MEDICAL UNIVERSITY

'PROF. DR. PARASKEV STOYANOV"

FACULTY OF MEDICINE
PEDIATRIC DEPARTMENT

NIKOLINKA YORDANOVA PEYKOVA, MD

„Metabolic risk and body composition in children, born small for gestational age with genetic syndroms (Prader-Willi, Silver-Russell syndrome and some other)"

THESIS SUMMARY
FOR AWARDING EDUCATIONAL AND SCIENTIFIC DEGREE "PhD"

Scientific supervisor:
prof. Violeta Mihova Iotova, MD, PhD, DSc

Varna, 2024

Scientific Jury:

Chair:

Assoc. Prof. Dr. Sonia Galcheva, PhD, University Hospital "St. Marina," Varna

External Members:

Prof. Dr. Daniela Avdzhieva-Tzavella, PhD, Specialized Hospital for Active Treatment of Pediatric Diseases "Prof. Dr. Ivan Mitev," Sofia

Assoc. Prof. Dr. Narcis Kaleva-Hodzheva, MD, University Hospital "Sv. Georgi," Plovdiv

Assoc. Prof. Dr. Chaika Petrova, PhD, University Hospital "Dr. Georgi Stranski," Pleven

Internal Members:

Assoc. Prof. Dr. Mira Siderova, PhD, University Hospital "St. Marina," Varna

Assoc. Prof. Dr. Sonia Galcheva, PhD, University Hospital "St. Marina," Varna

Alternate Members:

Assoc. Prof. Dr. Milena Belcheva, PhD, University Hospital "St. Marina," Varna

Assoc. Prof. Dr. Margarita Arshinkova, PhD, Specialized Hospital for Active Treatment of Pediatric Diseases "Prof. Dr. Ivan Mitev," Sofia

The dissertation consists of a total of 180 pages, illustrated with 30 tables and 8 figures.

The bibliography includes 382 references, 11 of them in Cyrillic and 371 in Latin script.

The research and laboratory analysis were conducted at the Clinical Laboratory of University Hospital "St. Marina" – Varna and the "Labor Express" laboratory. DXA scanning was performed at the Radiology Department of University Hospital "St. Marina" – Varna.

The doctoral candidate works in the First Pediatric Clinic with Intensive Care Unit at University Hospital "St. Marina" – Varna and is part of the multidisciplinary team for patiens with Prader-Willi syndrome at the Varna Expert Center for Rare Endocrine Disorders.

The dissertation was reviewed, accepted, and submitted for defense by the Departmental Council at the Department of Pediatrics, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, on October 26, 2024.

The dissertation defense will take place on December 12, 2024.

Materials for the defense are available in the Scientific Department of Medical University Varna and are published on the website of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AGV – adequate for gestational age
AND – Adiponectin
BGL – Blood Glucose Level
BMI – Body Mass Index
BMD – Bone Mineral Density
BP – Blood Pressure
CPP – Central Precocious Puberty
CRP – C-Reactive Protein
CVD – Cardiovascular Diseases
DBP – Diastolic Blood Pressure
DXA – Dual-Energy X-ray Absorptiometry
ELISA – Enzyme-Linked Immunosorbent Assay
FFM – Fat-Free Mass
FM – Fat Mass
FSH – Follicle-Stimulating Hormone
GDPR – General Data Protection Regulation
GnRHa – Gonadotropin-Releasing Hormone Agonist
HDL-C – High-Density Lipoprotein Cholesterol
HMW – High Molecular Weight
HOMA-IR – Homeostatic Model Assessment for Insulin Resistance
HR – Heart Rate
hrGH – Human Recombinant Growth Hormone
HST – Hormone-Substitution Therapy
IGF-1 – Insulin-Like Growth Factor 1
IFG – Impaired Fasting Glycemia
IOTF – International Obesity Task Force
IR – Insulin Resistance
LDL-C – Low-Density Lipoprotein Cholesterol
LH – Luteinizing Hormone
LM – Lean Mass
MS – Metabolic Syndrome
NH-CSS – Netchine-Harbisson Clinical Scoring System
OGTT – Oral Glucose Tolerance Test
PCR – Polymerase Chain Reaction
PWS – Prader-Willi Syndrome
QoL – Quality of Life
SBP – Systolic Blood Pressure
SDS – Standard Deviation Score (Z-Score)
SGA – Small for Gestational Age
SRS – Silver-Russell Syndrome
TC – Total Cholesterol
TG – Triglycerides
T2D – Diabetes Type 2
UA – Uric Acid
UPD – Maternal Uniparental Disomy
WC – Waist Circumference

PART I

Introduction

Prader-Willi syndrome (PWS) is characterized by muscular hypotonia in the neonatal period, excessive weight gain due to a lack of satiety after the age of 3-4 years, short stature, hypogonadism, and psychiatric disturbances. Children with Silver-Russell syndrome (SRS) are born small for gestational age (SGA, defined as birth weight and/or length below -2 SD for their gestational age) (Hokken-Koelega et al., 2023), exhibit postnatal growth failure, and present with distinctive dysmorphic features (triangular face, downturned corners of the mouth, micrognathia, prominent forehead, dental anomalies) and body asymmetry.

PWS and SRS share certain underlying mechanisms in the genetic defect pathogenesis, the so called genomic imprinting. In both conditions, some gene alleles are exclusively expressed from either the maternal or paternal genome, with the haploid genomes complementing each other to ensure a normal phenotype. Methylation of a specific parental chromosome region can result in "silencing" of genes in that region, leading to various pathologies (Gabriel et al., 1998). Other common characteristics include low birth weight and the development of early metabolic complications (Clayton et al., 2007). Between 20% and 65% of children with PWS are born SGA, and initially, it was believed that 100% of children with SRS were born SGA. In SRS patients, characteristics specific to the fetal origins hypothesis are observed—low birth weight is linked to an increased risk of cardiovascular disease, hypertension, dyslipidemia, obesity, insulin resistance, and type 2 diabetes mellitus.

Both PWS and SRS patients present with lower percentage of muscle mass compared to healthy controls. Treatment with recombinant human growth hormone (rhGH) in both groups leads to an increase in

muscle mass percentage. To date, no data is provided for associations between reduced muscle mass and metabolic parameters in these patients.

These similarities and unexplored relationships gives us the motivation to choose exactly PWS and SRS patients for our study. Moreover, in recent years, the concept of advancing clinical care and expanding knowledge in the field of rare diseases (such as these two syndromes) has become an increasingly important aspect of modern science and practice.

PART II. RATIONALE

1. Absence of a comprehensive assessment of the metabolic profile of PWS and SRS patients in Bulgaria and a lack of summerized data for comparison with international findings.
2. Scarcity of global data on the correlation between muscle mass and metabolic parameters in PWS and SRS patients.
3. The availability of a suitable, regularly followed cohort of patients with confirmed diagnoses, treated at University Hospital "St. Marina," Varna, from 2000 to the present moment. Since the designation of our Center as a National Expert Center for Rare Endocrine Diseases in 2016, all patient data have been systematically recorded in the National Registry for Rare Diseases.
4. Access to reimbursed recombinant human growth hormone (rhGH) therapy for PWS patients and availability of rhGH for SRS patients through the Presidential Charity Initiative “The Bulgarian Christmas.” Diagnostic and therapeutic support for these patients is well-supported by the National Health Insurance Fund.
5. Development of Networks for Rare Endocrine Diseases worldwide and the growing necessity for multidisciplinaryteams of highly specialized experts, aiming to improve and personalize care for patients with rare imprinting syndromes by considering desired treatment outcomes of patients and their families
6. Development of national collaboration on imprinting syndromes, with publication of a updated National Consensus on PWS

treatment in children and pharmacotherapeutic guidelines that include recommendations for managment for both syndromes.

PART III. OBJECTIVE AND AIMS OF THE STUDY:

Objective

To evaluate the cardiovascular (CV) and metabolic risk in patients with PWS and SRS and to seek for correletaion between CV and metabolic parameters and fat and muscle mass.

Aims:

1. To assess the clinical and laboratory profile of patients with PWS and SRS monitored at the Varna Expert Center for Rare Endocrine Diseases (VECRED).
2. To determine the prevalence of metabolic and CV disorders in patients with PWS and SRS, compared to gender-, age-, and BMI-matched healthy controls.
3. To analyze body composition in patients with PWS and SRS and its associations with CV and metabolic parameters.
4. To evaluate the quality of life in these two patient groups.

PART IV. RESEARCH

1. Participants and Methods

A. Participant Selection

The study strated two months after ethical approval was obtained from Ethics Committee for Scientific Research (July 1, 2021). To achieve the objective and aims of this scientific project, two parallel cross-sectional case-control sub-studies were conducted:

1. A cross-sectional case-control sub-study comparing patients with PWS to healthy controls matched by gender, age, and BMI.
2. A cross-sectional case-control sub-study comparing patients with RSS to healthy controls matched by gender, age, and BMI.

During the study period from September 2021 to September 2023, 82 participants were enrolled: 25 with genetically confirmed PWS, 18 with genetically confirmed or clinically diagnosed SRS, and 39 healthy controls matched by gender, age, and by BMI. Both patient groups (88% of those with PWS and 94,4% of those with SRS), with the exception of three PWS patients and one SRS, were assessed while on rhGH therapy. A total of 78 patients were evaluated during hospitalization at the First Pediatric Clinic, and the remaining 4, due to reaching adulthood, were assessed at the Clinic of Endocrinology and Metabolic Diseases at University Hospital "St. Marina."

Inclusion Criteria for Patients:

- Genetically confirmed PWS or clinically/genetically confirmed RSS.
- Age range from 1 month to 35 years.
- Signed informed consent from a parent or guardian for participants under 18 years of age, as well as a GDPR-compliant data protection declaration, following ethical approval from the Ethics Committee for Scientific Research.
- Signed informed consent from a personal assistant (parent) and a GDPR-compliant data protection declaration for participants over 18 years of age, following ethical approval from Ethics Committee for Scientific Research.
- Participating patients must have been systematically monitored (over 6 months) at the Expert Center, with exceptions of newly diagnosed in the early neonatal period.

Inclusion Criteria for Controls:

- Age range from 1 month to 35 years.
- Gender, age, and BMI matching with the included cases.
- Signed informed consent from individuals over 18 years, or from a parent or guardian for participants under 18 years of age, as well as a GDPR-compliant data protection declaration, following ethical approval from Ethics Committee for Scientific Research.

Exclusion Criteria for Patients and Controls:

- Severe comorbid somatic or psychiatric disorders.
- Acute infection at the time of the investigations or within the previous two weeks.
- Major surgical interventions within the previous six months.
- Refusal to sign the informed consent form.

To minimize risk during patient assessment, the following measures were implemented:

1. Scheduling and Hospitalization: A convenient day and time for routine, planned hospitalization were arranged for the patients' parents, during which all necessary tests were conducted. Control group patients were assessed during hospitalization for other reasons. After consultation with and approval from parents, additional protocol-required tests were performed.
2. Informed Consent and Anonymization: Detailed explanations regarding informed consent and the completion of questionnaires were provided directly by the researcher (N.Y.) and co-researcher (M.D.). Identification codes were used to prevent participant identification, and all information was securely stored at the Department of Pediatrics, Medical University – Varna, within the First Pediatric Clinic at St. Marina University Hospital.

3. Blood Test Protocol: Standard procedures for venous blood collection were followed, with a detailed explanation provided in advance to the parents and child regarding the steps involved in the procedure.

4. DXA Procedure Information: Additional information was provided to parents regarding the DXA scan process, including assurances about its lack of adverse health effects on the child.

B. Methods Used in the Prospective Study

The prospective study included an assessment of anthropometric measurments, metabolic markers, body composition, and quality of life among patients with PWS and SRS, seeking for correlations among these parameters and searching for novel predictive markers for cardiovascular and metabolic risk.

All patients and controls were enrolled in the study after parents or guardians were informed about the study's objectives and anticipated outcomes, followed by the signing of informed consent (two versions were provided: one for patients under 18 years and another for those at the age of 18 and older).

Participation by parents and their children was voluntary, with the option to withdraw from the study at any time without explanation and without any consequences for the participants. All collected information was anonymized and all documents were securely stored in First Pediatric Clinic.

After wide explamation for the patients and their parents about the study's purpose, upcoming procedures, and expected results on the day of signing the informed consent, an interview was conducted for providing medical history. Additionally, a

questionnaire for assessing quality of life was provided to the patient group.

B1. Questionnaire – On the day informed consent and the GDPR-compliant data protection declaration were signed, an interview was conducted with both patients and controls. For the patient group, two separate versions were adapted specifically for PWS and SRS, taking into account the unique characteristics of the patient groups. Both were thoroughly interviewed to collect information about family history of cardiovascular and metabolic diseases, growth rate data, parental and target height, prior and co-existing conditions (including precocious puberty), date of diagnosis, genetic results, taken medications (including rhGH therapy and GnRH agonist treatment), therapy initiation, and dosage.

For patients with PWS, the anamnesis included questions regarding the presence or absence of oligohydramnios, polyhydramnios, reduced fetal movement, conception method, delivery mechanism, gestational week, birth length and weight, Fenton growth chart for assessment of small for gestational age (SGA), and tube feeding requirement.

For patients with SRS, data were collected on the presence or absence of oligohydramnios, intrauterine growth restriction, reduced fetal movements, method of conception, mechanism of delivery, gestational age, birth length, weight, and head circumference, as well as an assessment of relative macrocephaly. Evaluation based on the Fenton growth curve determined whether the infant was small for gestational age (SGA), along with data on tube feeding, constipation, night sweats, and assessment of additional criteria per Netchine-Harbison criteria (postnatal growth restriction $\leq$ -2 SDS at 24 months of age or $\leq$ -2 SDS from mid-parental target height at 24 months; feeding difficulties and/or BMI $\leq$ -2 SDS at 24 months; prominent forehead between 1 and 3 years; and body asymmetry).

For the control group, a detailed medical history was recorded according to good clinical practice guidelines, including the general questions common to both patient groups outlined above.

B2. Quality of Life Assessment Questionnaire - Quality of Life Inventory–Disability (QI-Disability)

A quality of life questionnaire adapted for patients with intellectual disability (QI-Disability), was provided to the parents of the patients. The QI-Disability, validated by Downs et al. (2019) among 253 patients with intellectual disabilities (including Rett syndrome, Down syndrome, cerebral palsy, and autism spectrum disorder), was used after written permission from its author. The questionnaire evaluates key domains: physical health, positive and negative emotions, social interactions, leisure time, and independence. In conclusion, it was determined that the questionnaire is a reliable measure of quality of life across the spectrum of intellectual disabilities, facilitating identification of interventions that meet the specific needs of each patient and allowing for tracking of intervention outcomes. Data scoring was done by the principal investigator, each domain was scored separately according to Likert scale protocols, and results were subsequently transformed into percentages.

B3. Anthropometry

Anthropometric measurements were conducted in the morning, fasting, on the day of arrival at the Clinic. Height, weight, and waist circumference were measured for both patients and healthy controls. Weight was measured to an accuracy of 0.1 kg using a calibrated SECA 861 digital scale (SECA Ltd, Hamburg, Germany), with each child weighed barefoot and in light clothing, positioned at the center of the scale standing still with feet together.

Height was measured by using a standard wall-mounted HARPENDEN2000 stadiometer and recorded to the nearest 0.1 cm, with the participant in an upright stance and the head positioned along the horizontal Frankfurt plane, while the head and gluteuses in tight connection with the vertical component of the

stadiometer. Measurements were taken twice consecutively, with the mean value documented.

BMI was calculated using the standard formula: BMI = Weight (kg) / Height² (m^2). Patients and controls were classified according to age- and gender-specific international BMI references by Cole (2000) for the 85th and 95th percentiles in children and adolescents (for participants over 2 years of age) and according to diagnostic values for adults (Alberti et al., 2006).

Waist circumference was measured to the nearest 1 mm using a flexible, non-stretchable SECA 201 measuring tape. Measurements were taken with the tape placed directly on the skin along the midpoint of the mid-axillary line between the 10th rib and the anterior superior iliac crest, recorded twice consecutively, with the average value documented. Participants were classified according to age- and gender-specific waist circumference standards for Bulgarian children and adolescents (Galcheva et al., 2009) or according to IDF criteria for those aged 18 years and older (Zimmet et al., 2007).

B4. Physical Examination

After antropometric measurments was assessed, a comprehensive physical examination was performed, including blood pressure and heart rate assessment. Heart rate was measured twice at 5-minute intervals at the right radial artery (a. radialis) following 10 minutes of rest in a seated position, with the second reading recorded. Blood pressure (BP) was measured using a sphygmomanometer (Korotkoff method) in a seated position after 10 minutes of rest, taken twice at 5-minute intervals. The mean value of the systolic (SBP) and diastolic (DBP) blood pressure readings was used. Hypertension (systolic, diastolic, or both) was defined as a value above the 95-th percentile according to reference values based on gender, age, and height, as per the European Society of Hypertension guidelines for managing high blood pressure in children and adolescents (Lurbe et al., 2016).

Pubertal development staging was assessed based on secondary sexual characteristics according to the Tanner scale.

Any pathological deviations in somatic status relevant to the main characteristics of the two syndromes were recorded. For PWS, scoliosis and cryptorchidism and for SRS relative macrocephaly, micrognathia, low muscle mass, clinodactyly of the fifth finger, prominent heel, shoulder dimples, syndactyly of the second and third toes, body asymmetry, and scoliosis were specifically sought.

B5. Laboratory Biochemical and Hormonal Tests

After performance of physical examination, biochemical and hormonal analyses were conducted using a single venous blood sample collected after a 12-hour fasting period. Blood serum was stored at -80°C to ensure maximum analytical accuracy by performing additional tests in outer Laboratory. The evaluated parameters included fasting blood glucose (FBG) and serum insulin levels, both fasting and during a standard oral glucose tolerance test (OGTT), with a glucose load of 1.75 g per kilogram of body weight (up to a maximum of 75 g) for children, and values recorded at the 30th and 120th minutes. Additionally, lipid profile (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), uric acid, CRP, glycated hemoglobin (HbA1c), cortisol, SHBG, leptin, high-molecular-weight adiponectin, and irisin were measured.

Insulin resistance was assessed using the surrogate marker HOMA-IR, calculated with the following formula:

HOMA-IR = (FBG mmol/l x fasting inulin IU/ml) / 22,5

The following analytical methods were employed:

- Glucose – measured using the ADVIA 1800 biochemical analyzer through an enzymatic hexokinase method.

- Total Cholesterol – measured with the ADVIA 1800 biochemical analyzer using a three-step enzymatic reaction with Trinder endpoint.

- HDL-Cholesterol – measured with the ADVIA 1800 biochemical analyzer through a direct enzymatic elimination/catalase method.

- LDL-Cholesterol – measured with the ADVIA 1800 biochemical analyzer. For triglyceride levels < *4.5 mmol/L*, LDL cholesterol was calculated using Friedewald's formula:

LDL Cholesterol = Total Cholesterol - (HDL Cholesterol + Triglycerides/2.2)

For triglyceride levels > *4.5 mmol/L*, LDL cholesterol was measured directly using an enzymatic elimination/catalase method.

- Triglycerides – measured with the ADVIA 1800 biochemical analyzer using a three-step enzymatic reaction with Trinder endpoint.

- CRP – measured with the ADVIA 1800 biochemical analyzer through latex-enhanced immunoturbidimetric analysis.

- Uric Acid – measured with the ADVIA 1800 biochemical analyzer using an enzymatic uricase method.

- SHBG – measured with the Immulite 2000 analyzer using chemiluminescent immunoassay.

- IGF-1 – measured with the Immulite 2000 analyzer through chemiluminescent immunoassay.

- IGFBP-3 – measured with the Immulite 2000 analyzer using chemiluminescent immunoassay.

- Cortisol – measured with the ADVIA Centaur CP analyzer through chemiluminescent immunoassay.

- Insulin – measured with the Roche Cobas 6000 analyzer via electrochemiluminescent immunoassay.

- Glycated Hemoglobin (HbA1c) – measured with the Roche Cobas 6000 analyzer using a method standardized according to DCCT/NGSP guidelines. Results were presented as a percentage, calculated from the ratio of HbA1c to Total Hemoglobin (THb). HbA1c was measured by turbidimetric immunoassay, while THb was measured through a colorimetric method.

Leptin Immunoassay – Sandwich-type ELISA using two high-affinity specific antibodies for the quantitative measurement of human leptin. The leptin in samples binds to the primary antibody on a microtiter plate. In the next step, a secondary specific anti-leptin antibody binds to the immobilized leptin. This antibody is biotin-labeled and applied alongside a streptavidin-peroxidase enzyme conjugate. In the subsequent substrate reaction, the color change is catalyzed proportionally to the leptin levels in the samples. Absorbance is directly proportional to leptin concentration. Kit accuracy: intra-assay variability 6.3-7%, inter-assay variability 7.88–19.21%; sensitivity <0.25 ng/ml, range 0.25–100 μg/l.

High-Molecular-Weight (HMW) Adiponectin – reagent catalog no. EN4453 from Fine Test. This is a three-step sandwich-type ELISA. The anti-HMW adiponectin antibody is pre-coated on a 96-well plate (first reaction). Biotin-labeled anti-HMW adiponectin antibody is used as the detection antibody. Standards and samples are added to the wells and incubated, with unbound conjugates removed via wash buffer. The biotin-labeled detection antibody is then added to bound HMW adiponectin on the coated antibody (second reaction). After a second wash, streptavidin-peroxidase enzyme conjugate is added (third reaction). Following a final wash, tetramethylbenzidine (TMB) substrates are added to visualize the enzymatic reaction. TMB is catalyzed by the streptavidin-peroxidase conjugate, producing a blue color that turns yellow upon adding the stop solution. HMW adiponectin concentration in the sample is calculated based on absorbance at 450 nm, measured simultaneously with a standard solution. Kit accuracy: sensitivity up to 0.469 ng/ml, range 0.8–7.6 μg/ml.

Irisin – reagent catalog no. RAG018R from BioVendor. Competitive sandwich-type ELISA for the quantitative determination of irisin in human biological fluids (plasma, serum, cell culture supernatants). A polyclonal antibody recognizing irisin reacts with a sequence of pre-defined recombinant irisin standard proteins or competing samples on an irisin-coated plate. Relative reactivity is compared to that of standardized proteins. Kit accuracy: intra-assay variability 4.86–6.74%, inter-assay variability 9.67–9.71%; sensitivity up to 1 ng/ml, range 0.001 μg/ml–5 μg/ml.

B6. Dual-Energy X-ray Absorptiometry (DXA)

Following the aforementioned procedures, a DXA scan was conducted on participants to determine body composition and measure the quantities of lean (muscle), fat, and bone mass, with the exception of those who did not consent or were unable to complete the protocol (e.g., children under 1 year of age, due to their inability to remain still for the required period, or those exhibiting hyperactive behavior).

DXA is an imaging technique based on X-ray technology, where two energy peaks/beams are emitted from the X-ray tube. As these beams pass through tissues, they are attenuated differently depending on the type of substance (tissue) they traverse. The DXA scan provides data on three compartments: lean (muscle), fat, and bone mass. The attenuation values for different tissues are assumed to be constants and are derived using attenuation constants calculated for the so-called "standard human." Due to growth and development processes in children, comparison with a standard constant can lead to systematic errors, necessitating the use of specialized pediatric software (Lunar Prodigy).

The directly measured parameters from the device included: Bone Mineral Density (BMD) (g/cm^2), age-matched BMD Z-score, LBM/height (p), BMC/LBM (p), Lean Mass (LM) (g), Fat-Free Mass (FFM) (g), Bone Mineral Content (BMC) (g), and Fat Percentage. For data analysis, only those parameters relevant to bone density, lean mass, and fat mass, which are components of the specific body composition in the two patient groups, were used. The Z-score was calculated by the formula:

$z = (x - \mu) / \sigma,$

where “z” is the Z-score, “x” is the variable being examined (in this case, BMD), “μ” is the mean value for the specific age and sex, and “σ” is the standard deviation. The Z-score normalizes deviations, enabling comparisons regardless of the type of standard applied.

2. Statistical Methods for Data Analysis

The following analytical methods were used in the data processing:

2.1 Method of statistical data classification – Variables were organized according to their type into variation, interval, categorical, ordinal, and dynamic statistical series.

2.2 Statistical Estimation Method:

- Point estimates – used for calculating the arithmetic mean of continuous variables.

- Interval estimates:

  - Confidence level (significance level) – denoted as “p”. For p = 0.95 (95%), the Type I statistical error is 0.05 (5%).

  - Confidence intervals (CI) – 95% confidence intervals were used around the point estimate, interpreted as a 95% probability that the interval contains the true point value.

2.3 Graphical Method – Various graphical representations were utilized, including linear and plane charts, pie and pie-sector diagrams.

2.4 Nonparametric Analysis – For the evaluation of categorical variables, Pearson's Chi-square ($\chi 2$) test was applied. The Mann-Whitney and Kruskal-Wallis nonparametric tests were used for comparisons across gender and age groups.

2.5 Correlation Analysis – Univariate Pearson correlation coefficients were calculated. Partial multiple correlation analysis was also used in evaluating relationships between two variables while accounting for the influence of additional factors, such as gender and age.

- The correlation coefficient "r" can range from 0 to -1 for *inverse* relationships and from 0 to +1 for *direct* correlation.
- The strength of the correlation between two variables, as indicated by the "r" coefficient, is interpreted as follows:
  - If "r" is below 0.30 – weak correlation
  - If "r" is between 0.30 and 0.50 – moderate correlation
  - If "r" is between 0.50 and 0.70 – substantial correlation
  - If "r" is between 0.70 and 0.90 – strong correlation
  - If "r" is above 0.90 – very strong correlation

2.6 Analysis of Variance – For comparisons of continuous variables, the Student-Fisher ttt-test was used for independent samples, while paired t-tests were applied for data comparisons within the same participant. For multiple comparisons of variables with several categories, the p-value was adjusted using the Bonferroni correction according to the number of comparisons.

2.7 Linear Regression Analysis – For a comprehensive assessment of the independent effect of individual variables on a given continuous outcome, a multivariable linear regression analysis was performed.

All values are presented as mean ± standard deviation (SD). In all comparisons, $p < 0.05$ was considered statistically significant. Data analysis was conducted using the specialized statistical software package SPSS for Windows, version 25.0 (Chicago, IL, USA).

PART V. ORIGINAL RESULTS

After applying all inclusion and exclusion criteria outlined in the study protocol, the total number of participants was 82 (38 males), with a mean age of 10.3 ± 5.9 years (range: 0.25–33 years). To address the study objectives, participants were divided into four subgroups, as shown in Figure 1.

Twenty-five of the participants (15 boys) had a genetic diagnosis of PWS, and 18 (8 boys) had a genetic and/or clinical diagnosis of SRS, according to the established Netchine-Harbison Clinical Scoring System (NH-CSS) criteria. The remaining 39 healthy controls were divided into two subgroups: 24 healthy controls (11 boys) matched by sex, age, and BMI to the PWS patients (PWS Controls) and 15 healthy participants (4 boys) matched by the same criteria to the SRS patients (SRS Controls).

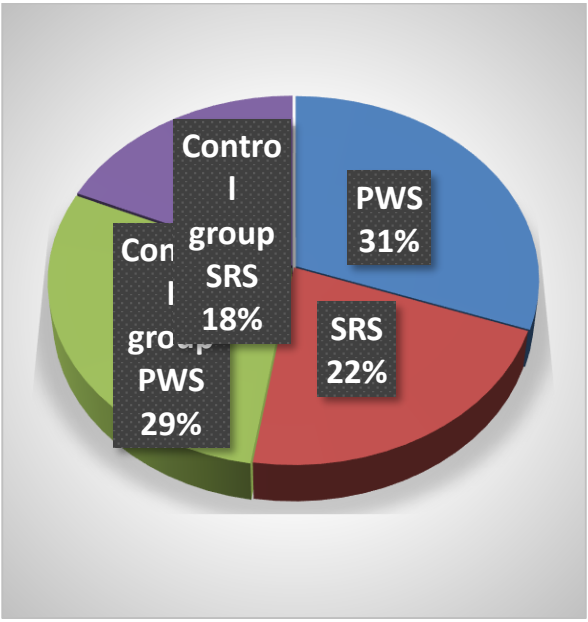

***Fig. 1*. Distribution of Study Participants by Group (%)**

V.A Cross-Sectional Case-Control Substudy Comparing PWS Patients with Healthy Controls Matched by Sex, Age, and BMI

1. Demographic, auxologicaL and clinical characteristics of the participants

In this substudy, a total of 49 patients/healthy controls (27 boys), mean age of 11.3 ± 6.4 years (range: 0.3–33.0 years) participated after providing informed consent. No significant differences were observed in sex and age distribution between participants in the two groups (Table 1).

Table 1. Demographic Characteristics of Participants.

Indicator	PWS (n=25)	Control group (n=24)	p
Sex N (%)			NS
Male	16 (64)	11 (45.8)	
Female	9 (36)	13 (54.2)	
Age (years)	11.3±8.2	11.3±3.9	NS

The results are presented as absolute numbers (percentage) or mean ± SD.

NS – non-significant difference.

The diagnosis of PWS was confirmed in all patients by genetic analysis, conducted at a mean age of 3.9 ± 5.0 years (range: 0.1–15.0 years). The distribution of identified genetic variants is shown in Figure 2. In our PWS group, the most frequent finding was a deletion on chromosome 15, while in three patients, the diagnosis was confirmed via a methylation test.

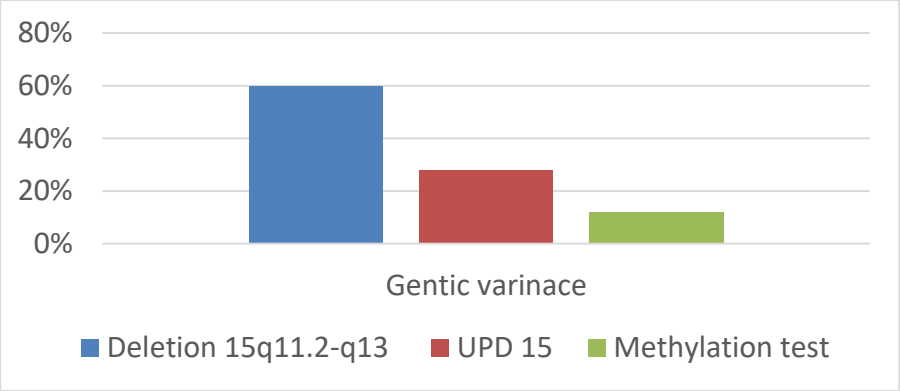

Fig. 2. *Genetic variants among the PWS patients (%).*

The mean age at the start of rhGH therapy was 5.1 ± 5.1 years. Eighty-eight % (n=22) of participants currently undergoing therapy at the time of the study, 4% (n=1) who had never received treatment, and 8% (n=2) who had discontinued therapy due to a lack of reimbursement after reaching adulthood. The average dose was 0.024 ± 0.001 mg/kg/day (range: 0.007-0.035), including patients receiving adult dosing (0.4-0.6 mg/day).

Following the protocol of this substudy, auxological measurements and clinical assessments were conducted for all participants (including SBP, DBP, HR, Tanner staging).

Table 2. Anthropometric, Clinical, and Pubertal Assessment of Participants by Group.

Parameter	PWS (n=25)	Control group (n=24)	p
Weight (kg)	57.0 (16.7-82.0)	69.7 (54.8-83.9)	NS
Height (cm)	137.4 (103.2-160.0)	156.2 (144.2-163.8)	0.017
BMI (kg/m^2)	24.1 (15.5-31.1)	29.4 (24.9-32.7)	NS
WC (cm)	79.8 (52.5-95.1)	90.0 (79-102.0)	NS
SBP (mmHg)	103.0 (95.0-113.5)	120.0 (115.0-125.5)	<0.001
DBP (mmHg)	75.0 (65.0-79.5)	80.0 (72.5-82.5)	0.046
HR (beats/min)	93.5 (81.5-105.3)	98.0 (88.8-104.0)	NS

Tanner staging N (%)			
1 st.	11 (44) (0.6-12.8)	7 (29.2) (3-8.2)	0.046
2 st.	5 (20) (8.6-16.6)	3 (12.5) (10.1-13.4)	
3 st.	4 (16) (13.4-22.8)	3 (12.5) (10-15.4)	
4 st.	4 (16) (16.3-33)	8 (33.3) (11.6-16.5)	
5 st.	0	3 (12.5) (15.2-16,8)	

The results are presented as median (25th-75th percentile) or absolute number (percentage).

NS – non-significant difference.

Among all anthropometric parameters, only height showed a significant difference between the two study groups (Table 2), with PWS patients being significantly shorter than healthy controls ($p=0.017$). Differences in generalized and abdominal obesity indicators (BMI and WC) did not reach statistical significance ($p>0.05$), although the median values in the PWS cases were lower than those of the controls. Using partial correlation analysis, after controlling for sex and age, a positive correlation between BMI and WC was observed in both PWS patients ($r=0.784$, $p<0.001$) and the control group ($r=0.757$, $p<0.001$).

Figure 3 shows the prevalence of normal weight, overweight and obesity among participants. It was found that 1/5 of PWS patients are overweight, while nearly half of them are obese.

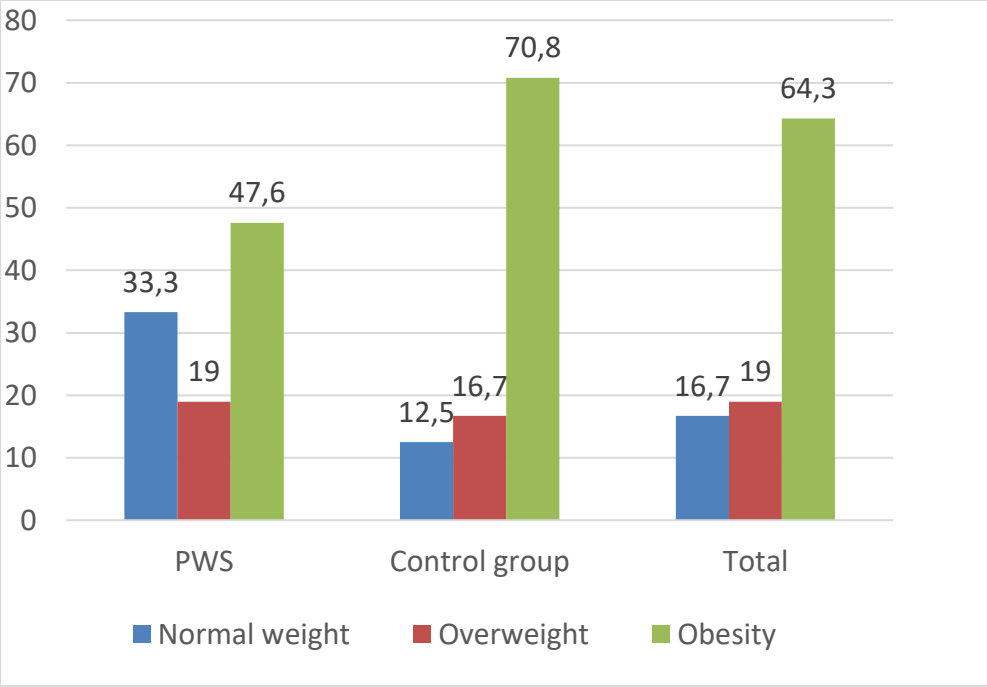

Fig. 3. Prevalence of Overweight and Obesity for all participants, group distribution (%)

Among the participants over 6 years of age, the relative proportion of abdominal obesity was also calculated. It was found that 62.7% of the PWS patients included in the analysis (n=22) and 66.7% of the corresponding controls had central obesity.

Data analysis showed significantly lower SBP and DBP values in PWS patients compared to controls ($p<0.05$), while the difference in measured HR between the two groups did not reach statistical significance.

Systolic hypertension was observed in 4.0% of PWS patients and 29.2% of the control group, while diastolic hypertension was found in 16.0% of the patients with the syndrome and 20.8% of the corresponding controls.

WC showed a good correlation with SBP for all participants (r=0.463, p=0.004). Correlations between adiposity indicators (BMI and WC), BP and HR, after controlling for age and sex in both groups, are presented in Table 3. After further controlling the analysis for BMI, only in PWS group a direct association between WC and SBP (r=0.864, p<0.001) and WC and DBP (r=0.534, p=0.033) was found. This indicates that increased abdominal fat accumulation in patients with syndromic obesity is associated with unfavorable hemodynamic changes, which is an independent predictor of developing CVD in adulthood.

Table 3. Correlation Coefficients between Obesity Indicators, Blood Pressure, and Heart Rate by Group, Controlling for Age and Sex.

	PWS				**CONTROL GROUP**			
	WC		**BMI**		**WC**		**BMI**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
SBP	0.638	**0.006**	0.140	NS	0.620	**0.022**	0.628	**0.022**
DBP	0.508	**0.037**	0.240	NS	0.263	NS	0.095	NS
HR	-0.170	NS	0.186	NS	0.376	NS	0.369	NS

NS – non-significant difference.

The findings indicate that PWS patients present with delayed pubertal development compared to healthy controls, despite there was no significant differences in sex and age between the two groups (Table 2). Nearly 2/3 of PWS patients are in the prepubertal or early stages of sexual development. Not a single patient had reached full pubertal development. Two PWS patients (14.3%) were diagnosed with central precocious puberty, which required treatment with GnRH analog. Even after discontinuation of treatment, they did not complete pubertal development.

Among all boys with PWS, 8 (53.3%) had unilateral/bilateral cryptorchidism, requiring orchiopexy, and 3 (30%) of the girls with PWS reported spontaneous or hormonally induced menarche.

All PWS patients demonstrated dysmorphic clinical features typical for the syndrome. Scoliosis is observed in 29.2% of cases. None of these characteristics were identified in any of the participants from the control group.

2. Diagnostic risk factors and family history

The prevalence of basic typical chracteristics, which are part of the diagnostic algorithm for assessing PWS patients, was analyzed in all participants. Data for both study groups are presented in Table 4.

Table 4. Diagnostic characteristics of participants.

Parameter	PWS	Control group	p
Birth weight (g)	2560±502	3084±548	**0,001**
Birth lenght (cm)	47.8±2.5	49.5±3.4	NS
Gestational age	37.6±2.8	38.0±2.2	NS
Olyiohydramnion (%)	32	0	**0.006**
Polihydramnion (%)	12	0	
Intrauterine hypotonia (%)	68	0	**<0.001**
Tube feeding (%)	60	0	**<0.001**

The results are presented as mean ± SD or percentage.

NS – non-significant difference.

A total of 48.0% of PWS group and 12.5% of corresponding controls were born SGA (p<0.001). PWS participants present with a significantly lower birth weight (p=0.001). They were, on average, 1.7 cm shorter at birth compared to controls, although the difference between the two groups for this parameter did not reach statistical significance. Analysis of the relationship between birth weight and length and the participants' current anthropometric measurements shows that only current height in the control group correlates significantly with birth anthropometric measurements (p<0.01) (Table 5).

Table 5. Correlation coefficients between current anthropometric indicators and birth measurements of participants by group, accounting for the influence of sex, age, and pubertal stage.

	PWS				CONTROL GROUP			
	Birth weight		Birth lenght		Birth weight		Birth lenght	
	r	p	r	p	r	p	r	p
Weight	-0.028	NS	0.039	NS	-0.064	NS	0.373	NS
Height	-0.072	NS	-0.099	NS	0.734	**0,007**	0.759	**0.004**
BMI	-0.047	NS	0.065	NS	-0.531	NS	-0.119	NS
WC	-0.070	NS	-0.015	NS	-0.283	NS	0.175	NS

In the PWS patient group, delivery by Cesarean section with strong indications was reported as significantly more common (64.0% vs. 26.1%, p=0.008). Notably, none of the control participants had documented cases of oligohydramnios or polyhydramnios, intrauterine hypotonia, or tube feeding requerment during the neonatal or infancy period (Table 4).

A crucial factor strongly linked to the development of cardiometabolic disorders is the presence of a family history of socially impactful diseases. This substudy assessed the proportion of participants with familial predisposition to CVD and MS among first- and/or second-degree relatives. Detailed anamnesis indicated a significantly higher incidence of CVD (84.0% vs. 33.3%, p<0.001) and MS (84.0% vs. 45.8%, p=0.001), affecting the PWS relatives compared to the ralatives of the control group.

3.Biochemical Metabolic and Hormonal Laboratory Parameters

The results concerning carbohydrate and lipid metabolism, as well as CRP levels, are detailed in Table 6.

Table 6. Biochemical parameters

Parameter	PWS	Контроли	p
BGL 0 min. (fasting) (mmol/l)	4.8±0.8	4.8±0.8	NS
BGL 120 min. (mmol/l)	6.9±1.5	6.6±1.0	NS
Insulin (mIU/L)	16.0±10.9	23.9±11.9	**0,025**
HOMA-IR	3.2±2.5	5.4±3.1	**0,012**

HbA1c (%)	5.6±0.4	5.4±0.4	NS
TC (mmol/l)	4.6±0.7	4.3±0.8	NS
TG (mmol/l)	1.1±0.5	1.1±0.6	NS
HDL-C (mmol/l)	1.3±0.3	1.2±0.2	NS
LDL-C (mmol/l)	2.8±0.7	2,6±0,7	NS
UA (µmol/l)	279.6±112.8	344.0±71.4	**0.030**
CRP (mg/l)	1.5±1.6	4.4±3.7	**0.020**

The results are presented as mean ± SD or percentage.

NS – non-significant difference.

Although no significant difference in the mean pre- and postprandial BGL was found between the two subgroups, PWS patients presented with more favorable glucose homeostasis profile, with significantly lower serum insulin levels and calculated HOMA-IR index compared to the controls ($p<0.05$).

Applying the IDF criteria for metabolic syndrome (Zimmet et al., 2007), 8 participants (16.3%) in the entire study group were found to have fasting hyperglycemia (blood glucose ≥5.6 mmol/L)—four with PWS (16.0%) and four from the control group (16.7%) ($p>0.05$), with no significant difference in mean BGL between the groups (6.0 ± 0.5 vs. 5.9 ± 0.3 mmol/L, $p>0.05$).

Data indicating IGT (BGL at 120 minute between 7.8-11.1 mmol/L) during OGTT was observed in 6 out of 36 participants (16.7%).Their characteristics are presented in Table 7. Although no significant difference was found in the tested parameters between the two subgroups, PWS patients with IGT demonstrated lower preprandial BGL, serum insulin, and calculated HOMA-IR index values ($p>0.05$).

Table 7. Characteristics and glucose metabolism parameters of participants with IGT.

Parameter	PWS	Control group	p
N (%)	3 (21.4)	3 (15.0)	NS
Sex (male/female)	2/1	1/2	NS
Age (y)	12.6±3.9	11.4±4.5	NS
BMI (kg/m^2)	27.9±7.9	26.7±4.3	NS
WC (cm)	82.8±14.4	86.5±9.2	NS
Glucose 0 min.$_{(fasting)}$(mmol/l)	5.2±0.7	5.5±0.9	NS
Glucose 120 min. (mmol/l)	9.2±0.8	8.2±0.3	NS
Insulin (mIU/L)	23.4±8.7	30.0±13.6	NS
HOMA-IR	5.6±2.7	8.1±4.4	NS
HbA1c (%)	6.1±0.6	5.7±0.2	NS

The results are presented as mean ± SD.

NS – non-significant difference.

The partial correlation analysis, adjusted for the effects of sex, age, and pubertal stage, reveals a positive correlation between WC and specific glucose homeostasis parameters only in PWS group (Table 8). Additionally, these patients demonstrate a very strong correlation between serum insulin and birth weight (r=0.971, p=0.001), birth length (r=0.906, p=0.013), as well as family history of metabolic syndrome (r=0.843, p=0.035).

Table 8. Correlation coefficients between anthropometric indicators and glucose homeostasis parameters.

	PWS				CONTROL GROUP			
	WC		BMI		WC		BMI	
	r	p	r	p	r	p	r	
ин.	-0.038	NS	-0.127	NS	0.089	NS	0.714	
мин.	-0.288	NS	-0.176	NS	-0.024	NS	0.567	
ин	0.796	**0.006**	0.588	NS	-0.134	NS	-0.651	
-IR	0.697	**0.025**	0.468	NS	-0.081	NS	0.121	

NS – non-significant difference.

No significant difference was found in the serum levels of TC, TG, HDL-C, and LDL-C between participants in the two subgroups (p>0.05) (Table 6).

In the entire study group, 6 participants (12.2%) were identified with fasting TG levels above 1.7 mmol/l, representing 12.0% (n=3) of all PWS patients and 12.5% (n=3) of all controls. The characteristics of these individuals are presented in Table 9. Although no significant differences were found in their distribution by sex, age, and adiposity parameters, PWS patients with "elevated TG" presented with significantly higher levels of cardioprotective HDL-C compared to the corresponding control participants (p=0.041).

Table 9. Lipid Metabolism Indicators of Participants with TG above 1.7 mmol/l.

Parameter	PWS	Control group	p
Sex (male/female)	1/2	2/1	NS
Age (y)	11.3±9.6	11.4±6.6	NS
BMI (kg/m^2)	27.1±11.3	29.2±0.8	NS
WC (cm)	96.5±7.6	89.5±14.2	NS
TC (mmol/l)	4.6±0.9	4.4±1.3	NS
TG (mmol/l)	2.4±0.4	2.2±0.5	NS
HDL-C (mmol/l)	1.5±0.3	0.9±0.04	**0.041**
LDL-C (mmol/l)	2.2±1.1	2.5±1.2	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

Among the participants, 9 individuals (18.4%) presented with HDL-C levels below 1.03/1.1 mmol/l (according to age), accounting for 12.0% of syndromic patients and 25.0% of controls (Table 10). Analysis showed that PWS patients with low "good" cholesterol levels had significantly lower serum TG concentrations (p=0.024) compared to controls, despite the absence of significant differences in BMI and WC.

Table 10. Lipid Metabolism Indicators of Participants with HDL-C Below 1.03 mmol/l.

Parameter	PWS	Control group	p
Sex (male/female)	0/2	4/2	NS
Age (y)	14.0±16.8	10.2±5.0	NS
BMI (kg/m^2)	30.0±29.4	31.2±2.9	NS

WC (cm)	97.5±57.3	93.8±12.4	NS
TC (mmol/l)	3.9±0.7	4.1±0.9	NS
TG (mmol/l)	1.1±0.3	1.8±0.5	**0.024**
HDL-C (mmol/l)	0.7±0.3	0.9±0.04	NS
LDL-C (mmol/l)	2.4±0.4	2.4±0.9	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

In Table 11, the correlation relationships between lipid metabolism parameters and adiposity are presented, accounting for the effects of sex, age, and pubertal development stage among participants from both subgroups. A significant positive correlation between WC, respectively BMI, and LDL-C ($p<0.05$) is demonstrated only in the PWS group.

Table 11. Partial Correlation analysis between adiposity and lipid metabolism parameters.

	PWS				**CONTROL GROUP**			
	WC		**BMI**		**WC**		**BMI**	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
TC	0.622	NS	0.662	**0.050**	0.089	NS	0.714	NS
TG	0.205	NS	-0.048	NS	-0.024	NS	0.567	NS
HDL-C	-0.522	NS	-0.380	NS	-0.134	NS	-0.651	NS
LDL-C	0.698	**0.037**	0.772	**0.015**	-0.081	NS	0.121	NS

NS – non-significant difference.

In a detailed subgroup analysis of the correlation coefficients among lipid profile markers, glucose homeostasis parameters, and blood pressure, a significant inverse association was found between HDL-C and serum insulin levels ($r = -0.537$, $p = 0.026$), as well as with the HOMA-IR index ($r = -0.477$, $p = 0.05$) only in the PWS group.

Serum UA concentration was found to be significantly lower in PWS patients (Table 6). According to more detailed anlysis in PWS subgroup,

UA demosntrated strong correlations with WC (r = 0.735, p = 0.002), BMI (r = 0.597, p = 0.019), SBP (r = 0.545, p = 0.036), serum insulin (r = 0.571, p = 0.026), HOMA-IR (r = 0.642, p = 0.010), and LDL-C (r = 0.522, p = 0.046). In contrast, within the control group, a significant correlation was observed only between UA and BMI (r = 0.763, p = 0.010).

To assess low-grade systemic inflammatory activity, serum CRP concentration was measured. It was significantly lower in PWS patients (p = 0.020), showing a direct correlation with WC (r = 0.600, p = 0.023), BMI (r = 0.560, p = 0.037), and serum TG (r = 0.589, p = 0.027).

Hormonal parameters (cortisol, IGF-1 (level/SDS), IGF BP3, and SHBG) are summarized in Table 12. Significantly elevated serum SHBG levels were identified in PWS patients (p = 0.011), which isin concordance with their favorable metabolic profile in comparison with the control subgroup. No evidence of adrenal dysfunction or substantial differences in IGF-1 and IGF BP3 levels were noted. However, the PWS cohort demonstrated a higher SDS IGF-1 value, potentially attributable to the fact that 88% were receiving rhGH therapy at the time of evaluation, as well as due to their increased body mass.

Table 12. Hormonal Analysis of Participants by Group.

Parameter	PWS	Control group	p
Cortisol (nmol/l)	350.7±188.9	379.2±203.8	NS
IGF-1 (ng/ml)	223.8±166.2	239.2±110.9	NS
IGF-1 SDS	0.6±2.6	-0.3±1.4	NS
IGF-BP3 (ug/ml)	5.0±2.0	6.3±2.0	NS
SHBG (nmol/l)	55.1±41.0	28.9±21.0	**0,006**
HMW Adiponectin (ug/ml)	2.1±1.3	2.4±1.2	NS
Leptin (ng/ml)	22.6±1.4	28.5±16.6	NS
Irsin (ug/ml)	7.0±2.9	7.8±2.2	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

When comparing serum levels of certain specific hormones (adipokines and adipo-myokines) between PWS patients and matched controls, the PWS group displayed lower concentrations, although the differences did not reach statistical significance (Table 12).

The correlation coefficients between selected hormonal, metabolic, and auxological parameters for the PWS group, adjusted for sex, age, and pubertal stage, are presented in Table 13.

Table 13. Relationships between hormonal, auxological, and metabolic parameters in PWS patients.

		IGF-1	SHBG	Adiponectin	Leptin	Irisin
BMI	***r***	0.665	-0.694	-0.939	0.700	-0.189
	p	**0.013**	**0.009**	NS	**0.008**	NS
WC	***r***	0.774	-0.772	-0.998	0.730	-0.121
	p	**0.002**	**0.002**	**0.044**	**0.005**	NS
SBP	***r***	**0.701**	-0.729	-0.999	0.224	0.084
	p	**0.008**	**0.005**	**0.026**	NS	NS
DBP	***r***	**0.613**	-0,596	-0.812	0.199	-0.106
	p	**0.026**	**0.032**	NS	NS	NS
BGL	***r***	-0.065	-0.252	-0.855	0.406	0.232
	p	NS	NS	**0.019**	NS	NS
insulin	***r***	0,558	-0.354	-0.150	0.394	-0.301
	p	**0,047**	NS	NS	NS	NS
HOMA-IR	***r***	0.622	-0.608	-0,366	0.487	-0.136
	p	**0.014**	**0.028**	NS	NS	NS
TG	***r***	0.075	0.134	-0.523	0.274	-0.597
	p	NS	NS	NS	NS	**0.031**
HDL-C	***r***	-0.251	-0.037	-0.082	0.155	-0.009
	p	NS	NS	NS	NS	NS
LDL-C	***r***	0.375	-0.262	0.993	0.115	-0.321
	p	NS	NS	NS	NS	NS
UA	***r***	0.657	-0.596	-0,943	0,494	-0.054
	p	**0.015**	**0.032**	NS	NS	NS
SHBG	***r***	-0.750	-	0,959	-0,426	-
	p	**0.003**	-	**0,041**	NS	-

Adiponectin	***r***	-0.690	-	-	-	-
	p	NS	-	-	-	-
Leptin	***r***	0.610	-0.426	-0.931	-	0.198
	p	**0.027**	NS	NS	-	NS
Irisin	***r***	0.138	-0.236	-0.764	-	-
	p	NS	NS	NS	-	-

In the PWS patient group, a strong positive correlation was observed between adiposity indicators (BMI and WC) and the concentrations of IGF-1 and leptin, while their associations with adiponectin and SHBG were significant but inversely correlated ($p<0.05$). Adiponectin showed a significant negative correlation with fasting BGL and SBP, whereas IGF-1 and SHBG levels were significantly associated with the presence of insulin resistance. The analyzed irisin demonstrated a significant negative correlation only with serum TG levels ($r = -0.597$, $p = 0.031$).

A partial correlation analysis in the control group, adjusted for sex, age, and pubertal stage, was not presented due to the substantial reduction in the number of controls after required selection. As a result, the correlations are artificially strengthened and become unreliable.

4. Body Composition Parameters and Fat/Lean Mass Distribution

As part of the protocol, body composition, FM/LM distribution, and BMD were analyzed in 32 participants (65.3%) through whole-body DXA scanning. Group-specific data are presented in Table 14. Results demosntrated a significantly lower bone density in PWS patients despite ongoing rhGH therapy, as well as lower height-adjusted LM (LM/height) ($p<0.05$). No significant differences were observed between the two subgroups for the remaining parameters.

Table 14. Body Composition of paticipants.

Parameter	**PWS (n=17)**	**Control group (n=15)**	**p**
BMD (g)	26889±13260	33913±8209	NS
FM (g)	28580±21802	30985±12767	NS

FM (%)	48.1±10.1	49.1±4.7	NS
LM/height	56.7±35.1	82.1±12.9	**0,022**
android/gynoid FM (%)	0.9±0.3	1.1±0.2	NS
LM (g)	26889±13260	33913±8209	NS
FM (g)	28580±21802	30985±12767	NS

The results are presented as mean ± SD.

NS – non-significant difference

BMD, bone mineral density; LM, lean mass; FM, fat mass

Partial correlation analysis was performed in the PWS group, after adjustment for sex, age, and pubertal stage and revealed a significant association between LM/height and BMD z-score ($r = 0.816$, $p = 0.025$). FM and %FM showed significant correlations with leptin concentration ($r = 0.985$, $p = 0.002$ and $r = 0.965$, $p = 0.008$, respectively) and UA ($r = 0.934$, $p = 0.020$ and $r = 0.903$, $p = 0.036$, respectively). In linear regression analysis accounting for factors such as age, sex, pubertal stage, BMI, WC, FM, %FM, LM, and LM/height, only %FM demonstrated predictive significance for leptin levels ($B = 0.889$ (95% CI 1.3–3.1), $p < 0.001$). No significant associations were found between body composition compartments and metabolic or auxological parameters.

Applying the same analysis in the control group a significant positive correlation of BMI with FM ($r = 0.723$, $p = 0.043$), %FM ($r = 0.958$, $p < 0.001$), and LM ($r = 0.712$, $p = 0.047$) was presented, while WC was significantly associated with %FM ($r = 0.885$, $p = 0.003$). In the same group, %FM showed an inverse relationship with HDL-C ($r = -0.846$, p

= 0.017) and a positive association with UA concentration (r = 0.860, p = 0.013) and leptin concentration (r = 0.950, p = 0.004).

4. Quality of Life (QoL) Assessment for PWS Patients

Analysis of the QoL questionnaire showed an average total QoL score of 69.0 ± 16.6 (range: 21.0–92.0), with a median (50th percentile) score of 72.0 points. Based on this result, the PWS participants were divided into two groups: the first group with a low QoL score (< 72.0 points) and the second group with a high QoL score (≥ 72.0 points) (Figure 4), which were subsequently analyzed comparatively (Table 15).

It was observed that participants with a high QoL score had lower WC, insulin, HOMA-IR, and TG levels compared to the low-score group, although the differences did not reach statistical significance (p > 0.05).

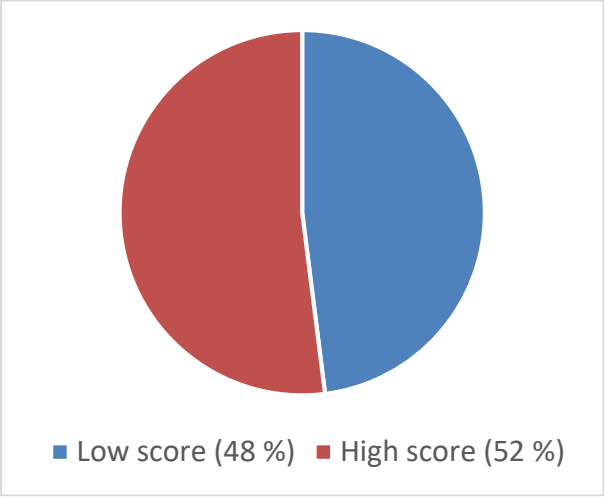

Figure 4. Distribution of PWS Participants by QoL (%).

Table 15. Laboratory and body composition indicators in PWS patients, presented by QoL scoring

Показател	PWS low score (n=12)	PWS high score (n=13)	p
Sex (f/m)	8/4	8/5	NS
Age (y)	10.4±9.1	12.1±7.8	NS
QoL score	56.6±15.0	80.4±7.2	**<0.001**
Wight (kg)	48.8±38.9	55.9±35.4	NS
Height (cm)	124.5±36.8	134.9±30.1	NS
BMI (kg/m^2)	25.6±14.1	25.5±11.7	NS
WC (cm)	79.7±29.7	77.3±24.5	NS
SBP (mmHg)	96.5±15.0	108.1±10.2	0.019
DBP (mmHg)	69.3±6.3	75.7±10.4	NS
HR (уд./мин.)	100.4±14.0	87.3±16.6	**0.050**
BG (mmol/l)	4.8±1.0	4.8±0.7	NS
Insulin (mIU/L)	18.4±13.1	13.6±8.0	NS
HOMA-IR	3.8±3.0	2.7±1.8	NS
TG (mmol/l)	1.2±0.6	1.0±0.5	NS
HDL-C (mmol/l)	1.3±0.3	1.4±0.4	NS
UA (µmol/l)	255.2±101.0	301.8±123.0	NS
IGF-1 (ng/ml)	230.0±210.6	218.2±120.5	NS
IGF-1 SDS	0.9±3.4	0.4±1.9	NS
SHBG (nmol/l)	66.5±52.2	43.7±22.8	NS
HMW Adiponectin (ug/ml	2.2±1.9	2.0±0.8	NS
Leptin (ng/ml)	22.4±19.6	22.7±23.5	NS
Irsin (ug/ml)	7.2±2.6	6.8±3.3	NS
BMD	0.80±0.29	0.82±0.29	NS
BMD z-score	0.3±1.1	0.2±0.6	NS
LM (g)	27901±14223	25990±13142	NS
FM (g)	29551±23435	27717±21641	NS

FM (%)	51.1±8.6	45.5±11.1	NS
LM/height	67.6±38.6	48.9±33.1	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

In a correlation analysis conducted on the entire PWS group, with adjustments for sex and age, no significant associations were observed between the calculated QoL score and the corresponding anthropometric, laboratory, and body composition indicators ($p > 0.05$). However, the analysis in the group of participants with high QoL score, a significant direct association was identified between the QoL score and IGF-1 SDS ($r = 0.662$, $p = 0.05$).

V.B Cross-Sectional Case-Control Substudy Comparing SRS Patients with Matched Healthy Controls by Sex, Age, and BMI

1. Demographic, auxological and clinical characteristics of the participants

This substudy included a total of 33 patients and healthy controls (11 boys) who participated after providing informed consent, with a mean age of 8.7 ± 4.9 years (range: 1.0–17.8 years). No significant differences in sex and age distribution were found between the two groups (Table 16).

Table 16. Demographic Characteristics of Participants.

Parameter	SRS (n=18)	Control group (n=15)	p
Пол N (%)			NS
Male	7 (38.9)	4 (26.7)	
Female	11 (61.1)	11 (73.3)	
Age (y)	8.5±4.7	9.0±5.3	NS

The results are presented as mean ± SD and absolute count.
NS – non-significant difference.

A genetic analysis and clinical evaluation according to Netchine-Harbison Clinical Scoring System (NH-CSS) were conducted for all participants in the SRS group to confirm the diagnosis of the syndrome. Genetic diagnosis was confirmed in 50% of these participants (n=9), with the distribution of identified genetic variants shown in Figure 5.

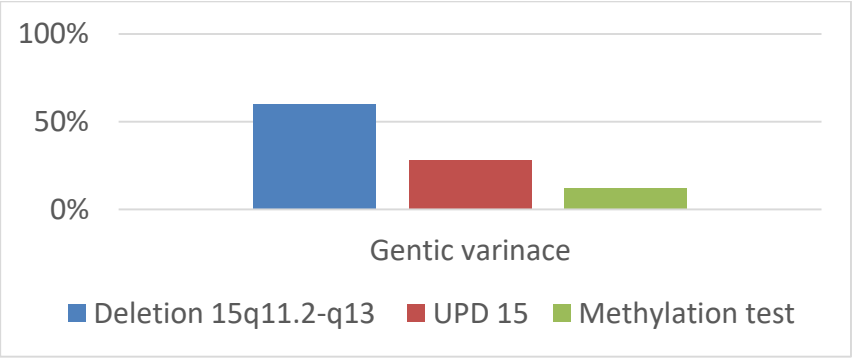

Figure 5. Genetic Variants in SRS Patients (%).

In SRS patients with a negative genetic result (n=9), the diagnosis was established solely on the basis of specific anamnestic and clinical features (clinical diagnosis), fulfilling ≥4 out of 6 criteria in the NH-CSS. Figure 6 shows the distribution of SRS patients according to NH-CSS, with half of the patients fully meeting the clinical criteria for diagnosis.

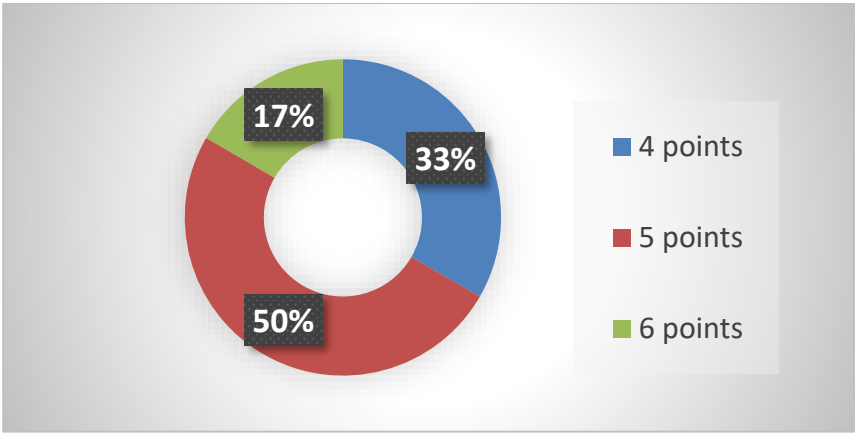

Figure 6. Distribution of Patients (%) According to Clinical Evaluation by NH-CSS.

The mean age at the start of rhGH therapy was 4.68 ± 3.3 years (range: 2.16–12.08), with 94.4% of patients receiving this treatment. The average dosage was 0.038 ± 0.009 mg/kg/day (range: 0.026–0.061).

All participants in this substudy underwent standard auxological measurements, a comprehensive clinical evaluation including SBP, DBP, and HR measurements according to protocol, and determination of pubertal stage according to the Tanner scale. Data for SRS participants and their matched healthy controls are presented in Table 17.

Table 17. Anthropometric, clinical, and pubertal assessment of the participants.

Parameter	SRS (n=18)	Control group (n=15)	p
Weight (kg)	21.3±9.8	28.1±17.4	NS
Height (cm)	**117.2±23.1**	**127.4±33.2**	NS

ИТМ (kg/cm^2)	**14.5±2.1**	**15.7±2.7**	NS
WC (cm)	**51.0±8.8**	**54.4±11.7**	NS
SBP (mmHg)	**96.1±13.8**	**103.3±13.0**	NS
DBP (mmHg)	**68.2±11.9**	**67.8±9.6**	NS
HR (уд./мин.)	**104.9±13.1**	**92.8±16.6**	**0.039**
Tanner staging N (%)			
1 st.	10 (55.6)	8 (57.1)	**0.046**
2 st.	3 (16.7)	0 (0)	
3 st.	0 (0)	2 (14.3)	
4 st.	4 (22.2)	2 (14.3)	
5 st.	1 (5.6)	2 (14.3)	

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

Although SRS patients presented with lower average weight and height compared to age-matched controls at the time of assessment, no statistically significant differences were identified in anthropometric parameters between the two groups. BMI and WC did not exhibit differences reaching significance ($p > 0.05$).

A partial correlation analysis, adjusted for sex and age, revealed a very strong direct correlation between body weight, BMI, and WC in both the SRS group and the control group (Table 18).

Table 18. Correlation between auxological parameters in SRS patients and healthy controls

		SRS		**Control group**	
		BMI	**WC**	**BMI**	**WC**
Weight	***r***	0.826	0.616	0.979	0.923
	p	**0.002**	**0.044**	**0.004**	**0.025**
WC	***r***	0.613	-	0.962	-
	p	**0.045**	-	**0.009**	-

Applying the selected methodology, none of the participants presented with overweight or obesity. Additionally, among participants older than 6 years, no cases of abdominal obesity were observed according to the specified criteria.

SRS patients showed a significantly higher prevalence of 2nd and 3rd degree underweight compared to healthy controls (p=0.035, Pearson Chi-square test) (Figure 7).

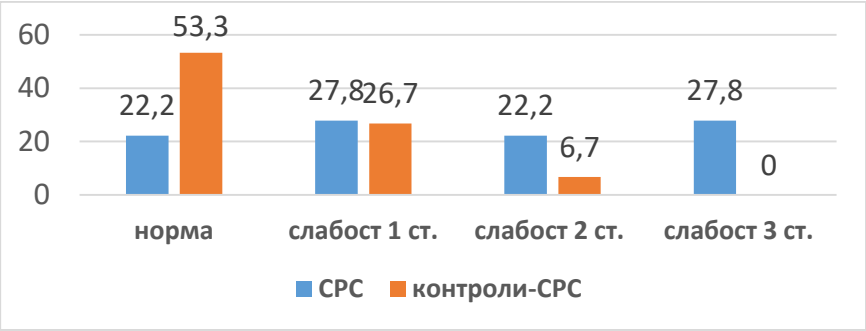

Figure 7. Distribution of participants by degree of underweight according to Cole (%).

Data analysis revealed no significant difference in SBP and DBP values between SRS patients and healthy controls ($p > 0.05$), whereas the difference in measured HR between the two groups reached statistical significance, favoring SRS patients ($p = 0.039$). Applying the selected criteria (Lurbe et al., 2016), 15.4% of SRS patients and 0% of controls were diagnosed with systolic hypertension, while diastolic hypertension was identified in 23.1% of syndromic patients and 10% of the control group.

According to bivariate analysis among all the participants, a strong positive correlation was observed between WC and SBP ($r = 0.603$, $p = 0.005$) as well as DBP ($r = 0.490$, $p = 0.028$). BMI showed a significant

inverse correlation with HR (r = -0.454, p = 0.013) and a positive correlation with SBP values (r = 0.532, p = 0.009).

Correlation coefficients between adiposity parameters, BP and HR, adjusted for age and sex, are presented in Table 19. Among syndromic patients, an inverse association was observed between HR and height (r = -0.676, p = 0.022).

Table 19. Correlation coefficients between adiposity, BP and HR parameters, adjusted for sex and age.

	SRS				CONTROL GROUP			
	WC		BMI		WC		BMI	
	r	p	r	p	r	p	r	p
SBP	0.295	NS	0.224	NS	-0.562	NS	-0.660	NS
DBP	0.180	NS	0.170	NS	-0.158	NS	-0.152	NS
HR	0.087	NS	0.104	NS	0.550	NS	0.680	NS

Approximately two-thirds of SRS patients are in the prepubertal or early stages of sexual development, while one-third are nearly or fully post-pubertal. Notably, early puberty considering their physical characteristics was confirmed in five of the syndromic patients (27.8%), requiring treatment with a GnRH analog in 80% of these cases. The mean age of onset of early pubertal signs (n=5) was 5.08 ± 5.3 years (range: 1.33–10.25 years), with all affected individuals being female. The youngest patient presented with isolated thelarche at 1.33 years, which was monitored without intervention, being the only one not treated with a GnRH analog. Excluding this patient, the adjusted mean age of onset was 8.62 ± 0.29 years (range: 6.41–10.25 years).

Among all boys with SRS, 28.6% were diagnosed with unilateral or bilateral cryptorchidism, requiring orchiopexy. Spontaneous menarche was reported in three girls with the syndrome, occurring at a mean age of 13.42 ± 0.58 years (range: 13–14 years).

Table 20 presents the frequency of additional dysmorphic clinical features observed only in the SRS patient group. None of these clinical characteristics were identified in any of the participants from the control group.

Table 20. Additional clinical characteristics of the participants.

Parameter	SRS (n=18)	Control group (n=15)	p
Micrognatia (n/%)	18/100.0	0	**<0.001**
Low muscle mass (n/%)	18/100.0	0	**<0.001**
Syndactyly (n/%)	3/16.7	0	**<0.001**
Clindactyly (n/%)	16/88.9	0	**<0.001**
Shoulder dimples (n/%)	2/11.1	0	**<0.001**
Prominent heels (n/%)	3/16.7	0	**<0.001**
Scoliosis (n/%)	4/22.2	0	**<0.001**
Autism/ADSD (n/%)	4/22.2	0	**<0.001**

The results are presented as absolute number (%).

1. Diagnostic risk factors and family history

The frequency of key risk factors, which are part of the diagnostic algorithm (NH-CSS) for evaluating SRS patients, was analyzed for all participants. Data for the two study groups are presented in Table 21.

Table 21. Diagnostic risk factors in the participants

Paramter	SRS	Control group	p
Birth weight (g)	1983±528	2735±921	**0.006**
Birth lenght (cm)	43.4±3.3	47.6±5.5	**0.013**
Head circumferance (cm)	33.0±3.7	32.0±4.4	NS
Relative macrocepahaly (%)	55.6	0	**<0.001**
Gestational age	37.1±2.4	37.3±3.7	NS
IVF (%)	16.7	0	NS
Oligohydramnios/ Polyhydramnios (%)	5.6	0	NS

IUGR (%)	72.2	6.7	**<0.001**
Intrauterine hypotonia (%)	22.2	0	NS
Delivery (%)			NS
Normal	22.2	46.7	
C-section	77.8	53.3	
Tube feeding (%)	11.1	0	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

A total of 94.4% of SRS patients were born small SGA, compared to 0% of the corresponding controls ($p<0.001$). SRS patients presented with significantly lower birth weight and length, with relative macrocephaly observed in over 55% of patients ($p<0.05$). Nearly ¾ of syndrome participants had been diagnosed with IUGR during pregnancy ($p<0.001$), and about ¼ of the mothers reported decresed fetal movement.

According to analysis between birth weight and length and current anthropometric measures, only in SRS patients the weight, BMI, and WC significantly correlated with birth anthropometric measurements ($p<0.05$) (Table 22). Data for the control group are not shown due to the substantial reduction in sample size following additional adjustments, which leads to introducing bias in the results.

Table 22. Correlation coefficients between current anthropometric parameters and birth measurements of SRS participants, adjusted for sex, age, and pubertal stage..

	CPC			
	Birthweight		**Birthlenght**	
	r	**P**	**r**	**p**
Weight	0.751	**0.002**	0.809	**<0.001**
Height	0.395	NS	0.442	NS
BMI	0.753	**0.002**	0.765	**0.001**

WC	0.651	**0.012**	0.566	**0.035**

Although not statistically significant, the SRS patient group reported a higher frequency of operative delivery when necessary, as well as occurrences of oligohydramnios or polyhydramnios and nasogastric tube feeding during the neonatal or infancy period (Table 22).

The presence of family history with cardiovascular disease (CVD) and metabolic syndrome (MS) as risk factors for the development of cardiometabolic disorders was analyzed separately for each group in this substudy. Findings indicate that the prevalence of family history with CVD was lower among the families of SRS patients compared to controls (6.3% vs. 20%), while family history with MS was more frequent among syndromic patients, although not statistically significant (8.3% vs. 6.7%) ($p > 0.05$).

2. Biochemical Metabolic and Hormonal Laboratory Indicators

For all participants in the substudy, glucose and lipid metabolism parameters, uric acid, and CRP were assessed, and the results are presented in Table 23.

Table 23. Biochemical Parameters.

Parameter	SRS	Control group	p
BGL 0 min. (fasting) (mmol/l)	4.3±1.0	4.5±1.0	NS
Insulin (mIU/L)	14.8±16.3	6.8±6.4	NS
HOMA-IR	3.3±4.3	1.6±1.4	NS
HbA1c (%)	5.3±0.4	5.4±0.4	NS
TC (mmol/l)	3.9±0.3	4.2±0.7	NS
TG (mmol/l)	0.8±0.3	0.6±0.2	NS
HDL-C (mmol/l)	1.5±0.6	1.5±0.1	NS
LDL-C (mmol/l)	1.7±0.6	2.4±0.5	**0.011**

UA (µmol/l)	258.2±40.3	223.9±29.1	**0.030**
CRP (mg/l)	2.0±4.1	0.6±0.1	NS

The results are presented as mean ± SD and absolute count.
NS – non-significant difference.

Although no significant difference was found in the mean values of preprandial BGL, insulin, and calculated HOMA-IR index between the two subgroups, SRS patients exhibited an impaired glucose homeostasis profile, with higher serum insulin concentrations and HOMA-IR index values compared to the respective controls ($p > 0.05$).
Applying the IDF criteria for metabolic syndrome (MS) across the entire study group (n=33), fasting hyperglycemia (BG ≥5.6 mmol/L) was observed in only one participant, who belonged to the SRS patient group (5.6%). No instances of impaired glucose tolerance (IGT) or diabetic BGL were found in any participants who consented to perform OGTT.
Partial correlation analysis, adjusted for sex, age, and pubertal stage, demonstrated a positive association between WC, BMI, and certain glucose homeostasis parameters exclusively in SRS patients (Table 24). In this group, a strong inverse correlation was also observed between fasting BGL and serum insulin with birth weight ($r = -0.645$, $p = 0.032$ for BG and $r = -0.782$, $p = 0.004$ for insulin, respectively) and birth length ($r = -0.621$, $p = 0.042$ for BG and $r = -0.604$, $p = 0.049$ for insulin, respectively). Data for the control group were not shown, for the same reasons stated previously.

Table 24. Correlation coefficients between anthropometric indicators and glucose homeostasis parameters in SRS patients.

	SRS			
	WC		**BMI**	
	r	**p**	**r**	**p**
BGL 0 min. (fasting)	0.366	NS	0.374	NS
Insulin	0.508	**0.05**	0.666	**0.007**
HOMA-IR	0.565	**0.028**	0.714	**0.003**

A comparative analysis of lipid metabolism parameters between participants in the two subgroups revealed a significant difference

only in LDL-C levels, with higher values observed in healthy controls (p = 0.011) (Table 24).

According to IDF criteria for dyslipidemia, no participants in the study had fasting TG levels exceeding 1.7 mmol/L. However, 9.1% of the 33 total participants, specifically from the confirmed SRS patient group (16.7%), presented with HDL-C levels below the age-specific threshold of 1.03/1.1 mmol/L. Notably, SRS patients with low HDL-C were younger and showed elevated LDL-C levels compared to those with normal HDL-C concentrations, although these observed differences did not reach statistical significance (Table 25).

Table 25. Lipid metabolism parameters in SRS participants by HDL-C Levels.

Parameter	SRS with low HDL-C	SRS with normal HDL-C	p
Sex (male/female)	1/2	6/9	NS
Age (years)	5.8±3.9	9.0±4.8	NS
BMI (kg/m^2)	13.1±0.9	14.8±2.2	NS
WC (cm)	47.0±7.8	51.8±9.0	NS
TC (mmol/l)	3.9±0.2	3.9±0.4	NS
TG (mmol/l)	0.7±0.1	0.8±0.4	NS
HDL-C (mmol/l)	0.4±0.2	1.7±0.3	NS
LDL-C (mmol/l)	2.1±0.3	1.6±0.7	NS

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

No significant correlation was identified between lipid metabolism indicators and adiposity parameters in participants from both subgroups, even after adjusting for sex, age, and pubertal stage (Table 26).

Table 26. Partial correlation between adiposity and lipid metabolism indicators in SRS patients.

	CPC			
	WC	**BMI**	**WC**	**BMI**
	r	**p**	**r**	**p**
TC	-0.056	NS	-0.056	NS
TG	0.012	NS	0.012	NS
HDL-C	0.294	NS	0.294	NS
LDL-C	-0,080	NS	-0.080	NS

In a further assessment of the correlation coefficients between lipid metabolism indicators, glucose homeostasis, and blood pressure, a significant positive association was observed in the SRS group between TG and serum insulin levels ($r = 0.638$, $p = 0.019$), as well as with the HOMA-IR index ($r = 0.636$, $p = 0.019$). Due to severe reducing the number of the participants in the control group after adjustments for sex, age, and pubertal stage, data were not shown because of introducing bias in the results.

Serum UA level was analyzed as an parameter of adverse metabolic profile, with a significantly higher mean value observed in the SRS group (Table 23). In this subgroup, UA was directly correlated with TC level ($r = 0.559$, $p = 0.047$).

Serum CRP concentration, as a marker of low-grade inflammatory activity and cardiovascular risk, was evaluated in all participants in this substudy. Although mean CRP levels were higher in SRS patients, the difference compared to the control group did not reach statistical significance ($p > 0.05$) (Table 23). Partial correlation analysis, adjusted for sex, age, and pubertal stage, demonstrated that CRP in SRS participants was significantly correlated with BMI ($r = 0.782$, $p = 0.001$), WC ($r = 0.645$, $p = 0.013$), serum insulin ($r = 0.904$, $p < 0.001$), and HOMA-IR ($r = 0.942$, $p < 0.001$).

Hormonal parameters, including cortisol, IGF-1 (absolute value/SDS), IGFBP3, and SHBG, were analyzed in all participants (Table 27). Significantly higher serum levels of IGF-1 and IGF-1 SDS were observed in SRS patients ($p < 0.05$), likely attributable to the fact that 94,4% of these patients were receiving rhGH therapy at the time of laboratory evaluation.

Table 27. Hormonal Analysis of Participants by Group.

Parameter	SRS	Control group	p
Cortisol (nmol/l)	398.3±169.1	412.8±232.1	NS
IGF-1 (ng/ml)	235.2±132.0	144.8±94.4	**0.034**
IGF-1 SDS	0.3±2.8	-0.9±0.9	**0.006**
IGF-BP3 (ug/ml)	5.7±1.9	4.8±1.5	NS
SHBG (nmol/l)	86.6±34.0	89.1±36.2	NS
HMW Adiponectin (ug/ml)	2.4±0.5	2.8±0.7	NS
Leptin (ng/ml)	3.0±4.8	3.4±4.3	NS
Irisin (ug/ml)	9.5±1.3	8.1±1.0	**0.001**

The results are presented as mean ± SD and absolute count.

NS – non-significant difference.

A significantly higher concentration of the myoadipokine irisin was presented in the SRS group, while serum levels of the other two specific adipokines (leptin and adiponectin) did not differ significantly from the values measured in the corresponding controls (Table 27).

Tables 28 and 29 present the correlation coefficients between selected hormonal, metabolic, and auxological indicators for the two subgroups in this substudy, adjusted for sex, age, and pubertal stage.

A strong positive correlation was observed between adiposity indicators (BMI and WC) and leptin concentration in the SRS group. Their association with SHBG was significant but inversely related ($p < 0.05$). Leptin levels were significantly correlated with HOMA-IR, insulin, CRP, and TG, while SHBG demonstrated a significant inverse correlation with insulin, HOMA-IR, and DBP. Adiponectin showed a significant negative

correlation with TG, whereas the analyzed irisin did not demonstrate significant associations with anthropometric or metabolic/hormonal parameters.

Table 28. Relationships between hormonal, auxological and metabolic parameters in SRS patients.

		IGF-1	SHBG	Adiponectin	Leptin	Irisin
BMI	***r***	-0.370	-0.579	0,554	0,907	-0.258
	p	NS	**0.012**	NS	**<0,001**	NS
WC	***r***	-0.017	-0.599	0,823	0,711	-0.085
	p	NS	**0.009**	NS	**0,004**	NS
SBP	***r***	0.541	-0.380	0,451	-0,022	-0.049
	p	NS	NS	NS	NS	NS
DBP	***r***	0,376	-0.553	0,929	-0,186	-0.344
	p	NS	**0.05**	NS	NS	NS
BGL	***r***	-0.312	-0.180	-0,040	0,397	-0.032
	p	NS	NS	NS	NS	NS
insulin	***r***	-0.233	-0.555	-0.611	0.585	-0.346
	p	NS	**0.039**	NS	**0.036**	NS
HOMA-IR	***r***	-0.291	-0.546	-0.570	0.621	-0.302
	p	NS	**0.043**	NS	**0.023**	NS
TT	***r***	-0.332	-0.274	-0.997	0.587	-0.494
	p	NS	NS	**0.047**	**0.013**	NS
HDL-C	***r***	0.150	0.009	0.960	0.037	0.440
	p	NS	NS	NS	NS	NS
LDL-C	***r***	-0.004	-0.076	-0.510	0.063	-0.314
	p	NS	NS	NS	NS	NS
UA	***r***	0.490	-0.147	-	0,288	0.048
	p	NS	NS	-	NS	NS
CRP	***r***	-0.584	-0.484	0,815	0.961	-0.381
	p	NS	NS	NS	**<0.001**	NS
SHBG	***r***	0.005	-	0.483	-0.539	0.418
	p	NS	-	NS	**0.047**	NS
Adiponectin	***r***	0.761	0.483	-	-0.084	0.987
	p	NS	NS	-	NS	NS
Leptin	***r***	0.583	-0.539	-0.084	-	-0.341
	p	NS	**0.047**	NS	-	NS

Irisin	***r***	0.855	0.418	0.987	-0.341	-
	p	NS	NS	NS	NS	-

4. Body Composition Parameters and Fat/Lean Mass Distribution

According to the study protocol, body composition, FM/LM distribution, and BMD were analyzed in 20 participants using whole-body DXA scan. Group-specific data are presented in Table 29. Although no statistically significant differences were found in the measured parameters between the two groups, SRS patients showed a trend towards a lower BMD z-score, lower LM content (g), and higher FM content (g) ($p > 0.05$).

Table 29. Body Composition of Participants by Group.

Parameter	SRS (n=16)	Control group (n=4)	p
BMD	0.6±0.1	0.6±0.3	NS
BMD z-score	-1.3±0.8	-0.3±1.3	NS
LBM (g)	12461±5022	15342±11388	NS
FM (g)	6036±6171	4546±2183	NS
FM (%)	24.4±8.1	24.9±4.2	NS
LBM/height	31.5±25.3	25.3±27.9	NS
Android/Gynoid FM (%)	0.6±0.2	0.5±0.1	NS

The results are presented as mean ± SD.
NS – non-significant difference.

Partial correlation analysis (adjusted for sex, age, and pubertal stage) performed for the SRS group demonstrated that FM was significantly correlated with BMI ($r = 0.980$, $p = 0.020$), insulin ($r = 0.935$, $p = 0.002$), HOMA-IR ($r = 0.932$, $p = 0.002$), SHBG ($r = -0.809$, $p = 0.027$), and leptin ($r = 0.947$, $p = 0.001$). Additionally, %FM was significantly associated with insulin ($r = 0.896$, $p = 0.006$), HOMA-IR ($r = 0.888$, $p = 0.008$), CRP ($r = 0.998$, $p = 0.039$), SHBG ($r = -0.847$, $p = 0.016$), and leptin ($r = 0.839$, $p = 0.018$).

5. Quality of Life (QoL) assessment in SRS patients.

A specialized questionnaire was administered to all participants in the syndromic group. Data analysis demonstrated that the mean total QoL score for the entire group was 74.3 ± 2.6 (range: 53.0–88.0), with a median (50th percentile) of 79.5 points. Based on this result, participants were provisionally divided into two groups: a low QoL score group (< 79.5 points) and a high QoL score group (≥ 79.5 points) (Figure 8).

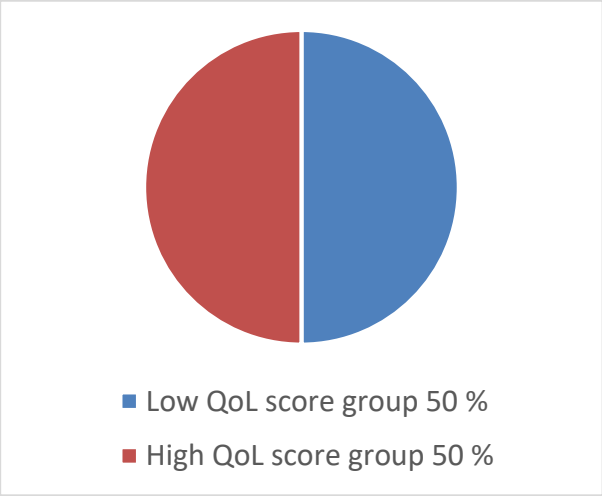

Figure 8. Distribution of SRS participants by QoL score (%).

The demographic characteristics, biochemical and hormonal tests, and body composition parameters of the two subgroups of patients, categorized by QoL score, are presented in Table 30.

Table 30. Key characteristics of SRS patients by QoL score.

Indicator	SRS Low Score QoL (n=9)	SRS High Score Qol (n=9)	p
Sex (m/f)	6/3	1/8	NS
Years (y)	7,4±4,2	9,5±5,1	NS
QoL Score	65,9±9,5	82,7±2,8	NS

Weight (kg)	19.1±8.8	23.4±10.7	NS
Height (cm)	111.8±20.0	122.6±25.8	NS
BMI (kg/m^2)	14.6±2.9	14.5±1.2	NS
WC (cm)	49.8±9.2	52.2±8.7	NS
SBP (mmHg)	92.3±13.3	100.5±14.2	NS
DBP (mmHg)	62.7±7.2	74.7±13.6	NS
HR (beats/min.)	103.9±16.5	105.7±10.2	NS
BGL (mmol/l)	4.5±1.2	4.1±0.9	NS
Insulin (mIU/L)	18.4±21.3	11.5±10.4	NS
HOMA-IR	4.4±5.9	2.4±2.3	NS
TG (mmol/l)	0.8±0.4	0.7±0.3	NS
HDL-C (mmol/l)	1.5±0.7	1.5±0.5	NS
UA (µmol/l)	275.9±33.8	233.7±39.9	NS
CRP (mg/l)	3.0±5.5	0.9±0.7	
IGF-1 (ng/ml)	205.9±127.4	264.4±137.4	NS
IGF-1 SDS	-0.4±3.7	0.9±1.2	NS
SHBG (nmol/l)	81.8±33.2	91.4±36.1	NS
HMW Adiponectin (ug/ml)	2,1±0,2	3.0±0.1	NS
Leptin (ng/ml)	4.1±6.4	1.8±1.4	NS
Irsin (ug/ml)	9.3±1.3	9.8±1.4	NS
BMD	0.6±0.1	0.6±0.2	NS
BMD z-score	-1.5±0.9	-1.2±0.8	NS
LBM (g)	11500±4134	13422±5903	NS
LBM (g)	4997±4021	7076±7934	NS
FM (%)	26.2±10.5	22.4±3.8	NS
LBM/height	25.5±30.9	35.5±23.1	NS

Results are presented as mean ± SD, absolute count, or %.

NS – non-significant difference

It was observed that participants with a high QoL score were taller and had higher IGF-1 levels, along with lower values for insulin, HOMA-IR, CRP, leptin, and %FM compared to the SRS subgroup with a low QoL score, although these differences did not reach statistical significance ($p > 0.05$).

Applying correlation analysis for the entire group of SRS patients, with adjustments for sex and age, a significant correlation was demonstrated between the calculated QoL score and both IGF-1 ($r = 0.584$, $p = 0.028$) and LM (g) ($r =$

0.832, p = 0.020). In an analysis restricted to participants with a high QoL score, a significant positive association was identified between QoL score and BMD (r = 0.929, p = 0.007), LM (g) (r = 0.891, p = 0.017), and IGF-1 (r = 0.878, p = 0.05).

SECTION V. DISCUSSION

According to the generally accepted European definition, a rare disease is considered one that has a frequency of no more than 5 per 10,000 people in the European Union (Moliner and Waligora, 2017). With the development of reference networks for rare endocrine diseases on a European and global scale, the knowledge about rare diseases is progressively increasing in the direction of improving the care of these patients and increasing their duration and quality of life. This reinforces the need to conduct similar types of studies at the national level. Prader-Willi syndrome (PWS) is a rare disease and according to the frequency quoted in the review about 80 patients should be found in Bulgaria. Due to the lack of a functional national register, their number is not clear. In the present work, 25 patients with PWS (about 1/3 of the expected number) from all over the country were examined. Silver-Russell syndrome (SRS) is also a rare disease and its frequency varies, with a molecular genetic defect identified in about 60% of cases (Wakeling et al., 2017). For this reason, the exact frequency worldwide remains unknown, and it is not possible to calculate the expected number for our country. 18 patients with SRS participated in the present work.

For the first time in Bulgaria, a study of the metabolic risk and body composition of these two groups of patients with rare diseases – PWS and SRS – is being conducted. Comparison with a control group of patients matched for sex, age, and BMI allows for the assessment of anthropometric indicators, diagnostic risk factors, cardio-metabolic and hormonal profiles in the participants. The detection of dependencies between the various indicators helps to derive an algorithm for the assessment of metabolic risk in patients with PWS and SRS. Included in this protocol is the assessment of the quality of life, which is one of the most popular concepts in modern science, not only in the field of psychology but also in the aspect of all sciences that are related to human life and its course. (Rozensztrauch and Śmigiel, 2022)

VA. Prader-Willi syndrome

Regarding the genetic distribution in this group, the highest percentage were patients who were diagnosed with deletion of chromosome 15 (60%), followed by mUPD15 (28%). A similar type of genetic distribution ratio is closer to data reported in the literature from more recent studies (Grootjen et al., 2022a). In most publications before 2015, the deletion rate has been thought to be close to 75%, possibly because of the more limited options for extensive genetic analysis to demonstrate uniparental disomy or the even rarer variants that require a targeted search. (Goldstone et al., 2008) In 12% of the patients in our group, only a methylation test was performed, on the grounds of which it was

not possible to differentiate the genetic variant. These are mostly patients over the age of 18 who were diagnosed a long time ago when genetic testing options were not widely available.

In a large-scale review, Passone et al. (2020) examined the benefits of recombinant human growth hormone (rhGH) treatment in patients with PWS. The authors concluded that in nine randomized trials (n=328) where height was monitored, treated patients reported an average increase of 1.67 SDS (in children younger than 3.5 years the difference was 1.08 SDS, and in larger it averaged 1.87 SDS). The benefits of starting therapy at 2 years of age are well established and documented, but recently there is increasing evidence for additional positives from starting treatment as early as between 6 and 12 months of age, particularly in terms of motor development, hypotonia, head circumference, and cognitive abilities (Goldstone et al., 2008). Some experts start rhGH treatment even earlier. The average age at the start of treatment with rhGH in our patient group is 5.1±5.1 years, and in the last decade, active efforts have been made to increase awareness among neonatologists about the disease, which leads to earlier diagnosis and, accordingly, early initiation of therapy. At the time of the survey, 88% of our patients with PWS were undergoing treatment with rhGH, and regardless of the lack of reimbursement and in accordance with current guidelines, most of the patients over 18 years of age continued the administration of growth hormone. The oldest patient (33 years) has never been treated, and two of the adults discontinued therapy due to a lack of reimbursement. Against this background, as expected, a significant difference was found in terms of height in favor of the controls (p=0.017).

Participants with syndromic disease demonstrated lower median values for BMI and waist circumference (WC) compared to controls, although statistical significance was not reached. The reason for this could be a greater number of participants (5 vs. 1) in the PWS group under 3 years of age who did not enter the 2b feeding phase, did not trigger hyperphagia, and were therefore not obese, as well as the ever earlier diagnosis, correspondingly early initiation of therapy and its positive impact on BMI and body composition.

Based on international age- and sex-specific Cole reference values for the 85^{th} and 95^{th} percentiles of child-adolescent BMI (for participants over 2 years of age) and the corresponding diagnostic values for adults, it was found that 1/5 of PWS patients are overweight and almost half have generalized obesity. In Bulgaria, there is a lack of previously systematically published observational data on weight control and the factors that influence this process. In comparison, a large-scale US remote study involving 165 PWS patients (mean age of 19.7 years, range of 12-48 years) found that there was minimal increase in BMI over a 6-month period (Vrana-Diaz et al., 2020). From the performed

multivariate analysis, the authors indicated time, sex, age, and duration of treatment with rhGH as statistically significant for changes in BMI.

Despite the follow-up of Bulgarian PWS patients in the Expert Center and systematic visits, weight control is still difficult to achieve, which is understandable after taking into account the factors listed above (diverse age groups, different ages of diagnosis, and, accordingly, of the beginning of treatment). This indicates the need to take additional measures, such as the use of specialized psychologists and nutritionists in the follow-up of patients, as well as longer-term monitoring. In this way, families could be better informed about the natural course of the disease in the absence of weight control and access to a structured diet and physical activity to prevent obesity.

In participants over 6 years of age, the relative share of abdominal obesity was also calculated, applying the normal rates for waist circumference for Bulgarian children and adolescents by gender and age and the criteria of the International Diabetes Federation (IDF) for those over 18 years of age. It was found that *62.7%* of the PWS patients included in the analysis and 66.7% of the matched controls had central obesity. Talebizadeh and Butler (2005) prove that adult PWS patients with more visceral adipose tissue are at higher risk of obesity-related complications compared to those with less visceral adipose tissue. In studies involving PWS patients of various age groups, excessive accumulation of visceral adipose tissue is associated with increased insulin resistance, increased serum lipids, and decreased adiponectin (ADN) levels, all markers of increased metabolic risk. Previous reports examining children, adults, as well as mixed groups of patients, have shown that obesity in PWS patients is predominantly subcutaneous and visceral adipose tissue volume is relatively low (Haqq et al., 2011), further confirming their more favorable metabolic status compared to obese controls. In our study, this trend is confirmed, although the amount of visceral fat in our participants was not measured by imaging methods, but reflected only by the anthropometric index for *WC*.

In the present study, WC showed a good correlation with systolic blood pressure (SBP) for all participants. In the patient group with PWS, the correlation analysis taking into account the role of BMI established the existence of a *significant direct relationship* between the WC and SBP and WC and diastolic blood pressure (DBP). Therefore, the more pronounced accumulation of abdominal adipose tissue in patients with syndromic obesity is associated with adverse hemodynamic changes, which are an independent predictor of cardiovascular diseases in adulthood, although as we described above, PWS is characterized by a more specific type of obesity. Such dependence has not been

found in the literature so far, and the increase in WC could serve as a *predictive marker for the development of hypertension in the syndromic group of patients*.

Data analysis showed *significantly lower values of SBP and DBP in patients with* PWS compared to healthy controls ($p<0.05$), while the difference in measured heart rate (HR) between the two groups did not reach a significance level. These results are of a confirmatory nature compared to those described so far in the literature. Brambilla et al. (2011) compared obese and non-obese children with PWS (n=109) to obese controls and found significantly lower SBP and DBP values in non-obese PWS patients compared to obese PWS patients and obese controls. They identified BMI as a predictor of metabolic complications in both groups of patients. The authors determined the incidence of arterial hypertension (BP $\geq 95^{th}$ percentile for age, sex, and height), which was again significantly lower in non-obese children with PWS (12%), compared to 32 and 35%, respectively, for the other two groups. Sinnema et al. (2011) reported an incidence of arterial hypertension (AH) in adolescents over 15 years of age and adult patients between 9 and 24%. In our patients, after applying the selected criteria, systolic hypertension was 4.0% of patients with PWS and 29.2% of the control group, while diastolic hypertension was found in 16.0% of participants with syndromic disease and 20.8% of the corresponding controls. This again confirms the more favorable metabolic profile of our patient group.

Expectedly, a delay in pubertal development was found in the study group in patients with PWS compared to healthy controls, despite the lack of a significant difference in gender and age between both groups of participants. Nearly 2/3 of patients with PWS are in the prepubertal or initial stage of sexual development, with no patient having fully completed puberty. Against this background, central precocious puberty (CPP) occurred in two of our male patients. There are reports in the literature of central precocious puberty in patients with PWS, which is a rare condition. Abreu et al. (Abreu et al., 2013) applied whole-exome sequencing to 40 individuals from 15 families (without genetic disease) with pronounced CPP. They identified four new heterozygous mutations in MKRN3 (the gene encoding makorin RING-finger protein 3, which is a paternally expressed, imprinted gene located in the critical region for PWS – chromosome 15q11-q13) in 5 of the 15 families. All affected individuals inherited the mutations from their fathers, indicating perfect segregation with the mode of inheritance expected for an imprinted gene. In 2022, in their publication, Kobayashi et al. (2022) summarized that, in addition to their presented case (a 7-year-old boy with a deletion in the 15q11.2 region), 15 more patients with CPP (7 boys and 9 girls in total) were described in the database, and it was not possible to clarify the relationship between the incidence of CPP and the genetic variant of PWS, as information on the genetic variant was provided for only five of the children. Unfortunately, to date, our patients have not been studied and we

cannot pinpoint the exact cause of their precocious puberty (PP). Unilateral/bilateral cryptorchidism was found in 53.3% of all boys with PWS, necessitating orchidopexy. According to literature data, this condition is more common – over 80% (Goldstone et al., 2008).

In the literature, scoliosis is reported in 23-40% of patients with PWS (Tauber and Hoybye, 2021). In our patient group, 29.2% reported this type of pathology, and that is against the background of a similar and even larger number of patients treated with rhGH. This finding supports the absence of a causal relationship between scoliosis and growth hormone treatment. This has been demonstrated by Grootjen et al. (2021) who examined 103 PWS patients treated long-term with rhGH (8 years) and in them also no worsening of the condition was observed.

In an analysis of the frequency of the leading risk factors that are part of the diagnostic algorithm for the assessment of patients with PWS, it was found that 48% of patients with PWS and 12.5% of matched controls were born small for their gestational age (SGA), with participants with the syndrome demonstrating significantly lower birth weight. This result correlates with the publications (Srebnik et al., 2020) in which SGA is defined as height and/or length at birth below the 10^{th} percentile, which we also used in our population. In the remaining studies that used height and/or birth length below the 2 SD (2^{nd} percentile), the frequency dropped to 20-21.1%, which also explains the wide percentage range for children with PWS born with SGA, mentioned in the Overview to the present work.

When analyzing the relationships between the birth weight and height of the participants with their current anthropometric indicators, it was found that only the current height in the control group correlated significantly with the anthropometric measurements at birth. A probable reason for the lack of correlation with height in the patient group could be the treatment with exogenous rhGH, and for weight – the influence of external factors (dietary culture and exercise habits of the family, social status, etc.).

In the group of patients with PWS, an emergency delivery by Cesarian section was significantly more often reported (64% vs. 26.1%, p=0.008), with no documented evidence of oligo- or polyhydramnios, intrauterine hypotension, or nasogastric tube feeding in the neonatal/infant age in the control group. In three other studies with a larger number of patients – Dutch (n=244), American (n=355), and Chinese (n=134), different frequencies of the remaining risk factors were derived (Grootjen et al., 2022b; Singh et al., 2018; Yang et al., 2020). Operative delivery was reported in 46, 54, and 82% of cases, respectively. Tube feeding was performed in 60% of the Bulgarian patients compared to 93.75 and 70% respectively in the Dutch, American, and Chinese patients with PWS. Regarding the presence of polyhydramnios, 12% was found in our patient group

versus 27, 18 and 57%. According to the data on olygohydramnios, its frequency in our group is the highest – 32%, against 5% in the American and 18.7% in the Chinese population with PWS. A reason for these differences could be the mother's lack of awareness or the suggestion through directed questions exercised by the researcher during history taking. Although we have not collected such data, we can also speculate that in our country the reduction of amniotic fluid did not lead to earlier delivery.

An important factor closely associated with the development of cardiometabolic disorders in the general population is the presence of a family burden of socially significant diseases. For this purpose, the present substudy assessed the relative proportion of participants burdened with cardiovascular disease (CVD) and metabolic syndrome (MS) among first- and/or second-generation relatives. On targeted questioning, there was a significantly higher incidence of familial CVD (84.0% vs. 33.3%) and MS (84.0% vs. 45.8%) in the families of patients with PWS compared with the group of controls. A French study including 39 patients with PWS aged 16.8 years (with a range of 11-24 years) found that individuals who developed early-onset type 2 diabetes (T2D) had a family history of T2D and were overweight. Parents of patients with PWS generally have a higher incidence of T2D compared to the general French population, similar to our case (in PWS the incidence of T2D was 34.5%, versus 5% in the general population). Although no other literature data on familial burden were found in this patient group, it could be concluded that a thorough history taking of this kind is necessary in order to detect early onset of T2D, which is important for timely initiation of treatment and prevention from complications.

When examining some biochemical and laboratory indicators, no significant difference in the average value of pre- and postprandial blood glucose (BG) between both subgroups was found. However, PWS patients showed a better glucose homeostasis profile with *significantly lower serum insulin concentration and calculated HOMA-IR index* compared to the corresponding controls, although no difference in body mass was found. These results are of a confirmatory nature compared to studies for the evaluation of metabolic indicators in different age groups. Haqq et al. (2011) compared 14 children with PWS to 14 obese controls (matched for sex, age, and BMI) and 14 lean children (matched for sex and age), confirming that fasting insulin and HOMA-IR in children with PWS were lower relative to obese controls and similar to lean controls. When comparing the same three groups, obese controls had 38% lower HDL-cholesterol and two-fold higher triglyceride levels than lean controls, but not in PWS patients. An Argentinian study of 75 children with PWS compared with obese controls showed lower basal insulin levels and HOMA-IR in the first group (Krochik et al., 2006). A publication involving elderly women with PWS

(n=13) revealed a similar pattern, with patients having reduced insulin resistance and milder hypertriglyceridemia compared to obese controls, after matching for age and overall adiposity (Goldstone et al., 2001). Talebizadeh and Butler (2005) confirmed these results in a mixed group of patients (n=37, aged 10.4 to 44 years).

After applying the IDF criteria for MS, 8 participants (16.3%) with fasting hyperglycemia (BG≥5.6 mmol/l) were found in the entire study group – four with PWS (16%) and four from the control group (16.7%), with no significant difference in mean BG between groups (6.0±0.5 vs. 5.9±0.3 mmol/l). In contrast to our results, Brambilla et al. (2011) reported lower preprandial BG levels in children from both groups with PWS. The study compared patients with PWS and normal weight, patients with PWS and obesity (n=109) versus obese controls. In 2011, this team of authors determined for the first time the frequency of metabolic syndrome in a representative sample of patients with PWS – 7.3%, as in none of the non-obese patients with PWS, the presence of MS was proven, in contrast to the obese PWS patients and obese controls. They present a hypothesis that obesity could play a major role in the formation of individual metabolic risk in the PWS population. The reason for the higher BG values in both patients and controls found in our study could lie in the presence of patients over 18 years of age.

There were 6 out of 36 participants (16.7%) with evidence of impaired glucose tolerance (with BG at 120th minute between 7.8-11.1 mmol/l) during the oral glucose tolerance test (OGTT). Although no significant difference was found in the studied parameters between the participants of both subgroups, patients with PWS and impaired glucose tolerance (IGT) demonstrated lower values of preprandial BG (Brambilla et al., 2011), serum insulin and calculated HOMA-IR index (Haqq et al., 2011), which is also a result of a confirmatory nature compared to the publications listed above.

Partial correlation analysis after accounting for the influence of sex, age, and stage of pubertal development showed the presence of a *direct relationship between WC (abdominal obesity) and insulin levels/HOMA-IR* only in patients with PWS, which once again sharpens our attention to WC as a metabolic risk assessment tool even in PWS patients.

Applying the IDF criteria for dyslipidemia, 6 participants (12.2%) with fasting thyroglobulin (TG) above 1.7 mmol/l were found in the entire studied group. This was 12.0% (n=3) of all PWS patients and 12.5% (n=3) of all controls. Although no significant difference was found in their distribution of sex, age, and adiposity indices, PWS patients with "elevated TG" demonstrated significantly higher values of cardioprotective HDL-C compared to the corresponding control participants. Similar results in PWS patients were reported by Haqq et al. (2011). With HDL-C below 1.03/1.1 mmol/l (according

to age) were 9 participants (18.4%), 12.0% of patients with the syndrome, and 25.0% of controls. Patients with low "good" cholesterol were found to have significantly lower serum TG concentrations compared to the control group, despite no significant difference in BMI and AC. Both results once again confirm the more favorable lipid profile in participants with PWS.

When examining the correlation dependences between the indicators of lipid metabolism and adiposity, after taking into account the influence of gender, age, and the stage of puberty development of the participants from both subgroups, only in the PWS group a *significant directly proportional correlation between WC, respectively BMI and LDL-C* was proven. A retrospective Italian study supports this dependence (Bedogni et al., 2020), which, however, included 45 adult patients and followed the percentage of fat tissue and BMI before and 6 years after carrying out a multidisciplinary rehabilitation program for weight loss. The authors reported a possibly clinically relevant reduction in LDL-C in the background of the reported weight reduction. Similar correlations were found in a mixed group of patients (Talebizadeh and Butler, 2005).

Upon additional examination (by subgroups) of the correlation coefficients between indicators of lipid metabolism, glucose homeostasis, and blood pressure, again only in the PWS group a reliable inverse relationship between HDL-C and serum insulin levels, respectively the HOMA-IR index was established, which again highlights that they are more insulin sensitive than obese controls. When assessing the cardiovascular risk indicator CRP, it was found that the latter was significantly lower in patients with PWS, correlating directly with WC, BMI, and serum TG of the participants in this subgroup. These results confirm those already described by Haqq et al. (2011).

Another component of the metabolic syndrome is serum uric acid (UA). Marzullo et al. (2020) proved that when comparing adult patients with PWS and obese controls, a significantly lower UA value was found in the first group. In our sub-study, UA level was also found to be significantly lower in PWS patients. It correlates reliably with WC, BMI, SBP, serum insulin, HOMA-IR, and LDL-C. Such correlations have been demonstrated for the first time in a mixed group of patients. This result is in contrast to previous findings showing that WC is not a reliable metabolic predictor in PWS due to a specific distribution of peripheral fat with predominant subcutaneous rather than visceral accumulation of abdominal adipose tissue (Talebizadeh and Butler, 2005).

Multiple studies from around the world in pediatric patients have indicated decreased serum SHBG levels as a predictor of MS onset (Agirbasli et al., 2009). From the hormonal analysis performed in our study, *statistical significance was demonstrated only for serum SHBG levels in* PWS *patients*, which were higher relative to controls. The fact that SHBG undergoes variations according to age should also be taken into account here. Lower SHBG values

than age reference ranges have been reported in the PWS patient population. Publications in this direction have mixed results. Eldar-Geva et al. (2010) found that in girls in the age group of 6 months to 7 years, 12 out of 20 of them had SHBG below the normal reference limits for the age. Hirsch et al. (2015) examined SHBG levels in 106 patients with PWS aged 1 month to 37 years. From the obtained results it is clear that in males from 2 to 15 years of age, the levels are below the lower limit of the normal range, and in the females, for the most part, they are within the normal limits, except in the childhood, when they move slightly below the normal. SHBG regulation is related to various factors such as age, puberty stage, degree of obesity, and diet. In our patient group, serum SHBG levels were higher and this was associated with their more favorable metabolic profile, as the reason for this could also be the lower BMI compared to healthy controls (24.1 vs. 29.4 kg/m^2).

Among participants, no significant difference in IGF-1 and IGFBP3 concentrations was found between subgroups. However, patients with the syndrome had a higher value of SDSIGF-1, which could be explained by the fact that 88% of them were undergoing treatment with rhGH at the date of the laboratory examination. Similar is the data of Bakker et al. (2015b) who compared 40 rhGH PWS patients with 41 age- and sex-matched healthy controls.

When comparing the serum levels of some specific hormones (HMW-adoponectin, leptin, and irisin) between PWS patients and the corresponding controls, the first demonstrated lower concentrations, although the differences did not reach significance. As already mentioned in the Overview, when comparing PWS patients with obese controls, leptin levels were similar in all age groups, with single reports of higher values. Regarding HMW adiponectin (ADN), higher levels are found both in adults with PWS and in children. Irisin levels are lower in adults and children with PWS, and there is a publication in a mixed group reporting similar irisn levels. The discrepancy between our results and those already published in the literature is most likely due to the age-disparate patient group, the small number of participants, as well as other additional factors that could influence these hormone levels (e.g. nutritional status, rhGH treatment, etc.).

When conducting a correlation analysis between some of the studied hormonal, metabolic, and auxological indicators of PWS patients after controlling for sex, age, and stage of puberty development, a *strong direct correlation between the indicators of adiposity (BMI and WC) and the concentration of IGF-1, respectively leptin*, was demonstrated. Leptin modifies hypothalamic regulation of UA secretion via hypothalamic receptors, respectively IGF-1 levels, and in severe malnutrition insufficient leptin reduces production of growth hormone (GH), subsequently IGF-1 and vice versa. According to Stejskal et al. (1997), in adults, leptinemia values correlated with

the percentage of subcutaneous adipose tissue. Lateva (2015) examined leptin levels in obese prepubescent children and confirmed a trend toward higher mean leptin values in those with BMI $\geq 85^{th}$ percentile for the relevant sex and age. Tanaka et al. (2013) emphasized that in PWS patients, with increasing age, the amount of adipose tissue increases and insulin levels rise. They found a positive correlation of leptin with subcutaneous adipose tissue and BMI, but no correlation with increased CVD risk and insulin resistance. Regarding leptin, our results are confirmatory. IGF-1 concentration is sensitive to short- and long-term changes in nutritional status in children (66.6% of our patients were overweight and obese) and interpretation of measurements should be done after taking a thorough nutritional history. A large part of our patient group is undergoing treatment with rhGH. Bakker et al. (2015b) investigated the performance evaluation parameters of rhGH treatment and demonstrated that IGF-1 levels were significantly higher in treated patients compared to levels in healthy controls. These dependencies are also confirmed in our PWS patients.

Partial correlation analysis in the patient group, controlled for sex, age, and stage of puberty development, showed *that the amount of adipose tissue (g) and adipose tissue as a percentage distribution were reliably associated with leptin concentration*. Weigle et al. (1997) investigated the effect of regional distribution of adipose tissue on plasma leptin levels by comparing 3 groups of patients – ethnically mixed, Japanese-American, and patients with genetically proven PWS who underwent computed tomography to measure the amount of adipose tissue. Similar to our results, the authors concluded that the level of circulating leptin reflects the *total fat mass* and not the combination of adipose tissue and its distribution, and the presence of PWS did not change the relationship between these two variables. The same association was reported by Myers et al. (2000b), as in their pediatric patients (n=27) the amount of adipose tissue decreased significantly after one year of administration of rhGH. They exclude leptin dysregulation as a cause of the pathophysiological mechanism of obesity in PWS. In a linear regression taking into account the influence of factors such as age, gender, stage of pubertal development, BMI, WC, fat mass (FM), %FM, lean body mass (LBM), and LBM/height showed that only FM as a percentage had a predictive value for leptin levels in our patient group, which once again confirms the above results.

In the same group, a relationship between BMI and WC with HMW ADN and SHBG was demonstrated, but with a negative sign. Expectedly, the lower the BMI and the smaller the WC, respectively the abdominal obesity, the higher the levels of these metabolic status protective markers. *HMW ADN demonstrated a significant negative correlation with fasting BG and SBP*. A large-scale Brazilian study by Cunha et al. (2023), including 353 non-syndromic children, confirmed HMW ADN as a metabolic marker for predicting the

development of hypertension in obese children. In previous publications including patients with PWS, a negative correlation of adiponectin was demonstrated only with HOMA-IR, insulin, and leptin, but not with SBP. The present results, despite the small number of patients studied, are a prerequisite for its future use in clinical practice as a predictive marker for the development of hypertension in patients with PWS, at least in a country like ours with a high incidence of hypertension.

More favorable glucose metabolism in general to be associated with hyperadiponectinemia was summarized in the review by Qian et al. (2022). By binding by its receptors, adiponectin regulates molecular pathways involving AMPK, PPAR-α, PPAR-γ, and others. PPAR-γ is critical for glucose metabolism and adipocyte differentiation, therefore increased ADN levels provide more favorable glucose metabolism and adipogenic differentiation in PWS patients, modulating insulin resistance by affecting the adipose tissue microenvironment. Adiponectin acts as a matrix-forming protein because it has structural similarity to collagens VII and X. Its high levels in PWS patients may contribute to a good glucose and fat metabolic microenvironment, while low ADN levels and general obesity may reflect microenvironmental dysfunction and "unhealthy" adipose tissue growth.

In our patient group, *IGF-1 values were significantly associated with a positive sign, and those of SHBG – with a negative sign, for the presence of insulin resistance*. A large Danish study examined the connection between IGF-1 and HOMA-IR in adults (n=3354) and found that low IGF-1 and high normal IGF-1 levels were associated with the development of insulin resistance (Friedrich et al., 2012). A Chinese team (Liang et al., 2016) examined 210 children and found no such correlation. In adult patients with PWS (n=15) no significant correlation between IGF-1 and HOMA-IR was found (van Nieuwpoort et al., 2018), in children with PWS no publications were found in the global database. Specifically for our study group, the positive correlation could be due to the small number of patients, a large proportion of whom were overweight and obese (66%), as well as the fact that 88% of them were treated with rhGH. The negative correlation with SHBG once again demonstrates its predictive effect on the tendency to develop MS, as discussed above.

The investigated irisin in the patient group showed a *significant negative correlation only with serum TG*. This dependence is the opposite of that demonstrated by Italian authors in 2020 (Mai et al., 2020), but their study group consisted only of elderly and obese patients (n=30, mean age 35.7±1.5 years, BMI 45.5±1.5 kg/m^2). According to the authors, *there was a positive association between irisn and percent fat mass after controlling for* PWS *status*, leading to the conclusion that different models of obesity suggest a potential relationship between circulating irisin, muscle mass, and obesity-related metabolic

dysfunction. Under normal metabolic conditions, it is considered that the muscle mass produces the main amount of irisin. Roca Rivada et al. (2013) hypothesized that in obesity, adipose tissue begins to produce the majority of circulating IS. The latter could be the reason for our mixed results. The same team, when examining children (n=25, age 6.6-17.8 years), found no associations between irisin and triglycerides. As in other patient populations, irisin does not currently show clear correlations and it is difficult to interpret its diagnostic and prognostic significance.

In our patient group, irisin levels also showed a reliable association with the amount of FM (g) and FM (%), and these data are confirmatory to the aforementioned Italian publication including adults with PWS (Mai et al., 2020). For the first time, we find such a correlation in a mixed group of patients. In children with PWS, the same team highlighted the potential reasons for the divergent results in establishing relationships between irisin and obesity. This could be due to the interaction between fat mass, muscle mass, and their ratio in peripuberty, as well as the potential of irisin to be secreted as a myokine in normal body weight and as an adipokine in obese individuals. Based on these results, the authors turned to examining fat-free mass, finding that patients with PWS had a significantly lower amount of FM and a smaller waist circumference compared to the control group. The lack of significant correlations between them suggests the minor role of FM in justifying the differences in circulating irisin between syndromic and non-syndromic patients. Although the correlation analysis in our patient group controlled for gender, age, and stage of pubertal development, the correlations of fat mass with adipokines could also be influenced by the presence/absence of rhGH treatment and/or hormone replacement therapy (HRT), as controlling for these parameters not possible due to the smaller number of participants, which could hypothetically have an impact on the results obtained above.

Among the participants with PWS in our patient group, there was no evidence of adrenal dysfunction assessed solely by cortisol level measured between 8 and 10 a.m., and such data were also reported in both other publications. The reported prevalence of central adrenal insufficiency varies from 0% to 60% when demonstrating cortisol deficiency based on different stimulation tests, which could be one reason for the wide variety of results. There is a lack of consensus among clinicians and an accurate algorithm for the diagnosis, follow-up, and treatment of this condition. The first analysis with the highest rate of hypoadrenalism (60%) was by de Lind van Wijngarden et al. (2008) who used a metyrapone test to demonstrate central adrenal insufficiency. In subsequent studies, other tests (synacthen, insulin, glucagon tolerance tests, etc.) were used in children and adults, the frequency varying between 5 and 15 %. In 2022 Angulo et al. (2020) measured morning plasma cortisol together with

ACTH among 128 children, suggesting that this method be used as a screening before appointment of stimulation tests. With the more widespread application of GH treatment in PWS, attention is increasingly being paid to another possible explanation for hypocortisolism – that GH may reduce the conversion of cortisone to cortisol by inhibiting 11-beta-hydroxysteroid dehydrogenase type 1 in adipose tissue, with yet to be studied in this direction. One recent report in adults with PWS (n=82) found an incidence of adrenal insufficiency of 1.2%. The authors argue against the routine prescription of corticosteroids in adults in order to prevent the consequences of their use – weight gain, osteoporosis, diabetes mellitus, and hypertension (Rosenberg et al., 2020). The diagnostic process and therapeutic approach to this endocrinopathy are yet to be unified worldwide in both adults and children.

In the analysis of body composition – distribution of FM/LBM and bone mineral density (BMD) by means of a whole-body DXA scan, a *significantly lower bone mineral density (BMD) was demonstrated in patients with* PWS *despite the treatment with rhGH*. As indicated in the literature review of this dissertation, numerous studies have confirmed the presence of reduced BMD and reduced bone mineral content in both adults and children with PWS. The question of the influence of rhGH treatment on BMD is controversial. Jorgensen et al. (2013) following 46 adults over 2 years of treatment found no improvement in BMD. The results of Longhi et al. (2015) (n=41, aged 29.4 ± 8.6 years) are in parallel with those described above, as they emphasized that rhGH affects bone mass and not BMD. However, a large-scale Dutch study of 102 adult patients with PWS showed that the incidence of osteoporosis was high among adults who did not receive rhGH treatment during adolescence (Sinnema et al., 2011). Four other articles, which included both children and adults, reported no difference in BMD before and after treatment. Carrel et al. (2022) and Myers et al. (2000a) reported an increase in BMD in children after long-term treatment with rhGH. Therefore, rhGH therapy in children with PWS affects bone parameters both directly and indirectly through the positive effects on body composition, the increase in muscle mass percentage, and hence, the improvement of physical capacity. More studies are needed to make a definitive conclusion about the effect of rhGH on BMD.

The same analysis also found a *lower LBM content adjusted for height (LBM/height)* in the PWS population. Our study also used adjustment for height because the patient group was significantly shorter compared to matched controls. It has been repeatedly confirmed in the literature that these patients have a higher percentage of fat mass and a lower percentage of lean mass, both with a characteristic redistribution compared to healthy controls, and this trend is observed at all ages. Regarding the treatment with rhGH, a Dutch collective proved that in an eight-year follow-up of 60 prepubescent children, a significant

increase in muscle mass was registered only in the first year of treatment with a standard dose – 0.035 mg/kg/day (-2.54 SDS at the beginning of the study to -1.5 SDS at the end of the eighth year) (Bakker et al., 2013). At a three-year follow-up of two groups (the first was on continuous treatment with rhGH, and in the second the treatment was suspended for 1 year after reaching full height, after which it was resumed) young adults with PWS (n=43, mean age 19 years, treated with 1/3 of the standard dose), found that the amount of muscle mass did not change significantly (-2.1 to -1.9 SDS) (Damen et al., 2020). Despite rhGH treatment, patients with PWS achieve muscle mass near the lower limit of normal or low normal level, which has been confirmed both in the literature and in our patient population. The importance of physical activity, which is not touched upon in the present work, is insufficiently highlighted.

Partial correlation analysis in the patient group after controlling for sex, age, and stage of pubertal development, showed that LBM/height correlated significantly with Bone density Z-score. A similar association of muscle mass, without controlling for height, was also confirmed in other European studies in prepubertal patients (n=77) on the background of rhGH treatment (Bakker et al., 2015a), young adults (n=27) in the background of rhGH and/or hormone replacement therapy (HRT) (Donze et al., 2018) and young adults (n=43) who are on combination therapy (rhGH + HRT) (Damen et al., 2021).

At this stage, the role of the particular body composition in PWS patients, the mechanism of the more favorable metabolic profile, and the relationship with the above-mentioned adipokines remain unclear.

The analysis of the results of the specialized questionnaire for the assessment of the quality of life (QoL) in the group of patients with PWS shows that the average total percentage of QoL for the entire studied group is 69.0±16.6 (21.0-92.0), and the median (50^{th} percentile) was estimated at 72.0%. Based on this result, *participants were conditionally divided into 2 groups, and participants with a high QoL score were found to have smaller waist circumference (WC), insulin, HOMA-IR, and TG levels compared to the low QoL score group, although the differences did not reach significance*. In a correlation analysis controlling for the studied dependencies by gender and age, including only the participants with a high QoL score, a reliable direct relationship between QoL and IGF-1 SDS was established. One of the factors described in the literature that significantly improves the quality of life in these patients is the treatment with rhGH (Höybye et al., 2005; Whitman et al., 2002). Based on our results, it could also be speculated that the implementation of the standard treatment with adequate doses and all its benefits has a positive impact on the QoL of patients with PWS and ensures their more favorable metabolic profile.

VB. Silver-Russell syndrome

It is assumed that the 18 patients with Silver-Russell syndrome (SRS) included in the study represent a significant part of the pediatric patients with the syndrome known in the country. The average age for diagnosis in patients with SRS was 4.6±3.7 years (0.9-12.0 years). Patients with SRS were compared with 15 controls, with no significant difference in gender and age distribution between participants in both groups being detected.

Despite the lack of previously published data at home and abroad, the age of diagnosis among the examined patients is satisfactory. Earlier detection and registration of children born SGA, the end spectrum of which are patients with SRS already in maternity hospitals, can greatly reduce this age. Such an approach in our country, however, needs the introduction of a structured program accepted by the national health insurance authorities and the Ministry of Health, as proved by a recently published large-scale study (Tanya Zlateva, 2024).

Fifty percent (n=9) of the patients had a positive genetic result. The percentage almost corresponds to the genetically confirmed variants reported in the literature (60%). The slightly lower incidence is probably related to the country's more limited genetic testing options. In SRS patients with a negative genetic result (n=9), the diagnosis was made solely on the grounds of specific anamnestic/clinical features *(clinical diagnosis)*, after meeting ≥4 out of 6 criteria of NH-CSS, without further detailed molecular genetic analysis to exclude rare monogenic variants as well as other clinically overlapping conditions (Temple syndrome, mUPD20, mUPD16, MLID), as state-of-the-art recommendations (Kurup et al., 2024). Half of all SRS patients met all (6/6) NH-CSS clinical criteria for diagnosis, one-third met 5 of 6, and the remaining 17% met the critical 4 of 6 criteria.

After the anthropometric, clinical, and pubertal assessment of the participants, the indicators of generalized and abdominal obesity (BMI and WC) did not demonstrate a difference that reached a level of significance. Applying partial correlation analysis, after controlling for gender and age, as expected, a very strong *directly proportional correlation between body mass, BMI, and WC* was evidenced in both the syndrome patients and the control group.

After using the IOTF values for BMI by Cole et al. to determine the degree of underweight, SRS patients demonstrated a significantly higher incidence of 2nd and 3rd grade compared to healthy controls.

Data analysis showed a *significant difference in measured HR between both groups* in favor of syndromic patients. According to the theory of metabolic programming in fetuses with intrauterine growth restriction, in order to preserve the important organs, the body adapts to an insufficient amount of oxygen, glucose, and amino acids by slowing down the basal metabolism, reducing the concentration of hormones and the sensitivity of tissues to them, as well as by redistribution of blood flow. Fetal stress as a result of impaired homeostasis in utero, as well as altered function of the hypothalamic-pituitary-adrenal axis, lead to impaired function of the autonomic nervous system in SGA children. In them, the action of the sympathetic component is enhanced, and as a result the heart rate increases. Zamecznik et al. (2017) examined 68 SGA patients aged between 5–10 years and proved that they have a faster heart rate and lower heart rate variability compared to AGA controls. This leads to a higher risk of developing CVD. In 2002 Iotova analyzed 78 SGA children and, for the first time in our country, assessed the effect of low birth weight and height on metabolic risk, comparing them with AGA controls. The author found that HR was significantly inversely proportional to size at birth, with the additional increase in HR observed in patients with the most pronounced postnatal growth and in those with a relatively higher BMI. As part of the SGA group, SRS patients in our study demonstrated a higher HR, which confirms their higher CVD risk.

After applying the selected criteria, 15.4% of patients with SRS were diagnosed with the presence of systolic hypertension, while diastolic hypertension was found in 23.1%. Lokulo-Sodipe et al. (2020) examined a mixed group of SRS patients (n=33, age 13.3-69.7 years), with the reported incidence of arterial hypertension being 27.6%, and in patients over 18 years of age being 33.3%. Takenouchi et al. (2015a) described the metabolic profile of three adult patients, with 100% of them having unlocked hypertension.

In a bivariate analysis of the dependencies of the data for all participants, a *strong direct correlation of WC with SBP and DBP* was demonstrated. Balomenou et al. (2023) examined the body composition of 106 SGA children (7-10 years). They found a significantly lower BMI and a similar size of WC compared to AGA controls, which indirectly

leads to the conclusion that SGA children in the studied group present with abdominal obesity. Both in our country and abroad, it has already been proven that in non-syndromic prepubescent children, a more pronounced accumulation of abdominal fat tissue is associated with adverse hemodynamic changes, which are an independent predictor of CVD. The same dependence was found in the patients with SRS from our studied group. As we have already shown, in the PWS patients of our study, as well as in the SRS patients, the increase in the size of the WC could serve as a predictive marker for the development of hypertension.

In five of the patients with the syndrome (27.8%), the presence of early puberty was proven, necessitating treatment with a depot form of a GnRH analog. According to Goedegebuure et al. (2018) the onset and progression of puberty in children with SRS (n=31) and those SGA-born were similar (11.5 years in boys with SRS vs. 11.6 years in SGA-born boys, and 10.5 years in girls with SRS and 10.7 years in SGA-born girls respectively). Patti et al. (2022) and Smeets et al. (2016) found that puberty started significantly earlier in patients with SRS compared to the matched SGA group (according to the second scientific collective, onset for girls with SRS at 10.2 years vs. 11.2 for SGA-born, respectively, and for boys with SRS at 11.4 years vs. 12.0 years for SGA-born).

According to Binder et al. (2017), early gonadarche follows early adrenarche in boys but not in girls. The age of gonadarchae found in the 2017 study in question is consistent with the data of Smeets et al. (2016) who studied a Dutch cohort of 62 SRS children with similar gonadarche age (11.4 years in boys and 10.2 years in girls). This age is similar to the general population, but the *physical parameters (height and weight) of those affected by the syndrome are much more unacceptable*. No cases of precocious puberty (defined as onset under 8 years for girls and under 9 years for boys) were reported in these two studies, suggesting that precocious puberty is actually *less common* in SRS than was previously believed (Binder et al., 2017). Both aforementioned studies reported a good effect on final growth prognosis in a two-year delay of puberty by adding GnRHa to rGH therapy, as this approach was used on a case-by-case basis in four of our patients. A benefit of such combined treatment has also been demonstrated in SGA-born patients who entered puberty before reaching a height of 140 cm, and therapy with both drugs had no long-term negative effects on the metabolic status of the patients compared to those of treatment with growth hormone alone. The mean

age of appearance of early pubertal marks in our study group was 5.08±5.3 years (with a range of 1.33-10.25 years). One of the female patients has a manifestation of isolated thelarche at 1.33 years of age, and to date, she has not been treated with a GnRH analog. Her participation in the analysis greatly reduced the average age of onset of early puberty marks. When excluding her from the group, the average age was 8.62±0.29, with a range of 6.41-10.25 years, and the age was again lower than that indicated by foreign authors. A reason for this could be the inclusion of patients with a diagnosis of "clinical SRS " who did not undergo additional molecular genetic analysis to confirm the presence of other SRS spectrum syndromes with an earlier onset of early puberty.

In 28.6% of all boys with SRS in our patient group, unilateral/bilateral cryptorchidism was found, with orchidopexy performed. In Wakeling et al. (2010) the incidence of genital abnormalities in boys was similar – 23%. In both our study and theirs, these were observed only in patients with a hypomethylated IG-DMR region (ICR1 hypomethylation), and the severity of the congenital condition is associated with the degree of hypomethylation (Bruce et al., 2009).

Regarding the additional markers in the study by Wakeling et al. (2010) found the following frequency:

Indicator (n/%)	SRS (n=18)	11p15LOM (n=6)	mUPD7 (n=3)	Clinical SRS (n=9)
Micrognathia(n/%)	18/100.0	6/100.0	3/100.0	9/100.0
Low MM (n/%)	18/100.0	6/100.0	3/100.0	9/100.0
Syndactyly(n/%)	3/16.7	3/50.0	0/0	0/0
Clinodactyly (n/%)	16/88.9	6/100.0	2/66.6	8/88.9
Shoulder dimples (n/%)	2/11.1	1/16.7	1/33.3	0/0
Prominent heel (n/%)	3/16.7	1/16.7	1/33.3	1/11.1
Scoliosis (n/%)	4/22.2	0/0	1/33.3	3/33.3
Autism/spectrum (n/%)	4/22.2	0/0	1/33.3	3/33.3

When comparing the percentage distribution of additional features and their appearance with those of the last review by Kurup et al. (2024), differences are found, which once again points to the role of these signs as non-pathognomonic and supportive rather than defining the main diagnosis.

Indicator (%)	11p15LOM Patient group	11p15LOM (Kurup et al.)	mUPDm Patient group	UPD7 (Kurup et al.)
Micrognathia	100,0	75,0	100,0	26,0
Low muscle mass	100,0	67,0	100,0	47,0

Syndactyly	50,0	42,0	0,0	17,0
Clinodactyly	100,0	81,0	66,6	56,0
Shoulder dimples	16,7	77,0	33,3	67,0
Prominent heel	16,7	26,0	33,3	100,0
Scoliosis	0/0	10,0	33,3	16,0

Regarding the leading risk factors for the diagnosis of SRS, 94.4% of SRS children are SGA-born, as expected the syndromic group demonstrated a significantly lower birth weight and length, with relative macrocephaly present in 55.6% of patients. Compared to other reports reporting the frequency of macrocephaly of both most common genetic variants (99% for 11p15LOM and 85% for mUPD7, identified by the first, and 70% and 90%, respectively, identified by the second cohort in our study), the percentage of macrocephaly is lower. A possible reason for this could be the lack of more detailed genetic analysis to confirm the rarer genetic variants or other SRS -like conditions in clinically diagnosed patients who could have normo- or microcephaly. Of participants with the syndrome, 72.2% were diagnosed with intrauterine growth restriction during pregnancy, and this rate correlates with data from the literature (Lokulo-Sodipe et al., 2020).

SRS patients demonstrated a worsened profile of glucose homeostasis with higher serum insulin concentration and HOMA-IR index, although no significant difference was found in the mean values of preprandial blood glucose (BG), insulin, and calculated HOMA-IR index with those of the control group. Such results were reported by Mericq et al. (2017) and Ibanez et al. (2006). According to them, SGA-born children, especially those with catch-up postnatal growth, are at risk of rapidly developing reduced insulin sensitivity compared to children born AGA. They recorded that initially, the average value of preprandial insulin was lower in SGA-born children compared to those born AGA, but in the next 3-4 years this changed and the average value became higher in the first group, with increasing insulin resistance.

A compensatory increase in insulin secretion ensures normoglycemia. According to Iñiguez et al. (2006), the observed changes in insulin sensitivity were due to changes in IGF-1 levels. In those born with SGA, they are lower at birth but increase by 3 years of age, compared to children born AGA. In SGA children at 1 year of age, the concentration of IGF-1 correlates positively with the increase in length

from birth and with insulin secretion. In contrast to 3 years of age with completed postnatal catch-up – IGF-1 levels correlate with BMI and IR. In conclusion, according to the latest internationally accepted SGA consensus (Hokken-Koelega et al., 2023), in SGA children, IR gradually increases, and in those with pronounced postnatal weight gain, it can be diagnosed at an early age (1-4 years of age). However, there is no evidence that T2D, IGT, clinically significant dyslipidemia, or hypertension occur more frequently in SGA children compared to AGA. The risk of metabolic disorders can be increased by the presence of factors such as overweight, obesity, ethnicity, and environmental factors. Therefore, routine evaluation of metabolic parameters is not recommended for all SGA children, but only for those with one or more risk factors. Adherence to these recommendations cannot be accepted unconditionally in children with SRS due to the need for long-term follow-up of larger groups of patients in order to understand the natural course of the disease and metabolic status. The internationally accepted consensus for patients with SRS recommends clinical and biochemical screening for insulin resistance in older children and adolescents, a healthy lifestyle, and an adequate diet in order to avoid excessive and sudden weight gain and as a consequence – an increase in insulin resistance.

After applying the selected criteria for metabolic syndrome (MS) in the entire study group, fasting hyperglycemia (BG $\geq$ 5.6 mmol/l) was found in only 1 participant who had SRS (5.6%). Smeets et al. (2017) compared the metabolic profiles of SRS adolescents and SGA children and found them to be similar, allowing us to use this fact in our study as well, using SGA patient reports as a comparison. Iotova (2002) reported that, although within the normal range, BG at minute zero was significantly higher in SGA-born adolescents (n=78) compared to their AGA peers. Her study in adolescents, as well as a survey of prepubescent SGA children (n=78) by Sas et al. (2001) found IGT in 8% and 7% of patients, respectively. Such a deviation was not registered in any patient from our group, as well as in a study among prepubescent SGA children (n=93) by Polish authors. Abnormalities in glucose homeostasis have been reported in adult patients with SRS, in a mixed group of patients, but not in children, and a comparison with them could not be made given the age range of our study group (8.5±4.7 years). Regarding the factor "accelerated postnatal growth" in our cohort, no conclusion can be drawn

due to the different study designs. As we summarized above, a systematic evaluation of the metabolic profile in SRS patients is necessary, with the aim of early detection of metabolic disorders and prevention of complications.

Partial correlation analysis, after accounting for the influence of gender, age, and stage of pubertal development in SRS patients, demonstrated a strong inverse correlation of fasting BG and serum insulin with birth weight and birth height. These dependencies have been confirmed in foreign publications in adults, adolescents, and pre-pubescent SGA children. In our country, Iotova (2002) found that the influence of birth size on fasting glucose was more pronounced than the influence of current BMI in SGA adolescents, with the combination of smallest birth size and highest BMI leading to the highest fasting BG and strongly increase the significance of the difference with AGA controls. Therefore, the children with SRS in our cohort, as part of the SGA group, obeyed the dependencies of Barker's theory. On the other hand in their review, Giabicani et al. (2018) summarized that fetal growth restriction may result from a combination of genetic and epigenetic defects, environmental factors, hormonal regulation, or vascular problems and their potential interaction. In contrast to previous reports, they postulated that the *accelerated postnatal growth* of low birth weight infants is a *more important factor* in the development of MS at a later age than low birth weight per se (*79.6%* of cardiovascular risk factors such as blood pressure, insulin resistance, hypertriglyceridemia, and LDL- and HDL-cholesterol levels reported in studies of the rapid catch-up growth hypothesis were statistically significant, while the corresponding figure was *58.5%* for the independent effect of low birth weight). More studies in this direction are needed specifically for patients with SRS. However, in both settings, systematic monitoring of metabolic parameters is required in patients with SRS as part of the SGA cohort. As we indicated above, regarding the possible dangers of rapid postnatal catch-up, a careful assessment of caloric intake and inclusion of appropriate motor activity are necessary.

Applying the IDF criteria for *dyslipidemia*, it was found that there were 3 of all 33 participants (9.1%) with HDL-C below 1.03/1.1 mmol/l (according to age), and they were only from the group of patients with proven SRS (16.7%). A significant decrease in HDL-C in prepubescent SGA children (n=93) compared to healthy controls was

also recorded by Zamojska et al. (2023). Patti et al. (2018) found decreased HDL-C levels in 28.5% of the examined group of adult patients with SRS. Smeets et al. (2017) found no significant difference in HDL-C levels in a mixed group of SRS patients compared to SGA controls. No publications were found determining the incidence of dyslipidemia in children with SRS.

When examining subgroups in the group of SRS participants, the presence of a *reliable direct relationship between TG and serum insulin, respectively the HOMA-IR index*, was established, which confirms the relationship between the increased cardiovascular and metabolic risk in these patients.

In the participants from both subgroups, no significant correlation was found between the indicators of lipid metabolism and adiposity, including after taking into account the influence of gender, age, and stage of puberty development.

All participants in the study were examined for the serum concentration of UA, the mean value of which was *significantly higher in the* SRS *patients* and correlated reliably with the concentration of total cholesterol in the same subgroup. Hyperuricemia was registered in one adult patient with SRS (Takenouchi et al., 2015b). Such a relationship has not been reported in the pediatric literature to date, and after validation, UA could be included in the mandatory panel of metabolic risk assessment endpoints in SRS.

Patients with the syndrome showed a higher mean CRP value, but the difference compared to the control group did not reach significance. Significantly higher high-sensitivity-CRP levels of SGA-born patients compared to AGA were reported by Trevisanuto et al. only in newborns. Applying partial correlation analysis with consideration of the influence of gender, age, and stage of puberty development, it was found that in SRS participants, *CRP correlated significantly with BMI, WC, serum insulin, and HOMA-IR*, as expected for a marker that is a screening component for MS in non-syndromic patients. Publications regarding CRP level and its correlations in SRS patients are not known in the literature, and further studies are needed to validate it as a risk marker in SRS patients.

When analyzing hormonal indicators, significantly higher values of serum IGF-1 and SDS_{IGF-1} were found in the patients with SRS, which can be explained by the fact that 94.4% of them were taking rGH

treatment at the date of laboratory examination. Another reason for this result that should be taken into account is that high IGF-1 levels do not always correspond to high IGF bioactivity in SGA children.

In the group of patients with the syndrome, a significantly *higher concentration of myokine-adipokine irisin* was found compared to the corresponding controls, which was associated with reduced insulin sensitivity. Considering these established correlations by Seppa et al. (2019) we can reconfirm that also in our cohort, children with SRS have a higher risk of developing MS as part of SGA patients. This is the first time in the literature that levels of this hormone in children with SRS have been investigated, and in our study with a relatively small number of included participants, it did not show a reliable relationship with anthropometric and metabolic/hormonal indicators.

After controlling the analysis for gender, age, and stage of pubertal development in the group of SRS patients, as expected, a *strong direct correlation between BMI and WC and leptin concentration was demonstrated, while their dependence on SHBG was reliable, but with a negative sign*. Leptinemia values have long been proven to correlate with the percentage of subcutaneous fat in adults and children, which was also seen in our patients. The lower the BMI and the smaller the WC of the patients in our syndrome group, the higher the SHBG and, accordingly, the healthier the metabolic profile they have. This result confirms that decreased serum SHBG levels are a predictor of the onset of MS in pediatric patients and this also applies in the SRS group. This statement is further supported by the *inverse significant correlation of SHBG with insulin, HOMA-IR, and DBP* established in the same patient group. In the group of SRS patients, *leptin levels were also significantly correlated with HOMA-IR, insulin, CRP, and TG*. Miras et al. (2010) investigated the relationship of insulin sensitivity to leptin in a population of 23 SGA children with postnatal catch-up, 26 SGA children without catch-up growth, and 48 prepubertal AGA controls. They established a significant correlation of leptin with parameters of insulin sensitivity in SGA children with postnatal catch-up, suggesting *serum leptin concentration as an early indicator of insulin resistance*. Given the confirmation of this dependence in our patient group, this assumption can also be taken into account for patients with SRS, although most likely not all have catch-up postnatal growth and in the current design it was not possible to make such an assessment.

As a result of the same analysis in the SRS group, it was found that *HMW ADN demonstrated a significant inverse correlation only with TG*, and our result is similar to that reported by Seppä et al. (2019) found in SGA children (n=192, 12 years of age). This once again confirms that low levels of this adipokine lead to a deterioration in the metabolic profile in healthy and SGA patients, as the indicator can be taken into account in SRS patients as well.

Within the protocol, body composition was analyzed in 20 participants. In SRS patients, a trend towards lower BMD Z-score, lower LBM content (g), and higher FM content (g) was found, which did not reach significance. In 2021 Offenheimer et al. (2021) emphasized sarcopenic obesity in healthy subjects, which is a combination of increased fat mass and decreased muscle mass. Although occurring in only 2.5% of the cohort (n=1394, 6-18 years), it is a prerequisite for higher health risk. This type of obesity may give the false impression of a mildly elevated BMI, which may falsely mask increased metabolic risk (Salton et al., 2022). Burrows et al. (2017) demonstrated that in adolescents (n=678, age of 16-17 years) less muscle mass was associated with a significantly higher risk of developing MS compared to non-obese controls with normal muscle mass. This also highlights the fact that patients presenting a combination of obesity and reduced muscle mass have the most unhealthy cardio-metabolic profile. Although our patients did not reach significance in terms of lower LBM, it could be speculated that they also present with a particular type of distribution of LBM and FM and accordingly have a more unfavorable metabolic profile.

Of the representatives of our patient group, 94.4% were undergoing treatment with rGH at the time of examination. In the literature so far, there are publications on the positive effect of rGH with different duration of follow-up on the body composition of SGA children and one for children with SRS. The authors of the latter compared the body composition of 29 patients with SRS versus SGA-born patients during and after 2 years of rGH therapy using DXA scanning. Fat-free mass before the start of treatment was in small amounts in both groups, which was more pronounced in patients with SRS, but without reaching statistical significance. Two years after stopping the therapy, a decrease in fat-free mass and an increase in the percentage of adipose tissue were found in both groups.

Two studies have been done in adults with SRS (Lokulo-Sodipe et al., 2024; Patti et al., 2018), both of which included treatment-naïve patients. Regarding LBM, all the above publications reported a lower amount of LBM in SRS patients, although the other authors based their results on SDS, and in our case, the amount was measured in grams. In both publications, a higher percentage of fat mass is found, while in our group this dependence applies to the amount of FM measured in grams. Our results are confirmatory, although a limiting factor for the discussion of this result is the small number of controls who consented to undergo DXA scanning. Regarding the BMD, the results are contradictory. Patti et al. (2018) reported normal BMD (whole body Z-score 0.44 ± 0.9) in patients with SRS, and Lokulo-Sodipe et al. (2024) found lower bone density compared to healthy controls. In our patient group, BMD was lower compared to controls despite ongoing rGH therapy. More studies are needed to clarify the specificity of body composition in SRS patients.

Partial correlation analysis in the patient group (after controlling for gender, age, and stage of pubertal development) showed that *FM correlated significantly with BMI, insulin concentration, HOMA-IR, and leptin, as well as with SHBG with an inverse sign*. *The percent of FM was significantly associated with insulin, HOMA-IR, CRP, and leptin as well as with SHBG with the opposite sign*. Such relationships are not found in the SRS literature but are expected results in the general population.

All participants from the syndromic disease group answered a specialized questionnaire to assess the QoL. Scoring was done analogously to the PWS patients, after which the result was converted to percentages. The mean overall QoL percentage for the entire SRS group was 74.3±2.6 (53.0-88.0), and the median (50^{th} percentile) was estimated at 79.5%. Based on this result, the participants were conditionally divided into 2 groups: the first group with a low QoL score (< 79.5 %) and the second group with a high QoL score (≥ 79.5%).

Participants with a high QoL score were found to have *higher height and IGF-1*, and lower insulin, HOMA-IR, CRP, leptin levels, and % of FM compared to the low-score SRS subgroup, although the differences did not reach significance. Applying a correlation analysis for the entire group of patients with SRS, controlling for gender and age, a *significant correlation was demonstrated between the calculated QoL score with IGF-1 and LBM (g)*. In an analysis including only the participants with a high QoL score, *a reliable direct correlation was*

found between the calculated QoL scores and BMD, LBM (g), and IGF-1. Lokulo-Sodipe et al. (2020) examined the quality of life in 33 elderly patients with SRS compared to healthy controls, finding a significant inverse correlation with BMI, i.e. the healthy body weight is a key to a better existence. In our group with a high QoL score, dependence was presented with BMD, LBM, and IGF-1, and it could be speculated that treatment with rGH and its indirect effect on these indicators and BMI improves QoL in these patients.

PART VII. MAIN CONCLUSIONS

Objective 1 reletad conclusions:

1.1. Among PWS patients:

- A more favorable metabolic profile was observed, with significantly lower serum insulin concentrations, HOMA-IR, CRP levels, and uric acid levels, as well as higher SHBG levels and a generally improved lipid profile.
- Elevated systolic and diastolic blood pressures were noted in tandem with a reduction in HMW adiponectin levels.
- Positive correlations were established between waist circumference and insulin levels, HOMA-IR, LDL-C, CRP, uric acid, IGF-1, and leptin, while SHBG and HMW adiponectin showed significant negative correlations.
- A significant inverse association between irisin levels and serum triglycerides was identified.

1.2. Among SRS patients:

- An impaired metabolic profile was observed, with significantly elevated uric acid levels, lower serum SHBG levels, a trend toward deteriorated glucose homeostasis, and higher average CRP levels.
- Significant positive correlations were identified between BMI, waist circumference, and leptin levels, along with a notable inverse correlation with SHBG.
- Irisin levels were significantly higher in the SRS group compared to controls.

Objective 2 reletad conclusions:

2.1. PWS patients exhibited lower HDL levels, reduced systolic and diastolic blood pressure values, fewer cases of impaired glucose tolerance, and a lower incidence of systolic and diastolic hypertension compared to controls.

2.2. In the SRS group, there was a higher prevalence of systolic and diastolic hypertension, increased heart rate, greater incidence of fasting hyperglycemia, and lower HDL levels.

Objective 3 reletad conclusions:

3.1. Analysis of body composition among PWS patients showed reduced bone density and lower lean mass adjusted for height (lean mass/height) relative to controls. A positive correlation was found between fat mass (both absolute and percentage) and leptin levels, while both indicators exhibited negative associations with SHBG.
3.2. In SRS patients, no significant differences were found in body composition or associations with metabolic indicators compared to healthy controls.

Objective 4 reletad conclusions:
4.1. Among PWS participants with a high QoL score, there was a strong positive association between the calculated QoL index and IGF-1 SDS. A trend was observed toward a more favorable metabolic profile (lower waist circumference, insulin levels, HOMA-IR, and triglycerides) in the group with higher QoL scores.
4.2. Across all SRS patients, a significant correlation was established between QoL score and IGF-1 levels. In those with the highest QoL scores, further associations were observed with bone density and lean mass. A trend was noted for increased IGF-1 levels, greater height, and lower insulin, HOMA-IR, CRP, leptin, and fat mass percentages in the group with the highest QoL scores.

PART VIII. CONTRIBUTIONS OF THE DISSERTATION

1. This study is the first in the accessible global literature to identify an association between increased abdominal circumference in both patient groups and decreased levels of high-molecular-weight adiponectin in PWS, indicating an elevated risk of hypertension.
2. For the first time in Bulgaria, the clinical and laboratory profiles and body composition of significant groups of patients with PWS and SRS are evaluated and analyzed, with these patients being long-term monitored in an Expert Center for Rare Endocrine Diseases.
3. This is the first national study to establish the prevalence of metabolic and cardiovascular disorders in patients with PWS and SRS, comparing them to healthy controls matched for sex, age, and partially by BMI.
4. This study provides the first national evaluation of quality of life in PWS and SRS patients, aligning with the contemporary focus in science on quality of life, especially within the field of rare diseases.

5. The study represents the first assessment of irisin levels in PWS and SRS patients in Bulgaria, examining correlations with metabolic indicators and body composition.
6. The established correlation of leptin with insulin and HOMA-IR in PWS and SRS patients suggests leptin as an early marker for the development of insulin resistance.
7. The study reaffirms the known relationship between low birth weight and length at birth with fasting blood glucose and serum insulin, indicating that SRS patients exhibit metabolic behaviors similar to SGA cohorts and can thus be managed using similar guidelines.
8. The necessity for routine assessment of metabolic parameters in PWS and SRS patients is emphasized, given the still unclear natural course of these disorders with or without the presence of one or more risk factors, to facilitate the early detection of abnormalities.

PART IX. FINAL CONCLUSION

PWS and SRS, as rare endocrine disorders, are characterized not only by distinct physical features and dysmorphisms but also by specific metabolic profiles and body composition, placing these patients at higher risk of early onset of socially significant diseases under certain conditions.

The commonalities between these two conditions, such as the underlying genetic defect mechanisms, low birth weight, unique body composition, and need for hrGH treatment, are insufficient to group them under a single clinical profile. Despite shared characteristics, the complex pathogenesis, predispositions, and subsequent complications associated with metabolic syndrome in both patient groups place them at opposite poles of metabolic health. PWS patients demonatrate a more favorable metabolic profile compared to controls matched for sex, age, and BMI, though the potential deterioration of these parameters due to uncontrolled weight gain warrants attention. Conversely, SRS patients demonstrate poorer metabolic parameters from early childhood, affirming their inclusion within the SGA cohort.

The results of this study suggest potential markers for heightened metabolic risk, which may be incorporated into routine clinical follow-ups for PWS and SRS patients. An increase in abdominal circumference across both groups, coupled with a decrease in high-molecular-weight

adiponectin levels in PWS, emerged as early markers for hypertension risk. Besides glucose homeostasis and lipid profile parameters, levels of uric acid, CRP, and SHBG should be part of regular assessments for these patient groups. Given the significant correlation of leptin with insulin and HOMA-IR in PWS and SRS patients, elevated leptin levels could serve as early indicators of insulin resistance. Discordant correlations observed between irisin and triglycerides in PWS patients, as well as elevated irisin levels in the SRS group, warrant further investigation.

The limited sample size and wide age range of our participants were restrictive factors in deriving more statistically significant correlations and conclusions. Further extensive and longitudinal studies on larger, homogenous patient groups are essential to explore the relationships between metabolic syndrome components, adipomyokines, and body composition in greater detail.

Attention must also be drawn to the importance of routine quality-of-life (QoL) assessments in patients with rare diseases. Such evaluations enable the early detection of declines, prompting interventions aimed at enhancing physical and mental health. Improved care for these two patient groups, through the prevention of type 2 diabetes and cardiovascular disease, will ultimately increase their QoL and life expectancy while reducing healthcare and societal costs.

Correlations between IGF-1 and QoL, as well as metabolic indicators, were identified in both patient groups, suggesting that hrGH treatment may positively influence these parameters. The necessity of hrGH administration for the physical and mental well-being of PWS and SRS patients is thus reinforced.

In summary, the findings of this dissertation, along with established data in the global literature, emphasize the need for systematic and comprehensive assessment of anthropometric parameters, physical status, metabolic and hormonal profiles, body composition, and QoL in these two rare endocrine diseases. Such an approach, integrated into daily clinical practice, is crucial for preventing obesity and its associated metabolic complications in PWS patients, and for early detection in the high-risk SRS patient group. Achieving these goals requires the support of expert centers for rare diseases and the consolidation of patient care under specifically trained specialists within these centers.

PART X. SCIENTIFIC PUBLICATIONS RELATED TO THE DISSERTATION

Publications:

1. Yordanova, N., Iotova, V., Bazdarska, Y., et al. Silver-Russell Syndrome – Presentation of Modern Consensus Guidelines for Diagnosis and Treatment, along with a Clinical Case Description, Pediatrics Journal 2020 (1), pp. 12-17. (in Bulg.)

2. Iotova, V., Yordanova, N., Bazdarska, Y., Galcheva, S., Kosev, P., Hinev, A., Popova, R., Balev, B., Mladenov, V., Boyadzhiev, V., Stoycheva, R. Prader-Willi Syndrome – What Makes It Different Today? Proceedings of the Union of Scientists – Varna 2016, Volume XXI, Issue 2: 48-54. (In Bulg.)

Participations:

1. Yordanova N., Iotova V., Galcheva S., Bazdarska Y., Mladenov V., Boyadzhiev V. Prader-Willi Patient with Rectal Bleeding – Experience in Center for Rare Endocrine Disordesrs in Varna, Bulagria. ESPE Annual Meeting, 27-30 Sept 2018, Athens, Greece. Horm Res Paediatr 2018; 90(suppl 1): P3-P250, 468; doi: 10.1159/000492307 (poster presentation)

2. Yordanova N, Bazdarska Y, Shishkov S, Halvadzhiyan I, Galcheva S, Iotova V. Evaluation of the first year of growth hormone treatment in Prader-Willi Syndrome Patients followed at an Expert Center of Rare Endocrine Diseases. 60th Annual Meeting of the ESPE, Rome, 15-17 Sep 2022. Horm Res Paediatr 2022; 95 (suppl 2): 514 (P2-160). DOI: 10.1159/000525606 (poster presentation)

ACKNOWLAGEMENTS

To all the patients and their families, as well as to the doctors, nurses, and laboratory technicians who participated in the study.

To my scientific supervisor, Prof. Violeta Iotova, for the idea, inspiration, and support throughout the entire work process.

To the Varna Society of Pediatric Endocrinology and its members, to St. Marina University Hospital - Varna, and to the Medical University - Varna and their staff for providing the conditions necessary for conducting the study.

To "Labor Express" Laboratory, Dr. Victoria Snegarova, and her team.

To my husband, my daughter, and our families for their patience and unconditional support.

In memory of my son, Yordan Atanasov Peykov, whom we lost irreversibly and tragically at the age of 50 days.