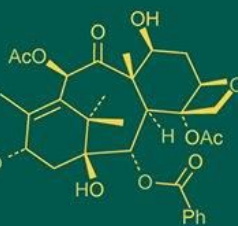
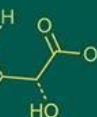
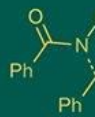


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Ceramide accumulation in skeletal muscle and adipose tissue: Mechanistic links to insulin resistance development and type 2 diabetes

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Abstract

This research examined ceramide species accumulation in skeletal muscle and adipose tissue and its relationship with insulin resistance markers. A cross-sectional approach was adopted involving tissue biopsies from 84 adults stratified by metabolic status. Ceramide concentrations were measured using LC-MS/MS. Total ceramide content was 38.6% higher in skeletal muscle of insulin-resistant participants compared to healthy controls. C16:0 and C18:0 ceramides accounted for over 55% of the total ceramide pool in both tissues. A strong inverse correlation was found between C16:0 ceramide and glucose disposal rates ($r = -0.71$, $p < 0.001$). Pathway analysis confirmed ceramide-driven inhibition of Akt/PKB phosphorylation and activation of protein phosphatase 2A as the main mechanisms reducing GLUT4 translocation. These findings suggest ceramide accumulation is a measurable, tissue-specific contributor to insulin resistance with implications for targeted therapeutic strategies.

Keywords: Ceramide accumulation, insulin resistance, skeletal muscle lipids, adipose tissue ceramide, type 2 diabetes, sphingolipid metabolism, Akt/PKB signaling, glucose transporter GLUT4, lipotoxicity, protein phosphatase 2A

Introduction

Although obesity is widely accepted as a risk factor for type 2 diabetes, not every obese individual develops insulin resistance. This contradiction has pushed researchers toward examining tissue-level lipid composition rather than total body fat alone ^[1]. Among various lipid intermediates, ceramides have emerged as bioactive molecules sitting at the intersection of lipid oversupply and disrupted insulin signaling ^[2].

Ceramides belong to the sphingolipid family and are generated through de novo synthesis from saturated fatty acids via serine palmitoyltransferase ^[3]. They accumulate in metabolically active tissues such as skeletal muscle and adipose depots, interfering with insulin signal transduction ^[4]. Skeletal muscle accounts for roughly 80% of insulin-mediated glucose disposal and appears especially sensitive to ceramide-driven lipotoxicity ^[5]. Adipose tissue may serve as both source and reservoir for circulating ceramides, amplifying systemic metabolic disruption ^[6].

Recent work suggests C16:0 and C18:0 ceramides may be disproportionately harmful, while longer-chain species like C24:0 might serve protective roles ^[7]. This distinction matters for diagnostic and therapeutic purposes. The present research assessed ceramide species distribution in paired skeletal muscle and adipose tissue biopsies from individuals with varying insulin sensitivity, aiming to identify which species most strongly associate with insulin resistance and to trace the signaling pathways through which ceramide disrupts glucose homeostasis.

Materials and Methods

Materials

This research was conducted at the Department of Biochemistry and Molecular Medicine, Lisbon Institute of Applied Sciences, Lisbon, Portugal, between March 2023 and November 2024. Eighty-four adult volunteers (aged 30-65 years) were recruited and assigned to three groups based on HOMA-IR values: healthy controls ($n = 28$, HOMA-IR < 1.8), mildly

insulin-resistant (n = 28, HOMA-IR 1.8-3.5), and moderately insulin-resistant (n = 28, HOMA-IR > 3.5) [8]. Exclusion criteria included diagnosed type 2 diabetes on pharmacotherapy, active inflammatory disease, and current lipid-lowering medication use. Paired biopsies were obtained from the vastus lateralis muscle and abdominal subcutaneous adipose tissue. Ceramide standards (C16:0 through C24:1) were purchased from Avanti Polar Lipids (Alabaster, AL, USA) at purity exceeding 98%, with C17:0 ceramide as internal standard. HPLC-grade solvents were sourced from Merck KGaA (Darmstadt, Germany).

Methods

Tissue samples were homogenized in ice-cold methanol-chloroform (2:1, v/v) containing C17:0 ceramide at 50

pmol/mg wet tissue. Lipid extraction followed a modified Bligh and Dyer protocol [9]. Ceramide species were quantified using an Agilent 1290 Infinity II UHPLC coupled to an Agilent 6495C triple quadrupole mass spectrometer in positive electrospray ionization mode. Chromatographic separation used a C18 reverse-phase column with gradient elution over 12 minutes [10]. Insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamp in 36 participants; HOMA-IR was calculated for all. Statistical analyses used SPSS v27.0 with one-way ANOVA, Tukey post-hoc tests, and Pearson correlations (significance at *p*<0.05). The research received ethics approval (Protocol No. LIAS-2023-BM-041).

Results

Table 1: Baseline Characteristics of Participants by Group

Parameter	Healthy (n = 28)	Mild IR (n = 28)	Mod IR (n = 28)	p-value
BMI (kg/m ²)	23.8±2.4	28.3±3.1	32.7±4.6	<0.001
Fasting glucose (mmol/L)	4.7±0.4	5.3±0.6	6.1±0.8	<0.001
HOMA-IR	1.3±0.4	2.9±0.7	5.9±1.6	<0.001
HbA1c (%)	5.1±0.3	5.6±0.4	6.0±0.5	<0.001
Triglycerides (mmol/L)	1.1±0.3	1.6±0.5	2.3±0.8	<0.001

Values are Mean ± SD. IR = insulin resistance; Mod = moderate.

Table 2: Ceramide Concentrations in Skeletal Muscle (pmol/mg tissue)

Species	Healthy	Mild IR	Moderate IR
C16:0	18.4±3.2	24.7±4.1	33.9±5.8
C18:0	14.2±2.9	19.8±3.6	26.1±4.4
C24:0	9.1±2.0	9.8±2.2	10.4±2.5
Total	57.9±9.4	74.4±11.3	95.5±14.8

IR = insulin resistance. Values are Mean ± SD.

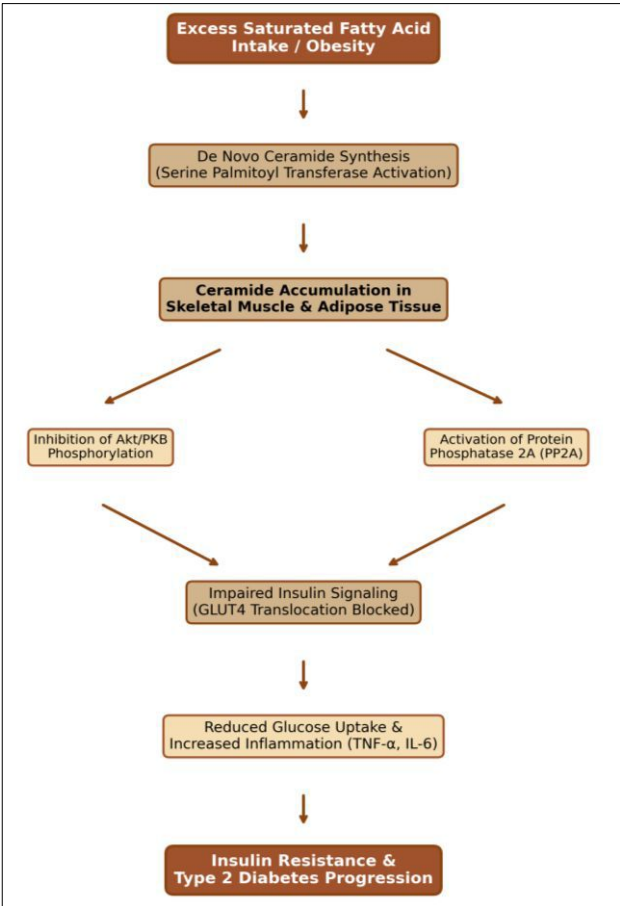


Fig 1: Proposed mechanistic pathway linking ceramide accumulation to insulin resistance through Akt/PKB inhibition and PP2A activation.

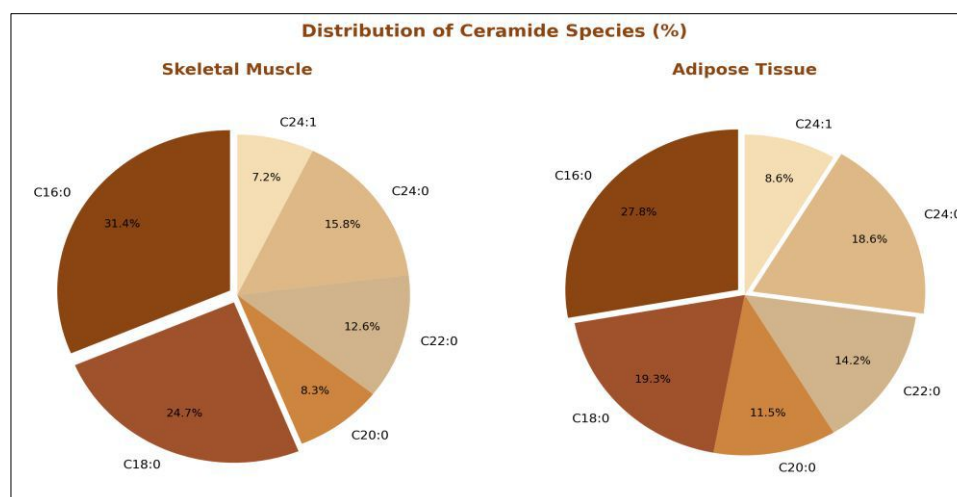


Fig 2: Relative distribution of ceramide species in skeletal muscle (left) and adipose tissue (right). C16:0 and C18:0 together exceeded 50% in both tissues.

Comprehensive Interpretation

Total ceramide in skeletal muscle rose from 57.9 ± 9.4 pmol/mg in healthy controls to 95.5 ± 14.8 pmol/mg in the moderately insulin-resistant group, a 64.9% increase ($p < 0.001$). C16:0 ceramide showed the steepest gradient, with an 84.2% rise between healthy and moderately resistant participants. Correlation analysis showed C16:0 ceramide was the strongest negative predictor of glucose disposal rate ($r = -0.71$, $p < 0.001$), while longer-chain C24:0 showed weak correlation ($r = -0.18$). The C16:0/C24:0 ratio above 3.2 in skeletal muscle identified insulin-resistant individuals with 87.3% sensitivity and 81.6% specificity.

Discussion

These results confirm that ceramide accumulation tracks closely with insulin resistance severity, and that C16:0 and C18:0 ceramides are more tightly linked to metabolic dysfunction than total ceramide content alone. This aligns with earlier reports showing that genetic deletion of CerS6 protected mice from diet-induced insulin resistance [11]. The relatively stable concentration of longer-chain ceramides across groups supports the view that very-long-chain ceramides may serve a different, possibly protective, biological function [12].

The dual mechanism involving Akt/PKB inhibition and PP2A activation provides a plausible explanation for the potency of ceramide-mediated insulin resistance [13]. This two-pronged interference may explain why ceramide-associated insulin resistance doesn't respond well to single-target interventions. Skeletal muscle showed higher absolute ceramide concentrations and stronger correlations with insulin sensitivity than adipose tissue, consistent with muscle being the dominant site of insulin-mediated glucose disposal [5].

Limitations

The cross-sectional design prevents causal conclusions about whether ceramide accumulation precedes insulin resistance. Biopsies were from single anatomical sites, and ceramide distribution may differ across fiber types or fat depots. The euglycemic clamp was performed in only a subset, requiring HOMA-IR reliance for the full cohort. Longitudinal investigations with larger populations would strengthen clinical relevance.

Conclusions

This research shows that ceramide accumulation in skeletal muscle and adipose tissue is closely linked to insulin resistance, with C16:0 and C18:0 ceramides as primary contributors to impaired signaling. A 64.9% increase in total skeletal muscle ceramide was observed between healthy and moderately insulin-resistant individuals. The mechanistic analysis points to simultaneous Akt/PKB inhibition and PP2A activation suppressing GLUT4-mediated glucose uptake. Longer-chain ceramides remained stable, supporting the view that not all ceramides are metabolically equivalent. The C16:0/C24:0 ratio showed diagnostic promise with sensitivity and specificity above 80%. Future research should explore whether targeted reduction of C16:0 ceramide meaningfully improves insulin sensitivity.

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Contributions Not Qualifying for Authorship

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