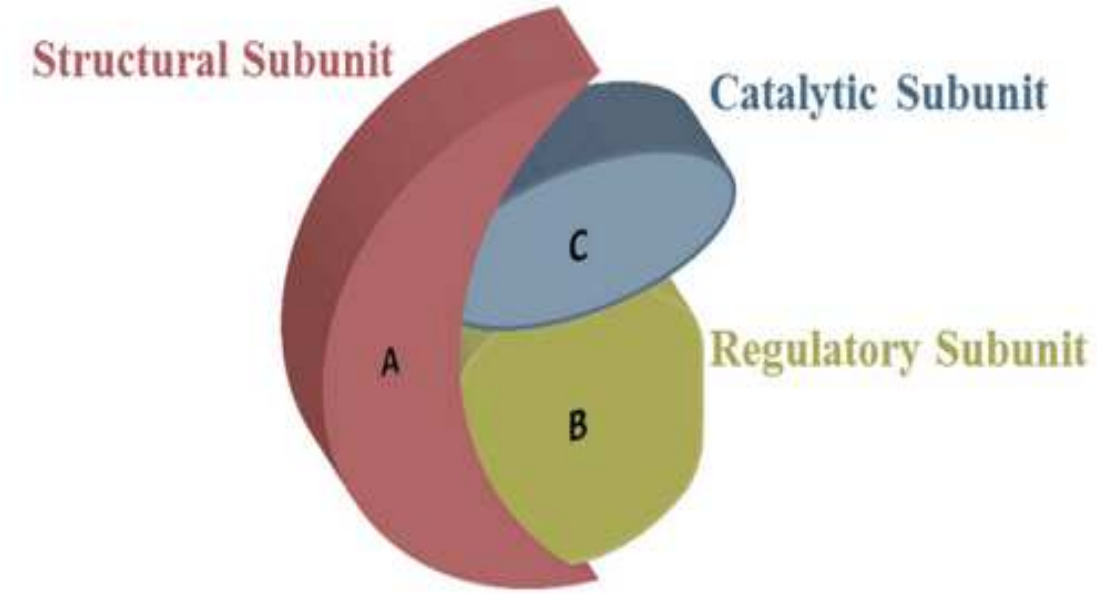


Figure 2. Structure of the heterotrimeric PP2A holoenzyme [88].

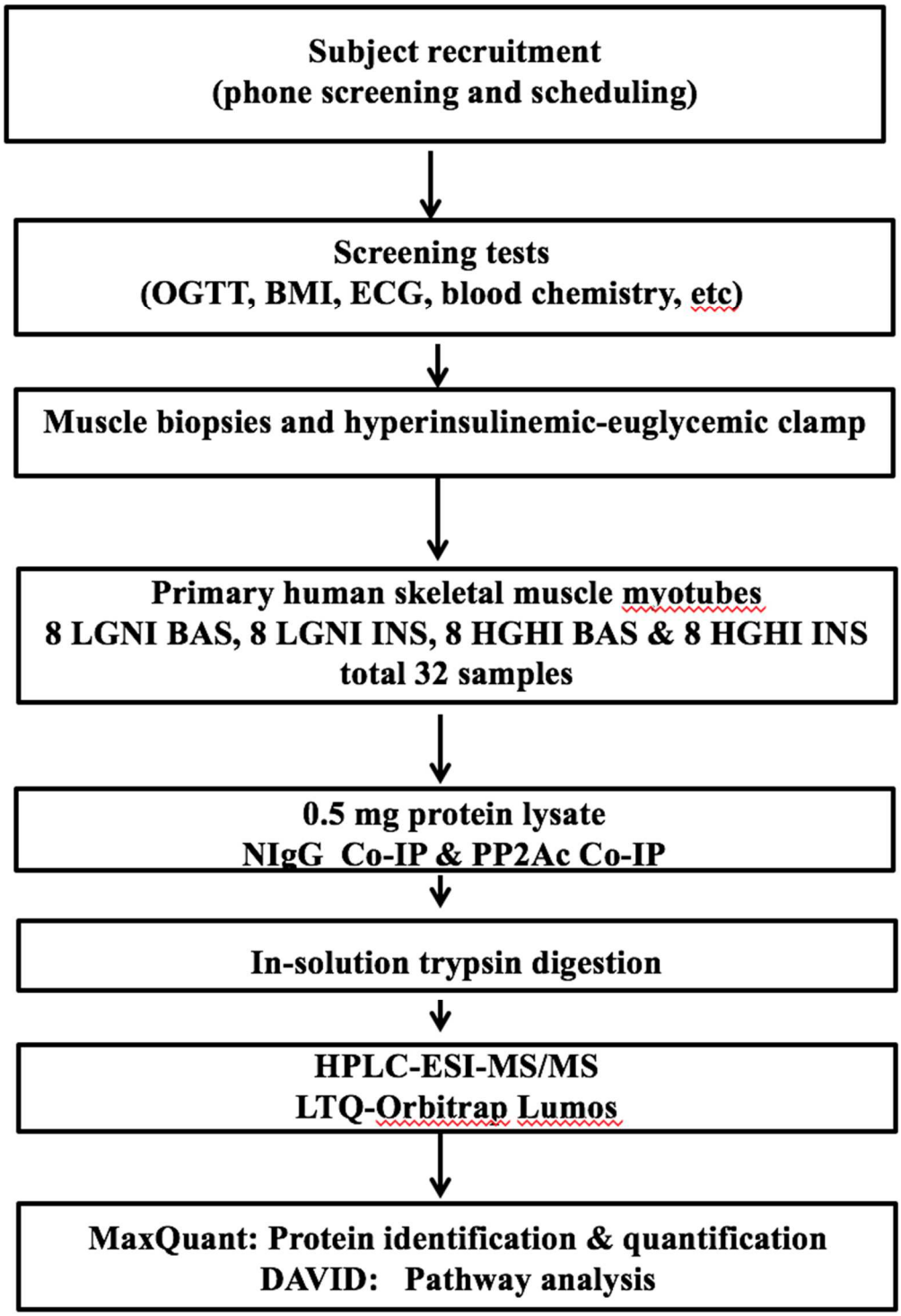


Figure 3. Clinical and proteomic data acquisition and analysis

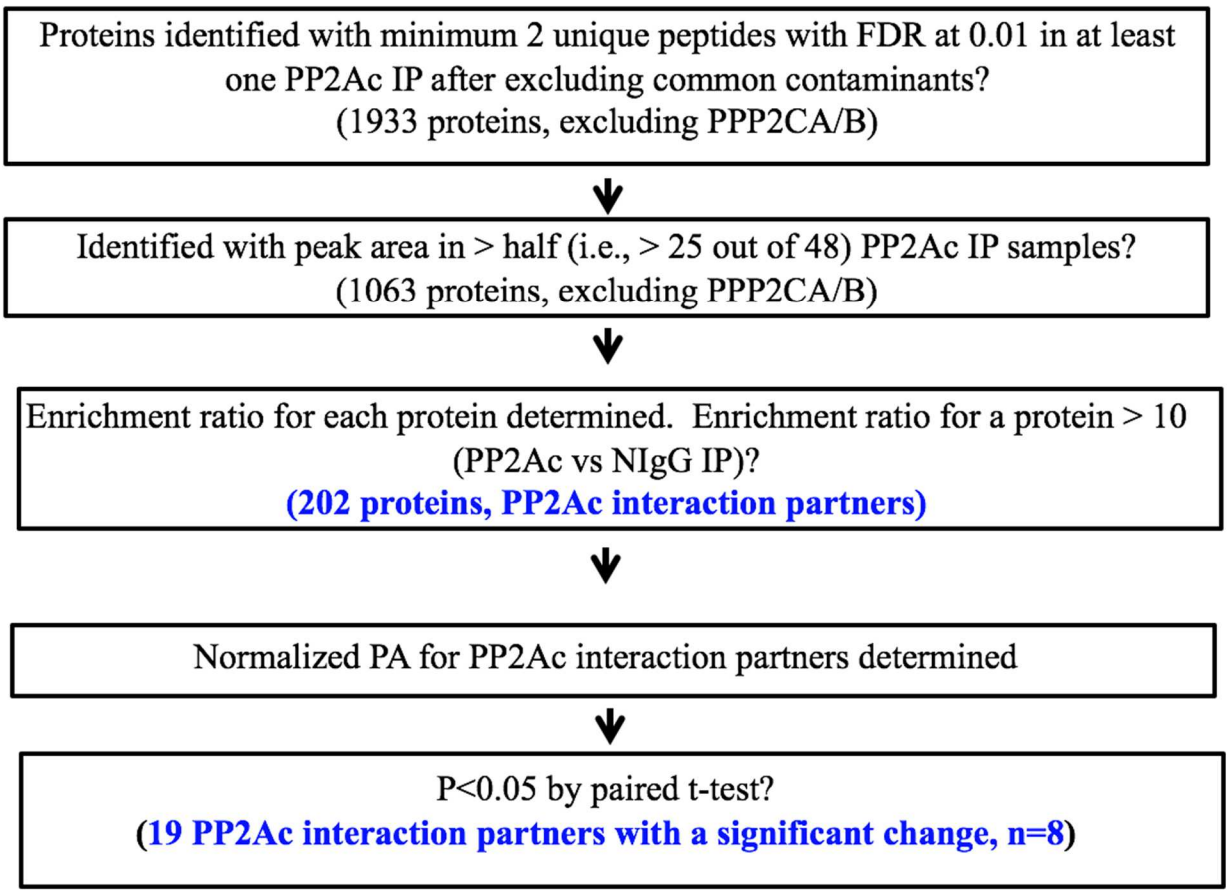


Figure 4. Proteomic data analysis

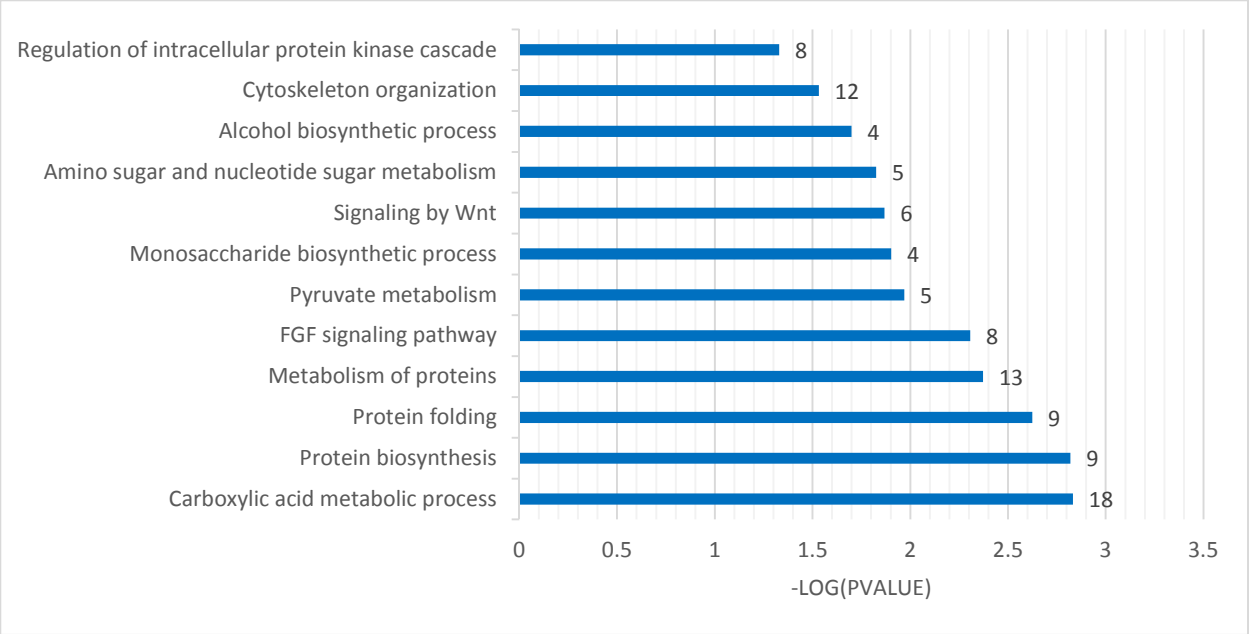
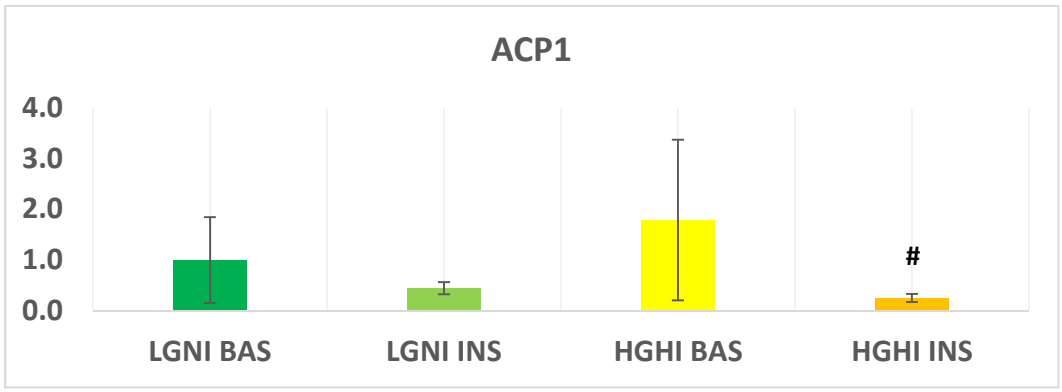
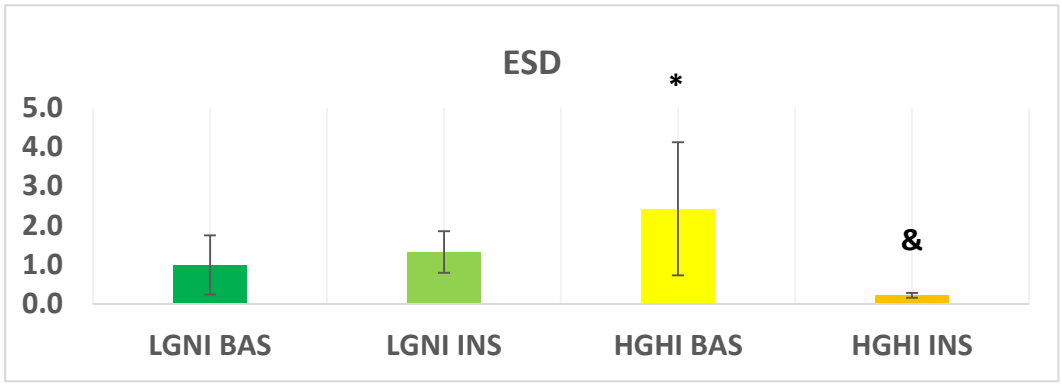
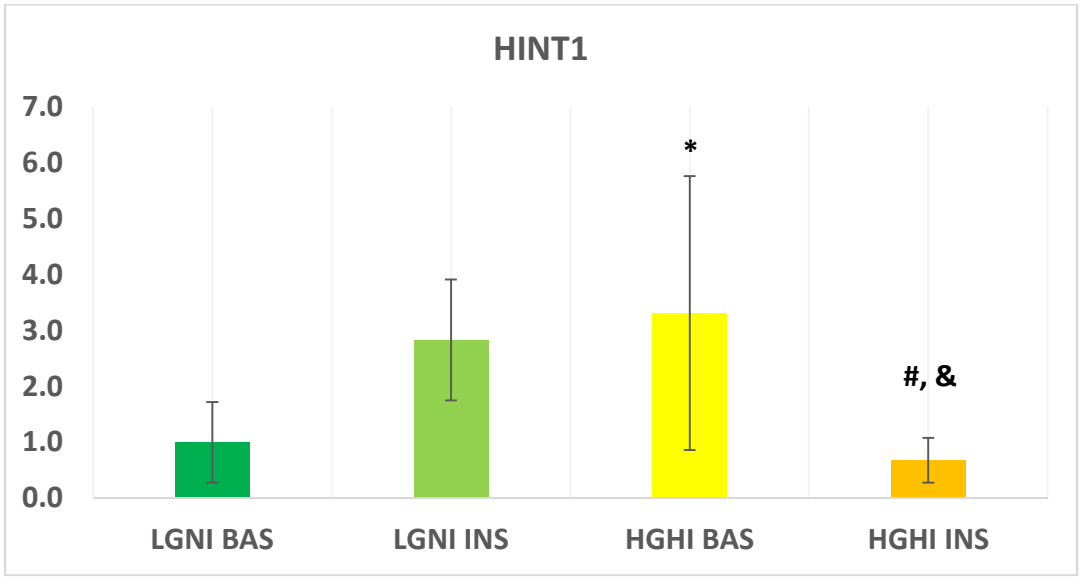


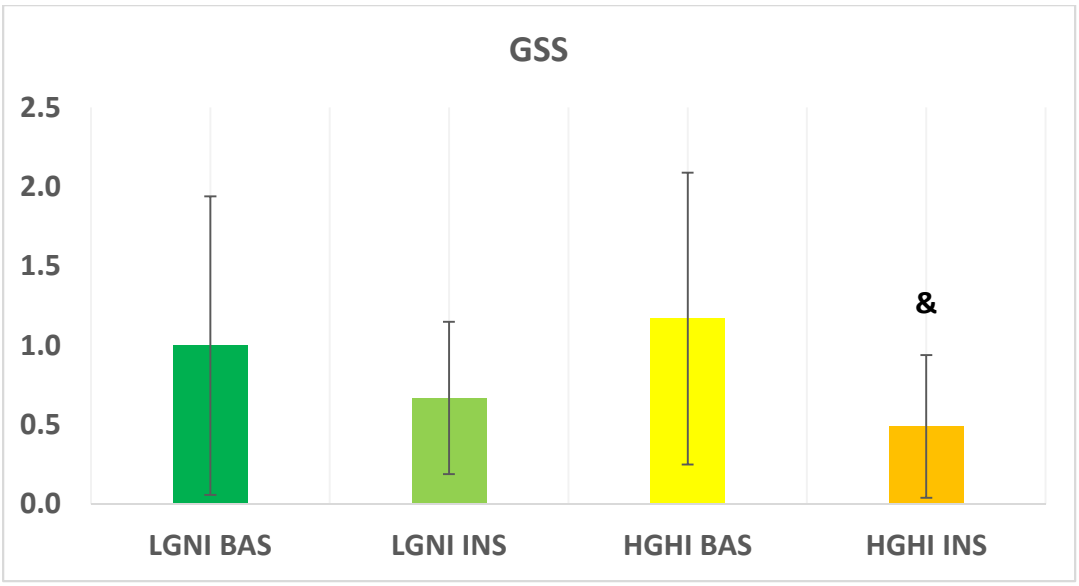
Figure 5. Significantly enriched pathways for the 202 interaction partners and PP2Ac in primary human skeletal muscle cells

Figure 6. Interaction partners that showed significant difference among 4 treatment groups. Mean of the normalized peak area for eachPP2Ac interaction partner in the LGNI BAS samples was set to 1.00, and all the fold changes were relative to LGNI BAS. *: p<0.05 vs. LGNI BAS, #: P<0.05 vs. HGHI BAS, and &: p<0,05 vs. LGLI INS





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&
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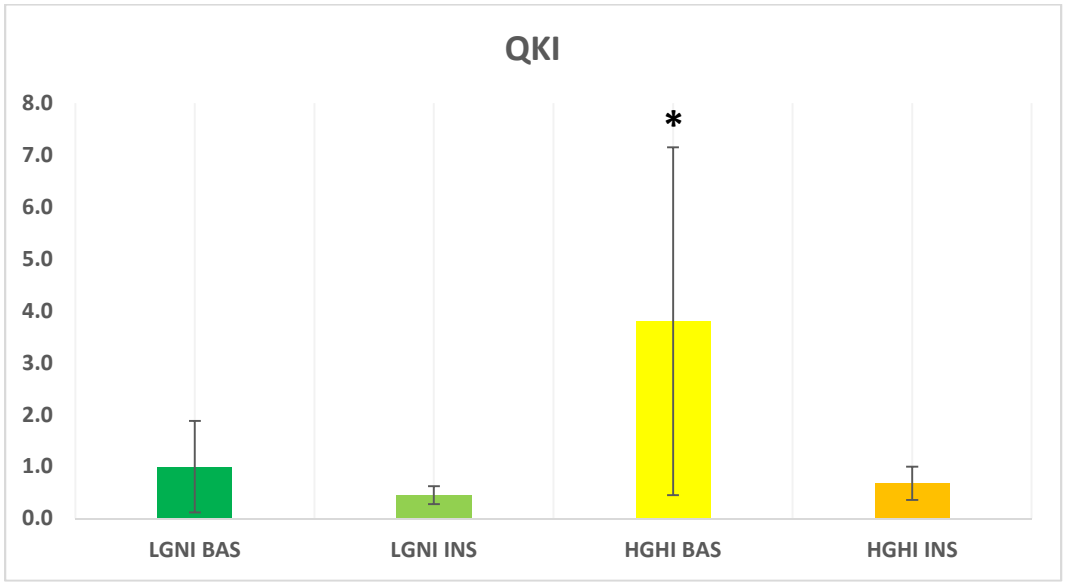
LGNI INS
HGHI BAS
HGHI INS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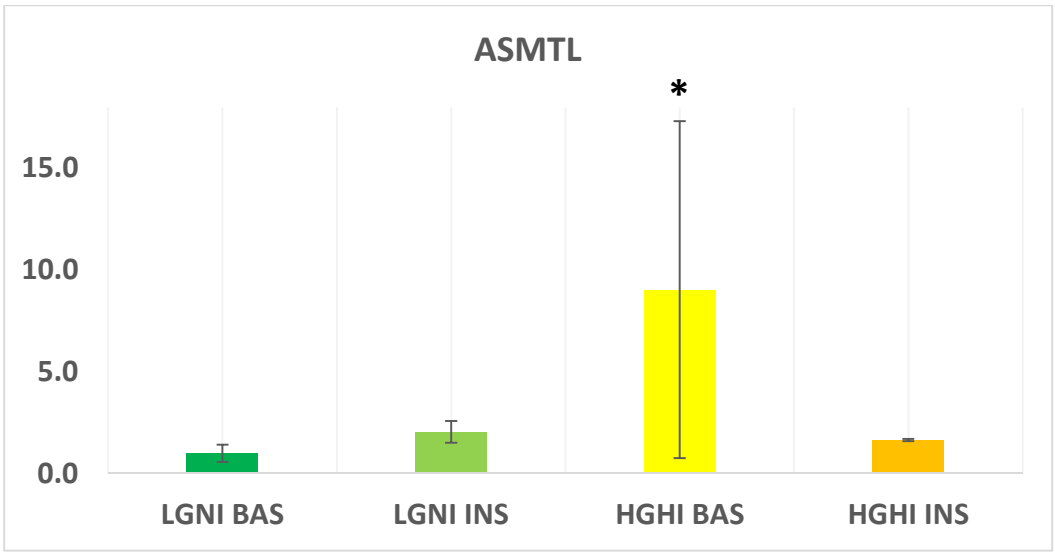
HGHI BAS
HGHI INS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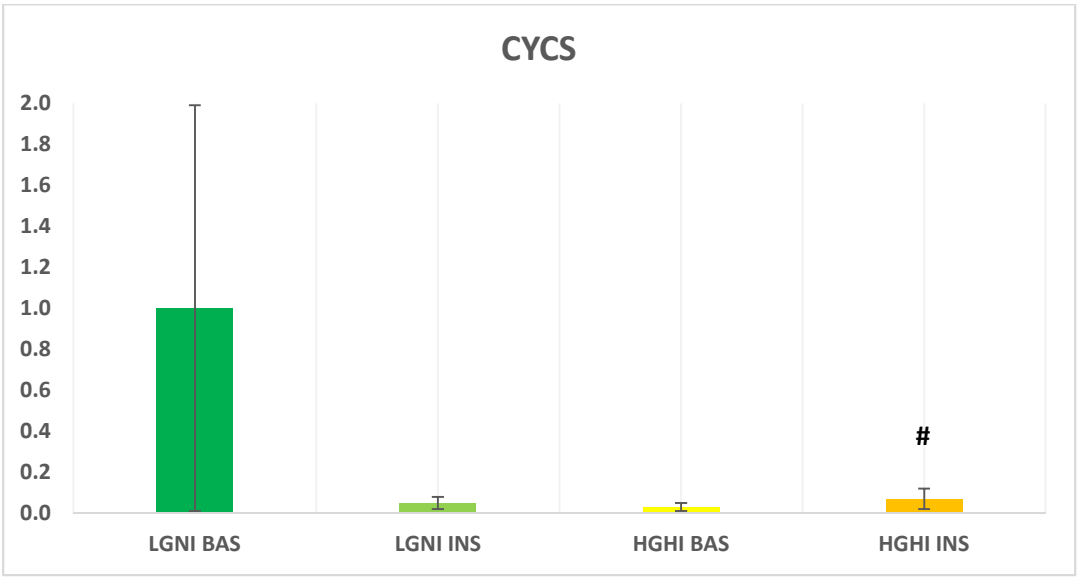
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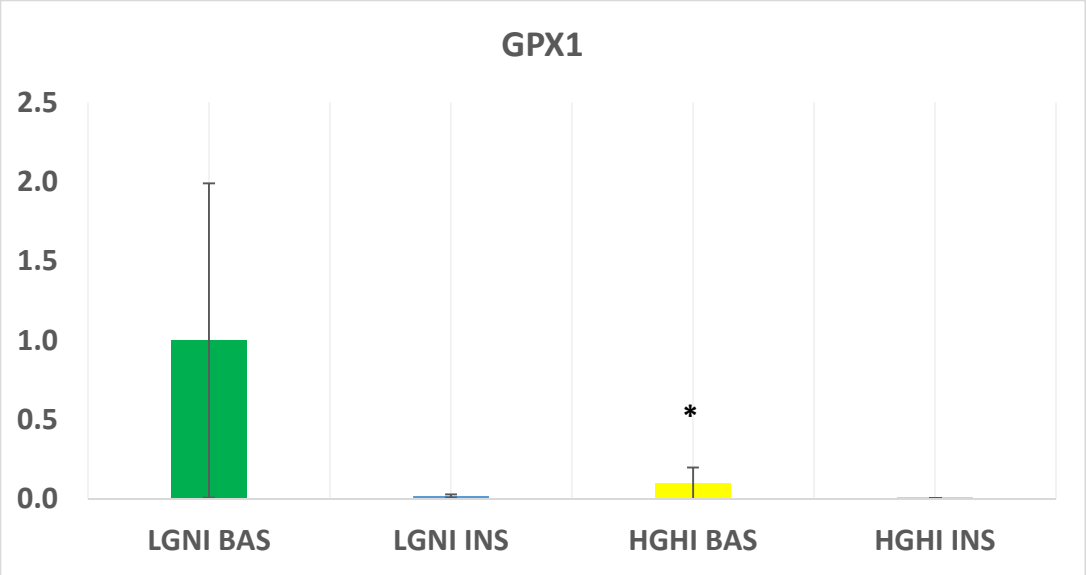
LGNI INS
HGHI BAS
HGHI INS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*
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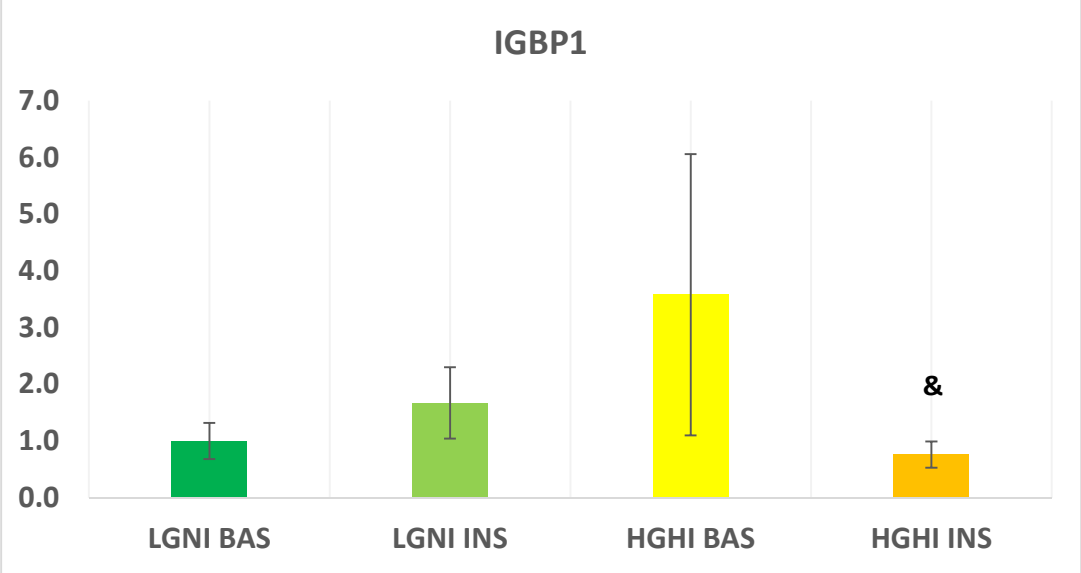
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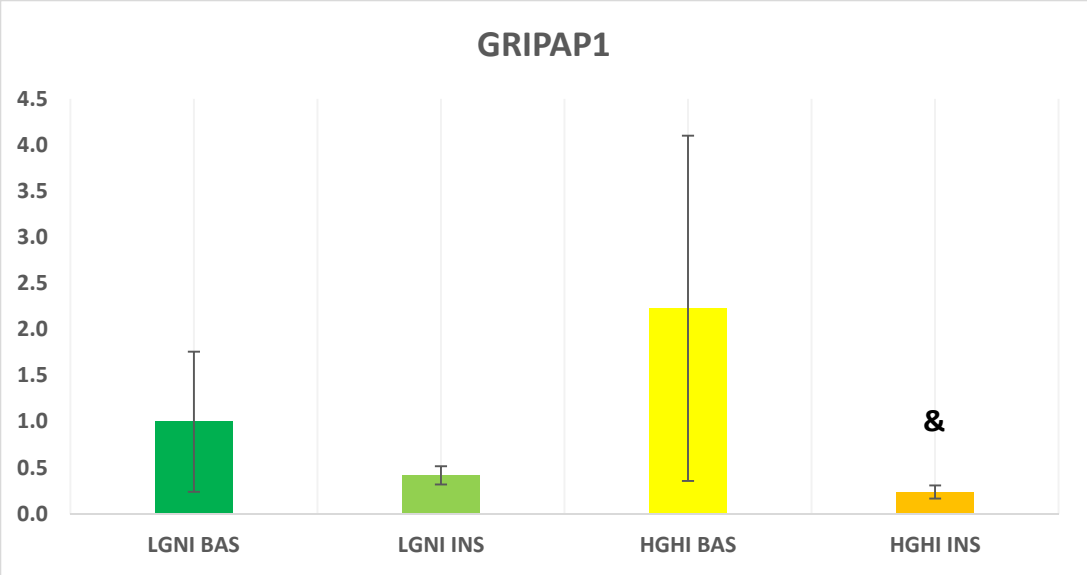
HGHI BAS
HGHI INS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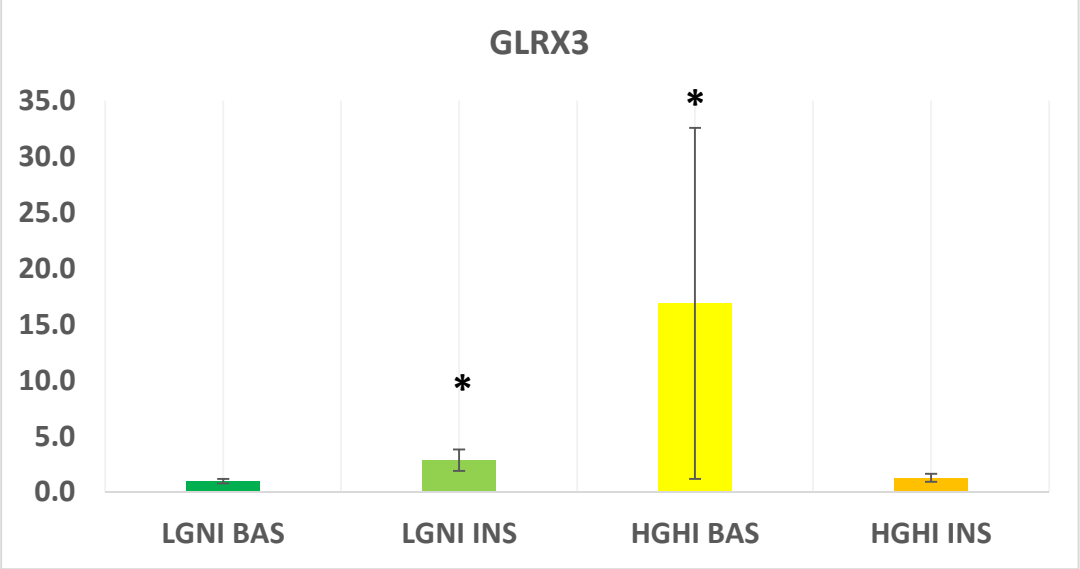
HGHI BAS
HGHI INS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*
*
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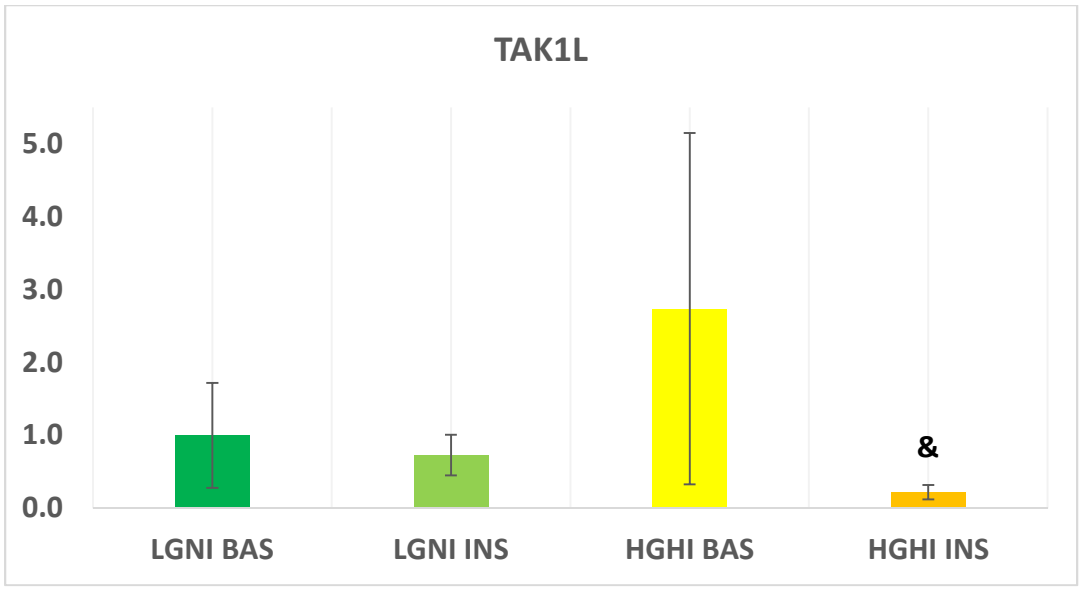
HGHI BAS
HGHI INS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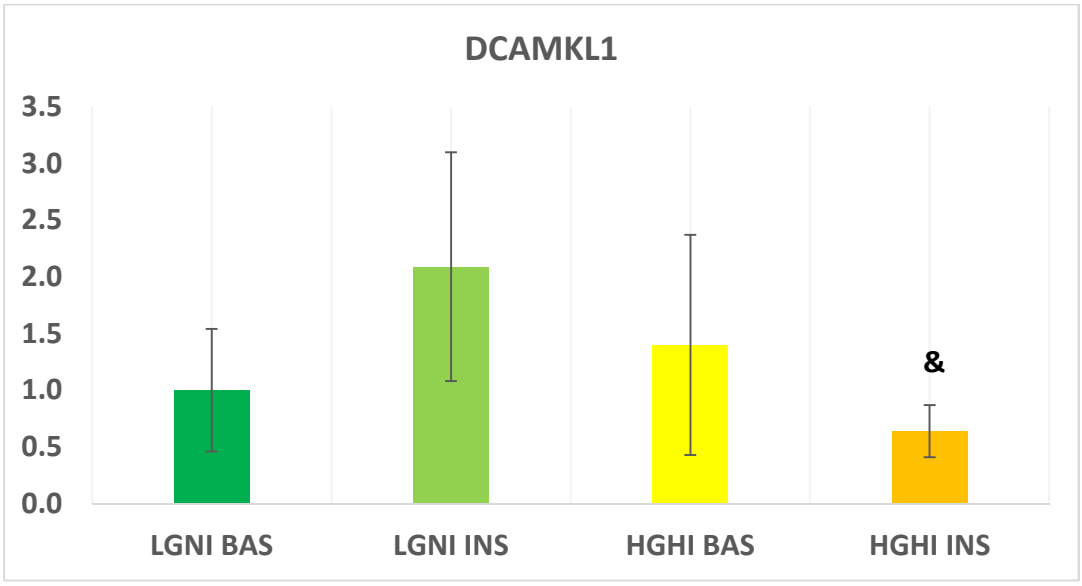
LGNI INS
HGHI BAS
HGHI INS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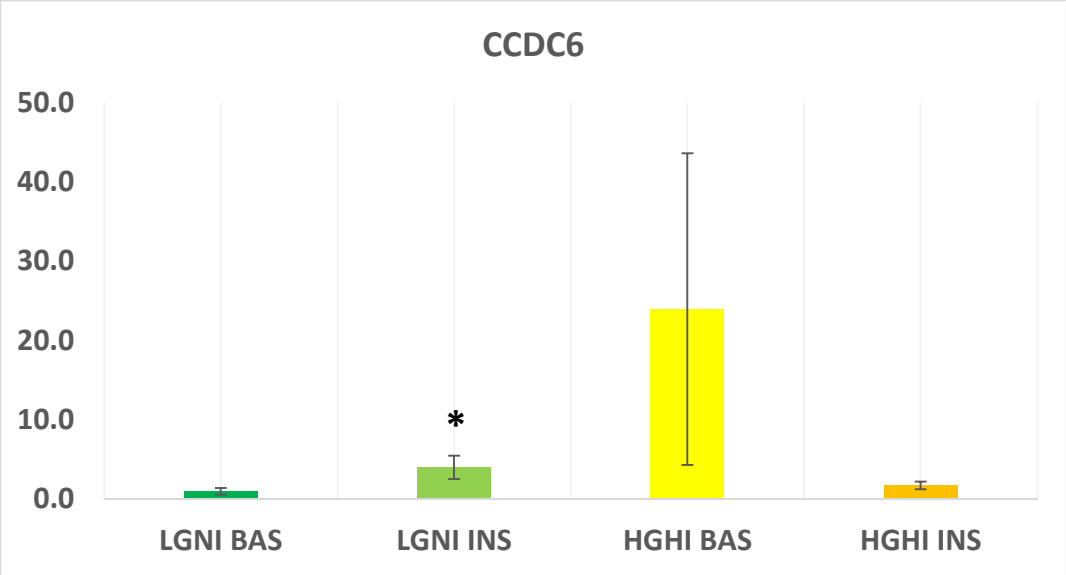
HGHI BAS
HGHI INS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*
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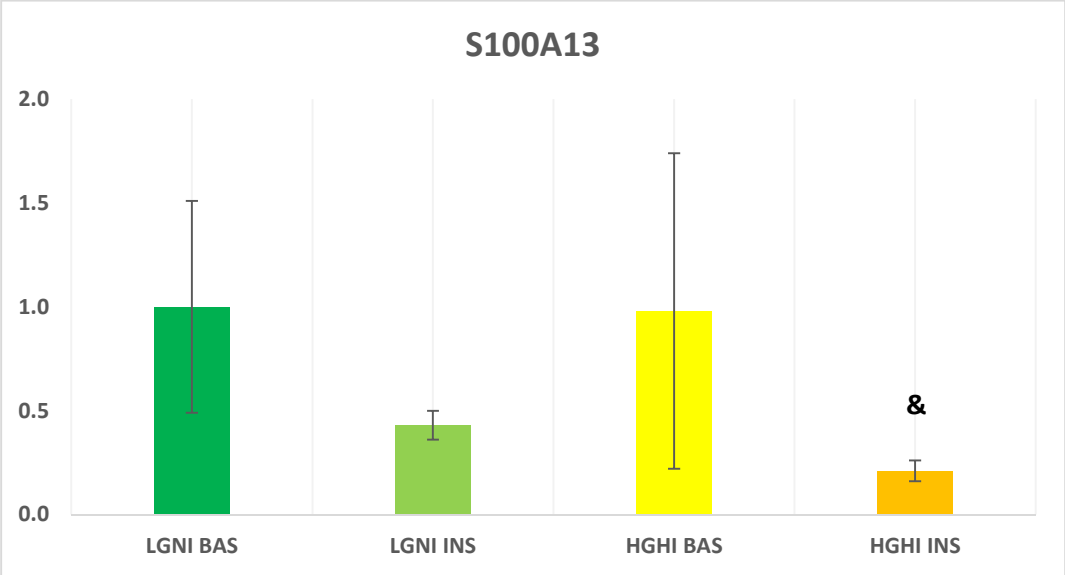
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HGHI BAS
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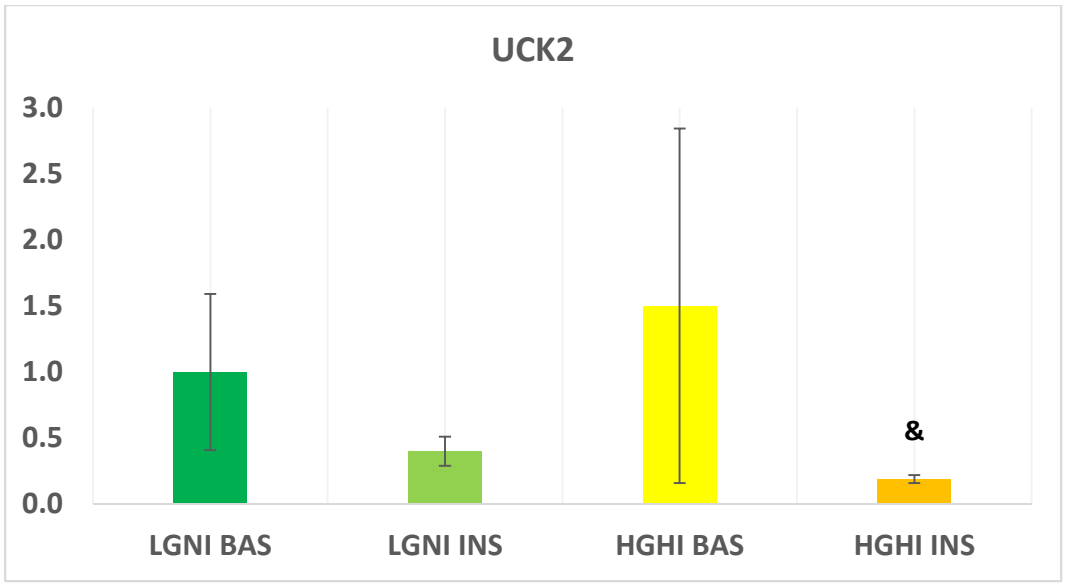
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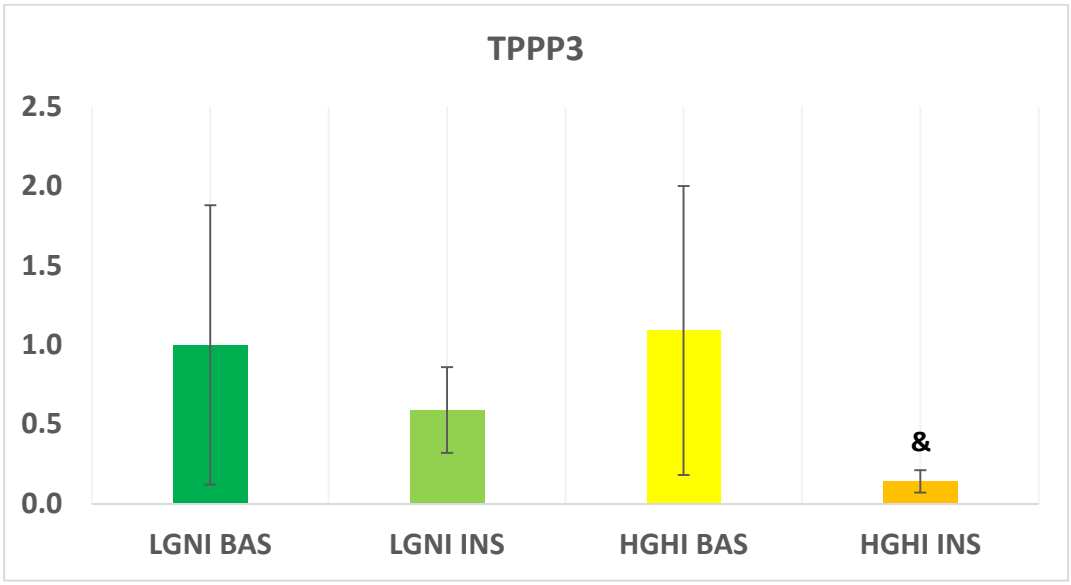
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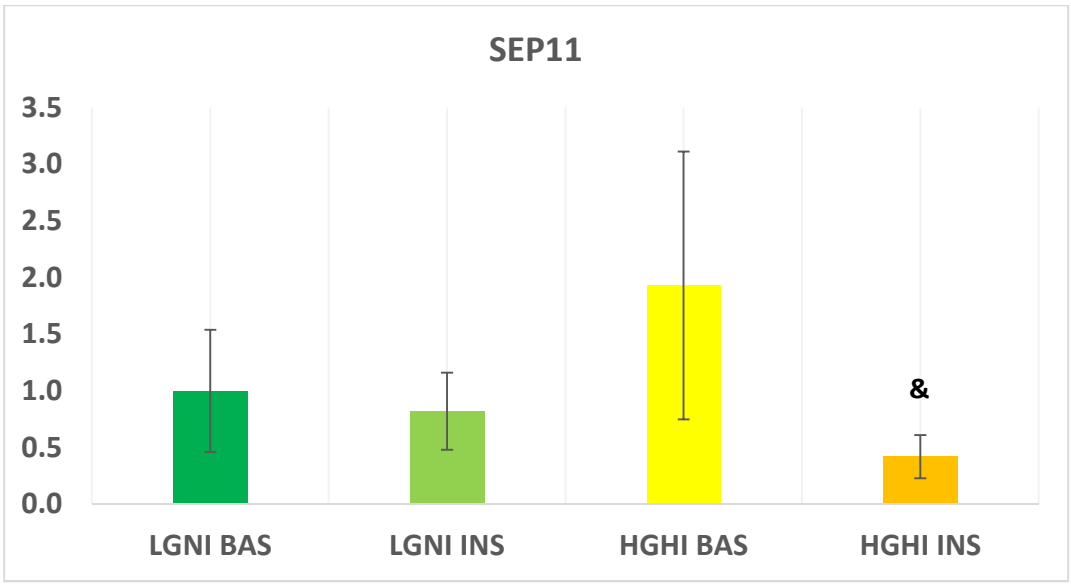
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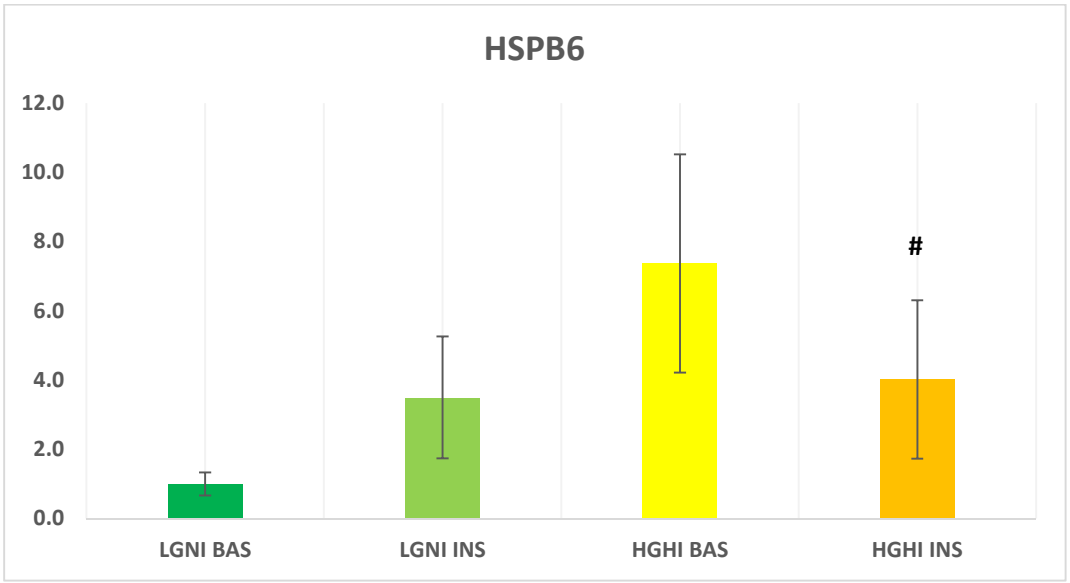
HGHI INS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#
LGNI BAS
LGNI INS
HGHI BAS
HGHI INS

TABLES

Table 1. Clinical characteristics for 8 lean participants in this study. Results were shown as mean ± SEM. [Cutoff values]

Gender (M/F)	(4/4)
Body Mass Index (kg/m^2)	21.3 ± 2.2 [25]
HbA1C (%)	5.4 ± 0.2 [5.7]
Fasting Plasma Glucose (mg/dl)	84.9 ± 5.6 [100]
2h OGTT (mg/dl)	106.6 ± 15.1 [140]
M-value (mg/kg·min)	11.0 ± 3.1 [5]

Table 2. The 202 protein groups that met the 2 criteria (See Methods for details) for classification as PP2Ac interaction partners in primary human skeletal muscle cells. (* indicates previously identified PP2A partners in INS-1 832/13 beta-cells).

Gene name	Protein name
PPP2R1A*	Serine/threonine-protein phosphatase 2A 65kDa regulatory subunit A alpha isoform
PPP2R2A*	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform
PPP2R5D	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform
PPP2R5E	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon isoform
A2M	Alpha-2-Macroglobulin
ACAT1	Acetyl-CoA Acetyltransferase 1, mitochondrial
ACP1*	Low molecular weight phosphotyrosine protein phosphatase; Acid phosphatase 1, soluble
ACTA1	Actin, alpha skeletal muscle
ACTB, ACTG1	Actin, cytoplasmic 1; Actin, cytoplasmic 1, N-terminally processed; Actin, cytoplasmic 2; Actin, cytoplasmic 2, N-terminally processed
ACYP2	Acylphosphatase-2;Acylphosphatase
ADA	Adenosine deaminase
AKR1B1	Aldose reductase

AKR1C1, AKR1C2, AKR1C3, AKR1C4	Aldo-keto reductase family 1 member C1;Aldo-keto reductase family 1 member C2;Aldo-keto reductase family 1 member C3;Aldo-keto reductase family 1 member C4
ALDH1A1	Retinal dehydrogenase 1
ALDH7A1	Alpha-aminoadipic semialdehyde dehydrogenase
ALDH9A1	4-trimethylaminobutyraldehyde dehydrogenase
AP2M1	AP-2 complex subunit mu
APOBEC2	Probable C->U-editing enzyme APOBEC-2
ASAH1	Acid ceramidase; Acid ceramidase subunit alpha; Acid ceramidase subunit beta
ASMTL	N-acetylserotonin O-methyltransferase-like protein
ATP5D	ATP synthase subunit delta, mitochondrial
ATXN2L	Ataxin-2-like protein
BAP18, C17ORF49	Chromatin complexes subunit BAP18
BCAS3	Breast carcinoma-amplified sequence 3
BCAT1	Branched-chain-amino-acid aminotransferase, cytosolic
BOD1	Biorientation of chromosomes in cell division protein 1
BOLA2, BOLA2B	BolA-like protein 2
BROX, C1ORF58	BRO1 domain-containing protein BROX
C1QTNF5	Complement C1q tumor necrosis factor-related protein 5
C21ORF7, TAK1L	TAK1-like protein
CAPRIN1	Caprin-1
CAT	Catalase

CCDC6*	Coiled-coil domain containing 6
CDV3	Protein CDV3 homolog
CHCHD3	Coiled-coil-helix-coiled-coil-helix domain-containing protein 3, mitochondrial
CHMP1A	Charged multivesicular body protein 1a
CIAPIN1	Anamorsin
CIP29, SARNP	SAP domain-containing ribonucleoprotein
CKB	Creatine kinase B-type
CLCC1	Chloride channel CLIC-like protein 1
COMT	Catechol O-methyltransferase
COPS8	COP9 signalosome subunit 8
CORO1B	Coronin-1B
CPPED1	Calcineurin-like phosphoesterase domain-containing protein 1
CRYZ	Quinone oxidoreductase
CTPS	CTP synthase 1
CTSA	Lysosomal protective protein; Lysosomal protective protein 32 kDa chain; Lysosomal protective protein 20 kDa chain
CTTN	Src substrate cortactin
CYCS	Cytochrome c
DBI	Acyl-CoA-binding protein
DCAMKL1	Doublecortin Like Kinase 1
DCTN3	Dynactin subunit 3
DDB1	DNA damage-binding protein 1

DDX19B	ATP-dependent RNA helicase DDX19B
DLST*	Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)
DR1	Protein Dr1
DYSF	Dysferlin
EIF2B1	Translation initiation factor eIF-2B subunit alpha
EIF4A3	Eukaryotic initiation factor 4A-III
EIF4H	Eukaryotic translation initiation factor 4H
ESD	S-formylglutathione hydrolase
ETF1	Eukaryotic peptide chain release factor subunit 1
EZR	Ezrin
FABP3	Fatty acid-binding protein, heart
FARSB	Phenylalanine--tRNA ligase beta subunit
FKBP1A	Peptidyl-prolyl cis-trans isomerase FKBP1A; Peptidyl-prolyl cis-trans isomerase
FKBP9	Peptidyl-prolyl cis-trans isomerase FKBP9
FUBP1	Far upstream element-binding protein 1
GLRX3	Glutaredoxin-3
GMPPB*	GDP-mannose pyrophosphorylase B
GORASP2	Golgi reassembly-stacking protein 2
GPI	Glucose-6-phosphate isomerase
GPX1	Glutathione peroxidase 1
GRIPAP1	GRIP1-associated protein 1

GSPT1	Eukaryotic peptide chain release factor GTP-binding subunit ERF3A
GSS	Glutathione synthetase
H2AFJ, H2AFV, H2AFX, H2AFZ, HIST1H2AA, HIST1H2AB, HIST1H2AC, HIST1H2AD, HIST1H2AG, HIST1H2AH, HIST1H2AJ, HIST2H2AA3, HIST2H2AB, HIST2H2AC, HIST3H2A	Histone H2A.x; Histone H2A type 1-A; Histone H2A type 1-B/E; Histone H2A type 1; Histone H2A type 1-D; Histone H2A type 2-A; Histone H2A type 3; Histone H2A type 1-C; Histone H2A type 2-B; Histone H2A type 2-C; Histone H2A.J; Histone H2A type 1-H; Histone H2A type 1-J; Histone H2A.Z; Histone H2A.V; Histone H2A
HINT1	Histidine triad nucleotide-binding protein 1
HIST1H1E	Histone H1.4
HIST1H4A	Histone H4
HMGCS1	Hydroxymethylglutaryl-CoA synthase, cytoplasmic
HN1	Hematological and neurological expressed 1 protein
HSPA4L	Heat shock 70 kDa protein 4L
HSPB6	Heat shock protein beta-6
HSPE1	10 kDa heat shock protein, mitochondrial

HTRA1	Serine protease HTRA1
IDH1	Isocitrate dehydrogenase [NADP] cytoplasmic
IGBP1*	Immunoglobulin (CD79A) binding protein 1
IMPA1	Inositol monophosphatase 1
IST1	IST1 homolog
ITGA5	Integrin alpha-5; Integrin alpha-5 heavy chain; Integrin alpha-5 light chain
IVD	Isovaleryl-CoA dehydrogenase, mitochondrial
KHSRP	Far upstream element-binding protein 2
LSS	Lanosterol synthase
LYPLA1	Acyl-protein thioesterase 1
MAN2B1	Lysosomal alpha-mannosidase; Lysosomal alpha-mannosidase A peptide; Lysosomal alpha-mannosidase B peptide; Lysosomal alpha-mannosidase C peptide; Lysosomal alpha-mannosidase D peptide; Lysosomal alpha-mannosidase E peptide
MATR3	Matrin-3
METTL11A	Alpha N-terminal protein methyltransferase 1A
MPI	Mannose-6-phosphate isomerase
MVD	Diphosphomevalonate decarboxylase
MYOM3	Myomesin-3
NAMPT*	Nicotinamide phosphoribosyltransferase
NASP	Nuclear autoantigenic sperm protein
NIT2	Omega-amidase NIT2

NME1, NME2, NME2P1	Nucleoside diphosphate kinase; Nucleoside diphosphate kinase B; Putative nucleoside diphosphate kinase
NSFL1C	NSFL1 cofactor p47
NUDCD2	NudC domain-containing protein 2
NUDT5	ADP-sugar pyrophosphatase
OSBPL9	Oxysterol-binding protein; Oxysterol-binding protein-related protein 9
OXCT1	Succinyl-CoA:3-ketoacid-coenzyme A transferase 1, mitochondrial
PCBP2*, PCBP3*	Poly(rC)-binding protein 2; Poly(rC)-binding protein 3
PCMT1	Protein-L-isoaspartate O-methyltransferase; Protein-L-isoaspartate(D-aspartate) O-methyltransferase
PDCD5	Programmed cell death protein 5
PDHX*	Pyruvate dehydrogenase protein X component, mitochondrial
PDLIM4	PDZ and LIM domain protein 4
PEBP1	Phosphatidylethanolamine-binding protein 1; Hippocampal cholinergic neurostimulating peptide
PEPD	Xaa-Pro dipeptidase
PFDN1	Prefoldin subunit 1
PFDN2	Prefoldin subunit 2
PFN1	Profilin-1
PGM3	Phosphoacetylglucosamine mutase
PHB	Prohibitin
PMPCA	Mitochondrial-processing peptidase subunit alpha
POLR2H	DNA-directed RNA polymerases I, II, and III subunit RPABC3

PPP4C*	Serine/threonine-protein phosphatase 4 catalytic subunit; Serine/threonine-protein phosphatase
PPP4R1	Serine/threonine-protein phosphatase 4 regulatory subunit 1
PPP4R2*	Serine/threonine-protein phosphatase 4 regulatory subunit 2
PRDX2	Peroxiredoxin-2
PRKACA, PRKACB	cAMP-dependent protein kinase catalytic subunit beta; cAMP-dependent protein kinase catalytic subunit alpha
PROSC	Proline synthase co-transcribed bacterial homolog protein
PSMA6*	Proteasome subunit alpha type; Proteasome subunit alpha type-6
PSMB4	Proteasome subunit beta type-4
PTER	Phosphotriesterase-related protein
PTK2	Focal adhesion kinase 1
PTMS	Parathymosin
QKI	Protein quaking
QTRT1	Queuine tRNA-ribosyltransferase
RAB11A, RAB11B	Ras-related protein Rab-11B;Ras-related protein Rab-11A
RABEP2	Rab GTPase-binding effector protein 2
RAD23A	UV excision repair protein RAD23 homolog A
RAPH1	Ras-associated and pleckstrin homology domains-containing protein 1
RPS21	40S ribosomal protein S21
RPSA, RPSAP58	40S ribosomal protein SA
RSU1	Ras suppressor protein 1
RTCD1	RNA 3-terminal phosphate cyclase

RTN1	Reticulon-1
S100A13	Protein S100-A13
S100A4	Protein S100-A4
S100Z	Protein S100-Z
SAR1A	GTP-binding protein SAR1a
SARS	Serine--tRNA ligase, cytoplasmic
SBDS	Ribosome maturation protein SBDS
SCRN1	Secernin-1
SEP11	Septin-11
SERPINB1	Leukocyte elastase inhibitor
SERPINB6	Serpin B6
SERPINC1	Antithrombin-III
SERPINE1	Plasminogen activator inhibitor 1
SET	Protein SET
SETD7	Histone-lysine N-methyltransferase SETD7
SF3A1	Splicing factor 3A subunit 1
SFPQ	Splicing factor, proline- and glutamine-rich
SLC9A3R1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
SNX1	Sorting nexin-1
SNX12	Sorting nexin-12
SNX17	Sorting nexin-17
SOD1	Superoxide dismutase [Cu-Zn]
SORD	Sorbitol dehydrogenase

SPAG7	Sperm-associated antigen 7
SRP9	Signal recognition particle 9 kDa protein
SSBP1*	Single-stranded DNA-binding protein, mitochondrial
ST13, ST13P5	Hsc70-interacting protein; Putative protein FAM10A5
STMN1	Stathmin
SWAP70	Switch-associated protein 70
SYNPO2L	Synaptopodin 2-like protein
TBCA	Tubulin-specific chaperone A
TCEB2	Transcription elongation factor B polypeptide 2
TFG	Protein TFG
THBS1	Thrombospondin-1
TMEM189, UBE2V1	Ubiquitin-conjugating enzyme E2 variant 1
TMF1	TATA element modulatory factor
TMSB10	Thymosin beta-10
TMSB4X	Thymosin beta-4; Hematopoietic system regulatory peptide
TPM2	Tropomyosin beta chain
TPP1	Tripeptidyl-peptidase 1
TPPPP3	Tubulin polymerization-promoting protein family member 3
TSG101	Tumor susceptibility gene 101 protein
TTC17	Tetratricopeptide repeat protein 17
TTC9	Tetratricopeptide repeat protein 9A

TUBA1A, TUBA1B	Tubulin alpha-1B chain; Tubulin alpha-1A chain
TUBB6*	Tubulin beta-6 chain
TXNDC5	Thioredoxin domain-containing protein 5
TXNL1	Thioredoxin-like protein 1
TXNRD1	Thioredoxin reductase 1, cytoplasmic
UAP1	UDP-N-acetylhexosamine pyrophosphorylase; UDP-N-acetylgalactosamine pyrophosphorylase; UDP-N-acetylglucosamine pyrophosphorylase
UBE2D3	Ubiquitin-conjugating enzyme E2 D3
UBL4A	Ubiquitin-like protein 4A
UCK2	Uridine-cytidine kinase 2
VPS25*	Vacuolar protein-sorting-associated protein 25
VTA1*	Vacuolar protein sorting-associated protein VTA1 homolog
VWA5A	von Willebrand factor A domain-containing protein 5A
ZNF157	Zinc finger protein 157

Table 3. Significantly enriched pathways for the PP2Ac interaction partners identified in this study revealed by DAVID pathway analysis

DAVID Pathways	-log(p-value)	Gene Names	Number of PP2Ac interaction partners in the study
Carboxylic acid metabolic process	2.83	BCAT1, DLST, PEPD, CTPS, SARS, LYPLA1, CKB, GSS, AKR1C3, AKR1C2, GPI, FARSB, FABP3, QKI, IDH1, PDHX, AKR1C1, ALDH9A1	18
Protein biosynthesis	2.82	RPSA, EIF4A3, GSPT1, EIF4H, SARS, RPSAP58, FARSB, ETF1, RPS21, EIF2B1	9
Protein folding	2.63	ST13, PFDN2, FKBP9, PFDN1, TBCA, HSPA4L, TTC9, HSPE1, FKBP1A	9
Protein metabolism	2.37	ACTB, FKBP9, RPSA, ETF1, EIF2B1, PFDN2, PFDN1, TBCA, RPSAP58, EIF4H, TUBB6, TUBA1A, RPS21, TUBA1B	13

FGF signaling pathway	2.31	PPP2R1A, PPP4R1, PPP2CA, PPP2R5D, PPP2CB, PEBP1, PPP2R5E, PPP2R2A	8
Pyruvate metabolism	1.97	ALDH7A1, AKR1B1, ACYP2, ACAT1, ALDH9A1	5
Monosaccharide biosynthetic process	1.90	PGM3, GPI, UAP1, SORD	4
Signaling by Wnt	1.87	PSMB4, PPP2R1A, PSMA6, PPP2CA, PPP2R5D, PPP2CB	6
Amino sugar and nucleotide sugar metabolism	1.83	GMPPB, PGM3, GPI, UAP1, MPI	5
Alcohol biosynthetic process	1.69	PGM3, GPI, UAP1, SORD	4
Cytoskeleton organization	1.53	ACTG1, PFN1, TPPP3, PTK2, EZR, ACTA1, TMSB4X, TMSB10, STMN1, SOD1, PPP4C, TUBA1B	12

Regulation of intracellular protein kinase cascade	1.33	PPP2R1A, GPX1, PPP2CA, UBE2V1, TFG, PEBP1, FKBP1A, CAT, TMEM189	8

Table 4. Interaction partners that showed significant difference upon insulin stimulation.

Gene Name	**Protein Name**	**Fold change LGNI BAS**	**Fold change LGNI INS**	**Fold change HGHI BAS**	**Fold change HGHI INS**
ACP1	Low molecular weight phosphotyrosine protein phosphatase	1.00±0.85	0.44±0.12	1.79±1.59	0.25±0.08$^{\#}$
ASMTL	N-acetylserotonin O-methyltransferase-like protein	1.00±0.43	2.05±0.54	9.04±8.28*	1.65±0.05
TAK1L	TAK1-like protein	1.00±0.72	0.73±0.28	2.74±2.41	0.22±0.1$^{\&}$
CCDC6	Coiled-coil domain-containing protein 6	1.00±0.42	4.03±1.46*	23.95±19.63	1.74±0.49
CYCS	Cytochrome c	1.00±0.99	0.05±0.03	0.03±0.02	0.07±0.05$^{\#}$

DCAMKL1	Serine/threonine-protein kinase DCLK1	1.00±0.54	2.09±1.01	1.40±0.97	0.64±0.23[&]
ESD	S-formylglutathione hydrolase	1.00±0.75	1.33±0.53	2.43±1.69*	0.23±0.06[&]
GLRX3	Glutaredoxin-3	1.00±0.21	2.87±0.96*	16.89±15.68*	1.29±0.36
GPX1	Glutathione peroxidase 1	1.00±0.99	0.02±0.01	0.10±0.10*	0.01±0
GRIPAP1	GRIP1-associated protein 1	1.00±0.76	0.42±0.1	2.23±1.87	0.24±0.07[&]
GSS	Glutathione synthetase	1.00±0.94	0.67±0.48	1.17±0.92	0.49±0.45[&]
HINT1	Histidine triad nucleotide-binding protein 1	1.00±0.72	2.83±1.08	3.31±2.45*	0.68±0.40[#,&]
HSPB6	Heat shock protein beta-6	1.00±0.33	3.5±1.76	7.37±3.15	4.02±2.29[#]
IGBP1	Immunoglobulin-binding protein 1	1.00±0.32	1.67±0.63	3.58±2.48	0.76±0.23[&]
QKI	Protein quaking	1.00±0.88	0.45±0.17	3.8±3.35*	0.68±0.32
S100A13	Protein S100-A13	1.00±0.51	0.43±0.07	0.98±0.76	0.21±0.05[&]
SEP11	Septin-11	1.00±0.54	0.82±0.34	1.93±1.18	0.42±0.19[&]

TPPP3	Tubulin polymerization-promoting protein family member 3	1.00±0.88	0.59±0.27	1.09±0.91	0.14±0.07[&]
UCK2	Uridine-cytidine kinase 2	1.00±0.59	0.4±0.11	1.5±1.34	0.19±0.03[&]

Data are given as fold changes (means ± SEM). Peak area for each protein identified in a specific sample was normalized against the peak area for PP2Ac identified in the same sample (See Methods). The normalized peak area for each PP2Ac interaction partner was compared among the 4 sets of samples to assess effects of acute insulin stimulation or effects of hyperinsulinemia hyperglycemia on protein-protein interactions involving PP2Ac. Mean of the normalized peak area for eachPP2Ac interaction partner in the LGNI BAS samples was set to 1.00, and all the fold changes were relative to LGNI BAS. *: $p<0.05$ vs. LGNI BAS, #: $P<0.05$ vs. HGHI BAS, and &: $p<0,05$ vs. LGLI INS.

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ABSTRACT

PROTEIN PHOSPHATASE 2A: FUNCTION AND REGULATION IN INSULIN RESISTANT HUMAN SKELETAL MUSCLE MYOTUBES

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Protein Phosphatase 2A (PP2A), a major serine/threonine phosphatase involved in insulin signaling pathway, plays a critical role in the development of insulin resistance and type 2 diabetes (T2D), which is characterized with hyperinsulinemic hyperglycemic condition. Moreover, majority of the glucose disposal takes place in the skeletal muscle which is also the main tissue responsible for insulin resistance. The catalytic subunit of PP2A (PP2Ac) can interact with multiple regulatory subunits and other regulatory proteins as well as substrates. These interactions are important for maintain normal PP2A function and subsequent cell signaling. We hypothesized that hyperinsulinemic hyperglycemic condition and insulin stimulation would result in differentially changed protein-protein interactions involving PP2Ac in primary human skeletal muscle cells derived from lean healthy participants.

Using UPLC-nanoESI-MS/MS, 202 PP2Ac interaction partners were identified in primary human skeletal muscle cells from 8 lean heathy participants. Out of which 18 partners were previously identified in the islet beta cells by our group. In addition, 19 interaction partners of PP2Ac showed a significant difference among 4 treatment groups: low glucose no insulin without acute 15-min insulin treatment (LGNI BAS), low glucose no insulin with acute 15-min insulin treatment (LGNI INS), high glucose high insulin without acute 15-min insulin stimulation (HGHI BAS) and high glucose high insulin with acute 15-min insulin stimulation (HGHI INS). Under basal conditions (i.e., without acute 15-minute insulin stimulation), 6 proteins showed a significant change in PP2Ac interaction between the low glucose no insulin and chronicle hyperinsulinemia and hyperglycemia conditions (LGNI BAS vs HGHI BAS, $p<0.05$). In addition, 2 proteins showed significant difference in PP2Ac interaction in LGNI condition in response to insulin stimulation (LGNI BAS vs LGNI INS, $p<0.05$). In HGHI conditions, 4 proteins showed significant difference in PP2Ac interaction in response to insulin stimulation (HGHI Bas vs HGHI INS, $p<0.05$). Upon acute insulin stimulation, 11 proteins showed significant difference in PP2Ac interaction in HGHI condition when compared to the LGNI condition (LGNI INS vs HGHI INS, $p<0.05$). These differential changes of PP2Ac interaction partners among the 4 treatments provide new information regarding PP2A in primary human skeletal muscle cells under hyperinsulinemic hyperglycemic conditions.

AUTOBIOGRAPHICAL STATEMENT

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ABSTRACTS

- Protein Phosphatase 2A: unction and Regulation in Insulin Resistant Human Skeletal Muscle Myotubes