Analysis of the association between mitochondrial dysfunction and common female reproductive endocrinological disorders in a Hungarian cohort

Ph.D. thesis

Vera Várhegyi MD

János Szentágothai Neurosciences Division, Doctoral College
Semmelweis University

Supervisors: Anikó Gál, PhD
Szabolcs Várbíró, MD, DSc

Official reviewers: Sándor Valent, MD, PhD
Szilvia Juhász, PhD

Head of the Complex Examination Committee:
Ilona Kovalszky, MD, DSc

Members of the Complex Examination Committee:
Barbara Molnár-Érsek, MSc, PhD
Gábor László Kovács, MD, PhD

Budapest, 2026

1. Introduction

Insulin resistance (IR) is a central metabolic disturbance driving a global epidemic of non-communicable diseases, including obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome. In reproductive medicine, IR acts as a primary catalyst for spectrum disorders such as polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI). The molecular landscape of IR includes overexpression of protein tyrosine phosphatase 1B (PTP1B), chronic low-grade inflammation (increased TNF-α and IL-6), and endoplasmic reticulum (ER) stress via the Unfolded Protein Response (UPR). Mitochondria are the nexus of these pathologies. As the primary site for oxidative phosphorylation (OXPHOS) and ATP production, mitochondrial integrity is essential for glucose sensing in pancreatic β-cells and oocyte maturation. Oocytes contain the highest mitochondrial density in the human body; thus, mitochondrial dysfunction – characterized by increased reactive oxygen species (ROS) and genomic instability – leads to accelerated follicular depletion and impaired steroidogenesis. In PCOS, a "vicious circle" emerges where hyperinsulinemia synergizes with LH to enhance androgen biosynthesis, while hyperandrogenism promotes visceral adiposity, further worsening IR. Circulating biomarkers are essential for the non-invasive assessment of this "mitonuclear stress". Growth differentiation factor 15 (GDF-15), a stress-responsive mitokine, and mitochondrial DNA (mtDNA) deletions provide complementary insights into functional stress signaling and structural genome damage. However, an integrated assessment of these markers across the IR-PCOS-POI spectrum has been lacking. This study characterizes the "mitochondrial phenotype" of these disorders, addressing the knowledge gap regarding how mitochondrial dysfunction drives systemic symptoms and accelerated reproductive aging.

2. Objectives

The aims of our study were:

1. To determine the prevalence, pattern, and clinical relevance of mitochondrial DNA (mtDNA) deletions and elevated plasma GDF-15 levels in women with insulin resistance (IR), IR-PCOS, and IR-POI, compared with healthy controls. This aim establishes the fundamental mitochondrial stress profile underlying these endocrine-metabolic disorders.
2. To investigate the relationship between GDF-15, mtDNA deletions, and metabolic burden, including BMI, reactive hyperinsulinemia, OGTT-derived insulin dynamics, and HOMA index, as well as the required intensity of metabolic therapy (metformin and GLP-1 receptor agonists). The objective is to evaluate whether GDF-15 functions as a quantitative biomarker of metabolic stress and treatment demand.
3. To examine the associations of GDF-15 and mtDNA deletions with key reproductive and thyroid hormone parameters (AMH, FSH, LH, estradiol, testosterone, prolactin, TSH, free T4, etc.). The purpose of this aim is to investigate the endocrine-mitochondrial regulatory axis in insulin resistance-related disorders.
4. To assess the link between mitochondrial dysfunction (mtDNA deletions and/or elevated GDF-15) and markers of reproductive aging, including AMH levels, AMH/FSH ratio, and age-dependent ovarian reserve trajectories. The goal is to determine whether mitochondrial dysfunction contributes to accelerated reproductive aging in women with insulin resistance.

5. To characterize multisystem clinical involvement (musculoskeletal, gastrointestinal, neuropsychiatric, autoimmune, and additional endocrine manifestations) in relation to GDF-15 levels and mtDNA deletion status, thereby defining the systemic phenotype associated with mitochondrial dysfunction in IR-related endocrine disorders. This objective examines the extent to which IR, PCOS, and POI exhibit a broader "mitochondrial phenotype".

3. Methods

3.1. Study Design and Population

This retrospective observational study utilized the **POMODORI cohort** (PCOS, Mitochondrial Dysfunction, Obesity, Insulin Resistance, Infertility; ClinicalTrials.gov: NCT06167135). The study included 81 patients: isolated IR (n=49), IR-PCOS (n=19), and IR-POI (n=13). An age-matched control group (n=41) of healthy women with normal BMI (20-25 kg/m²) was utilized for comparison.

3.2. Clinical and Laboratory Assessment

Metabolic status was assessed via 75g Oral Glucose Tolerance Tests (OGTT), measuring glucose and insulin at 0, 60, and 120 minutes. HOMA-IR was calculated to define insulin sensitivity. A structured clinical questionnaire evaluated multisystem symptoms across 12 organ domains, including neuropsychiatric and autoimmune conditions.

Hormonal parameters included serum AMH, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, prolactin, total testosterone, thyroid-stimulating hormone (TSH), free thyroxine (T4), free triiodothyronine (T3), and vitamin D3. AMH, FSH, LH, estradiol, and prolactin were measured between days 2 and 4 of the menstrual cycle, while progesterone was assessed between days 22 and 24, where applicable.

3.3. Molecular Procedures

- **DNA Isolation:** Total DNA was extracted from peripheral blood leukocytes and urine epithelial cells using the DNeasy® Blood & Tissue Kit. Urine cells were prioritized due to their established concordance with skeletal muscle heteroplasmy levels.
- **mtDNA Deletion Analysis:** Long-range PCR targeted an 8600-bp region (nucleotide positions 8232–16,496) to detect the 4977-bp "common deletion" and multiple deletions. Primers used: Forward 5′-TAAAAATCTTTGAAATAGGGC-3′ and Reverse 5′-CGGATACAGTTTTCACTTTAGCT-3′.
- **GDF-15 Measurement:** Plasma GDF-15 concentrations were measured via ELISA (BMS2258). Age-adjusted cut-offs were applied per international standards (e.g., <30 years: 2195 pg/mL; 30–39 years: 1950 pg/mL; 40–49 years: 1804 pg/mL).

3.4. Statistical Analysis

Data were analyzed using Python (SciPy). Tests included Mann-Whitney U, Kruskal-Wallis (with Dunn’s post hoc), and

Multiple Linear Regression models adjusted for age and BMI. Significance was set at $p < 0.05$.

4. Results

4.1 Biomarker Prevalence

Plasma GDF-15 levels were significantly elevated in patients compared to controls (1,213.6 ± 83.6 pg/mL vs. 572.8 ± 82.8 pg/mL, **$p < 0.001$**). The highest levels occurred in the IR-only subgroup (1,343.2 ± 105.9 pg/mL). MtDNA deletions were detected in 61.5% of patients, comprising 10.4% single and **51.1% multiple deletions**. The IR-PCOS group, despite being significantly younger (30.6 ± 1.2 years), showed a deletion prevalence of 31.5%.

4.2 Metabolic Correlations

GDF-15 levels strongly correlated with reactive hyperinsulinemia. Patients with elevated GDF-15 exhibited significantly higher insulin at 60 min (130.45 ± 90.54 vs. 90.38 ± 58.63 μU/mL) and 120 min (88.67 ± 39.65 vs. 59.87 ± 37.92 μU/mL). HOMA-IR was significantly higher in the elevated GDF-15 group (**4.4 ± 2.7 vs. 2.9 ± 2.5, $p < 0.05$**). Both GDF-15 and mtDNA deletion frequency increased progressively with BMI, with deletions exceeding 70% in patients with BMI >30 kg/m².

4.3 Treatment Effects

A positive correlation was identified between daily metformin dose and GDF-15 levels ($R^2 = 0.1723$). Patients with elevated GDF-15 required significantly higher doses (1,805.6 ± 634.6 mg/day) compared to those with normal levels (1,284.1 ± 786.3 mg/day, $p < 0.05$). This association was notably more linear in patients without mtDNA deletions ($R^2 = 0.307$ vs. $R^2 = 0.008$).

4.4 Multisystem Involvement

MtDNA deletion-positive patients exhibited a significantly higher burden of multisystem involvement ($\chi^2 = 6.94$, $p = 0.01$). Specifically, 26% (13/50) of deletion-positive patients had >5 affected organ systems, compared to only 3.2% (1/31) of deletion-negative patients. Key manifestations included musculoskeletal symptoms, GI disturbances, and autoimmune conditions such as Hashimoto's thyroiditis, SLE, and vitiligo.

4.5 Endocrine Interplay

Multiple linear regression identified free thyroxine (T4) as an **independent predictor** of GDF-15 (**$\beta = 88.4$, $p = 0.035$**; Model R^2=0.139). Notably, the T4 regression slope was **two-fold steeper** in deletion-positive patients ($\beta = 114.9$) than in deletion-negative patients (β=41.5). Deletion-positive patients also showed higher estradiol and significantly lower total testosterone (**$p<0.05$**).

4.6 Reproductive Aging

In the IR-only subgroup, deletion-positive patients with elevated GDF-15 showed a significantly reduced AMH/FSH ratio ($p < 0.05$). Linear regression confirmed that mtDNA deletion carriers exhibit a steeper age-related decline in AMH levels ($\beta = -2.25$ for deletion-positive vs. $\beta = -1.93$ for deletion-negative), indicating accelerated depletion of the ovarian reserve.

5. Conclusions

This thesis demonstrates that mitochondrial stress, as indicated by increased plasma levels of GDF-15 and the presence of mitochondrial DNA deletions, represents a unifying biological framework that links insulin resistance with associated

reproductive endocrine disorders, such as PCOS and POI. The integration of metabolic, hormonal, and clinical data from multiple systems indicates that mitochondrial dysfunction may contribute to reproductive decline and the burden of systemic symptoms rather than merely being an associated feature.

Women with insulin resistance exhibited significantly higher prevalence rates of mtDNA deletions and elevated plasma GDF-15 levels than healthy controls. The combined presence of these markers defines a characteristic mitochondrial stress profile, suggesting that mitochondrial involvement emerges early in the disease process and may precede the manifestation of reproductive dysfunction.

Plasma GDF-15 levels were closely associated with metabolic burden, including BMI, reactive hyperinsulinemia, altered insulin dynamics, and the requirement for insulin-sensitizing therapies. Together with the frequent occurrence of mtDNA deletions, these findings suggest that functional stress signaling and structural mitochondrial genome damage are interconnected components of metabolic dysregulation in insulin resistance.

Carriers of mtDNA deletions exhibited notably lower AMH/FSH ratios and steeper age-related declines in ovarian reserve markers, even among insulin-resistant women without PCOS or POI. This provides strong support for the concept of accelerated reproductive ageing associated with mitochondrial genome instability.

Beyond reproductive parameters, individuals with mtDNA deletions showed an increased prevalence of musculoskeletal, gastrointestinal, neuropsychiatric, autoimmune, and endocrine manifestations, delineating a broader multisystem phenotype associated with mitochondrial impairment. Taken together, these findings suggest that insulin resistance is linked to a distinct phenotype of accelerated mitochondrial and reproductive ageing, characterized by cumulative mitochondrial genome damage and stress-responsive mitokine signaling. This

has implications for risk stratification, metabolic management, and fertility counseling.

6. Bibliography of the candidate's publications

Publications related to the thesis:

Varhegyi V, Modos A, Trager D, Gerszi D, Horvath EM, Sipos M, Acs N, Molnar MJ, Varbiro S, Gal A. GDF-15 and mtDNA Deletions Are Useful Biomarkers of Mitochondrial Dysfunction in Insulin Resistance and PCOS. Int J Mol Sci. 2024 Oct 10;25(20):10916. DOI: 10.3390/ijms252010916. PMID: 39456699; PMCID: PMC11507876.
IF: 4.9

Varhegyi V, Banfi B, Trager D, Gerszi D, Horvath EM, Sipos M, Acs N, Molnar MJ, Varbiro S, Gal A. Mitochondrial DNA Deletions and Plasma GDF-15 Protein Levels Are Linked to Hormonal Dysregulation and Multi-Organ Involvement in Female Reproductive Endocrine Disorders. *Life*. 2025; 15(11):1744.
IF: 3.4

Publications not related to the thesis:

Várhegyi V, Molnár V, Gézsi A, Sárközy P, Antal P, Molnár MJ. A Magyar Genomikai Egészségtárház az egészséges hosszú élet kutatásának szolgálatában [Hungarian Genomic Data Warehouse supporting the healthy ageing research]. Orv Hetil. 2021 Jul 4;162(27):1079-1088. Hungarian.
DOI: 10.1556/650.2021.32131. PMID: 34224396.
IF: 0.7

Molnár JM, Molnár V, László I, Szegedi M, **Várhegyi V**, Grosz Z. A betegek által riportált kimeneti mutatók jelentősége Pompe-kórban [The importance of patient reported outcome measures in Pompe disease]. Ideggyogy Sz. 2021 Mar 30;74(3-4):105-115. Hungarian.
DOI: 10.18071/isz.74.0105. PMID: 33938668.
IF: 0.7

∑IF: 9.715