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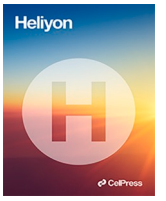

Research article

Aging related obesity and type 2 diabetes mellitus suppress neuromuscular communication and aggravate skeletal muscle dysfunction in rhesus monkeys

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ABSTRACT

Age-related functional deterioration in skeletal muscle raises the risk for falls, disability, and mortality in the elderly, particularly in obese people or those with type 2 diabetes mellitus (T2D). However, the response of the skeletal muscle to transitioning from obesity to diabetes remains poorly defined, despite that obesity is classified as a stage of pre-diabetes. We screened and selected spontaneously obese and diabetic rhesus monkeys and examined altered protein expression in skeletal muscle of healthy aging (CON), obesity aging (OB), and type 2 diabetes mellitus aging (T2D) rhesus monkeys using Tandem Mass Tags (TMT)-based quantitative proteomic analysis. In total, we identified 142 differentially expressed proteins. Muscle-nerve communication proteins were firstly suppressed at obese-stage. With the disintegration of skeletal muscle, mitochondrial complex I and other energy homeostasis relate proteins were significantly disordered at T2D stage. Indicating that aging related obesity suppressed muscle-nerve communication and contribute to T2D related functional deterioration of skeletal muscles in elderly rhesus monkeys. Some alterations of muscular functional regulator are detected in both obesity and T2D samples, suggesting some T2D related skeletal muscular hypofunctions are occurring at obesity or pre-obesity stage. Muscle-nerve communication proteins and muscular function related proteins could be potential therapy target or early diagnose marker of for skeletal muscular hypofunctions in aging obesity populations.

1. Introduction

Typically, aging is defined as the gradual deterioration of physical functioning and is associated with a high incidence of chronic disease, such as cardiovascular diseases and T2D [1,2]. Aging research in the past few decades suggest that a decline in body function, which is often triggered by cellular senescence and organ aging, contribute to worsen of quality of life in elderly [3]. With the

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accelerated growth of the global aging population, research and development on healthy aging strategies remain critical. Muscle mass gradually decreases during the aging process, followed by a reduction in contractile function of skeletal muscle [4]. Meanwhile, alternated lipid metabolism in the elderly could deteriorate the muscle function and arise the risk of T2D. It has been reported that patients with diabetes exhibit reduced lower extremity muscle strength (8–45% weaker) when compared with that of age-matched non-diabetic controls [5]. Hence, prevention of fall and fracture in elderly require a deep understanding of crosstalk among aging related obesity, T2D and decline in muscular function.

In 2020, the overweight and obesity rates for adults were 35.0 and 14.6% in China, respectively, revealing an increase of 2.3 and 4.1% from 2014; in the elderly, the rates were 41.7 and 16.7% [6]. In the United States, the prevalence of obesity in adults over 60 years is growing, with more than 37% deemed obese [7]. As a global epidemic, the number of aging and obese people is steadily increasing [8,9].

Obesity was reported associated with accelerated aging process [10], probably due to increased risk of metabolic diseases, such as T2D [11], which affect 10% of the global population. According to the International Diabetes Federation, an estimated 693 million individuals worldwide will suffer from diabetes by 2045 [11]. Falls are a major cause of morbidity and mortality among the elderly [12], and T2D increase the risk of falls and fractures in elderly [13]. Various potential complications from diabetes mellitus, such as diabetic retinopathy and peripheral neuropathy exhibiting as diabetic foot ulcer or inadequate glycemic control resulting in hypoglycemia, could be the cause of falls [14–16]. Skeletal muscle is one of the major tissues for the development of T2D, and muscle function decline with increased age is a well-known phenomenon leading to sarcopenia and decreased daily activity. Obesity-induced skeletal muscle contractile disturbance may result in negative obesity cycles: reduced exercise capacity may lower activity levels and energy expenditure, resulting in additional weight gain and, as a result, a lower quality of life [17].However, there is currently a lack of effective medicinal intervention for functional decline with age in skeletal muscle. Since the interplay among skeletal muscle health, obesity, and T2D has yet to be defined.

Since rhesus monkeys are genetically, anatomically, physiologically, and behaviorally similar to humans [18]. The use of lab monkeys is a plus as compared to similar human studies as they are raised under controlled conditions. In this study, we compared skeletal muscle from obese and T2D aging monkeys to control samples from healthy aging monkeys using Tandem Mass Tags (TMT) quantitative proteomic approach. The study revealed skeletal muscle proteome changes and enhanced existing evidence of interaction between muscular function, obesity, and T2D in elderly.

2. Materials and methods

2.1. Spontaneous obese and diabetic rhesus monkeys (Macaca mulatta) generation and study approval

Spontaneously obese and diabetic rhesus monkeys were screened as previously described [18]. Briefly, obesity was defined as body mass index (BMI) $\geq$ 40 kg/m^2, and T2DM was defined as plasma levels of fasting blood glucose $\geq$7.0 mmol/L. Finally, the study comprised the following groups (n = 3/group; 12–18 years): healthy female monkeys (CON), obese female monkeys (OB), and T2DM female monkeys (T2D). The monkeys were anesthetized using 20 mg/kg ketamine, followed by harvesting skeletal muscle (gastrocnemius) samples (100 mg/animal) [19]. All the experimental protocols were approved by the National Care and Use of Animals approved by the National Animal Research Authority (People's Republic of China) and the Institutional Animal Care and Use Committee of the Kunming Institute of Zoology of Chinese Academy of Sciences. The nonhuman primate care and experimental procedures were approved by the Ethics Committee of Kunming Institute of Zoology and the Kunming Primate Research Center, of the Chinese Academy of Sciences (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care). All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines.

2.2. Protein digestion

SDT method (4% sodium dodecyl sulfate [SDS], 100 mmol/L Tris/HCl, 0.1 mol/L DTT, pH 7.6) was used for protein extraction. An appropriate amount of protein was subjected to trypsin digestion using FASP (Filter aided proteome preparation). The peptides were desalted with C18 Cartridge, and lyophilized peptides were reconstituted with 40 μL of 0.1% formic acid solution. The same protein in different samples was labeled with TMT; subsequently, the BCA method was used to reconstitute peptides for quantitative analysis.

2.3. TMT labeling

Briefly, 100 μg of peptides was used to label each sample using the TMTsixplex™ Isobaric Label Reagent Set (#90111, Thermo Fisher Scientific) according to the kit instructions.

2.4. Reversed-phase fractionation

High pH Reversed-Phase Peptide Fractionation Kit (#84868, Thermo Fisher Scientific) was used to fractionate TMT-labeled peptides. The column was equilibrated with acetonitrile and 0.1% trifluoroacetic acid (TFA), followed by loading a mixed sample of TMT-labeled peptides, desalted at low-speed centrifugation. Then, the bound peptides were subjected to gradient elution with gradient concentrations of high pH acetonitrile solution. After vacuum drying, reconstitution was performed using 12 μL of 0.1% fatty acid (FA), and the peptide concentration was measured based on ultraviolet (UV) absorbance at 280 nm.

2.5. Cation exchange column (SCX) fractionation

After blending using AKTA Purifier 100 (GE, Uppsala, Sweden), each TMT-labeled peptide set was subjected to fractionation. Buffer A (10 mmol/L KH2PO4, 25% acetonitrile; pH 3.0) and buffer B (10 mmol/L KH2PO4, 500 mmol/L KCl, 25% acetonitrile; pH 3.0) were prepared. The chromatographic column was equilibrated with buffer A, and the relevant sample was loaded onto the chromatographic column using an injector, with the flow rate set to 1 mL/min. The gradient of buffer B was follows: 0%, 25 min; 0–10%, 25–32 min; 10–20%, 32–42 min; 20–45%, 42–47 min; 45–100%, 47–52 min; 100%, 52–60 min. After 60 min, buffer B was reset to 0%. UV absorbance was monitored at 214 nm during the elution process, and the elution fractions were collected every 1 min. After lyophilization, desalting was achieved using a C18 Cartridge.

2.6. Liquid chromatography with tandem mass spectrometry (LC-MS/MS)

Each graded sample was separated using a high-pressure liquid chromatography liquid phase system, Easy nLC, with a nanoliter flow rate. Buffer A was 0.1% formic acid aqueous solution, and buffer B was 0.1% formic acid-acetonitrile (acetonitrile was 84%). The column was equilibrated with 95% buffer A, and the sample was loaded to the C18 reversed-phase analytical column (Thermo Scientific Acclaim PepMap100, 100 μm × 2 cm, nanoViper C18) using an injector. Buffer B, used for linear gradient separation, was flown

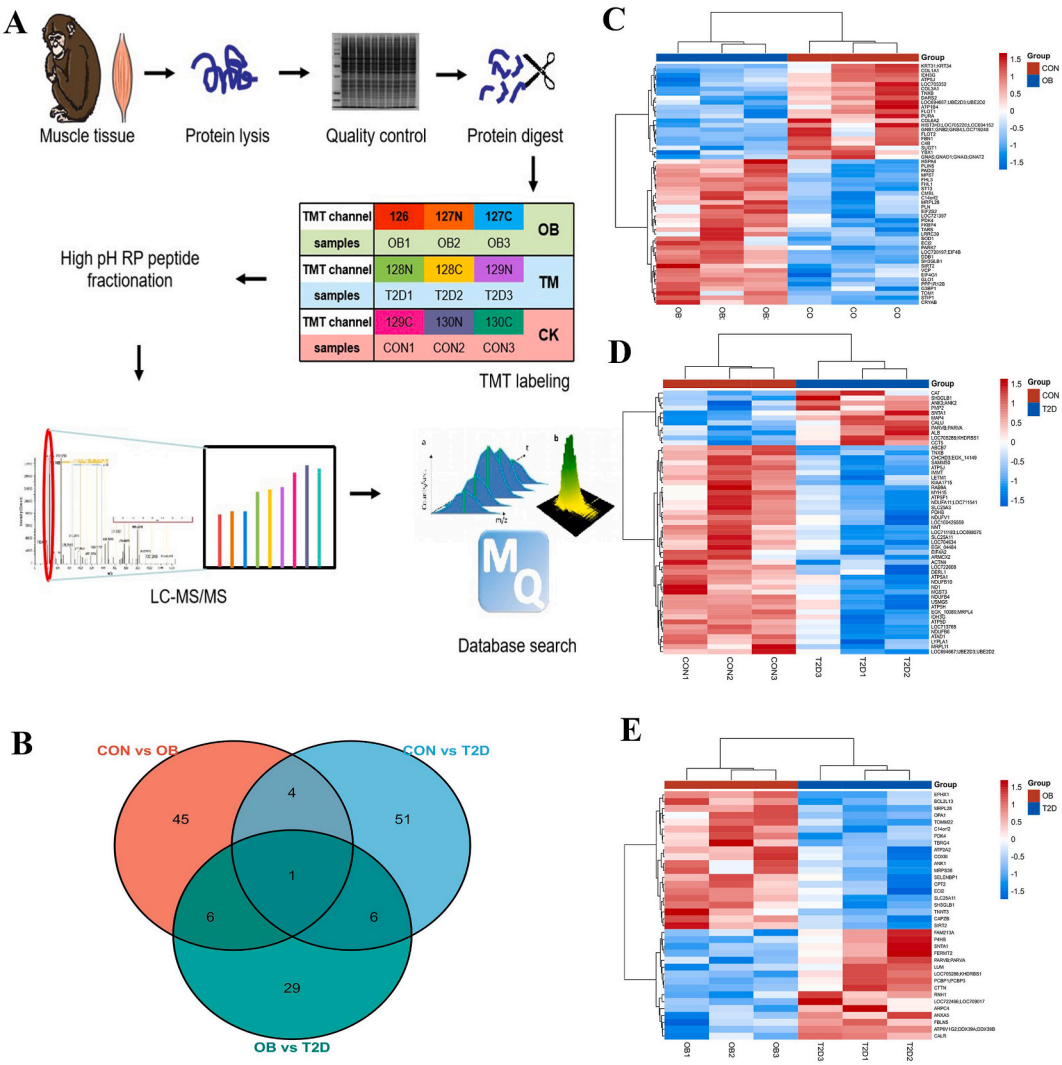

Fig. 1. Skeletal muscle proteomic profiles of healthy aging (CON), obese aging (OB), and T2DM aging (T2D) rhesus monkeys. A: Schematic diagram of experimental design. B: Venn diagram of proteins with altered expression levels in the three monkey groups. C: Dendrogram of the hierarchical clustering analysis of expression values for 56 proteins in the CON and OB rhesus monkeys. D: Dendrogram of the hierarchical clustering analysis of expression values for 62 proteins in the CON and T2D rhesus monkeys. E: Dendrogram of the hierarchical clustering analysis of expression values for 42 proteins in OB and T2D rhesus monkeys. n = 3/group. T2DM, type 2 diabetes mellitus.

through an analytical column (Thermo Scientific EASY column, 10 cm, ID75 μm, 3 μm, C18-A2) at a flow rate of 300 nL/min. Then, mass spectrometric analysis was performed using a Q-Exactive mass spectrometer.

2.7. Data analysis

Peptide identification and quantitation were performed using Maxquant software (http://www.coxdocs.org/doku.php?id=maxquant:start, version 1.5.1.0). Proteins were considered significantly altered between experimental groups if a p-value of < 0.05 was observed on assessing their corresponding mean of TMT ratios using the student's t-test. Differentially expressed proteins were subjected to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and biological process enrichment using the DAVID database (http://david.abcc.ncifcrf.gov/). Gene Ontology (GO) terms and pathways with a p-value <0.05 were deemed significantly enriched. Results are shown as mean and standard errors (mean ± SE). Figures were prepared using GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA) and "pheatmap" in language R.

3. Results

3.1. Obesity and T2D changing skeletal muscle proteomic profiling

To validate the affection of obesity and T2D on skeletal muscle health at a proteome level, TMT-based quantitative proteomic analysis performed in the present study (Fig. 1A). A total of 1368 proteins were identified. Among these, 142 differentially expressed proteins were significantly altered based on established criteria of a $p < 0.05$ on comparing two groups (CON vs. OB, CON vs. T2D, and OB vs. T2D), as listed in Supplemental Table S1. The Venn diagram illustrates the distinct and overlapping proteins with altered expression among CON, OB, and T2D groups (Fig. 1B). Compared with the CON group, expression levels of 56 and 62 proteins were altered in OB and T2D groups, respectively (Fig. 1B). Meanwhile, the expression of 42 proteins significantly differed between OB and

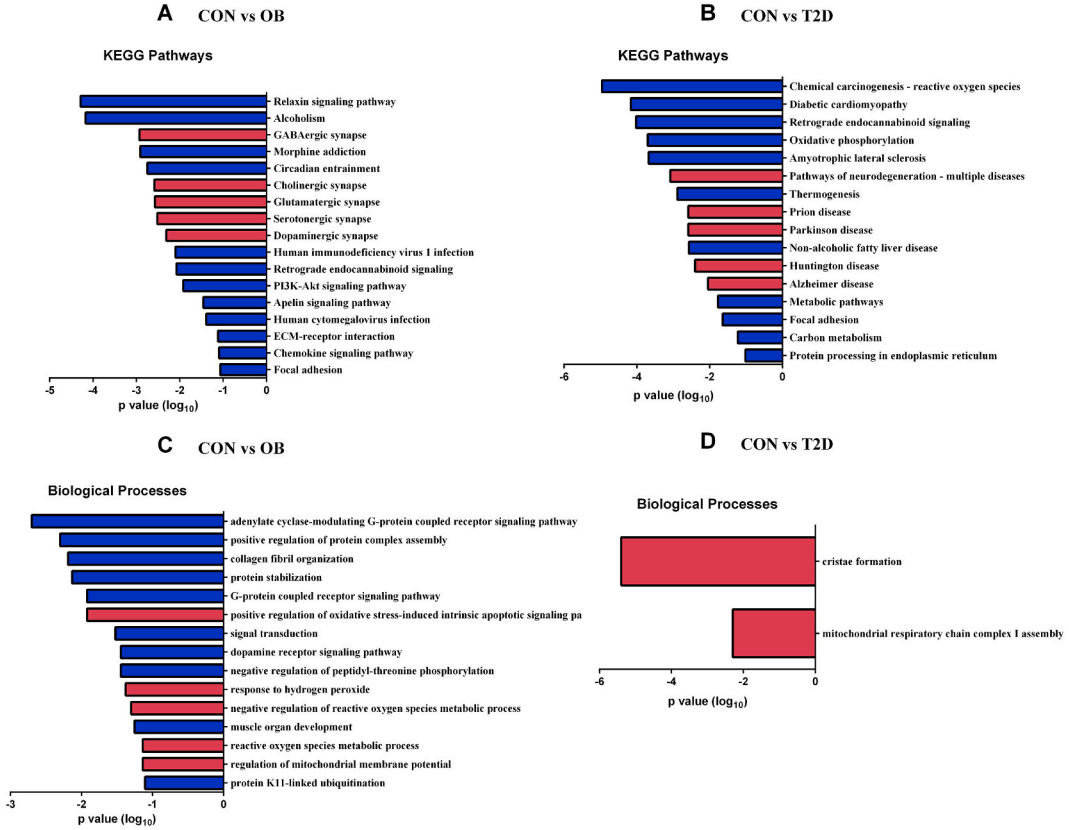

Fig. 2. KEGG pathways and biological processes enriched between healthy aging and disease-aging rhesus monkeys based on significantly altered skeletal muscle protein levels. A: KEGG pathway enrichment analysis of altered proteins between the CON and OB monkeys. B: KEGG pathway enrichment analysis of altered proteins between CON and T2D monkeys. C: Biological process enrichment analysis of altered proteins between CON and OB monkeys. D: Biological process enrichment analysis of altered proteins between CON and T2D monkeys. $p < 0.05$, n = 3/group. , CON, healthy aging monkeys; OB, obese aging monkeys; T2D, T2DM aging monkeys; T2DM, type 2 diabetes mellitus; KEGG, Kyoto Encyclopedia of Genes and Genomes.

T2D groups (Fig. 1B). Overlapping proteins were limited between experimental groups. Pairwise heatmaps indicate the overall differences in protein expression (Fig. 1C–E).

3.2. Skeletal muscle proteome revealed a distinct metabolic signature

The results revealed significantly enriched KEGG pathways and biological processes based on the p-value. Proteins associated with the "Relaxin signaling pathway" and "adenylate cyclase-modulating G-protein coupled receptor signaling pathway" showed high enrichment in the CON vs. OB comparison, while proteins involved in "Chemical carcinogenesis-reactive oxygen species" and "Cristae formation" were highly enriched in the CON vs. T2D comparison. Compared to the CON group, the OB group exhibited enrichment in KEGG pathways related to synapse functions, including "GABAergic synapse," "Cholinergic synapse," "Glutamatergic synapse," "Serotonergic synapse," and "Dopaminergic synapse" (Fig. 2A). Conversely, the T2D group showed enrichment in KEGG pathways associated with various aging-related neurological diseases such as "Parkinson's disease," "Prion disease," "Huntington's disease," and

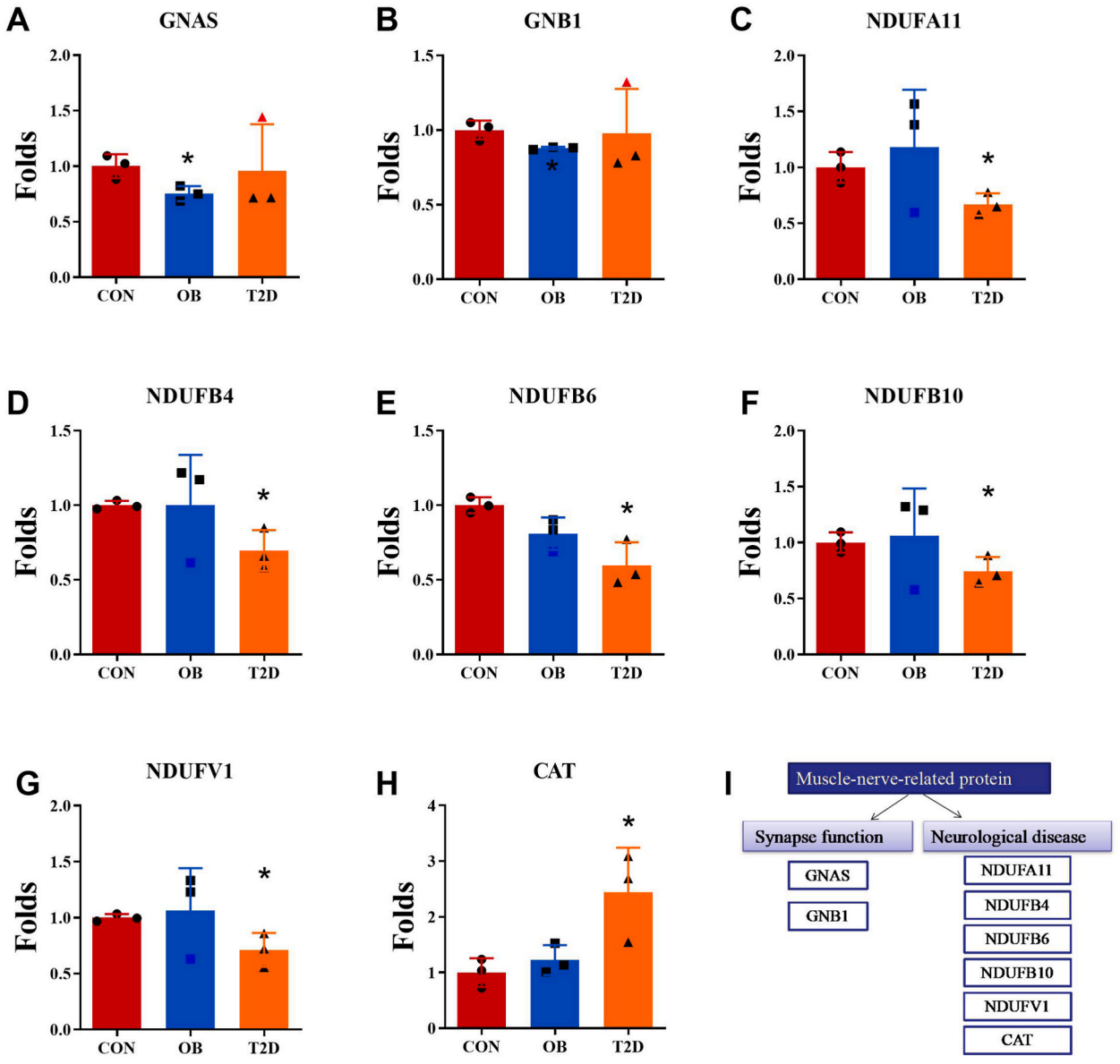

Fig. 3. Muscle-nerve-related protein alterations among healthy aging (CON), obese aging (OB), and diabetic aging (T2D) monkeys. A: GNAS; B: GNB1; C: NDUFA11; D: NDUFB4; E: NDUFB6; F: NDUFB10; G: NDUFV1; H: CAT; I: The list of related proteins function. The red triangle indicates the abnormal increasing levels belonging to the same monkey, and the blue square indicates an abnormal decreasing level belonging to the same monkey * means $p < 0.05$ by *t*-test, n = 3/group.

"Alzheimer's disease" (Fig. 2B). Furthermore, biological processes highly enriched in both groups included "Positive regulation of oxidative stress-induced intrinsic apoptotic signaling pathway," "response to hydrogen peroxide," "negative regulation of reactive oxygen species metabolic process," "regulation of mitochondrial membrane potential," "reactive oxygen species metabolic process," "mitochondrial respiratory chain complex I assembly," and "cristae formation" (Fig. 2C and D).

3.3. Muscle-nerve-related protein alterations in healthy, obese, and diabetic monkeys

Compared with the CON group, OB monkeys exhibited reduced levels of synapse-related proteins. Expression levels of guanine nucleotide binding protein alpha stimulating complex locus (GNAS) and G protein subunit beta 1 (GNB1) were significantly decreased in OB monkeys (Fig. 3 A and B, $p < 0.05$). Compared with the CON group, NADH:ubiquinone oxidoreductase subunit (NDUF) family proteins were broadly suppressed in T2D group. Expression levels of NDUFA11, NDUFB4, NDUFB6, NDUFB10 and NDUFV1 were significantly decreased in T2D monkeys (Fig. 3 C-G, $p < 0.05$), however, NDUFA11, NDUFB4, NDUFB10 and NDUFV1 were increased in the 2 OB monkeys. Catalase (CAT) was significantly increased in T2D monkeys (Fig. 3H, $p < 0.05$). In summary, synthase functional related proteins are down-regulated in the OB group and neurological disease related proteins are disordered in the T2D group (Fig. 3I).

3.4. Muscle function and metabolism related protein disturbed in obese, and diabetic monkeys

To investigate whether suppression of muscle-synthase communication proteins leads to reduced muscular function, we analyzed alterations in the expression of proteins involved in muscle contraction, energy balance, collagen, and other factors related to muscle health in the aging population. Hierarchical clustering analysis revealed that both obesity and T2D influenced the expression of proteins related to muscle health (Fig. 4A), indicating that the functional decline of skeletal muscle in aging may initiate during the obesity or pre-diabetes stage, rather than at the T2D stage. Furthermore, we observed that 8 out of 10 proteins related to muscle disorders and muscle contraction were elevated in both the OB and T2D monkeys (Fig. 4B and C). Among them, FHL1, a protein involved in sarcomere formation, showed the most significant upregulation. Additionally, phospholamban (PLN), a muscle-specific sarcoplasmic reticulum (SR) Ca-ATPase inhibitor, exhibited a 2.2-fold increase in the OB group and a 1.8-fold increase in the T2D group (Fig. 4B and C). ATP synthesis-related proteins were significantly decreased in the T2D monkeys, with the exception of ATP5J, which showed reductions of 52% and 55% in the OB and T2D monkeys, respectively (Fig. 4D). Corresponding to the decreased ATP generation proteins, RAB9A, a GDP binding protein, was significantly downregulated in the T2D group. Interestingly, PMP2, a lipid transport/binding protein in Schwann cells, was found to be significantly upregulated in the T2D group (Fig. 4E). In addition, expression of 3 protein folding related proteins (VCP, FKBP4 and STIP1) were detected significantly increased in the OB when

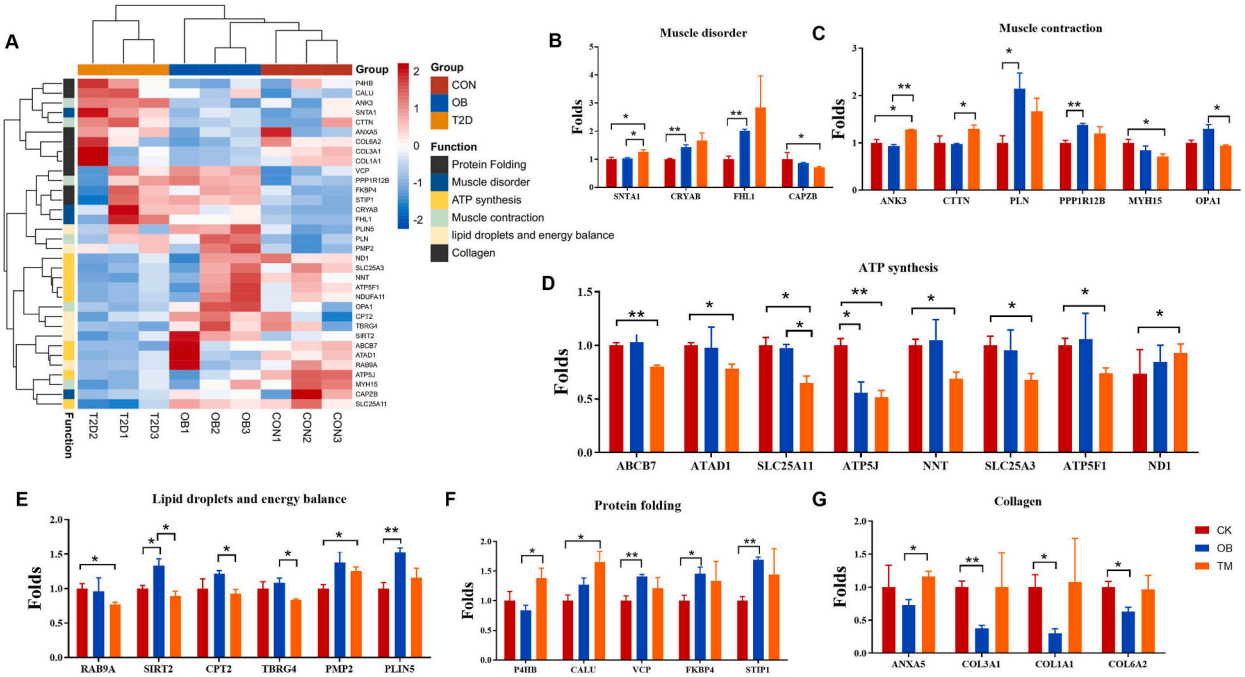

Fig. 4. Comparison of muscle function-related protein expression in healthy aging (CON), obese aging (OB), and diabetic aging (T2D) monkeys. A: Dendrogram of hierarchical clustering analysis of nine samples from CON, OB, and T2D monkeys. B:Proteins related to muscle disorders. C: Proteins related to muscle contraction. D: Proteins related to ATP synthesis. E: Proteins related to lipid droplets and energy balance. F: Proteins related to protein folding. G: Proteins related to collagen and blood clotting. * means $p < 0.05$ by *t*-test. ** means $p < 0.01$ by *t*-test; n = 3/group.

compared with the CON group. Calumenin (CALU), an ER located calcium-binding protein, upregulated stepwise from the CON to the OB and T2D (Fig. 4F, $p < 0.05$). Expression levels of collagen- and blood clotting-related proteins were significantly decreased in OB monkeys, which were recovered in T2D monkeys (Fig. 4G).

4. Discussion

4.1. Deterioration of neuromuscular junction (NMJ) function

In this study, we detected the downregulation of GNAS and GNB1 in skeletal muscle of obese aging monkeys. GNAS and GNB1 are highly expressed in neuromuscular junction (NMJ) and play a role in maintenance of normal function of NMJ by establishing functional communication between motor neurons and skeletal muscle. Mutation of GNAS results in Pseudohypoparathyroidism (PHP), which is characterized by steppage gait, myalgia atrophies in the lower legs and other neuromuscular symptoms [20]. GNB1 is reported that involved in intracellular signal transduction of NMJ. A disorder in expression of GNB1 may contribute to progression of amyotrophic lateral sclerosis [21].

Further, the respiratory complex I is affected in skeletal muscle of T2D monkeys. 6 of complex I subunits are found reduced, including NUDFA11, B4, B6, B10, V1 and ND1. Mutations in subunits of complex I lead to various neuromuscular and metabolic disorders. Suggesting a deterioration of NJM function in skeletal muscle of T2D aging monkeys compared with obese monkeys. It looks as though a consequence from disorder of NJM proteins at obesity stage. Suppression of NJM proteins complex I related neuromuscular disorder could be a contributor of highly prevalence of diabetic peripheral neuropathy (DPN), characterized by marked axonal degeneration and segmental demyelination, affecting up to 50% of individuals with diabetes [22,23]. Our study suggests that DPN, as well as other aging associated skeletal muscle disorder, may originate in obese stage or pre-diabetes stage. NJM proteins may be potential therapy targets for aging related skeletal muscle disorder. Meanwhile, obese aging individuals should pay attention to muscle function.

4.2. ATP synthesis and aggregation of ROS

The skeletal muscle disorder is detectable in obese-aging stage, through the ATP produce is still maintained. Only ATP5J was detected down regulated in skeletal muscle of obese monkeys. Along with the progress of obesity and deteriorated insulin sensitivity, ATP generating proteins were significantly inhibited in skeletal muscle at the T2D stage. ATP5F1 and ATP5J, subunits of ATP synthase in mitochondrial, are significantly downregulated in T2D stages. Meanwhile, some important transporters in mitochondrial, including ABCB7, SLC25A3 and SLC25A11, are significantly suppressed at the T2D stage. ABCB7 responsible for transporting various molecules across extra- and intra-cellular membranes. SLC25A3 and A11 function in transport phosphate ion and malate for oxidative phosphorylation, respectively. Previous studies showed that point mutation in SLC25A3 leads to ATP synthase deficiency in muscle cells. Suggesting that T2D fuel the dysfunction of various “cargo” transportation and ATP synthesis in muscular mitochondria.

On the other hand, suppression of respiratory complex I could elevate ROS level in skeletal muscle of aging T2D patients [24]. Then, the elevated ROS level deteriorated by decrease of NNT, a protein involved in antioxidant in the mitochondrial. Suppression of complex I and NNT leads to more ROS production and limited ROS clearance capacity, and finally the aggregation of ROS.

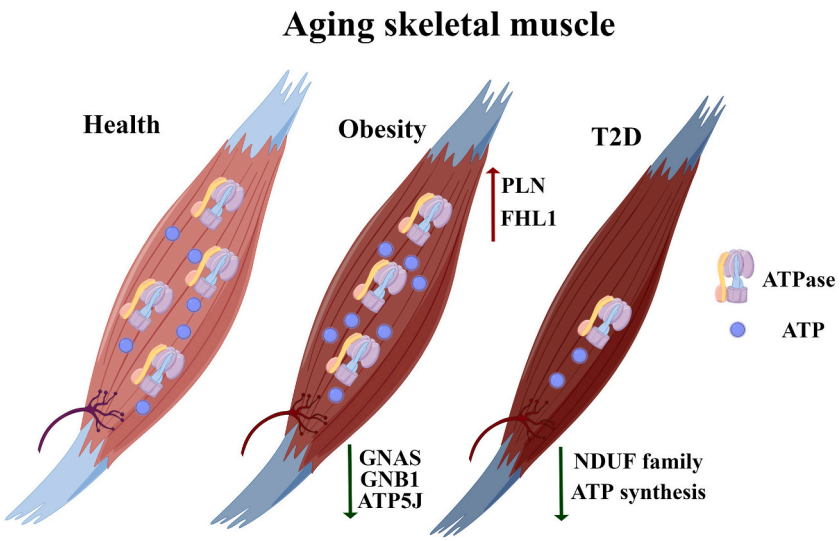

Fig. 5. Schematic diagram of skeletal muscle alteration in health, obesity, and type 2 diabetic (T2D) monkeys. The red arrow indicates increase, and green arrow indicates decrease.

Accompanied with the disorder of NMJ related proteins, PLN and FHL1 are significantly upregulated in obesity groups. PLN is an inhibitor of sarco (endo)plasmic reticulum Ca2+-ATPases (SERCAs). Overexpression of PLN results in reduced Ca2+ uptake and Centronuclear myopathy in rodent models [25]. This may be helpful to explain the crosstalk between calcium metabolic disorder and reduced muscle function in aging individuals. FHL1 is a 280-amino-acid protein, which containing 4 LIM domains and heavily expressed in skeletal muscle. Mutations in FHL1 associated with scapuloperoneal myopathy, X-linked myopathy with postural muscle atrophy and reducing body myopathy. Upregulation of PLN and FHL1 is a sign that disorder of muscle function in obese aging individuals.

In summary, aging obese individuals harbors a suppression of NJM proteins and mildly decrease of ATP synthase. Then the mitochondrial complex I components are down regulated at T2D stage and leads to elevated ROS level in skeletal muscle. Finally impair the ATP synthase capacity and contractile function of skeletal muscle (Fig. 5).

However, this study only focuses on the profile of muscle disorder from obesity to T2D, and limitation on the number of experimental monkeys can not provide the detailed mechanism about how the altered proteins in obesity are interconnected with the altered proteins in T2D monkeys. The proteins we highlighted should be deeply study soon.

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Data availability statement

All data from this article are available from the corresponding authors on request.

CRediT authorship contribution statement

Shaoxia Pu: Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization. **Yaowen Liu:** Writing – review & editing, Data curation. **Wenjun Wu:** Writing – original draft, Project administration, Data curation. **Fei Sun:** Writing – original draft, Data curation. **Hongsheng Lu:** Project administration, Methodology. **Xiaocui Xu:** Writing – original draft, Project administration, Methodology. **Yanhua Su:** Writing – review & editing, Methodology. **Wenming Cheng:** Writing – review & editing, Supervision, Methodology. **Haizhen Wang:** Funding acquisition, Methodology, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Shaoxia Pu reports financial support was provided by Yunnan Fundamental Research Projects (grant NO. 202101AU070095). Haizhen Wang reports financial support was provided by Yunnan Fundamental Research Projects (grant NO. 202201AS070081) and National Natural Science Foundation of China (grant NO. 32060206). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28549.

References

[1] D.G. Burton, R.G. Faragher, Obesity and type-2 diabetes as inducers of premature cellular senescence and ageing, Biogerontology 19 (2018) 447–459.
[2] J. Campisi, P. Kapahi, G.J. Lithgow, S. Melov, J.C. Newman, E. Verdin, From discoveries in ageing research to therapeutics for healthy ageing, Nature 571 (2019) 183–192.
[3] R.D. Micco, V. Krizhanovsky, D. Baker, F. d'Adda di Fagagna, Cellular senescence in ageing: from mechanisms to therapeutic opportunities, Nat. Rev. Mol. Cell Biol. 22 (2021) 75–95.
[4] A.J. Cruz-Jentoft, J.P. Baeyens, J.M. Bauer, Y. Boirie, T. Cederholm, F. Landi, F.C. Martin, J.P. Michel, Y. Rolland, S.M. Schneider, E. Topinková, M. Vandewoude, M. Zamboni, Sarcopenia: European consensus on definition and diagnosisReport of the European working group on sarcopenia in older people, age, Age (Chester) 39 (2010) 412–423.
[5] G. Orlando, M. Sacchetti, V. D'Errico, J. Haxhi, G. Rapisarda, G. Pugliese, S. Balducci, Muscle fatigability in patients with type 2 diabetes: relation with long-term complications, Diabetses-Metab, Res. 6 (2020) e3231.

[6] Z. Liu Agudamu, T. Bu, S. Akpinar, B. Jabucanin, The association between the China's economic development and the passing rate of national physical fitness standards for elderly people aged 60–69 from 2000 to 2020, Front. Public Health 10 (2022) 857691.
[7] J.A. Batsis, A.B. Zagaria, Addressing obesity in aging patients, Med. Clin. 102 (2018) 65–85.
[8] E. Vásquez, J.A. Batsis, C.M. Germain, B.A. Shaw, Impact of obesity and physical activity on functional outcomes in the elderly: data from NHANES 2005-2010, J. Aging Health 26 (2014) 1032–1046.
[9] F.B.C. Pimenta, E. Bertrand, D.C. Mograbi, H. Shinohara, J. Landeira-Fernandez, The relationship between obesity and quality of life in Brazilian adults, Front. Psychol. 6 (2015) 1–7.
[10] R.N. Baumgartner, S.J. Wayne, D.L. Waters, I. Janssen, D. Gallagher, J.E. Morley, Sarcopenic obesity predicts instrumental activities of daily living disability in the elderly, Obes. Res. 12 (2004) 1995–2004.
[11] N.H. Cho, J.E. Shaw, S. Karuranga, Y. Huang, J.D. da Rocha Fernandes, A.W. Ohlrogge, B. Malanda, IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045, Diabetes, Res. Clin. Pr. 138 (2018) 271–281.
[12] L.Z. Rubenstein, Falls in older people: epidemiology, risk factors and strategies for prevention, Age, Age (Chester) 35 (2006) 37–41.
[13] E. Phielix, M. Mensink, Type 2 diabetes mellitus and skeletal muscle metabolic function, Physiol. Behav. 94 (2008) 252–258.
[14] E.W. Gregg, G.L. Beckles, D.F. Williamson, S.G. Leveille, J.A. Langlois, M.M. Engelgau, K.M. Narayan, Diabetes and physical disability among older U.S. adults, Diabetes Care 23 (2000) 1272–1277.
[15] C. Wallace, G.E. Reiber, J. LeMaster, D.G. Smith, K. Sullivan, S. Hayes, C. Vath, Incidence of falls, risk factors for falls, and fall-related fractures in individuals with diabetes and a prior foot ulcer, Diabetes Care 25 (2002) 1983–1986.
[16] V. Rashedi, A. Iranpour, M. Mohseni, V. Borhaninejad, Risk factors for fall in elderly with diabetes mellitus type 2, Diabetes, Metab. Syndr. 13 (2019) 2347–2351.
[17] R. Busutil, O. Espallardo, A. Torres, L. Martínez-Galdeano, N. Zozaya, Á. Hidalgo-Vega, The impact of obesity on health-related quality of life in Spain, Health Qual. Life Outcome 15 (2017) 1–11.
[18] J. Wang, S. Xu, J. Gao, L. Zhang, Z. Zhang, W. Yang, Y. Li, S. Liao, H. Zhou, P. Liu, B. Liang, SILAC-based quantitative proteomic analysis of the livers of spontaneous obese and diabetic rhesus monkeys, Am. J. Physiol-Endoc. M. 315 (2018) E294–E306.
[19] H. Baş, A.O. Baş, S. Ceylan, M. Güner, M. Koca, M. Hafızoğlu, Z. Şahiner, Y. Öztürk, C. Balcı, B.B. Doğu, M. Cankurtaran, M.G. Halil, Lower gastrocnemius muscle stiffness, derived from elastography, is an independent factor for falls in older adults, Aging Clin. Exp. Res. 35 (2023) 2979–2986.
[20] K. Nagasaki, S. Tsuchiya, A. Saitoh, T. Ogata, M. Fukami, Neuromuscular symptoms in a patient with familial pseudohypoparathyroidism type Ib diagnosed by methylation-specific multiplex ligation-dependent probe amplification, Endocr. J. 60 (2013) 231–236.
[21] S. Apolloni, S. Amadio, P. Fabbrizio, G. Morello, A.G. Spampinato, E.C. Latagliata, I. Salvatori, D. Proietti, A. Ferri, L. Madaro, S. Puglisi-Allegra, S. Cavallaro, C. Volonté, Histaminergic transmission slows progression of amyotrophic lateral sclerosis, J. Cachexia. Sarcopeni. 10 (2019) 872–893.
[22] R. Pop-Busui, A.J.M. Boulton, E.L. Feldman, V. Bril, R. Freeman, R.A. Malik, J.M. Sosenko, D. Ziegler, Diabetic neuropathy: a position statement by the American Diabetes Association, Diabetes Care 40 (2017) 136–154.
[23] G. Said, Diabetic neuropathy—a review, Nat. Rev. Neurol. 3 (2007) 331–340.
[24] S.X.H. Mthembu, P.V. Dludla, T.M. Nyambuya, A.P. Kappo, E. Madoroba, K. Ziqubu, T.A. Nyawo, B.B. Nkambule, S. Silvestri, C.J.F. Muller, S.E. Mazibuko-Mbeje, Experimental models of lipid overload and their relevance in understanding skeletal muscle insulin resistance and pathological changes in mitochondrial oxidative capacity, Biochimie 196 (2022) 182–193.
[25] V.A. Fajardo, E. Bombardier, E. McMillan, K. Tran, B.J. Wadsworth, D. Gamu, A. Hopf, C. Vigna, I.C. Smith, C. Bellissimo, R.N. Michel, M.A. Tarnopolsky, J. Quadrilatero, A.R. Tupling, Phospholamban overexpression in mice causes a centronuclear myopathy-like phenotype, Dis. Model. Mech. 8 (2015) 999–1009.