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Stage dependent alterations in PBMC mitochondrial bioenergetics in pediatric obesity: from insulin resistance to type 2 diabetes

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ABSTRACT

Background: Pediatric obesity is associated with early-onset cardiometabolic disorders related to metabolic syndrome (MetS), including insulin resistance (IR) and type 2 diabetes (T2DM). Mitochondrial dysfunction plays a key role in their pathogenesis, but evidence on mitochondrial health in children and adolescents with obesity remains limited.

Methods: In this cross-sectional study, mitochondrial oxygen consumption rates (OCR, pmol/min/3 × 10^5 cells) and ATP production (pmol ATP/min/3 × 10^5 cells) were assessed using extracellular flux analysis in peripheral blood mononuclear cells (PBMCs) from 212 children and adolescents (6–18 years) with obesity, stratified by pubertal stage and degree of IR or T2DM.

Results: In the prepubertal group, IR participants had higher total (126.82 ± 7.92 vs. 109.38 ± 35.31, p = 0.028) and mitochondrial (86.04 ± 20.29 vs. 73.84 ± 23.55, p = 0.028) ATP production than insulin-sensitive (IS) peers. Postpubertal adolescents with T2DM showed lower basal OCR (14.21 ± 4.03 vs. 19.61 ± 7.51, p = 0.049), ATP-linked OCR (11.51 ± 3.69 vs. 16.90 ± 6.83, p = 0.016), and mitochondrial ATP production (64.35 ± 34.39 vs. 91.63 ± 34.92, p = 0.040) compared to IS, as well as lower coupling efficiency than the IR group (80.11 ± 8.87 vs. 86.70 ± 5.14 %, p = 0.002).

Conclusion: PBMC-based mitochondrial bioenergetic profiling suggests mitochondrial differences across different stages of IS, IR, and T2DM in pediatric obesity and may serve as a minimally invasive biomarker of early metabolic impairment.

1. Introduction

Early-in-life obesity is one of the most critical public health challenges of the 21st century, with prevalence rates continuing to rise globally. According to the World Health Organization (WHO), the prevalence of overweight among European children aged 5–9 years has reached 29.5 %, while obesity affects 11.6 %, marking a substantial increase over the past three decades [1]. Childhood obesity is associated with a wide range of serious physical and psychological complications. It constitutes a major risk factor for metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM), cardiovascular disease, and other chronic conditions [2,3]. Obesity and associated comorbidities in childhood may lead to persistent health impairments extending into adulthood, underscoring the need for early identification and intervention [4].

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Globally, the incidence of youth-onset T2DM has risen by approximately 30 % over the past twenty years, paralleling the increase in pediatric obesity and influenced by lifestyle factors such as high intake of sugar-sweetened beverages. The insidious and often prolonged subclinical course of T2DM, combined with limited awareness among patients and caregivers, can contribute to delayed diagnosis [5]. Although a T2DM diagnosis remains relatively rare in adolescents and the number of affected youths being treated in specialized centers is still low. The SWEETS e.V. registry registered 614 new cases of youth onset of T2DM in the EU between 2012 and 2021 [6]. The disease course in this age group appears more aggressive, with earlier and more severe comorbidities compared to adult-onset T2DM [7].

Insulin resistance (IR) is a critical pathophysiological link between obesity and its metabolic consequences. IR is a metabolic tipping point where compensatory mechanisms of glucose and lipid homeostasis start failing [8]. Declining insulin sensitivity triggers a cascade of systemic disturbances, progressively impairing metabolic health. These processes set the course for the development or progress of MetS and T2DM [9]. Understanding the molecular and cellular determinants of IR is essential for early risk stratification and intervention in pediatric populations.

Mitochondria are essential organelles responsible for cellular energy metabolism, providing ATP through oxidative phosphorylation and harboring or regulating numerous metabolic pathways. Mitochondrial dysfunction has been recognized as a pivotal factor in the development of cardiometabolic diseases, particularly through its contribution to insulin resistance, the MetS, and chronic low-grade inflammation [10].

Chronic energy surplus and excessive body fat accumulation can impair mitochondrial function [11,12]. Mitochondrial dysfunction manifests as reduced oxidative phosphorylation capacity, increased production of reactive oxygen species (ROS) and consequent oxidative stress, leading to cellular damage and progressive metabolic deterioration [13].

A growing body of evidence links mitochondrial dysfunction to the onset and progression of T2DM, primarily by promoting IR. Impaired oxidative phosphorylation and mitochondrial metabolic pathway disruption compromise insulin signaling and glucose utilization. These defects reflect alterations in mitochondrial dynamics and respiratory chain efficiency, resulting in decreased ATP production and elevated ROS levels [14].

Oxidative stress arising from mitochondrial dysfunction, together with systemic inflammation, constitutes a central component of MetS. The close relationship between mitochondrial impairment and metabolic dysregulation underscores the importance of mitochondrial integrity in preventing and treating metabolic disorders. Clarifying these underlying mechanisms will help develop new therapeutic strategies and enhance early detection of metabolic dysfunction [15].

Previous studies have measured mitochondrial dysfunction in adipocytes together with skeletal or liver cells [16]. Accessing those cells, especially in pediatric cohorts, is very invasive and therefore limited. Peripheral blood mononuclear cells (PBMCs), which include lymphocytes and monocytes, can be used as a minimally invasive model for investigating mitochondrial function. The accessibility of PBMCs through standard blood testing makes them an excellent choice for pediatric research applications [17]. Research has demonstrated that mitochondrial respiration in PBMCs correlates with skeletal muscle respiration and other known clinical parameters [18,19]. Comparative analyses between PBMCs and other cell types have further validated their use in pediatric populations. In a study investigating mitochondrial function across different human cell types, PBMCs were used to evaluate age-related reductions in mitochondrial activity, highlighting their potential in clinical research [20].

Altered PBMC metabolism has been observed in adults with obesity and related metabolic conditions, including T2DM, supporting the potential of PBMCs as indicators of systemic metabolic health [21,22]. Nevertheless, early-in-life studies investigating metabolic health in PBMCs from children and adolescents across different age groups and pubertal stages remain limited.

This study aims to identify differences in mitochondrial respiration in freshly isolated PBMCs among children and adolescents with obesity and varying degrees of insulin resistance, taking into consideration the physiological insulin resistance during puberty, as well as in adolescents with T2DM.

2. Materials and methods

2.1. Study design

This study is part of an Horizon Europe funded multicenter project PAS GRAS *„Derisking metabolic, environmental and behavioral determinants of obesity in children, adolescents and young adults",* which investigates individual risk factors for the development of obesity and its consequential diseases from early childhood to adulthood.

Written consent was obtained from the Ethical Commission of University Medicine Halle, Germany (#2023–238). The study was conducted in accordance with the Declaration of Helsinki and the STROBE criteria. Written informed consent was obtained from the parents or legal guardians of all participants. Children aged 6 to 16 provided written assent to participate, while participants aged 16 and older also provided informed consent.

In this monocenter study, two groups of participants were recruited between December 2023 and October 2025:

Group A): Children and adolescents aged 6–18 years with obesity (BMI $\geq$ 90th percentile according to German reference values [23].

Group B): Adolescents with obesity and manifest T2DM according to WHO guidelines [24], aged 6–19 years with obesity (BMI $\geq$ 90, percentile) [23].

Exclusion criteria were syndromic obesity, pregnancy, and chronic disease preventing study participation.

Participants of group A) were stratified into three groups based on pubertal development: prepubertal (Tanner stage 1), peripubertal (Tanner stages 2–3), and postpubertal (Tanner stages 4–5). Individuals of group B) were all in the postpubertal stage (Tanner stages 4–5). Participants older than 12 years with disturbed glucose tolerance (i.e., serum glucose $>$ 7.8 mmol/l $<$ 11.1 mmol/l) or severe insulin resistance (i.e., HOMA-IR $>$ 97th percentile) were offered treatment with metformin with a starting dose of 500 mg per day and escalation to 500 mg twice daily after 2 weeks.

For analysis of mitochondrial respiration, participants were further stratified based on insulin sensitivity. Insulin resistance (IR) was defined using the homeostasis model assessment of insulin resistance (HOMA-IR) with a cutoff at the 90th percentile adjusted for age [25]. Three subgroups were formed: (1) insulin-sensitive children (HOMA-IR $<$ 90th percentile) (IS), (2) insulin-resistant (HOMA-IR $\geq$ 90th percentile) (IR), and (3) participants with T2DM.

2.2. Anthropometric measurements and biochemical parameters

Body height and weight were measured according to standardized procedures in all participants wearing light underwear. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and converted to standard deviation scores (BMI-SDS) according to German reference data [23]. Overweight was defined as BMI-SDS $\geq$ 1.28 (90th percentile), obesity as BMI-SDS $\geq$ 1.88 SDS (97th percentile), and extreme obesity as BMI-SDS $\geq$ 2.58 SDS (99.5th percentile) [26].

Waist circumference was measured with a flexible tape measure according to standard procedures, and the waist-to-height ratio (WHtR) was calculated by dividing waist circumference (cm) by height (cm). WHtR is an established measure of visceral obesity, with values $\geq$ 0.5 indicating increased cardiometabolic risk [27,28]. Pubertal development was assessed by trained clinicians according to standard Tanner staging criteria [29].

Fasting venous blood samples were collected for the determination of glucose, insulin, HbA1c, uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), total cholesterol, HDL-cholesterol and triglycerides at the clinical laboratory of the University clinic Halle (Saale), Germany using standardized analytic procedures (Supplementary Table S1). HOMA-IR was calculated by multiplying fasting glucose [mmol/l] by fasting insulin [mU/l] and dividing by 22.5 [30].

2.3. Isolation of PBMCs

PBMCs were isolated from fasting venous blood samples using Leucosep tubes (Greiner Bio-One, cat. no. 163290). 10 ml Leucosep tubes were prefilled with 3 ml Pancoll human 1.077 g/ml density gradient medium (PAN Biotech, cat. no. P04-60500) at room temperature (RT, 21°C) and spun for 1 min at 1000 × g with the brake on. 7 ml of whole blood were then layered onto the porous barrier, and the tube was spun at 1000 × g for 10 min at RT with the brake off. The enriched mononuclear cell layer (buffy coat) was collected using Pasteur pipettes. The harvested cells were washed with 10 ml phosphate-buffered saline (PBS) and spun at RT for 10 min at 1000 × g, brakes on. Residual erythrocytes were removed by incubation with 3 ml erythrocyte lysis buffer (c-c-pro GmbH, cat. no. PL-29-L). After washing the cells again with 13 ml PBS, cells were counted and viability assessed with trypan blue exclusion using a Neubauer improved counting chamber. PBMCs were immediately used for extracellular flux analysis as described below.

2.4. Measurement of bioenergetics in PBMCs

Measurements of PBMC oxygen consumption and ATP production rates were carried out using a Seahorse XF96 Analyzer (RRID: SCR_019545) controlled by Wave software (Agilent, Santa Clara, USA, RRID: SCR_024491).

The day before running the assay, a 96-well plate was coated with poly-D-lysine (Gibco™, cat. no. A3890401) and stored at 4°C, upside down. On the day of measurement, freshly isolated PBMCs were resuspended in Seahorse XF RPMI Medium (pH 7.4) supplemented with 10 mM glucose, 1 mM sodium pyruvate and 2 mM glutamine (Agilent, Santa Clara, USA, cat. no. 103576–100). A total of 300,000 cells per well were seeded in 80 µl assay medium, with 5 technical replicates per sample and up to 10 samples per plate. The plate was centrifuged at 50 × g, RT, brakes off, for 5 min in one direction, then at 80 × g, RT, brakes off, for 5 min in the opposite direction. Each well was gently brought up to a total volume of 175 µl. The plate was incubated at 37°C without CO_2 for 60 min before starting the assay.

The Agilent Seahorse XF Cell Mito Stress Test and the Agilent Seahorse XF Real-Time ATP Rate Assay (Agilent, Santa Clara, USA, cat. no. 103015–100) were applied to separate halves of each plate according to the manufacturer's instructions to assess oxygen consumption rates (OCR) and ATP production [31]. The sensor cartridges, hydrated the day before the experiment, were loaded with the compound solutions of the mitochondrial respiration modulators. For the Cell Mito Stress Test, port A was filled with 20 µl oligomycin (final concentration 2 µM), port B with 22 µl Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) (final concentration 2 µM), and port C with 25 µl rotenone and antimycin A (final concentration 0.5 µM). For the Real-Time ATP Rate Assay, port A was filled with 20 µl oligomycin (final concentration 2 µM), port B with 22 µl rotenone and antimycin A (final concentration 0.5 µM), and port C with 25 µl assay medium. After sensor calibration, the cell plate was loaded into the Seahorse Analyzer, and measurements were acquired over 4 repeated cycles.

Mitochondrial parameters of the Mito Stress Test were calculated from normalized raw data using the manufacturer's analysis guidelines [31]. Based on these data, the Bioenergetic Health Index (BHI) was calculated as described by Chacko et al. using the formula: BHI = log ((reserve capacity) * (ATP-linked oxygen consumption) / (non-mitochondrial oxygen consumption) * (proton leak)) [32]. The BHI combines favorable components and potential deleterious effects of mitochondrial respiration into a single composite measure of mitochondrial bioenergetic health. Higher BHI values indicate better mitochondrial respiratory function and have previously been shown to be sensitive to bioenergetic impairment [33].

The parameters of the ATP Rate Assay were calculated using the Oxygen Consumption Rates (OCR) and Extracellular Acidification Rate (ECAR) in the Agilent Seahorse Wave Software as described before [34].

2.5. Statistical analyses

Descriptive statistics were used to summarize demographic, anthropometric, and biochemical characteristics. Continuous variables are reported as mean ± standard deviation (SD) and as median with interquartile ranges (Q1, Q3), whereas categorical variables are presented as absolute frequencies and percentages.

Differences in clinical parameters between pubertal subgroups, defined by Tanner stage, were examined using one-way analysis of variance (ANOVA), followed by post hoc tests where appropriate. For mitochondrial respiration and ATP production, unpaired two-tailed t-tests were used for comparisons between two groups (IR vs IS), and one-way ANOVA with post hoc tests was used for comparisons among three groups (IR, IS, T2DM).

In children without manifest T2DM, Pearson correlation analyses were performed between mitochondrial respiration and ATP production parameters and variables related to IR and T2DM, as well as the treatment with metformin. A p-value < 0.05 was considered statistically significant for all analyses.

All statistical analyses were carried out using IBM SPSS Statistics version 28.0 and GraphPad Prism version 10.2.0.

3. Results

A total of 200 participants from group A) were stratified into three groups according to pubertal stage: prepubertal (Tanner stage 1, n = 69), peripubertal (Tanner stages 2–3, n = 64), and postpubertal (Tanner stages 4–5, n = 67). Group B) with n = 12 T2DM participants were compared with postpubertal peers from group A).

The sex distribution did not differ significantly between groups ($p = 0.260$). BMI-SDS increased during puberty ($p = 0.051$), peaking in the postpubertal group (2.91 ± 0.68). HOMA-IR scores demonstrated a significant increase across groups ($p < 0.001$), with the highest values observed in participants with T2DM (10.4 ± 8.3). The percentage of participants with IR (HOMA-IR ≥ 90th percentile) compared to IS participants rose from 65.2 % in the prepubertal to 82.1 % in the postpubertal group. The percentage of participants treated with metformin increased from 11.6 % (prepubertal) to 42.2 % (peripubertal) and 74.6 % (postpubertal). All participants with T2DM were treated with metformin. Detailed group characteristics are summarized in Table 1.

Fasting glucose and HbA1c levels were significantly elevated in the T2DM group compared to participants without manifest diabetes (both $p < 0.001$). Fasting insulin concentrations steadily increased across puberty ($p < 0.001$).

Lipid parameters showed a significant decline in HDL cholesterol during puberty ($p = 0.024$), while triglyceride levels did not vary between pubertal stages ($p = 0.057$). Uric acid levels increased significantly with pubertal progression ($p < 0.001$). Liver enzymes displayed minimal variations between groups, with AST being highest in the prepubertal group (0.52 ± 0.15, $p = 0.013$), and GGTP peaking in the postpubertal T2DM group (0.54 ± 0.41, $p < 0.001$) (Table 1).

3.1. Mitochondrial oxygen consumption rates (OCR) and ATP production

Mitochondrial OCR and ATP production were measured at each pubertal stage, comparing IR and IS children. In the postpubertal group,

Table 1
Descriptive statistics from study population. P-values for continuous variables from one-way ANOVA, and for categorical variables from Chi-square test.

Variables	Prepubertal Tanner 1 N = 69	Peripubertal Tanner 2 & 3 N = 64	Postpubertal Tanner 4 & 5 N = 67	T2DM Tanner 4 & 5 N = 12	P −Value
Sex					
Female	29 (42.0 %)	28 (43.8 %)	34 (50.7 %)	3 (25 %)	0.260
Male	40 (58.0 %)	36 (56.2 %)	33 (49.3 %)	9 (75 %)	
Age [years]					
Mean ± SD	9.7 ± 1.8	12.5 ± 1.6	15.2 ± 1.6	15.1 ± 2.4	<0.001
Median (Q1, Q3)	9.9 (8.1, 11.0)	12.6 (11.3, 13.6)	15.3 (13.3, 16.3)	15.1 (13.1, 16.9)	
BMI-SDS					
Mean ± SD	2.60 ± 0.46	2.57 ± 0.56	2.86 ± 0.67	2.53 ± 0.77	0.051
Median (Q1, Q3)	2.60 (2.25; 2.98)	2.59 (2.21, 2.93)	2.76 (2.35, 3.26)	2.65 (1.99, 2.99)	
WHtR					
Mean ± SD	0.57 ± 0.06	0.59 ± 0.07	0.60 ± 0.08	0.59 ± 0.07	0.176
Median (Q1, Q3)	0.58 (0.53, 0.62)	0.58 (0.54, 0.64)	0.60 (0.54, 0.66)	0.59 (0.52, 0.65)	
HOMA-IR					
Mean ± SD	3.7 ± 2.2	5.5 ± 3.0	6.0 ± 3.0	10.4 ± 8.3	<0.001
Median (Q1, Q3)	3.1 (2.3, 4.5)	5.1 (3.3, 7.2)	5.6 (3.9, 7.9)	6.5 (4.0, 18.5)	
Insulin-Resistance (HOMA-IR ≥ 90. Percentile)	45 (65.2 %)	50 (78.1 %)	56 (83.6 %)		
Metformin					
Treatment	8 (11.6 %)	27 (42.2 %)	50 (74.6 %)	12 (100 %)	
No Treatment	61 (88.4 %)	37 (57.8 %)	17 (25.4 %)	0 (0 %)	
Fasting Glucose [mmol/l]					
Mean ± SD	4.83 ± 0.38	5.01 ± 0.43	4.91 ± 0.35	6.66 ± 1.58	<0.001
Median (Q1, Q3)	4.79 (4.58, 5.10)	5.00 (4.76, 5.23)	4.91 (4.65, 5.12)	6.56 (5.44, 6.96)	
Fasting Insulin [mU/l]					
Mean ± SD	17.04 ± 9.61	24.39 ± 12.34	27.14 ± 12.80	33.34 ± 24.90	<0.001
Median (Q1, Q3)	15.10 (10.55, 19.55)	23.70 (15.35, 30.60)	26.05 (17.75, 32.45)	22.00 (14.30, 47.55)	
HbA1c [%]					
Mean ± SD	5.4 ± 0.3	5.4 ± 0.3	5.4 ± 0.3	6.4 ± 0.7	<0.001
Median (Q1, Q3)	5.5 (5.3, 5.6)	5.4 (5.1, 5.6)	5.4 (5.2, 5.6)	6.4 (5.8, 6.9)	
HbA1c [mmol/mol]					
Mean ± SD	36 ± 3	36 ± 3	35 ± 3	47 ± 7	<0.001
Median (Q1, Q3)	36 (34, 38)	35 (33, 38)	35 (33, 37)	46 (40, 52)	
Total Cholesterol [mmol/l]					
Mean ± SD	4.23 ± 0.61	3.82 ± 0.73	4.11 ± 0.75	4.00 ± 0.80	0.013
Median (Q1, Q3)	4.25 (3.78, 4.64)	3.75 (3.32, 4.41)	4.05 (3.65, 4.51)	4.03 (3.38, 4.56)	
HDL-Cholesterol [mmol/l]					
Mean ± SD	1.23 ± 0.34	1.13 ± 0.26	1.12 ± 0.21	1.07 ± 0.28	0.024
Median (Q1, Q3)	1.24 (1.0, 1.43)	1.10 (0.97, 1.27)	1.07 (0.99, 1.27)	1.06 (0.83, 1.23)	
Triglyceride [mmol/l]					
Mean ± SD	1.12 ± 0.47	1.18 ± 0.74	1.32 ± 0.76	1.28 ± 0.60	0.196
Median (Q1, Q3)	0.96 (0.73, 1.42)	1.04 (0.72, 1.39)	1.13 (0.93, 1.43)	1.16 (0.79, 1.77)	
Uric Acid [µmol/l]					
Mean ± SD	302.0 ± 62.8	352.8 ± 83.2	369.2 ± 83.0	333.5 ± 46.7	<0.001
Median (Q1, Q3)	304.4 (262.0, 345.8)	341.6 (299.2, 406.0)	364.6 (300.5, 430.6)	330.8 (297.7, 363,7)	
AST [µkat/l]					
Mean ± SD	0.52 ± 0.15	0.48 ± 0.13	0.43 ± 0.12	0.44 ± 0.18	0.013
Median (Q1, Q3)	0.51 (0.43, 0.60)	0.45 (0.38, 0.55)	0.41 (0.33, 0.52)	0.37 (0.32, 0.51)	
ALT [µkat/l]					
Mean ± SD	0.48 ± 0.20	0.51 ± 0.27	0.52 ± 0.32	0.59 ± 0.36	0.580
Median (Q1, Q3)	0.42 (0.33, 0.59)	0.41 (0.31, 0.57)	0.43 (0.30, 0.61)	0.46 (0.35, 0.74)	
GGPT [µkat/l]					
Mean ± SD	0.31 ± 0.08	0.32 ± 0.14	0.40 ± 0.19	0.54 ± 0.41	<0.001
Median (Q1, Q3)	0.30 (0.25, 0.37)	0.28 (0.23, 0.36)	0.36 (0.26, 0.49)	0.37 (0.21, 0.99)	

these were additionally compared with adolescents with T2DM. Descriptive statistics for PBMC mitochondrial bioenergetic parameters in each group and pubertal stage are shown in Supplementary Table S2a, and the corresponding mean group differences (CI 95 %) and effect sizes are presented in Supplementary Table S2b.

In the prepubertal group (Fig. 1) IR participants showed increased BHI (1.85 ± 0.24 vs. 1.69 ± 0.29, p = 0.018), total (126.82 ± 27.92 vs. 109.38 ± 35.31 pmol ATP/min/3 × 10^5 cells, p = 0.028) and mitoATP (86.04 ± 20.29 vs. 73.84 ± 23.55 pmol ATP/min/3 × 10^5 cells, p = 0.028) ATP production compared to IS participants.

In the peripubertal group (Fig. 2), IR participants showed elevated glyco ATP compared to IS peers (50.66 ± 28.10 vs. 34.40 ± 8.94 pmol ATP/min/3 × 10^5 cells, p = 0.038). The other bioenergetic parameters did not differ significantly between groups.

In the postpubertal group (Fig. 3), the BHI in T2DM (1.61 ± 0.19) was lower than in IR (1.89 ± 0.24, p = 0.004) and IS (1.92 ± 0.42, p = 0.015). Coupling efficiency was reduced in T2DM compared with IR (80.11 ± 8.87 vs. 86.70 ± 5,14 %, p = 0.002). T2DM values were reduced compared to IS in basal respiration (14.21 ± 4.03 vs. 19.61 ± 7.51 pmol/min/3 × 10^5 cells, p = 0.049), ATP production-related OCR (11.51 ± 3.69 vs. 16.90 ± 6.83 pmol/min/3 × 10^5 cells, p = 0.016) and mito ATP (64.35 ± 34.39 vs. 91.63 ± 34.92 pmol ATP/min/3 × 10^5 cells, p = 0.040).

PBMC OCR parameters did not correlate with anthropometric parameters, including BMI-SDS and WHtR, or with sex. Primary respiratory parameters showed no significant correlations to IR-related blood parameters. However, BHI correlated weakly with age (r = 0.172, p = 0.015), WHtR (r = 0.156, p = 0.027), and fasting glucose (r = 0.170, p = 0.017). Correlation analyses, taking into account mitochondrial respiration and ATP production rates in association to pharmacological treatment with metformin, did not reveal any significant correlations (Table 2).

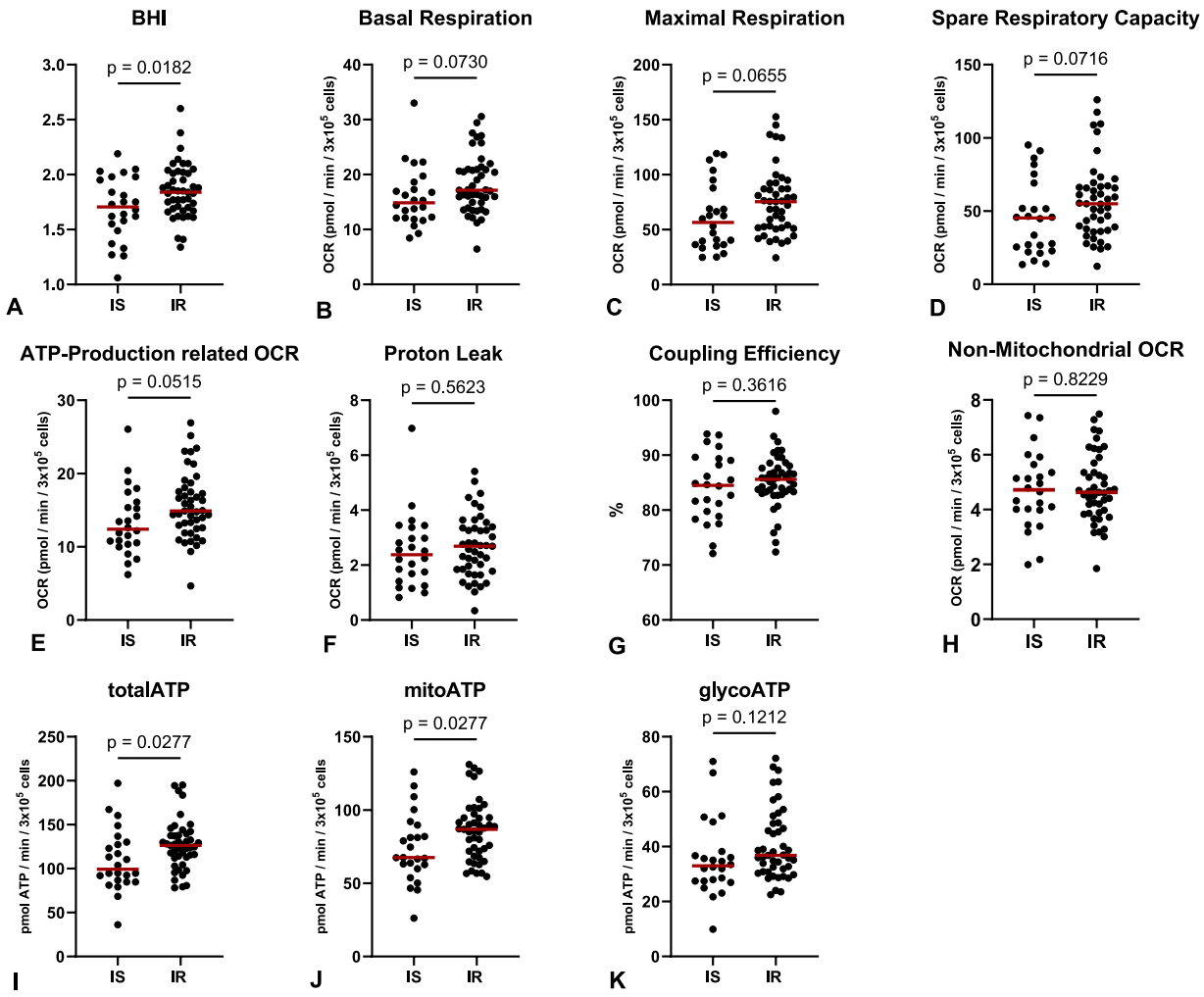

Fig. 1. Prepubertal participants: mitochondrial respiration parameters in insulin-sensitive (IS) and insulin-resistant (IR) children. (A) Bioenergetic Health Index (BHI). (B-H) mitochondrial respiratory parameters measured as oxygen consumption rate (OCR), including basal respiration, maximal respiration, spare respiratory capacity, ATP production-related OCR, proton leak, coupling efficiency and non-mitochondrial OCR. (I-K) Total ATP production, mitochondrial ATP production (mitoATP) and glycolytic ATP production (glycoATP), expressed as pmol ATP per minute per 3×10^5 cells. Points represent individual values with group means; p values from unpaired two-tailed *t* test.

4. Discussion

To the best of our knowledge, this study is the first to examine mitochondrial respiration in freshly isolated PBMCs from pre-, peri-, and postpubertal children and adolescents with obesity and varying degrees of insulin resistance or manifest T2DM. The aims of this study were a) to determine whether obesity-related metabolic impairment, defined by IR or manifest T2DM, is associated with alterations in mitochondrial respiration and b) to evaluate the potential of PBMCs as a minimally invasive biomarker of metabolic health in pediatric populations.

These data offer several important insights into the relevance of mitochondrial bioenergetic profiling in PBMCs in the context of pediatric obesity and metabolic disease:

First, our data show that mitochondrial OCR, ATP production, and BHI in fresh PBMCs, as markers of bioenergetic health can be surrogates of the degree of metabolic impairment in childhood obesity.

Second, in the prepubertal group, mitochondrial respiration was, interestingly, elevated in IR children. BHI, total ATP production, and mitoATP were significantly higher. Basal and maximal respiration, spare respiratory capacity, and OCR-related ATP production showed trends towards elevated values in IR. This finding might reflect mitochondrial adaptation or compensation in response to chronic overnutrition or elevated blood glucose levels. The elevated mitochondrial respiration in PBMCs, which include immune cells from IR participants, may also be attributed to increased systemic inflammation. This observation of increased inflammation and oxidative metabolism has actually been already described in animal models of metabolic dysfunction-associated steatotic liver disease [35]. Previous studies have shown a strong correlation between HOMA-IR and inflammatory markers, such as interleukin-6 and C-reactive protein [36]. However, this hypothesis does not account for the observed alteration in mitochondrial respiration following the onset of T2DM, despite the continued progression in inflammation [37]. Böhm et al. reported similar findings in adipocytes from adults: subjects with insulin resistance had elevated mitochondrial respiration rates compared with participants with insulin-sensitive obesity [16,38].

Third, in peripubertal children and adolescents with obesity, mitochondrial respiration parameters showed no differences except for glycoATP, which was higher in IR. GlycoATP indicates the amount of ATP that is produced from glycolysis rather than the more effective oxidative phosphorylation. In our study population, only 21.9 % of peripubertal participants were classified as IS, even though the age-corrected HOMA-IR percentiles from Hammel et al. show a HOMA-IR of 3.6 at the 90th percentile [25]. Children who were not considered IR still showed elevated fasting glucose and insulin values. Puberty is a critical window during which the risk for MetS and other metabolic diseases increases in

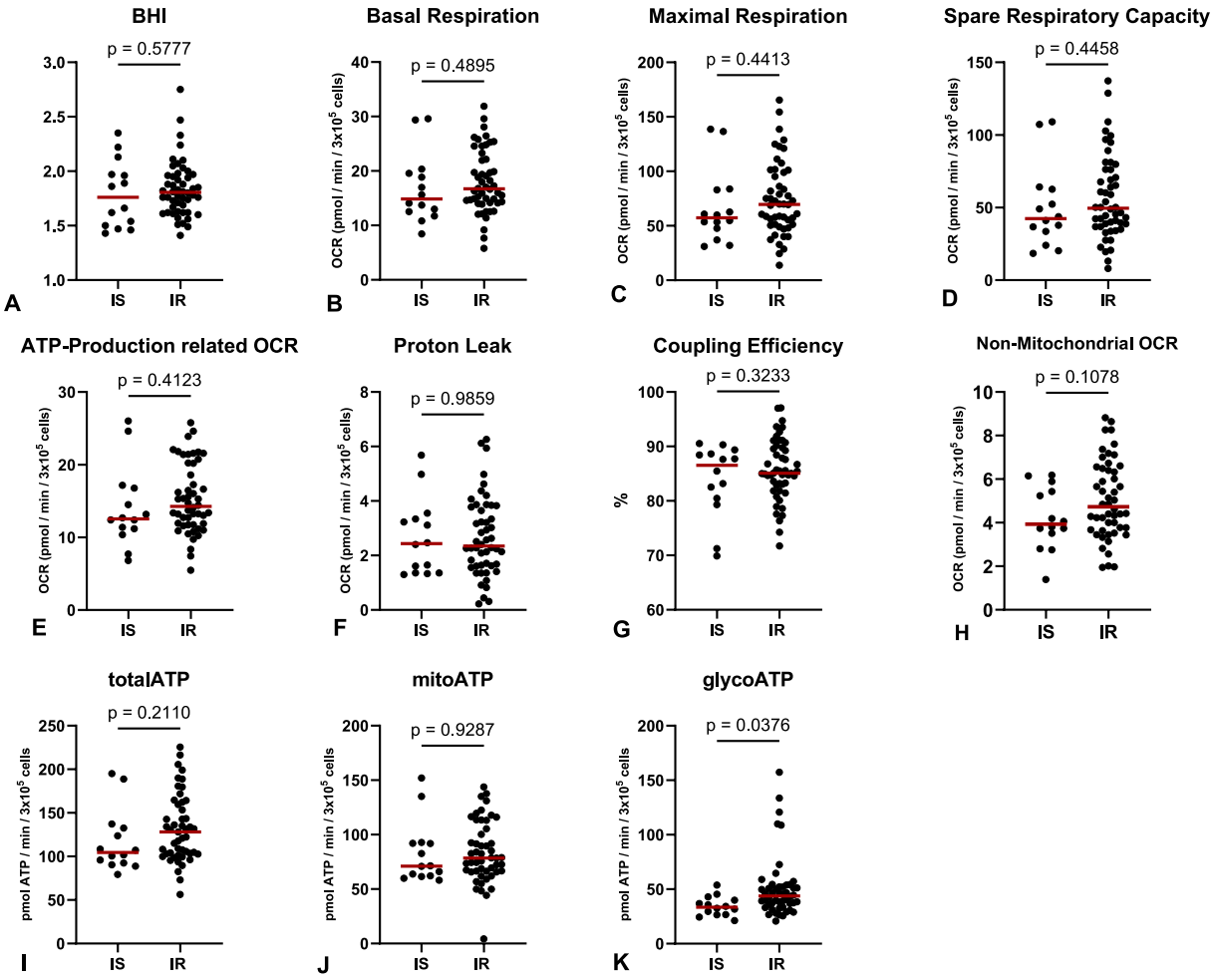

Fig. 2. Peripubertal participants: mitochondrial respiration parameters in insulin-sensitive (IS) and insulin-resistant (IR) children. (A) Bioenergetic Health Index (BHI). (B-H) mitochondrial respiratory parameters measured as oxygen consumption rate (OCR), including basal respiration, maximal respiration, spare respiratory capacity, ATP production-related OCR, proton leak, coupling efficiency and non-mitochondrial OCR. (I-K) Total ATP production, mitochondrial ATP production (mitoATP) and glycolytic ATP production (glycoATP), expressed as pmol ATP per minute per 3×10^5 cells. Points represent individual values with group means; p values from unpaired two-tailed *t* test.

adolescents [39]. In our study, mitochondrial function did not differ between the peripubertal group and the pre- and postpubertal groups.

Fourth, and most importantly, the findings for the T2DM group show lower coupling efficiency than in IR postpubertal adolescents, reflecting reduced efficiency of oxygen utilization for ATP production. This indicates a reduced ability to handle metabolic stress, suggesting mitochondrial alterations, impaired energy production, and reduced metabolic flexibility. Possible biological implications include oxidative stress, cellular senescence, or chronic metabolic disease. Basal respiration, ATP production-related OCR, and mitochondrial ATP were significantly lower in T2DM compared to the IS group in the same pubertal stage. The BHI in T2DM was significantly lower than in both the IS and IR groups. None of the primary parameters used to calculate the BHI showed significant differences between IR and T2DM postpubertal youths. This result suggests that the BHI, a composite index reflecting multiple aspects of mitochondrial respiration, may serve to amplify small differences in parameters, serving as an better early indicator for mitochondrial alterations than isolated respiratory parameters [32,33]. Simultaneously, the BHI does not indicate which process of mitochondrial respiration is altered, although several studies have shown its usefulness in mitochondrial research [18]. These results are consistent with earlier research demonstrating associations between PBMC bioenergetics and metabolic status in adults [18,22]. The alterations in mitochondrial performance likely reflect increased oxidative stress, diminished ATP production efficiency, and altered mitochondrial function, all of which have been implicated in insulin resistance and the progression of metabolic syndrome development [15,40].

However, in the postpubertal group, no differences in mitochondrial respiration were observed between IS and IR participants. To further examine the relationship between PBMC mitochondrial respiration parameters and metabolic status beyond categorical groups, a Pearson correlation analysis was conducted between respiratory parameters and clinical markers of IR and T2DM. Overall, primary mitochondrial respiratory parameters did not show strong correlations, whereas the BHI demonstrated only weak positive associations with age, WHtR, and fasting glucose. These findings suggest that mitochondrial respiration captures aspects of metabolic health that are not fully reflected by common anthropometric or biochemical parameters.

Carvalho et al. have also investigated mitochondrial respiration in fresh PBMCs from children with normal weight, overweight, or obesity; however, analyses were limited to prepubertal children (5–10 years). The authors showed – among other results – that PBMC mitochondrial respiration positively correlated with BMIz, HOMA-IR, fasting glucose and insulin, but was negatively correlated with inflammatory cytokines in prepubertal children. In line with our results, the authors concluded that PBMCs from young children with overweight/obesity may already exhibit adaptations to the metabolic stressors associated with IR and that PBMC metabolism correlates with whole-body metabolism [41].

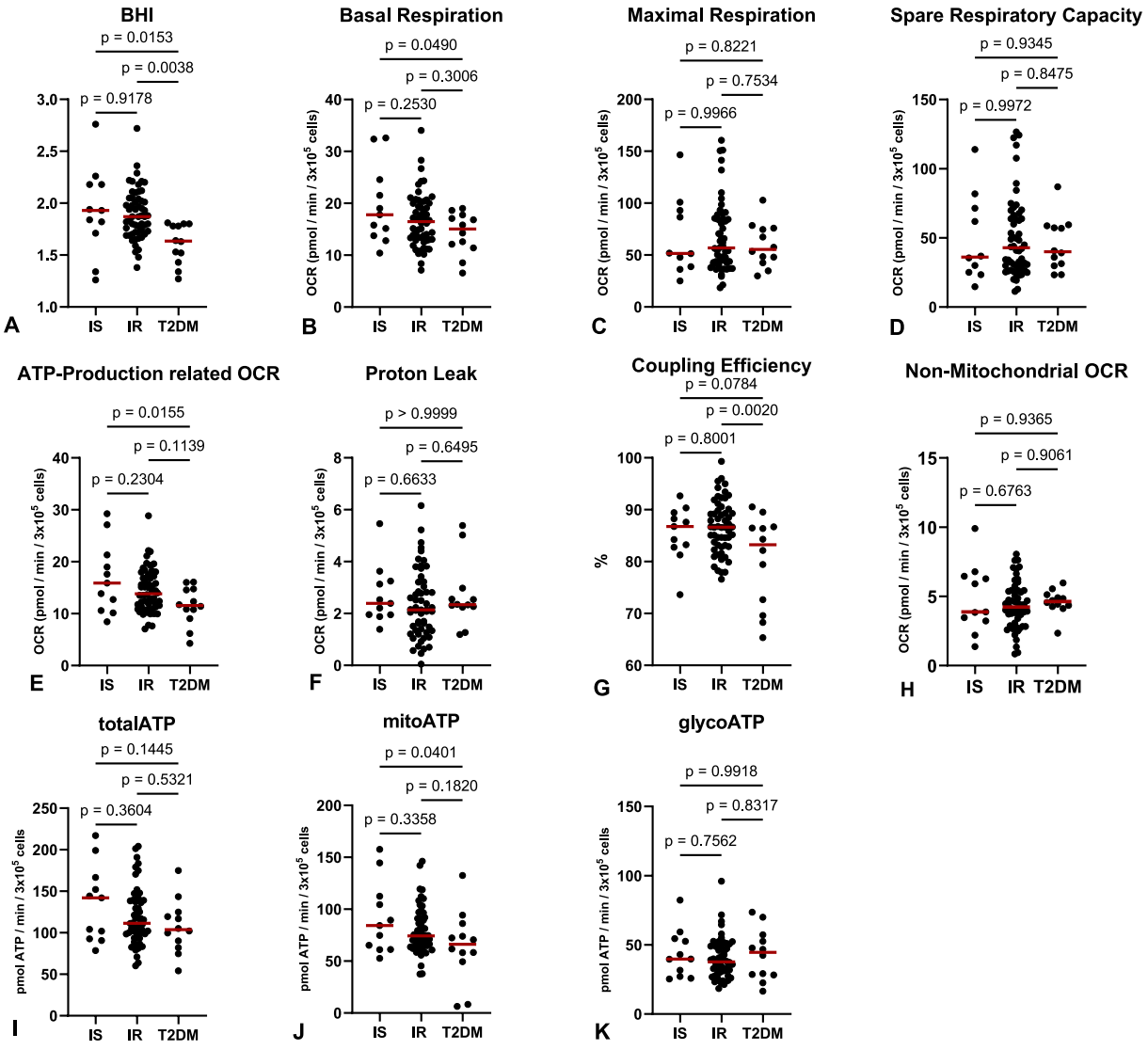

Fig. 3. Postpubertal participants: mitochondrial respiration parameters in insulin-sensitive (IS), insulin-resistant (IR), and children with type 2 diabetes (T2DM). (A) Bioenergetic Health Index (BHI). (B-H) mitochondrial respiratory parameters measured as oxygen consumption rate (OCR), including basal respiration, maximal respiration, spare respiratory capacity, ATP production-related OCR, proton leak, coupling efficiency and non-mitochondrial OCR. (I-K) Total ATP production, mitochondrial ATP production (mitoATP) and glycolytic ATP production (glycoATP), expressed as pmol ATP per minute per 3×10^5 cells. Points represent individual values with group means; p values from ANOVA and post-hoc tests.

Thus, we confirmed and extended these findings by showing that mitochondrial respiration parameters were elevated in the presence of IR and exhibited significant disturbances in youth with obesity and manifest T2DM.

Similar results in adults were reported by Pinho et al. and Barbosa et al. [16,42]. These studies evaluated and compared bioenergetics and energy substrate preference by omental and subcutaneous adipose tissue from 40 adult subjects with obesity at distinct metabolic stages. In accordance with our results, the authors showed that the oxidative phosphorylation capacity of adipose tissue differs with the progression of metabolic disease and that subjects with obesity and diabetes had the lowest mitochondrial respiratory capacity in both subcutaneous and omental adipose tissues [42].

Taken together, our findings confirm and extend previous work by showing for the first time that mitochondrial adaptation to chronic overnutrition is already present in pre-, peri-, and postpubertal children with obesity, and that these compensatory mechanisms fail once T2DM develops, despite the young age of the participants. The pubertal-stage-specific patterns suggest that strategies to preserve or restore metabolic and mitochondrial health may need to be tailored to different phases of puberty. Targeting IR and obesity early in puberty could be critical to prevent mitochondrial decline. This underscores the importance of early, proactive prevention strategies in children and adolescents at high risk [39].

4.1. *Strengths and limitations*

Our study population comprises an anthropometrically, clinically, and metabolically well-characterized sample of children and adolescents with obesity, spanning all pubertal stages from Tanner stage 1 (prepubertal) to Tanner stage 5 (postpubertal). A total of 212 participants were included, representing a markedly larger sample than in previously published studies. The subdivision into groups based on age-adjusted IR, using HOMA-IR percentiles, or on the presence of T2DM provides a clearer picture of the relationship between mitochondrial alteration and metabolic impairment, while also minimizing confounders such as puberty and age. To the best of our knowledge, this is also the first study to include a well-defined group of adolescent patients with T2DM in such

Table 2
Pearson Correlation Analysis between mitochondrial respiration / ATP production parameters and IR / T2DM related parameters, including metformin treatment, in pre-, peri- and postpubertal participants with obesity, without T2DM (n = 200).

	Mito Stress Test							ATP Rate Assay		
	Basal Respiration	ATP production-related OCR	Maximal Respiration	Proton Leak	Spare Respiratory Capacity	Coupling Efficiency (%)	BHI	glycoATP Production	mitoATP Production	Total ATP
Sex										
r	0.129	0.129	0.110	0.085	0.106	0.006	0.138	−0.054	0.091	0.056
p	0.069	0.069	0.120	0.229	0.139	0.929	0.051	0.446	0.200	0.436
Metformin treatment										
r	−0,025	−0,005	−0,022	−0,088	−0,018	0,064	0,099	−0,052	0,033	0,011
p	0,728	0,942	0,754	0,217	0,799	0,366	0,161	0,462	0,640	0,875
Age										
r	0.009	−0.020	−0.046	−0.097	−0.020	0.125	**0.172**	0.028	−0.026	0.004
p	0.897	0.778	0.173	0.173	0.553	0.079	**0.015**	0.696	0.710	0.953
BMI-SDS										
r	0.009	0.024	0.086	−0.048	0.096	0.019	0.103	0.095	0.042	0.074
p	0.897	0.734	0.227	0.499	0.176	0.789	0.145	0.181	0.557	0.298
WHtR										
r	0.051	0.068	0.313	−0.027	0.140	0.057	**0.156**	0.092	0.048	0.071
p	0.469	0.336	0.065	0.704	0.048	0.419	**0.027**	0.193	0.499	0.319
fasting Glucose										
r	0.088	0.095	0.091	0.031	0.089	0.092	**0.170**	0.074	0.068	0.080
p	0.220	0.181	0.201	0.662	0.214	0.199	**0.017**	0.299	0.263	0.263
fasting insulin										
r	0.079	0.091	0.101	0.007	0.100	0.078	0.107	0.029	0.055	0.057
p	0.268	0.199	0.155	0.919	0.159	0.273	0.132	0.686	0.442	0.422
HOMA-IR										
r	0.010	0.025	0.043	−0.047	0.046	0.095	0.114	0.011	−0.017	−0.004
p	0.884	0.728	0.549	0.515	0.526	0.186	0.110	0.877	0.813	0.957
HbA1c										
r	0.015	0.007	−0.012	0.041	−0.051	−0.083	−0.020	0.004	0.045	0.020
p	0.828	0.920	0.864	0.567	0.839	0.245	0.781	0.956	0.527	0.779

analyses. Another strength is the use of fresh PBMCs, a minimally invasive and easily accessible surrogate tissue, to assess mitochondrial respiration in a pediatric population. Prior work has described that PBMCs can mirror metabolic activity in less accessible tissues such as muscle and liver, supporting their suitability for this purpose [17,19]. The findings further demonstrate the practical value of using circulating cell mitochondrial respiration to distinguish different stages of dysmetabolism in pediatric cohorts, particularly for identifying metabolic disturbances at an early stage. We also applied the BHI, a well-established and validated marker of metabolic health, in pediatric patients with varying degrees of metabolic impairment. We show, for the first time and regard this as a major strength of our study, that mitochondrial respiration analysis in children and adolescents with obesity and T2DM can serve as a novel diagnostic tool, providing essential insights into mitochondrial bioenergetics during early metabolic disease progression. The use of minimally invasive sampling of PBMCs from venous blood makes this approach ethically and logistically feasible in pediatric research. It supports its potential translation into routine clinical practice for early detection and risk assessment.

The main limitation of this study is the relatively small sample size of participants with manifest T2DM, as T2DM still remains a rare condition in adolescents. Furthermore, although we adjusted for key confounders, such as age and puberty, other factors known to influence insulin sensitivity and mitochondrial function, such as physical activity levels and dietary intake, were not systematically captured. In addition, our cross-sectional design has compared different individuals at each pubertal stage rather than the same individual followed over time. Baseline differences, including birth weight, family medical history and other factors may differ between groups and could be a potential source of bias. Another potential limitation of this study, as with many studies conducted in PBMCs, is that, although it offers a convenient, minimally invasive model, this cell population is inherently heterogeneous [43]. PBMCs are composed predominantly of lymphocytes – including T cells, B cells, and natural killer (NK) cells – with monocytes representing a smaller fraction and dendritic cells only a minor population [43]. PBMCs are functionally heterogeneous and, importantly, can also differ metabolically: quiescent immune-cell subsets, particularly naive lymphocytes, generally rely more on oxidative phosphorylation (OXPHOS), whereas activation is associated with metabolic reprogramming [44,45]. In addition, interindividual variability may arise from the donor's physiological status and other biological differences [46].Despite this variability, PBMC respiration is a recognized readout of personalized bioenergetic fitness [47].

5. Conclusion

We demonstrate that markers of mitochondrial respiration and ATP production, which serve as indicators of bioenergetic health, are associated with the extent of metabolic impairment in childhood obesity. Thus, PBMC-based mitochondrial bioenergetic profiling may represent a sensitive biomarker of early metabolic impairment in obese children and adolescents with varying degrees of insulin resistance. In prepubertal participants with insulin resistance, mitochondrial respiration was increased. Bioenergetic parameters in postpubertal participants with obesity and T2DM were significantly lower compared to postpubertal peers with obesity alone. Further studies are required to confirm the clinical utility of this approach and to investigate the progression of mitochondrial function across different stages of metabolic deterioration. Future work should incorporate immunophenotyping or single-cell methodologies to better account for PBMC heterogeneity and to determine whether immune cell subsets drive the observed mitochondrial alterations.

CRediT authorship contribution statement

Marlene Rechtsteiner: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Susanne Kröber:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Samiya Al-Robaiy:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hans Zischka:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Andreas Simm:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **Paulo J. Oliveira:** Writing – review & editing, Methodology, Funding acquisition, Data curation, Conceptualization. **Eugénia Carvalho:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Susann Weihrauch-Blüher:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors 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.diabres.2026.113300.

References

[1] Kluge HHP. WHO european regional obesity report 2022. Regional Office for Europe, Copenhagen: World Health Organization; 2022.
[2] Abiri B, Valizadeh M, Amini S, Kelishadi R, Hosseinpanah F. Risk factors, cutoff points, and definition of metabolically healthy/unhealthy obesity in children and adolescents: a scoping review of the literature. Obes Rev 2023;24. https://doi.org/10.1111/obr.13548.
[3] Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome-a critical look on the discrepancies between definitions and its clinical importance. Int J Obes (Lond) 2021;45:12–24. https://doi.org/10.1038/s41366-020-00713-1.
[4] Zeljkovic A, Vekic J, Stefanovic A. Obesity and dyslipidemia in early life: impact on cardiometabolic risk. Metabolism 2024;156:155919. https://doi.org/10.1016/j.metabol.2024.155919.
[5] Lynch JL, Barrientos-Pérez M, Hafez M, Jalaludin MY, Kovarenko M, Rao PV, et al. Country-specific prevalence and incidence of youth-onset type 2 diabetes: a narrative literature review. Ann Nutr Metab 2020;76:289–96. https://doi.org/10.1159/000510499.
[6] Gesuita R, Eckert AJ, Besançon S, Crimmins NA, Cavallo F, Kim J, et al. Frequency and clinical characteristics of children and young people with type 2 diabetes at diagnosis from five world regions between 2012 and 2021: data from the SWEET Registry. Diabetologia 2024;68:82–93. https://doi.org/10.1007/s00125-024-06283-5.
[7] Lin B, Coleman RL, Bragg F, Maddaloni E, Holman RR, Adler AI. Younger-onset compared with later-onset type 2 diabetes: an analysis of the UK prospective Diabetes Study (UKPDS) with up to 30 years of follow-up (UKPDS 92). Lancet Diabetes Endocrinol 2024;12:904–14. https://doi.org/10.1016/S2213-8587(24)00242-0.
[8] Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The crucial role and mechanism of insulin resistance in metabolic disease. Front Endocrinol (Lausanne) 2023;14:1149239. https://doi.org/10.3389/fendo.2023.1149239.
[9] Boutari C, DeMarsilis A, Mantzoros CS. Obesity and diabetes. Diabetes Res Clin Pract 2023;202:110773. https://doi.org/10.1016/j.diabres.2023.110773.
[10] Zong Y, Li H, Liao P, Chen L, Pan Y, Zheng Y, et al. Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduct Target Ther 2024;9:124. https://doi.org/10.1038/s41392-024-01839-8.
[11] Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, Esquivel-Chirino C, et al. Inflammation, oxidative stress, and obesity. Int J Mol Sci 2011;12:3117–32. https://doi.org/10.3390/ijms12053117.
[12] Al-Kataan MA, Fawzi MM. Obesity and mitochondrial function in children: a case–control study, open access maced. J Med Sci 2022;10:1153–7. https://doi.org/10.3889/oamjms.2022.8614.
[13] Wang J, Lin X, Zhao N, Dong G, Wu W, Huang K, et al. Effects of mitochondrial dynamics in the pathophysiology of obesity. Front Biosci (Landmark Ed) 2022;27:107. https://doi.org/10.31083/j.fbl2703107.
[14] Prasun P. Mitochondrial dysfunction in metabolic syndrome. Biochim Biophys Acta Mol basis Dis 2020;1866:165838. https://doi.org/10.1016/j.bbadis.2020.165838.
[15] Cojocaru K-A, Luchian I, Goriuc A, Antoci L-M, Ciobanu C-G, Popescu R, et al. Mitochondrial dysfunction, oxidative stress, and therapeutic strategies in diabetes, obesity, and cardiovascular disease. Antioxidants (Basel) 2023;12. https://doi.org/10.3390/antiox12030658.
[16] Pinho ACO, Lazaro A, Barbosa P, Porter C, Tralhão JG, Carvalho E. Impact of the metabolic disease status in obesity and surgical weight loss on human adipose tissue bioenergetics. J Physiol 2025;603:2583–617. https://doi.org/10.1113/JP286103.
[17] Garrafa E, Segala A, Vezzoli M, Bottani E, Zanini B, Vetturi A, et al. Mitochondrial dysfunction in peripheral blood mononuclear cells as novel diagnostic tools for non-alcoholic fatty liver disease: visualizing relationships with known and potential disease biomarkers. Diagnostics (Basel) 2023;13. https://doi.org/10.3390/diagnostics13142363.
[18] Braganza A, Annarapu GK, Shiva S. Blood-based bioenergetics: an emerging translational and clinical tool. Mol Aspects Med 2020;71:100835. https://doi.org/10.1016/j.mam.2019.100835.
[19] Tyrrell DJ, Bharadwaj MS, van Horn CG, Marsh AP, Nicklas BJ, Molina AJA. Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults. Exp Gerontol 2015;70:84–91. https://doi.org/10.1016/j.exger.2015.07.015.
[20] Ehinger JK, Westerlund E, Frostner EÅ, Karlsson M, Paul G, Sjövall F, et al. Mitochondrial function in peripheral blood cells across the human lifespan. NPJ Aging 2024;10:10. https://doi.org/10.1038/s41514-023-00130-4.
[21] Janssen JJE, Lagerwaard B, Porbahaie M, Nieuwenhuizen AG, Savelkoul HFJ, van Neerven RJJ, et al. Extracellular flux analyses reveal differences in mitochondrial PBMC metabolism between high-fit and low-fit females. Am J Phys Endocrinol Metab 2022;322:E141–53. https://doi.org/10.1152/ajpendo.00365.2021.
[22] Shirakawa R, Nakajima T, Yoshimura A, Kawahara Y, Orito C, Yamane M, et al. Enhanced mitochondrial oxidative metabolism in peripheral blood mononuclear cells is associated with fatty liver in obese young adults. Sci Rep 2023;13:5203. https://doi.org/10.1038/s41598-023-32549-w.
[23] Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V, et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschr Kinderheilkd 2001;149:807–18. https://doi.org/10.1007/s001120170107.
[24] World Health Organisation, Definition and diagnosis of Diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF Consultation, Switzerland, 2006.
[25] Hammel MC, Stein R, Kratzsch J, Vogel M, Eckert AJ, Triatin RD, et al. Fasting indices of glucose-insulin-metabolism across life span and prediction of glycemic deterioration in children with obesity from new diagnostic cut-offs. Lancet Reg Health Eur 2023;30:100652. https://doi.org/10.1016/j.lanepe.2023.100652.
[26] Deutsche Adipositas-Gesellschaft e.V., Deutsche Gesellschaft für Kinder- und Jugendmedizin e.V., S3-Leitlinie: Therapie und Prävention der Adipositas im Kindes- und Jugendalter. Stand 31.08.2019, AWMF-Registernummer 050-002 (2019).
[27] Ukegbu TE, Wylie-Rosett J, Groisman-Perelstein AE, Diamantis PM, Rieder J, Ginsberg M, et al. Waist-to-height ratio associated cardiometabolic risk phenotype in children with overweight/obesity. BMC Public Health 2023;23:1549. https://doi.org/10.1186/s12889-023-16418-9.
[28] Zong X, Kelishadi R, Hong YM, Schwandt P, Matsha TE, Mill JG, et al. Establishing international optimal cut-offs of waist-to-height ratio for predicting cardiometabolic risk in children and adolescents aged 6-18 years. BMC Med 2023;21:442. https://doi.org/10.1186/s12916-023-03169-y.
[29] Ulijaszek SJ, editor. The Cambridge encyclopedia of human growth and development. Cambridge: Cambridge University Press; 2000.

[30] Tahapary DL, Pratisthita LB, Fitri NA, Marcella C, Wafa S, Kurniawan F, et al. Challenges in the diagnosis of insulin resistance: focusing on the role of HOMA-IR and Tryglyceride/glucose index. Diabetes Metab Syndr 2022;16:102581. https://doi.org/10.1016/j.dsx.2022.102581.
[31] A. Technologies, Inc., Seahorse XF Cell Mito Stress Test Kit User Guide.
[32] Chacko BK, Kramer PA, Ravi S, Benavides GA, Mitchell T, Dranka BP, et al. The bioenergetic health index: a new concept in mitochondrial translational research. Clin Sci (Lond) 2014;127:367–73. https://doi.org/10.1042/CS20140101.
[33] Chacko BK, Zhi D, Darley-Usmar VM, Mitchell T. The Bioenergetic Health Index is a sensitive measure of oxidative stress in human monocytes. Redox Biol 2016;8:43–50. https://doi.org/10.1016/j.redox.2015.12.008.
[34] Desousa BR, Kim KK, Jones AE, Ball AB, Hsieh WY, Swain P, et al. Calculation of ATP production rates using the Seahorse XF Analyzer. EMBO Rep 2023;24:e56380. https://doi.org/10.15252/embr.202256380.
[35] Satapati S, Kucejova B, Duarte JAG, Fletcher JA, Reynolds L, Sunny NE, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. J Clin Invest 2015;125:4447–62. https://doi.org/10.1172/JCI82204.
[36] Kim KE, Heo JS, Han S, Kwon S-K, Kim S-Y, Kim JH, et al. Blood concentrations of lipopolysaccharide-binding protein, high-sensitivity C-reactive protein, tumor necrosis factor-α, and Interleukin-6 in relation to insulin resistance in young adolescents. Clin Chim Acta 2018;486:115–21. https://doi.org/10.1016/j.cca.2018.07.042.
[37] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. J Am Med Assoc 2001;286:327–34. https://doi.org/10.1001/jama.286.3.327.
[38] Böhm A, Keuper M, Meile T, Zdichavsky M, Fritsche A, Häring H-U, et al. Increased mitochondrial respiration of adipocytes from metabolically unhealthy obese compared to healthy obese individuals. Sci Rep 2020;10:12407. https://doi.org/10.1038/s41598-020-69016-9.
[39] de Lamas C, Kalén A, Anguita-Ruiz A, Pérez-Ferreirós A, Picáns-Leis R, Flores K, et al. Progression of metabolic syndrome and associated cardiometabolic risk factors from prepuberty to puberty in children: the PUBMEP study. Front Endocrinol (Lausanne) 2022;13:1082684. https://doi.org/10.3389/fendo.2022.1082684.
[40] Rovira-Llopis S, Bañuls C, Diaz-Morales N, Hernandez-Mijares A, Rocha M, Victor VM. Mitochondrial dynamics in type 2 diabetes: pathophysiological implications. Redox Biol 2017;11:637–45. https://doi.org/10.1016/j.redox.2017.01.013.
[41] Carvalho E, Landes RD, Cotter M, Delhey LM, Børsheim E, Rose S. Enhanced mitochondrial respiration in peripheral blood mononuclear cells (PBMCs) from young children with overweight/obesity and insulin resistance. Eur J Clin Invest 2025:e70090. https://doi.org/10.1111/eci.70090.
[42] Barbosa P, Pinho A, Lázaro A, Paula D, Tralhão JG, Paiva A, et al. Bariatric surgery induces alterations in the immune profile of peripheral blood T cells. Biomolecules 2024;14. https://doi.org/10.3390/biom14020219.
[43] Monaco G, Lee B, Xu W, Mustafah S, Hwang YY, Carré C, et al. RNA-Seq signatures normalized by mRNA abundance allow absolute deconvolution of human immune cell types. Cell Rep 2019;26:1627–1640.e7. https://doi.org/10.1016/j.celrep.2019.01.041.
[44] Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol 2014;2:206–10. https://doi.org/10.1016/j.redox.2013.12.026.
[45] Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. Immunity 2013;38:633–43. https://doi.org/10.1016/j.immuni.2013.04.005.
[46] Maes E, Landuyt B, Mertens I, Schoofs L. Interindividual variation in the proteome of human peripheral blood mononuclear cells. PLoS One 2013;8:e61933. https://doi.org/10.1371/journal.pone.0061933.
[47] Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Shively C, Andrews RN, et al. Blood-based bioenergetic profiling reflects differences in brain bioenergetics and metabolism. Oxid Med Cell Longev 2017;2017:7317251. https://doi.org/10.1155/2017/7317251.