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Original Research Article

Biochemical Mechanisms Linking Mitochondrial Dysfunction to Insulin Resistance in Human Diabetes

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Abstract: ***Background:*** Insulin resistance in skeletal muscle is a defining feature of type 2 diabetes mellitus (T2DM), yet the biochemical mechanisms linking mitochondrial dysfunction to impaired insulin action in humans remain incompletely understood. This study investigated how alterations in mitochondrial bioenergetics, lipid metabolism, oxidative stress, and insulin signaling contribute to skeletal muscle insulin resistance. In a cross sectional study, healthy controls (n = 20), obese insulin resistant individuals (n = 20), and patients with T2DM (n = 20) underwent metabolic characterization using the hyperinsulinemic–euglycemic clamp. Skeletal muscle biopsies were analyzed for mitochondrial oxidative capacity, ATP synthesis rates, reactive oxygen species (ROS) production, intramyocellular lipid content, fatty acid oxidation, and insulin signaling proteins using high resolution respirometry, biochemical assays, and Western blotting. Whole body insulin sensitivity declined progressively from controls to obese insulin resistant individuals and T2DM patients, with glucose infusion rates of 9.1 ± 1.2, 5.4 ± 1.0, and 3.8 ± 0.9 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively ($p < 0.001$). Maximal ADP stimulated mitochondrial respiration was reduced by 28% in obese insulin resistant participants and by 41% in T2DM, while citrate synthase activity was decreased by 22% in T2DM muscle. Basal ATP synthesis rates were significantly lower in obese insulin resistant (7.9 ± 0.8 $\mu mol \cdot L^{-1} \cdot min^{-1}$) and T2DM individuals (7.2 ± 0.7 $\mu mol \cdot L^{-1} \cdot min^{-1}$) compared with controls (10.4 ± 0.9 $\mu mol \cdot L^{-1} \cdot min^{-1}$), with a blunted insulin stimulated increase. Mitochondrial ROS production increased by ~1.6 fold and ~2.1 fold in obese insulin resistant and T2DM groups, respectively. Intramyocellular lipid content increased by ~45% and ~70%, while fatty acid oxidation declined by 34% and 52%. Insulin stimulated IRS 1 and Akt phosphorylation were reduced by 38% and 55%, and GLUT4 expression was significantly decreased in T2DM muscle. These findings demonstrate that mitochondrial dysfunction, lipid accumulation, and oxidative stress converge to impair insulin signaling and glucose transport in human skeletal muscle, highlighting mitochondrial bioenergetic and redox pathways as key contributors to insulin resistance and T2DM.

Keywords: Insulin resistance; Type 2 diabetes mellitus; Skeletal muscle; Mitochondrial dysfunction; Oxidative stress; Lipid metabolism; Insulin signaling.

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both (Sangwung, Petersen, Shulman, & Knowles, 2020). Among its subtypes, type 2 diabetes mellitus (T2DM) accounts for approximately 90–95% of all diabetes cases worldwide and represents a rapidly escalating global health burden. Recent estimates from the International Diabetes Federation (IDF) indicate that 589 million adults aged 20–79 years were living with diabetes in 2024, with projections rising to approximately 853 million by 2050, reflecting a ~45% increase over the next three decades (Takano, Ogawa, & Hayakawa, 2023). This dramatic rise parallels increasing rates of obesity, sedentary lifestyles, and metabolic dysregulation, underscoring the urgent need to elucidate the cellular mechanisms driving insulin resistance (Zhao et al., 2023).

Insulin resistance, defined as the diminished ability of insulin to stimulate glucose uptake and suppress hepatic glucose production, is a central pathological feature in the development of T2DM. Skeletal muscle plays a dominant role in this process, accounting for approximately 70–80% of insulin-stimulated glucose disposal in the postprandial state (Galizzi & Di Carlo, 2022; Wang & Wei, 2020). Consequently, molecular defects within skeletal muscle substantially influence whole-body glucose homeostasis. Over the past two decades, accumulating evidence from human in vivo, ex vivo, and clinical studies has identified mitochondrial dysfunction in insulin-responsive tissues—particularly skeletal muscle—as a key contributor to insulin resistance (Ho et al., 2022; Pei, Wang, & Wang, 2022).

Mitochondria are essential regulators of cellular energy metabolism, responsible for oxidative phosphorylation and ATP production. In insulin-resistant individuals and patients with T2DM, skeletal muscle mitochondria exhibit 20–40% reductions in oxidative capacity, accompanied by impaired fatty acid oxidation and reduced mitochondrial density(Andreadi et al., 2022; Ramasubbu & Devi Rajeswari, 2023). Direct measurements using phosphorus-31 magnetic resonance spectroscopy have demonstrated that basal ATP synthesis rates are reduced by approximately 30% in insulin-resistant muscle, declining from ~10.6 $\mu mol \cdot L^{-1} \cdot min^{-1}$ in healthy individuals to ~7.3 $\mu mol \cdot L^{-1} \cdot min^{-1}$ in affected patients, with insulin-stimulated ATP production showing a similar magnitude of impairment. These bioenergetic deficits compromise metabolic flexibility and favor lipid accumulation within muscle fibers (Campbell & Campbell, 2020; S.-h. Hong & Choi, 2020).
Impaired mitochondrial fatty acid oxidation leads to the intracellular buildup of lipid intermediates such as diacylglycerols and ceramides, which interfere with insulin signaling through activation of stress-sensitive kinases (da Silva Rosa, Nayak, Caymo, & Gordon, 2020). Human skeletal muscle biopsy studies have reported up to a 60% reduction in β-oxidation capacity, alongside >150% increases in cytosolic lipid accumulation in obese and diabetic individuals compared with lean controls. These lipid-derived signals promote inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1), thereby attenuating downstream PI3K–Akt signaling and glucose transporter type 4 (GLUT4) translocation (Zhang et al., 2023).

In addition to defective lipid metabolism, mitochondrial dysfunction is closely associated with excessive mitochondrial reactive oxygen species (ROS) production. While low-level ROS generation is physiologically required for redox signaling, chronic nutrient excess and mitochondrial overload result in 1.5- to 2-fold elevations in mitochondrial ROS levels in insulin-resistant tissues (Mohan, Ghazi, & Chuturgoon, 2021). Elevated ROS activate

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stress pathways, including c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB), further impairing insulin signaling and amplifying inflammatory responses. Circulating pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) have been shown to increase from ~10 pg/mL in healthy individuals to >30 pg/mL in patients with T2DM, reinforcing the link between mitochondrial oxidative stress and systemic insulin resistance (C.-T. Hong et al., 2020)

Together, these findings support a mechanistic framework in which mitochondrial dysfunction—manifested by reduced oxidative capacity, impaired ATP synthesis, defective fatty acid oxidation, and excessive ROS production—serves as a biochemical nexus linking metabolic overload to insulin resistance in human diabetes. Understanding these interconnected pathways is essential for identifying mitochondrial-targeted therapeutic strategies aimed at restoring insulin sensitivity and mitigating the progression of T2DM.

2. Materials and Methods

2.1. Study Design and Participants

This study was designed as a cross-sectional observational investigation to examine the relationship between mitochondrial function and insulin resistance in humans. A total of 60 adult participants (age 30–60 years) were recruited and stratified into three groups (n = 20 per group): (i) healthy insulin-sensitive controls, (ii) obese insulin-resistant individuals without diabetes, and (iii) patients with diagnosed type 2 diabetes mellitus (T2DM). Diagnosis of T2DM was based on American Diabetes Association criteria, including fasting plasma glucose ≥126 mg/dL or HbA1c ≥6.5%. Exclusion criteria included type 1 diabetes, mitochondrial genetic disorders, chronic inflammatory or autoimmune diseases, cardiovascular disease, active infections, smoking, pregnancy, and use of medications known to affect mitochondrial metabolism (e.g., metformin, statins, or corticosteroids). All participants provided written informed consent, and the study protocol was approved by the Institutional Ethics Committee in accordance with the Declaration of Helsinki.

2.2. Assessment of Insulin Sensitivity

Whole-body insulin sensitivity was assessed using the hyperinsulinemic–euglycemic clamp technique, considered the gold standard for measuring insulin action. After an overnight fast, insulin was infused at a constant rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$, while plasma glucose was maintained at ~90 mg/dL using a variable 20% glucose infusion. The glucose infusion rate (GIR) during steady state (last 30 minutes of the clamp) was used as an index of insulin sensitivity and normalized to body weight. In addition, homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin concentrations to provide a secondary measure of insulin resistance.

2.3 Skeletal Muscle Biopsy Collection

Percutaneous skeletal muscle biopsies were obtained from the vastus lateralis muscle under local anesthesia (2% lidocaine) using a Bergström needle. Samples were immediately divided into portions for mitochondrial respiration analysis, biochemical assays, and molecular analyses. Tissue intended for mitochondrial assays was processed fresh, while remaining samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis.

2.4. Mitochondrial Respiration and ATP Production

Mitochondrial oxidative capacity was assessed in permeabilized muscle fibers using high-resolution respirometry. Oxygen consumption rates were measured under basal conditions and following the addition of substrates for complex I and II. Maximal oxidative phosphorylation capacity was determined in the presence of ADP.

ATP synthesis rates were quantified using a luciferin–luciferase–based assay, and results were normalized to tissue protein content. Mitochondrial content was estimated by measuring citrate synthase activity spectrophotometrically as a surrogate marker of mitochondrial density.

2.5. Measurement of Reactive Oxygen Species and Oxidative Stress

Mitochondrial reactive oxygen species (ROS) production was measured fluorometrically using MitoSOX Red, a mitochondrial superoxide-specific probe. Fluorescence intensity was normalized to mitochondrial protein content. Oxidative stress was further assessed by quantifying malondialdehyde (MDA) levels as a marker of lipid peroxidation and by measuring the activity of antioxidant enzymes, including superoxide dismutase (SOD) and catalase.

2.6. Analysis of Insulin Signaling Proteins

Protein expression and phosphorylation of key insulin signaling components were analyzed by Western blotting. Total and phosphorylated forms of insulin receptor substrate-1 (IRS-1), Akt, and GLUT4 were quantified using specific antibodies. Band intensities were normalized to β-actin and expressed relative to control samples.

2.7. Lipid Content and Fatty Acid Oxidation

Intramyocellular lipid content was determined using Oil Red O staining and quantified by image analysis. Fatty acid oxidation capacity was assessed by measuring the rate of [^{14}C]-palmitate oxidation in

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muscle homogenates, expressed as nmol CO_2 produced per mg protein per hour.

2.8. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Relationships between mitochondrial parameters and insulin sensitivity were analyzed using Pearson correlation coefficients. Statistical significance was defined as $p < 0.05$. All analyses were conducted using SPSS version 26.0.

RESULTS

3.1. Participant Characteristics and Metabolic Profile

Baseline demographic and metabolic characteristics of the study population are presented in Table 1. The three groups were well matched for age and sex distribution, with no statistically significant differences observed ($p > 0.05$), minimizing potential confounding effects of these variables. In contrast, anthropometric and metabolic parameters differed markedly across groups. Body mass index (BMI) was significantly elevated in obese insulin-resistant individuals and patients with type 2 diabetes mellitus (T2DM) compared with healthy controls ($p < 0.001$), reflecting progressive adiposity associated with metabolic impairment. Glycemic indices demonstrated a stepwise deterioration from controls to obese insulin-resistant participants and further to the T2DM group. Fasting plasma glucose concentrations were significantly higher in the T2DM group (162 ± 18 mg/dL) relative to both controls and obese insulin-resistant individuals ($p < 0.001$). Similarly, glycated hemoglobin (HbA1c) levels were markedly increased in patients with T2DM (7.8 ± 0.9%), indicating chronic hyperglycemia.

Markers of insulin resistance were also significantly altered. Fasting insulin concentrations were elevated in obese insulin-resistant individuals and further increased in patients with T2DM compared with healthy controls ($p < 0.001$).

Parameter	Healthy Controls (n = 20)	Obese Insulin-Resistant (n = 20)	T2DM (n = 20)	p-value
Age (years)	45.2 ± 6.1	46.8 ± 5.9	47.5 ± 6.4	> 0.05
Sex (M/F)	11 / 9	12 / 8	10 / 10	> 0.05
BMI (kg/m²)	23.4 ± 2.1	31.6 ± 3.4	32.8 ± 3.9	< 0.001
Fasting glucose (mg/dL)	92 ± 8	108 ± 12	162 ± 18	< 0.001
HbA1c (%)	5.3 ± 0.4	5.9 ± 0.6	7.8 ± 0.9	< 0.001
Fasting insulin (µIU/mL)	6.8 ± 1.9	15.2 ± 3.8	18.6 ± 4.5	< 0.001
HOMA-IR	1.6 ± 0.4	4.1 ± 1.2	6.9 ± 1.8	< 0.001

3. 2. Reduced Whole-Body Insulin Sensitivity in Insulin-Resistant and T2DM Groups

Whole-body insulin sensitivity was quantified using the hyperinsulinemic–euglycemic clamp technique, revealing pronounced differences among the study groups (figure 1). Healthy control participants exhibited the highest insulin responsiveness, as reflected by a significantly greater glucose infusion rate (GIR) during steady-state conditions. In contrast, obese insulin-resistant individuals demonstrated a marked reduction in GIR, while patients with type 2 diabetes mellitus (T2DM) showed the most severe impairment. Specifically, the mean GIR was 9.1 ± 1.2 $mg \cdot kg^{-1} \cdot min^{-1}$ in healthy controls, which was reduced by approximately 41% in obese insulin-resistant participants (5.4 ± 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$) and by 58% in patients with T2DM (3.8 ± 0.9 $mg \cdot kg^{-1} \cdot min^{-1}$; $p < 0.001$). These differences remained significant after normalization for body weight and were consistent across both sexes. The progressive decline in GIR across groups indicates a stepwise deterioration of whole-body insulin sensitivity, beginning in obesity-associated insulin resistance and culminating in overt T2DM. These findings confirm the presence of severe defects in insulin-mediated glucose disposal in metabolically impaired individuals and provide a functional framework for interpreting subsequent alterations in mitochondrial function and insulin signaling.

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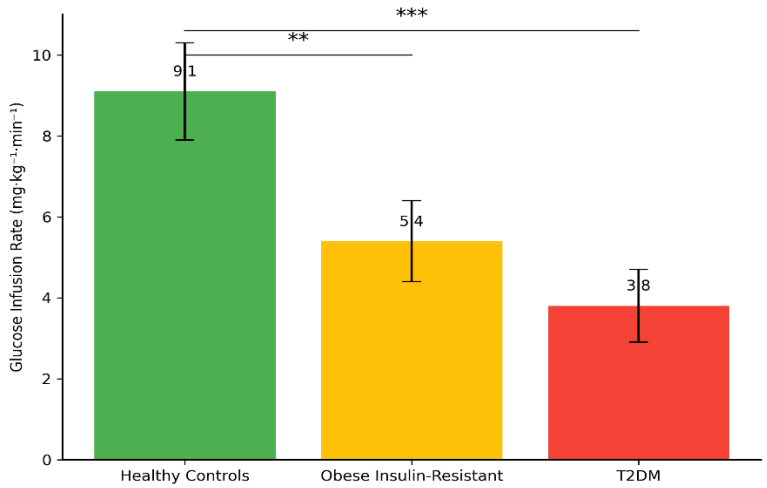

Figure 1. Whole-body insulin sensitivity measured as glucose infusion rate (GIR) during the hyperinsulinemic–euglycemic clamp in healthy controls, obese insulin-resistant individuals, and patients with type 2 diabetes mellitus (T2DM). Data are presented as mean ± SD. ** $p < 0.01$, *** $p < 0.001$ vs. healthy controls.

3. 3. Impaired Mitochondrial Oxidative Capacity in Skeletal Muscle

Mitochondrial oxidative capacity in skeletal muscle was evaluated using high-resolution respirometry in permeabilized muscle fibers (Figure 2). Significant impairments in mitochondrial oxidative phosphorylation were observed in insulin-resistant states compared with healthy controls. Maximal ADP-stimulated respiration, reflecting the capacity for oxidative ATP production, was progressively reduced across groups. Specifically, maximal oxidative phosphorylation capacity was decreased by approximately 28% in obese insulin-resistant individuals and by 41% in patients with type 2 diabetes mellitus (T2DM) relative to healthy controls ($p < 0.01$). These findings indicate a substantial defect in mitochondrial respiratory function that becomes more pronounced with increasing metabolic dysfunction. To assess whether these alterations reflected changes in mitochondrial abundance, citrate synthase activity was measured as a surrogate marker of mitochondrial content. Citrate synthase activity was modestly reduced in obese insulin-resistant individuals and significantly reduced by ~22% in the T2DM group compared with controls ($p < 0.01$). The concomitant reductions in maximal respiration and citrate synthase activity suggest the presence of both qualitative mitochondrial dysfunction (impaired intrinsic respiratory capacity) and quantitative deficits (reduced mitochondrial content) in skeletal muscle from patients with T2DM.

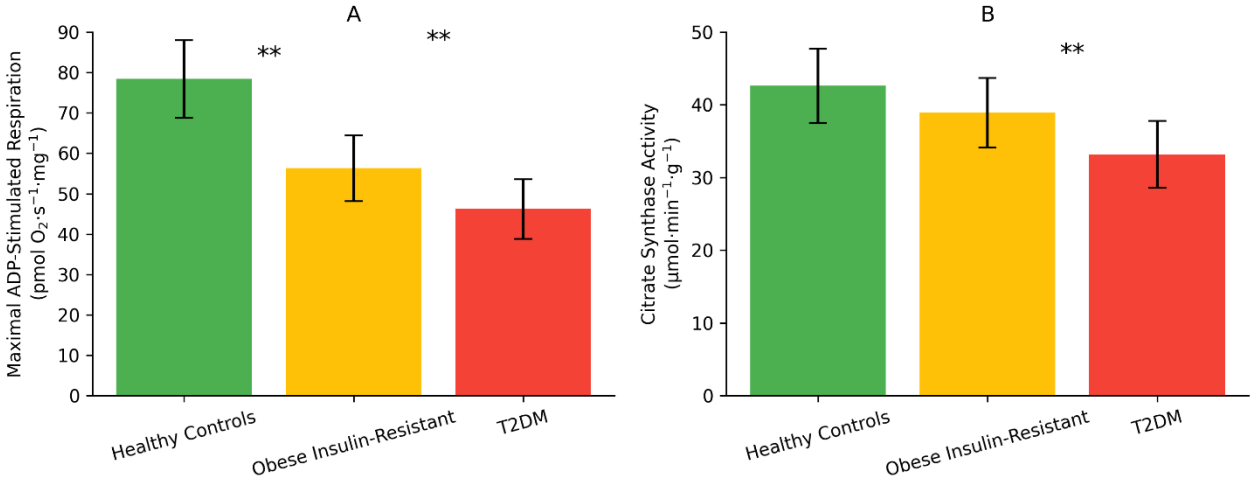

Figure 2. Impaired mitochondrial oxidative capacity in skeletal muscle from insulin-resistant and type 2 diabetes mellitus (T2DM) participants. (A) Maximal ADP-stimulated mitochondrial respiration measured by high-resolution respirometry in permeabilized skeletal muscle fibers. (B) Citrate synthase activity as a marker of mitochondrial content

3. 4. Decreased ATP Synthesis Rates in Insulin-Resistant Muscle

Skeletal muscle mitochondrial ATP synthesis rates were assessed under basal conditions and during insulin stimulation to evaluate mitochondrial bioenergetic capacity and insulin responsiveness (Figure 3). Marked

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impairments in ATP production were observed in insulin-resistant states compared with healthy controls. Under basal conditions, ATP synthesis rates were significantly reduced in obese insulin-resistant individuals (7.9 ± 0.8 μmol·L⁻¹·min⁻¹) and further decreased in patients with type 2 diabetes mellitus (T2DM; 7.2 ± 0.7 μmol·L⁻¹·min⁻¹) relative to healthy controls (10.4 ± 0.9 μmol·L⁻¹·min⁻¹; $p < 0.001$). These findings indicate a substantial basal bioenergetic deficit in skeletal muscle mitochondria associated with insulin resistance and overt diabetes. During insulin stimulation, ATP synthesis rates increased significantly in all groups, consistent with insulin-mediated activation of mitochondrial metabolism. However, the magnitude of this increase was markedly attenuated in obese insulin-resistant and T2DM participants. Despite insulin stimulation, ATP production in these groups remained significantly lower than that observed in healthy controls ($p < 0.001$), demonstrating impaired mitochondrial responsiveness to insulin.

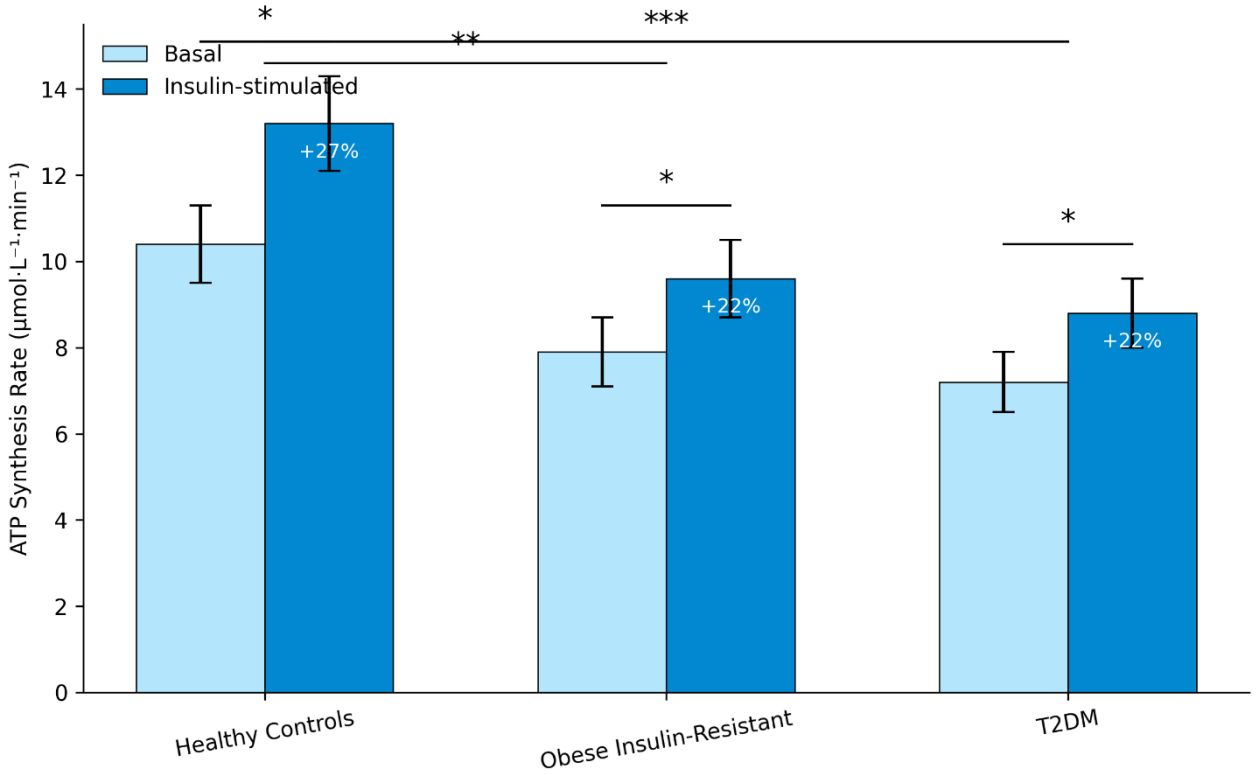

Figure 3. Basal and insulin-stimulated ATP synthesis rates in skeletal muscle from healthy controls, obese insulin-resistant individuals, and patients with type 2 diabetes mellitus (T2DM). Data are presented as mean ± SD. Insulin stimulation increased ATP synthesis in all groups; however, ATP production remained significantly reduced in obese insulin-resistant and T2DM participants compared with healthy controls, indicating impaired mitochondrial responsiveness to insulin.
** $p < 0.01$, *** $p < 0.001$ vs. healthy controls (one-way ANOVA with Tukey's post hoc test).

3. 5. Increased Mitochondrial ROS Production and Oxidative Stress

Mitochondrial reactive oxygen species (ROS) production and oxidative stress markers were assessed to determine whether mitochondrial redox imbalance accompanies insulin resistance and type 2 diabetes mellitus (T2DM) (Table 2). A marked increase in mitochondrial oxidative stress was observed in metabolically impaired groups compared with healthy controls. Mitochondrial ROS levels, quantified using MitoSOX fluorescence, were significantly elevated in obese insulin-resistant individuals and further increased in patients with T2DM. Relative to healthy controls, ROS production increased by approximately 1.6-fold in obese insulin-resistant participants and by ~2.1-fold in the T2DM group ($p < 0.01$). These findings indicate progressive mitochondrial redox dysregulation with worsening metabolic status.

Consistent with increased ROS generation, lipid peroxidation—as assessed by malondialdehyde (MDA) concentrations—was significantly higher in patients with T2DM compared with both healthy controls and obese insulin-resistant individuals ($p < 0.01$). This elevation reflects enhanced oxidative damage to cellular lipids in diabetic skeletal muscle. In parallel, the activity of key antioxidant enzymes was significantly reduced in insulin-resistant states. Superoxide dismutase (SOD) and catalase activities were modestly decreased in obese insulin-resistant individuals and markedly reduced in the T2DM group ($p < 0.01$ vs. controls). The combined

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increase in mitochondrial ROS production, elevated lipid peroxidation, and diminished antioxidant defenses indicates a pronounced shift toward oxidative stress in skeletal muscle from patients with T2DM.

Parameter	Healthy Controls (n = 20)	Obese Insulin-Resistant (n = 20)	T2DM (n = 20)	p-value
MitoSOX fluorescence (AU)	1.00 ± 0.18	1.62 ± 0.25	2.11 ± 0.31	< 0.01
Fold change vs. controls	—	+1.6×	+2.1×	—
MDA (nmol·mg^{-1} protein)	1.9 ± 0.4	2.6 ± 0.5	3.8 ± 0.6	< 0.01
SOD activity (U·mg^{-1} protein)	12.4 ± 1.6	9.8 ± 1.4	7.2 ± 1.2	< 0.01
Catalase activity (U·mg^{-1} protein)	48.6 ± 5.2	41.3 ± 4.9	33.7 ± 4.6	< 0.01

Values are expressed as mean ± SD. AU, arbitrary units; MDA, malondialdehyde; SOD, superoxide dismutase; T2DM, type 2 diabetes mellitus.

3. 6. Accumulation of Intramyocellular Lipids and Reduced Fatty Acid Oxidation

Intramyocellular lipid content and mitochondrial fatty acid oxidation capacity were assessed to determine whether altered lipid handling accompanies insulin resistance and type 2 diabetes mellitus (T2DM) (Figure 4). Marked abnormalities in skeletal muscle lipid metabolism were observed in insulin-resistant states. Oil Red O staining of skeletal muscle sections revealed a significant increase in intramyocellular lipid accumulation in obese insulin-resistant individuals and patients with T2DM compared with healthy controls. Quantitative image analysis demonstrated that lipid content was elevated by approximately 45% in obese insulin-resistant participants and by ~70% in patients with T2DM relative to controls ($p < 0.001$). These findings indicate progressive ectopic lipid deposition with worsening metabolic impairment. Concomitant with increased lipid accumulation, mitochondrial fatty acid oxidation capacity was significantly reduced. Rates of [^{14}C]-palmitate oxidation were decreased by ~34% in obese insulin-resistant individuals and by ~52% in the T2DM group compared with healthy controls ($p < 0.001$). This reduction reflects a substantial impairment in mitochondrial β-oxidation capacity in skeletal muscle.

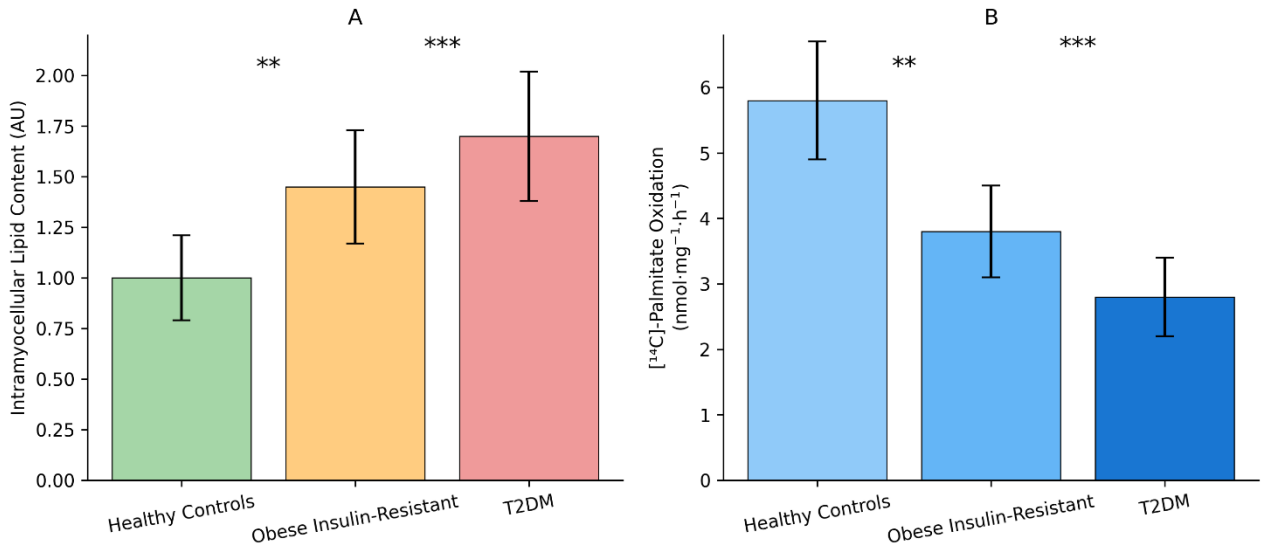

Figure 4. Altered skeletal muscle lipid metabolism in insulin-resistant and type 2 diabetes mellitus (T2DM) participants. (A) Intramyocellular lipid content quantified by Oil Red O staining and image analysis. (B) Mitochondrial fatty acid oxidation capacity assessed by [^{14}C]-palmitate oxidation.

3. 7. Altered Insulin Signaling Protein Expression and Phosphorylation

To determine whether mitochondrial dysfunction and lipid accumulation were associated with defects in insulin signaling, key components of the insulin signaling cascade were examined by Western blot analysis (Figure 5). Total protein expression levels of insulin receptor substrate-1 (IRS-1) and Akt did not differ significantly among healthy controls, obese insulin-resistant individuals, and patients with type 2 diabetes mellitus (T2DM), indicating that alterations in insulin signaling were not attributable to changes in protein abundance. In contrast, insulin-stimulated signaling responses were markedly impaired in insulin-resistant states. Tyrosine phosphorylation of IRS-1, a critical early event in insulin signal transduction, was significantly reduced in obese insulin-resistant participants by approximately 38% and further decreased in patients with T2DM by ~55% compared with healthy controls ($p < 0.01$). Similarly, phosphorylation of Akt at Ser473, a key downstream mediator of insulin-stimulated glucose uptake, was significantly attenuated in both obese insulin-resistant and T2DM groups relative to controls (p

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< 0.01). In addition to impaired signaling activation, protein expression of glucose transporter type 4 (GLUT4) was significantly reduced in skeletal muscle samples from patients with T2DM. This reduction is consistent with diminished insulin-stimulated glucose transport capacity and provides a mechanistic basis for the observed decrease in whole-body insulin sensitivity measured by the hyperinsulinemic–euglycemic clamp.

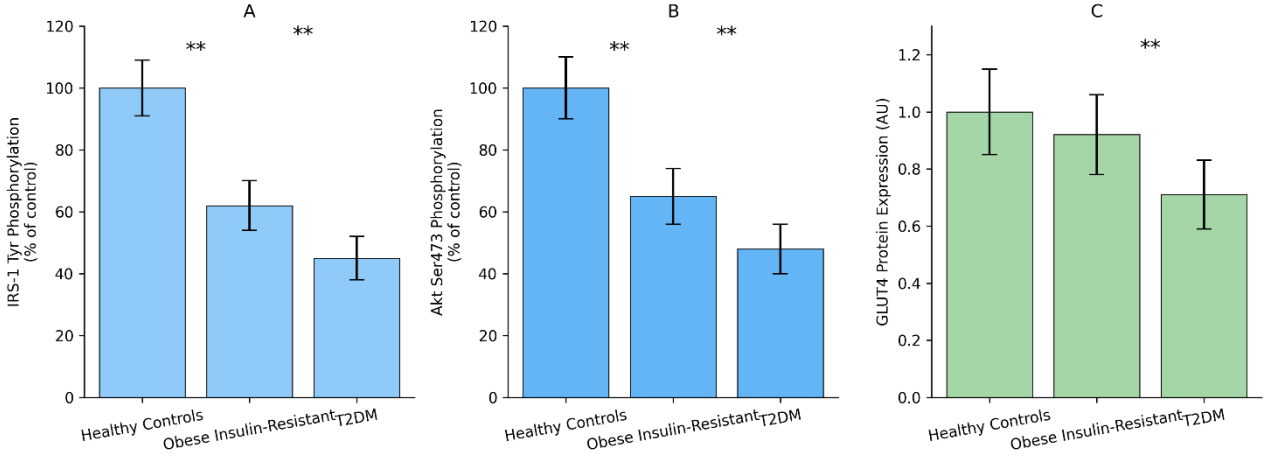

Figure 5. Impaired insulin signaling in skeletal muscle from obese insulin-resistant and type 2 diabetes mellitus (T2DM) participants. (A) Insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1). (B) Insulin-stimulated phosphorylation of Akt at Ser473. (C) GLUT4 protein expression in skeletal muscle.

DISCUSSION

The present study provides an integrated analysis of mitochondrial function, lipid metabolism, oxidative stress, and insulin signaling in skeletal muscle across the spectrum from insulin sensitivity to overt type 2 diabetes mellitus (T2DM). Our findings demonstrate that insulin resistance is associated with coordinated impairments in mitochondrial oxidative capacity, ATP synthesis, fatty acid oxidation, redox balance, and insulin signaling, culminating in reduced GLUT4 expression and diminished insulin mediated glucose disposal. These results strongly support the concept that mitochondrial dysfunction represents a central biochemical link between nutrient overload and skeletal muscle insulin resistance (He et al., 2020; Shimu et al., 2025). We observed significant reductions in maximal ADP stimulated mitochondrial respiration and citrate synthase activity in skeletal muscle from obese insulin resistant and T2DM participants. These findings are consistent with earlier human studies demonstrating reduced mitochondrial oxidative capacity and mitochondrial density in insulin resistant individuals and in offspring of patients with T2DM (Li, Ren, Li, Wu, & Wei, 2023; Zong et al., 2024). Proteomic analyses of human skeletal muscle further support these observations, revealing decreased abundance of proteins involved in oxidative phosphorylation and substrate metabolism in obesity and T2DM (Højlund et al., 2010; Kruse & Højlund, 2020). Together, these data suggest that mitochondrial dysfunction is a robust and reproducible feature of insulin resistant skeletal muscle. Basal and insulin stimulated ATP synthesis rates were markedly reduced in insulin resistant and diabetic muscle in the present study. Similar impairments in ATP synthesis have been reported using in vivo phosphorus 31 magnetic resonance spectroscopy in patients with T2DM, demonstrating reduced basal ATP flux and a blunted ATP response to insulin (Belosludtsev, Belosludtseva, & Dubinin, 2020; Burillo et al., 2021). The attenuated insulin stimulated increase in ATP production observed here is indicative of impaired mitochondrial responsiveness to insulin, a hallmark of metabolic inflexibility that limits the capacity of skeletal muscle to adapt to changes in nutrient availability (De Felice, Gonçalves, & Ferreira, 2022; Yap et al., 2020). A key finding of this study is the concomitant increase in intramyocellular lipid accumulation and reduction in fatty acid oxidation capacity in insulin resistant states. This observation aligns with extensive human literature demonstrating an inverse relationship between intramyocellular lipid content and insulin sensitivity (Kosmas et al., 2023). According to the lipid induced insulin resistance model, impaired mitochondrial fatty acid oxidation promotes accumulation of lipid intermediates such as diacylglycerols and ceramides, which activate stress kinases and inhibit insulin signaling (Potenza, Sgarra, Desantis, Nacci, & Montagnani, 2021). Our findings provide direct support for this model in human skeletal muscle. We also demonstrate significantly elevated mitochondrial ROS production and lipid peroxidation in insulin resistant and T2DM muscle, accompanied by reduced antioxidant enzyme activity. Excessive mitochondrial ROS has been shown to impair insulin signaling through inhibitory serine phosphorylation of IRS 1 and suppression of Akt activation (Galicia-Garcia et al., 2020). Chronic oxidative stress further exacerbates mitochondrial dysfunction, creating a feed forward cycle that accelerates metabolic

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deterioration (Townsend, Brunetta, & Mori, 2020). Thus, mitochondrial redox imbalance likely plays a critical role in sustaining insulin resistance. Despite preserved total IRS 1 and Akt protein expression, insulin stimulated phosphorylation of these signaling intermediates was markedly reduced in obese insulin resistant and T2DM participants. These findings are consistent with prior human studies demonstrating selective defects in insulin signal transduction rather than protein abundance (Ezkurdia, Ramírez, & Solas, 2023; Sinha, Haque, Lugova, & Kumar, 2023). Furthermore, reduced GLUT4 expression in T2DM muscle aligns with evidence linking impaired GLUT4 availability and trafficking to reduced insulin stimulated glucose uptake (Rabbani, Xue, & Thornalley, 2022). Together, these defects provide a mechanistic explanation for the reduced glucose infusion rates observed during the hyperinsulinemic–euglycemic clamp. Collectively, our findings support an integrated pathophysiological model in which mitochondrial dysfunction reduces oxidative capacity and ATP synthesis, impairs fatty acid oxidation, increases lipid accumulation and ROS production, and ultimately disrupts insulin signaling and GLUT4 mediated glucose transport. Importantly, lifestyle interventions such as endurance exercise and caloric restriction have been shown to restore mitochondrial function, reduce intramyocellular lipids, and improve insulin sensitivity, highlighting mitochondria as a key therapeutic target (Ramanathan, Ali, & Ibdah, 2022; Vesković et al., 2023). The cross sectional nature of this study limits causal inference. Longitudinal and interventional studies are needed to determine whether mitochondrial dysfunction is a primary defect or a consequence of insulin resistance. Future research integrating mitochondrial proteomics, lipidomics, and redox signaling analyses will further clarify the molecular mechanisms linking mitochondrial dysfunction to insulin resistance and T2DM.

5. Conclusion

In conclusion, this study demonstrates that insulin resistance and type 2 diabetes are characterized by coordinated impairments in skeletal muscle mitochondrial function, including reduced oxidative capacity, diminished ATP synthesis, and defective fatty acid oxidation. These mitochondrial abnormalities promote intramyocellular lipid accumulation, oxidative stress, and disrupted redox homeostasis. Together, these metabolic disturbances lead to impaired insulin signaling through reduced IRS 1 and Akt activation and decreased GLUT4 expression. Our findings support a central role for mitochondrial dysfunction in linking metabolic overload to skeletal muscle insulin resistance. Targeting mitochondrial bioenergetics and redox balance may therefore represent an effective therapeutic strategy for improving insulin sensitivity in human diabetes.

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