MEDICAL UNIVERSITY

“PROF. DR. PARASKEV STOYANOV” – VARNA

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“NON-CLASSICAL FORM OF CONGENITAL ADRENAL HYPERPLASIA – A MOSAIC OF THE KNOWN AND THE UNKNOWN“

AUTHOR’S ABSTRACT

of a dissertation submitted for the award of the educational and scientific degree **“Doctor”**

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The public defense of the dissertation will take place on November 6, 2025, at 1:00 p.m. in Hall 501, 5th floor, at University Hospital "St. Marina," during an open session of the Scientific Jury.

The defense materials are available at the Research Department of the Medical University – Varna and have been published on the official website of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

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LIST OF ABBREVIATIONS

3βHSD – 3β-hydroxysteroid dehydrogenase

17βHSD – 17β-hydroxysteroid dehydrogenase

17OHP – 17-hydroxyprogesterone

21-OH – 21-hydroxylase

A – Asceticism

AA – Androgenic alopecia

AP – Affective problems

AH – Arterial hypertension

FFM – Fat-free mass

VAT – Visceral adipose tissue

CAH – Congenital adrenal hyperplasia

GC – Glucocorticoids

DBP – Diastolic blood pressure

ED – Emotional dysregulation

ID – Interoceptive deficits

IR – Insulin resistance

BMI – Body mass index

BMD – Bone mineral density

CS – Corticosteroids

PA – Personal alienation

LH – Luteinizing hormone

IA – Interpersonal alienation

FM – Fat mass

IP – Interpersonal problems

AT – Adipose tissue

AG – Adrenal glands

NCAH – Non-classic congenital adrenal hyperplasia

IRO – Insecurity in interpersonal relations

LS – Low self-esteem

GPM – General psychological maladjustment

WC – Waist circumference

TC – Total cholesterol

P – Perfectionism

PP – Premature pubarche

C – Overcontrol

SBP – Systolic blood pressure

FMF – Fear of maturity

WHO – World Health Organization

PCOS – Polycystic ovary syndrome

CVD – Cardiovascular disease

T – Testosterone

TG – Triglycerides

FSH – Follicle-stimulating hormone

ACTH – Adrenocorticotropic hormone

AMH – Anti-Müllerian hormone

CRP – C-reactive protein

DXA – Dual-energy X-ray absorptiometry

DHEA – Dehydroepiandrosterone

DHEA-S – Dehydroepiandrosterone sulfate

Fat % – Percentage of fat mass

HDL – High-density lipoprotein cholesterol

LBM – Lean body mass

LDL – Low-density lipoprotein cholesterol

LM – Lean mass (muscle mass)

QoL – Quality of life

SHBG – Sex hormone-binding globulin

PART I. INTRODUCTION

Congenital adrenal hyperplasia (CAH) constitutes a heterogeneous group of autosomal recessive enzymatic disorders that fundamentally impair the synthesis of glucocorticoids and/or mineralocorticoids within the adrenal cortex. In more than 95% of all reported cases, the underlying defect is attributable to a deficiency of the steroidogenic enzyme 21-hydroxylase (21-OH), which is crucial in cortisol and aldosterone biosynthesis. The absence or insufficiency of this enzyme results in the accumulation of intermediate precursors, most notably 17-hydroxyprogesterone (17-OHP), with a consequent diversion of steroidogenesis toward enhanced adrenal androgen production. The resultant state of hyperandrogenism underlies the broad spectrum of clinical manifestations observed across the CAH continuum, ranging from the severe classical phenotypes that present in the neonatal period to the milder, non-classical variants that typically become clinically apparent later in life.

The non-classical form of CAH (NCCAH) was initially described by Decourt and colleagues in 1957, and subsequently genetically and molecularly characterized in 1984 by White, New, and Dupont. NCCAH is recognized as a monogenic autosomal recessive disorder, predominantly arising from mutations in the **CYP21A2** gene, which is located on the short arm of chromosome 6 (6p21). Epidemiological data indicate that the prevalence of NCCAH varies between 1:100 and 1:500 live births, with more than 90% of cases being the consequence of recombination events between the functional gene and its adjacent pseudogene. Less frequently, gene conversion events and uniparental isodisomy have also been implicated as pathogenic mechanisms.

The clinical expression of CAH is highly variable and is largely determined by the degree of residual 21-OH enzymatic activity. In classical forms ("salt-wasting" or "simple virilizing"), in which residual activity is typically below 2%, the clinical presentation is characterized by severe symptomatology evident already in the neonatal period. In contrast, in NCCAH the enzymatic activity is only partially compromised (approximately 20–70% of normal activity), which accounts for the generally milder and later clinical onset. Symptomatology, predominantly driven by hyperandrogenism, may arise during prepubertal years, at puberty, or in adulthood, and encompasses premature pubarche, hirsutism, acne, menstrual irregularities, anovulation, polycystic ovarian morphology, and disturbances in growth velocity and bone maturation. In adult women, the predominant clinical features are hirsutism and reduced fertility potential, often accompanied by significant psychological distress. A central challenge in the

management of NCCAH lies in the well-documented poor correlation between genotype and phenotype, which frequently results in delayed or erroneous diagnosis, with the condition being misclassified as other hyperandrogenic disorders. This diagnostic ambiguity underscores the conceptualization of NCCAH as a "mosaic of the known and the unknown."

In recent years, increasing scientific attention has been directed toward elucidating the long-term sequelae of chronic hyperandrogenism in women with NCCAH. Accumulating evidence suggests a potential association between androgen excess and the development of several components of the metabolic syndrome, including insulin resistance, dyslipidemia, arterial hypertension, central obesity, and type 2 diabetes mellitus. It has been hypothesized that visceral adiposity, at least partially driven by androgen excess, contributes to adverse alterations in body composition, thereby conferring an elevated cardiometabolic risk in affected women.

Beyond the hormonal and metabolic dimensions, the diagnosis, management, and long-term follow-up of women with NCCAH carry substantial psychosocial implications. Patients frequently report diminished self-esteem, impaired social integration, and reduced quality of life, particularly in the context of delayed diagnosis and inadequate therapeutic control. Consequently, chronic hyperandrogenism, alterations in body image, and fertility challenges (both current and anticipated) emerge as key determinants of compromised psycho-emotional well-being. Nevertheless, systematic investigations that comprehensively explore the complex interplay between hormonal dysregulation, metabolic status, body composition, and psychosocial adaptation in NCCAH remain sparse.

Against this background, the necessity for more profound and integrative research into NCCAH as a chronic multisystem condition, with significant potential for long-term complications, becomes evident. The scientific and clinical relevance of this topic is determined not only by the relatively high prevalence of the disorder but also by its under-recognition and the existing opportunities for early intervention, which may substantially improve patient outcomes and overall quality of life. A deeper understanding of the pathophysiological interrelations among hyperandrogenism, metabolic risk, alterations in body composition, and psycho-emotional health is expected to provide the foundation for the development of optimized diagnostic algorithms and therapeutic strategies. Such knowledge will facilitate the implementation of a multidisciplinary, patient-centered approach to the management of this complex, yet frequently underestimated, clinical entity.

PART II. RATIONALE, AIM, AND SCIENTIFIC OBJECTIVES OF THE STUDY

1. Rationale for the selection of the research topic

1.1 The limited number of scientific studies in the international literature addressing various aspects of non-classical congenital adrenal hyperplasia (NCCAH), particularly during childhood and adolescence, in combination with the presence of contradictory findings.

1.2 The small number of studies, often reporting inconsistent results, regarding the relationship between hyperandrogenism in NCCAH and the presence of metabolic and cardiovascular disturbances.

1.3 The extremely scarce data in the international literature concerning body composition in patients with NCCAH and its association with hyperandrogenism.

1.4 The very limited evidence available worldwide with respect to psychological assessment and quality of life in patients with NCCAH.

1.5 A comprehensive and systematic evaluation of female patients with NCCAH would represent a pioneering effort at the national level and one of the few such investigations on a global scale.

1.6 Conducting this dissertation as an integrative and systematic study of women with NCCAH will significantly enhance current knowledge of this disorder, which frequently remains under-recognized or is misclassified as other hyperandrogenic conditions.

2. Aim

The aim of the present dissertation is to evaluate the cardiometabolic risk, body composition, psychological perception, and quality of life in women diagnosed with non-classical congenital adrenal hyperplasia (NCCAH), by examining their association with the presence of clinical and/or biochemical hyperandrogenism and comparing the results with those obtained from healthy controls.

3. Scientific Objectives

3.1 To evaluate the clinical and laboratory profile of adolescent girls and women up to 30 years of age diagnosed with NCCAH.

3.2 To determine the prevalence of overweight and obesity among adolescent girls and women up to 30 years of age diagnosed with NCCAH.

3.3 To identify the type and frequency of cardiometabolic abnormalities (dyslipidemia, insulin resistance, hypertension, and disturbances in glucose homeostasis) in adolescent girls and women up to 30 years of age diagnosed with NCCAH, in comparison with healthy controls.

3.4 To analyze the association between cardiometabolic risk and the presence of clinical and/or biochemical hyperandrogenism in adolescent girls and women up to 30 years of age diagnosed with NCCAH.

3.5 To compare serum levels of selected adipokines (adiponectin and leptin) between patients with NCCAH and healthy controls, and to explore their potential associations with indicators of cardiometabolic risk and hyperandrogenism.

3.6 To assess body composition in adolescent girls and women up to 30 years of age diagnosed with NCCAH, in comparison with healthy controls, and to investigate its association with the clinical and laboratory characteristics of adrenal hyperandrogenism as well as with indicators of cardiometabolic disturbances.

3.7 To evaluate psychosocial and psycho-emotional adaptation and behavior in patients with NCCAH in comparison with healthy controls.

3.8 To assess quality of life in adolescent girls and women up to 30 years of age diagnosed with NCCAH, in comparison with healthy controls.

PART III. STUDY DESIGN, PARTICIPANTS, AND METHODS

1. Study design and participant selection

The clinical investigation was conducted according to a **case–control study protocol**, targeting adolescent girls and young women diagnosed with non-classical congenital adrenal hyperplasia (NCCAH) and age- and sex-matched healthy controls. Over a three-year period (2019–2022), a comprehensive evaluation of potential participants was carried out at the First Pediatric Clinic, the Department of Pediatric Endocrinology, and additional clinical and research units of the University Hospital "St. Marina" Varna, including the Department of Imaging Diagnostics and the Clinical Laboratory.

Participant recruitment was performed through systematic review of the patient registries of the Pediatric Endocrinology Clinic at University Hospital "St. Marina" Varna, as well as through targeted referral of patients and healthy controls by pediatric endocrinologists and metabolic disease specialists working in outpatient settings in Varna, Dobrich, Ruse, and Burgas. After receiving detailed information about the study procedures, potential participants were screened according to predefined inclusion and exclusion criteria for both the "NCCAH patient" group and the corresponding "healthy control" group.

Inclusion criteria for NCCAH patients:

- Age between 8 and 30 years
- Confirmed diagnosis of NCCAH
- Signed informed consent (by parent/guardian for participants under 18 years of age, or by the participant herself if ≥18 years)

Exclusion criteria for NCCAH patients:

- Age below 8 or above 30 years
- Presence of another hyperandrogenic disorder
- Refusal to provide informed consent

Inclusion criteria for controls:

- Age between 8 and 30 years
- Absence of endocrine or chronic disease

- Signed informed consent (by parent/guardian for minors, or by the participant herself if ≥18 years)

Exclusion criteria for controls:

- Age below 8 or above 30 years
- Presence of underlying endocrine or chronic disease
- Refusal to provide informed consent

The study protocol was reviewed and approved by the **Research Ethics Committee** of the Medical University of Varna (Decision No. 88, 28.11.2019) prior to initiation of participant enrollment (Appendix 1). Partial funding was provided through Project No. 20026/2021–2023 of the "Science Fund" of the Medical University of Varna (Appendix 2).

Participation in the study was entirely voluntary, with the option to withdraw at any time without providing a reason. All participants received both oral and written information about the study and the planned procedures, and were encouraged to ask questions, which were answered by the investigators. The study process for each participant commenced upon signing the **Informed Consent Form** (Appendix 3).

To ensure compliance with ethical requirements regarding data anonymity, each participant was assigned an individual, anonymized identification code.

2. Study methods

2.1 Structured interview (Appendix 4)

During their scheduled visit at the University Hospital "St. Marina" Varna, each participant (accompanied by a parent/guardian if under 18 years of age) was interviewed using a structured questionnaire covering the following:

- Date of birth
- Maternal and perinatal history (pregnancy, delivery, gestational age, birth weight, neonatal period)
- Infant feeding practices and introduction of complementary foods

- History of previous or concomitant chronic diseases
- Family history of socially significant diseases, hyperandrogenic conditions, or disorders among first- and second-degree relatives
- Family history of reproductive failures among first- and second-degree relatives
- Current and past medication use
- Age at diagnosis (for NCCAH patients)
- Clinical symptoms (hirsutism, acne, menstrual disorders, etc.), with detailed data on age of onset and progression

2.2 Anthropometric measurements

All anthropometric measurements were performed in the morning of the study visit by the same trained investigator using standardized equipment.

- **Weight (W)** was measured to the nearest 0.1 kg using a calibrated digital scale SECA 861 (SECA Ltd, Hamburg, Germany), with participants barefoot and in light clothing.
- **Height (H)** was measured twice to the nearest 1 mm using a certified medical stadiometer Harpenden 2000 (Hamburg, Germany), in a standing position with the head aligned in the horizontal Frankfurt plane, without shoes or outerwear.
- **Waist circumference (WC)** was measured twice to the nearest 0.1 cm using a non-stretchable SECA 201 measuring tape (SECA Ltd, Hamburg, Germany), placed at the midpoint between the lower margin of the 10th rib and the iliac crest along the mid-axillary line. Measurements were performed at the end of a gentle expiration, with participants standing upright and without upper clothing.

To assess overweight (OW) and obesity, the **Body Mass Index (BMI)** was calculated using the standard formula:

$$\text{BMI} = \text{Weight (kg)} / \text{Height (m}^2\text{)}$$

The classification of overweight and obesity was based on **International Obesity Task Force (IOTF)** reference values for children and adolescents, adjusted for age and sex. Overweight was defined as a BMI between the 85th and 95th percentile, corresponding to BMI values of

25.00–29.99 kg/m² in adults. Obesity was defined as a BMI above the 95th percentile, corresponding to a BMI ≥30 kg/m² in adults (Quetelet index) (216).

Abdominal obesity was defined as a waist circumference ≥90th percentile for age (217).

2.3 Clinical examination

All participants underwent a comprehensive physical examination with particular attention to clinical manifestations of hyperandrogenism, such as hirsutism, acne, alopecia, excessive scalp seborrhea, clitoromegaly, and others.

- **Assessment of hirsutism** was performed using a modified version of the Ferriman–Gallwey scoring system (Appendix 5), applying a semi-subjective analysis of hair growth in nine androgen-sensitive body areas. Each area was graded from 0 (absence of terminal hair) to 4 (extensive terminal hair growth). A total score ≥8 was considered indicative of clinical hirsutism.
- **Pubertal development** was staged according to the Tanner method, with additional recording of age at menarche and genital examination for the presence of clitoromegaly.
- **Blood pressure (BP)** was measured with a sphygmomanometer using a cuff size appropriate for the participant's age. The auscultatory method of Korotkoff was applied, measuring BP on the right arm, with the participant seated, after 30 minutes of rest, and with the cuff positioned at heart level (American Heart Association, 2019). Systolic BP (SBP) was recorded at the appearance of the first clear sound (Korotkoff phase I), and diastolic BP (DBP) at the disappearance of sounds (Korotkoff phase V). Measurements were taken three times at 2-minute intervals; the arithmetic mean of the last two readings was used for analysis for both SBP and DBP.
- **Heart rate (HR)** was determined by palpation of the radial artery in the seated position, recorded over a period of one minute.

2.4 Laboratory biochemical and hormonal analyses

Biochemical and hormonal laboratory assessments were performed at the **Central Clinical Laboratory of University Hospital "St. Marina" Varna**. All blood samples were obtained from peripheral venous blood under standardized conditions — in the morning, after a 12-hour

overnight fast, and with minimal discomfort for the participants. Serum was isolated using gel-separator vacutainers and centrifuged for 15 minutes at 2500 g. For assays of 17-hydroxyprogesterone (17-OHP), adiponectin, and leptin, sera were aliquoted into additive-free vacutainers and stored at –80 °C until analysis. All assays were conducted after a single freeze–thaw cycle by the specialized laboratory team.

The following parameters were measured:

- **Blood glucose (mmol/L):** enzymatic hexokinase method, analyzed using ADVIA Chemistry 1800 (Siemens). Reference range: 3.3–5.6 mmol/L.
- **Lipid profile:**
    - Total cholesterol (mmol/L): enzymatic determination with cholesterol esterase, cholesterol oxidase, and peroxidase (CHE-CHOD-POD method) and colorimetric Trinder reaction. Reference range: 2.7–5.2 mmol/L.
    - Triglycerides (TG, mmol/L): enzymatic hydrolysis with lipase, followed by colorimetric determination of glycerol using glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase (GPO-POD Trinder method). Reference range: 0.4–1.77 mmol/L.
    - High-density lipoprotein cholesterol (HDL-C, mmol/L): determined after precipitation of LDL and VLDL, analyzed from the supernatant. Reference range: 1.03–1.55 mmol/L.
    - Low-density lipoprotein cholesterol (LDL-C, mmol/L): calculated using the Friedewald formula. Reference range: ≤2.6 mmol/L.
      All lipid parameters were analyzed on ADVIA Chemistry 1800 (Siemens).
- **C-reactive protein (CRP, mg/L):** measured on ADVIA Chemistry 1800 (Siemens) using an immunoturbidimetric assay. Reference range: 0–5 mg/L.
- **Human insulin:** determined using the DRG Insulin Enzyme Immunoassay Kit (sandwich ELISA method) for quantitative measurement in human serum/plasma. The assay detection range was 0–100 μIU/mL with a sensitivity of 1.76 μIU/mL. Reference range: 2–29.1 μIU/mL.

Values of fasting blood glucose and insulin were used to calculate the **Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)**, according to the formula:

HOMA-IR=Fasting glucose (mmol/L)×Fasting insulin (μIU/mL)/22.5. HOMA-IR values >2.6 were considered indicative of the presence of insulin resistance (IR).

Hormonal assays

Hormonal parameters were also measured in the morning after an overnight fast, during the follicular phase of the menstrual cycle (days 3–5). In patients receiving hormonal therapy, treatment was discontinued at least 48 hours prior to blood sampling. The following assays were performed:

- **Luteinizing hormone (LH, IU/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); limit of detection (LOD) = 0.05 IU/L. Reference values in the follicular phase: 1.1–11.6 mIU/mL. Analyzer: Immulite 2000 Xpi, Siemens.
- **Follicle-stimulating hormone (FSH, IU/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.1 IU/L. Reference values in the follicular phase: 2.8–11.3 mIU/mL. Analyzer: Immulite 2000 Xpi, Siemens.
- **Estradiol (pmol/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.39 pmol/L. Reference values: ≤529 pmol/L. Analyzer: ADVIA Centaur CP, Siemens.
- **Testosterone (T, nmol/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.5 nmol/L. Reference values: 0–1.38 nmol/L. Analyzer: Immulite 2000 Xpi, Siemens.
- **Androstenedione (nmol/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.3 nmol/L. Reference values: 1–11.5 nmol/L. Analyzer: Immulite 2000 Xpi, Siemens.
- **Dehydroepiandrosterone sulfate (DHEA-S, μmol/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.08 μmol/L. Reference values: 0.95–11.67 μmol/L. Analyzer: Immulite 2000 Xpi, Siemens.
- **17-hydroxyprogesterone (17-OHP, ng/mL):** NovaTec, Germany; LOD = 0.05 ng/mL. Cross-reactivity: 11-deoxycortisol (0.846%), progesterone (0.590%), pregnenolone (0.250%), testosterone (0.017%); with aldosterone, androstenedione, cortisol, DHEA, prednisolone, cholesterol <0.01%. Applicable for both basal and stimulated serum concentrations. Reference values: children 0.2–0.9 ng/mL; women in follicular phase 0.2–1.3 ng/mL; luteal phase 1.0–4.5 ng/mL.

- **Sex hormone-binding globulin (SHBG, nmol/L):** chemiluminescent immunoassay (non-isotopic antibody labeling); LOD = 0.02 nmol/L. Reference values: 18–144 nmol/L. Analyzer: Immulite 2000 Xpi, Siemens.
- **Anti-Müllerian hormone (AMH, pmol/L):** electrochemiluminescent immunoassay; LOD = 0.21 pmol/L. Reference values: 2.1–84.1 pmol/L. Analyzer: Cobas e 601, Roche.
- **Prolactin (mIU/L):** chemiluminescent immunoassay; LOD = 10.6 mIU/L. Reference values: 40–530 μIU/mL. Analyzer: Immulite 2000 Xpi, Siemens.
- **Cortisol (nmol/L):** chemiluminescent immunoassay; LOD = 0.5 nmol/L. Reference values: 115.7–451.2 nmol/L. Analyzer: ADVIA Centaur CP, Siemens.
- **Adiponectin (μg/mL):** measured by ELISA (Human Adiponectin ELISA, BioVendor, Czech Republic). LOD = 0.026 μg/mL. Reference values (women): BMI <25.0 kg/m²: 13.6 ± 5.4 μg/mL; BMI 25.0–29.99 kg/m²: 13.9 ± 8.6 μg/mL; BMI ≥30.0 kg/m²: 11.4 ± 3.8 μg/mL.
- **Leptin (ng/mL):** measured by ELISA (Leptin-ELISA Kit, DIAsource, Belgium). LOD = 0.04 ng/mL. Reference values (women):
    - BMI 14–17.99 kg/m²: 0.5–0.7 ng/mL
    - BMI 18.0–24.99 kg/m²: 0.5–7.9 ng/mL
    - BMI 25.0–29.99 kg/m²: 4.1–14.5 ng/mL
    - BMI ≥30.0 kg/m²: 5.5–40.4 ng/mL

2.5 Ultrasound examination of the ovaries and uterus

Each participant underwent ultrasonographic evaluation of the ovaries and uterus in order to exclude the presence of abnormal structures or tumors. Examinations were performed using an ALOKA Prosound SSG-3500 ultrasound machine with a transabdominal transducer operating at a variable frequency of 3.5–6 MHz. All examinations were conducted by the same investigator, who was blinded to the participants' clinical status and laboratory results.

2.6 Densitometric assessment by dual-energy X-ray absorptiometry (DEXA)

DEXA scanning was performed at the Department of Imaging Diagnostics, using a LUNAR Prodigy Pro iDXA device (GE Healthcare, USA) equipped with CoreScan™ software (GE

Healthcare, USA). Daily calibration of the equipment was carried out using a phantom in accordance with the manufacturer's instructions.

DEXA is an X-ray imaging technique in which the X-ray tube emits radiation at two distinct energy peaks; as the beams pass through body tissues, differential attenuation occurs depending on the type of tissue traversed. The result of DEXA densitometry is the acquisition of data on the three principal body compartments: lean (muscle) mass, fat mass, and bone mass.

Whole-body osteodensitometry (total body less head, TBLH) was performed, with measurement of bone area (BA, cm^2), bone mineral density (BMD, g/cm^2), and bone mineral content (BMC, g). Using the integrated software, body composition parameters were automatically assessed, including total body fat mass (FM, g and %), lean body mass (LBM, g, comprising organs, skin, and muscle tissue), and regional fat distribution. Fat distribution was analyzed according to android and gynoid compartments, with calculation of the android/gynoid fat ratio.

- The android region was defined by a horizontal line at the upper edge of the iliac crest (lower boundary), and a second horizontal line cranially located at a distance corresponding to 20% of the distance between the iliac crest and the base of the skull (upper boundary).
- The gynoid region encompassed the hips and upper thighs, with the upper boundary located caudally to the iliac crest at a distance 1.5 times the height of the android region, and the lower boundary defined caudally at a distance equal to twice the height of the android region from the upper gynoid line.

2.7 Psychological assessment

Psychological evaluation was performed using the standardized **EDI-3 questionnaire** (Eating Disorder Inventory-3), consisting of 91 items organized into 12 primary scales (https://www.eat-26.com/eating-disorder-inventory-3) (Appendix 6). Nine of these scales are psychological in nature and assess: low self-esteem (LSE), personal alienation (PA), interpersonal insecurity (II), interpersonal alienation (IA), interoceptive deficits (ID), emotional dysregulation (ED), perfectionism (P), asceticism (A), and fear of maturity (FM). Three scales assess attitudes toward eating and body image: drive for thinness (DT), bulimia (B), and body dissatisfaction (BD).

The instrument also provides five composite psychological indices:

- Ineffectiveness (INE) – combining low self-esteem and personal alienation
- Interpersonal problems (IP) – combining interpersonal insecurity and interpersonal alienation
- Affective problems (AF) – combining interoceptive deficits and emotional dysregulation
- Overcontrol (O) – combining perfectionism and asceticism
- General psychological maladjustment (GPM) – integrating the nine psychological scales.

The results were presented through an interpretative graphical report in standardized **T-scores**, normalized for ages 10–73 years. Higher scores were interpreted as indicative of poorer psychological functioning and deteriorated mental health status.

2.8 Quality of life assessment

Quality of life was evaluated using the validated **WHOQOL-BREF 26 questionnaire** developed by the World Health Organization (Appendix 7) (https://www.who.int/tools/whoqol/whoqol-bref). The questionnaire assesses four domains: physical health (D1), psychological health (D2), social relationships (D3), and environment (D4).

- **Domain 1 (D1):** mobility, daily activities, functional capacity, energy, pain, and sleep.
- **Domain 2 (D2):** self-perception, negative thoughts, positive attitudes, self-esteem, outlook, learning ability, memory and concentration, religion, and overall psychological state.
- **Domain 3 (D3):** personal relationships, social support, and sexual life.
- **Domain 4 (D4):** financial resources, safety, health and social care services, opportunities for acquiring new skills and knowledge, leisure, physical environment (e.g., noise, air pollution), and transportation.

Each item was rated on a five-point ordinal scale, with subsequent linear transformation of results to a 0–100% scale. Higher percentages corresponded to higher perceived quality of life.

3. Medical-statistical data analysis

Statistical analysis was performed using the specialized software package **SPSS for Windows, version 25.0** (Chicago, IL, USA). Statistical significance was accepted at $p \leq 0.05$.

The following statistical methods were applied:

3.1. Statistical grouping method – variables were organized according to type into variational, interval, categorical, ordinal, and dynamic statistical series.

3.2. Statistical estimation methods:

- *Point estimates* – used to calculate arithmetic mean values of continuous variables.
- *Interval estimates:*
    - Confidence probability (significance level, *p*). At a confidence coefficient of 0.95 (95%), the type I error was 0.05 (5%).
    - Confidence intervals (CI). Ninety-five percent CIs around point estimates were used, interpreted as the probability that the interval contains the true value in 95% of cases.

3.3. Graphical methods – line graphs, surface diagrams, bar charts, and other graphical representations were employed.

3.4. Descriptive analysis – measures of central tendency and measures of dispersion were calculated.

3.5. Variance analysis (ANOVA):

- For comparison of continuous variables, Student–Fisher *t*-test was applied for independent samples.
- For multiple comparisons of categorical variables, Bonferroni correction of *p*-values was applied (ANOVA test).

3.6. Non-parametric analysis:

- For categorical variables, Pearson's chi-square (χ^2) test was used.

- Mann–Whitney and Kolmogorov–Smirnov tests were applied where appropriate, providing higher statistical power for non-normally distributed variables.

3.7. Correlation analysis:

- Univariate correlation was assessed using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation for non-normally distributed data.
- Partial multiple correlation analysis was applied when evaluating the relationship between two variables while adjusting for confounding factors.
- Correlation coefficients (r) were interpreted as follows:
    - $r < 0.30$ – weak correlation
    - $0.30 \leq r < 0.50$ – moderate correlation
    - $0.50 \leq r < 0.70$ – substantial correlation
    - $0.70 \leq r < 0.90$ – strong correlation
    - $r > 0.90$ – very strong correlation

3.8.Linear regression analysis:

To assess the independent effect of selected variables on a continuous dependent outcome, multivariate linear regression analysis was applied. Regression models expressed the dependent variable (y) as a function of independent predictors ($x1, x2, \ldots xn$) according to the formula:

$y=\beta 0+\beta 1x1+\beta 2x2+\ldots+\beta nxn$ where:

- y – dependent variable
- β_0 – intercept
- $\beta_1 \ldots \beta_n$ – regression coefficients of independent predictors
- $x1 \ldots xn$ – independent variables under evaluation.

A backward elimination method was used to exclude non-significant predictors from the regression equation, with entry and removal significance levels set at 0.05 and 0.1, respectively.

PART IV. OWN RESULTS

1. General characteristics of the study participants

After application of all inclusion and exclusion criteria defined in the study protocol and following the provision of informed consent, a total of **68 adolescent girls and young women**, aged between 10 and 27 years, were enrolled in the study.

For the purposes of addressing the research objectives, participants were allocated into two groups:

a) **Patients with NCCAH** – adolescent girls and young women with a protocol-confirmed diagnosis of non-classical congenital adrenal hyperplasia, hereafter referred to as "patients" or "NCCAH" (n = 34).

b) **Controls** – adolescent girls and young women matched to the patients by demographic and weight status (n = 34).

The mean age of all participants was **15.6 ± 3.3 years** (median: 15.5 years; range: 13.0–17.0 years). No statistically significant difference was observed in the age distribution between the two groups (p = 0.147) (**Table 1**).

Table 1. Age characteristics of the participants by study groups

Parameter	***NCCAH*** *(n=34)*	***CONTROLS*** *(n=34)*	***p***
Age (years)	16,2±3,4 {16,0 (13,7-17,7)}	15,0±3,1 {15,0 (13,0-17,0)}	ns

The results are presented as mean ± SD {median (25th–75th percentile)}; ns – non-significant difference.

The mean age at diagnosis of NCCAH among the adolescent girls and young women included in the study was 14.4 ± 4.0 years (range: 6–24 years). At the time of evaluation, approximately half of the patients were receiving chronic therapy with hydrocortisone (44.1%, n = 15) or another oral medication **(**5.9%, n = 2).

Pubertal development was assessed in all participants using **the Tanner staging system**. It was established that **60.3%** of the examined girls and young women had completed pubertal

development, **35.3%** were in Tanner stage IV, and only 3 participants were classified as stage III. None of the participants were in prepubertal (stage I) or early pubertal (stage II) development.

When data were analyzed according to group allocation, a significantly higher proportion of adolescent girls and young women with NCCAH were found to have completed pubertal development compared with controls (p = 0.029) (**Table 2**).

Table 2. Comparison of pubertal development stage of participants by study groups

Tanner stage	***NCCAH** (n=34)*	***CONTROLS** (n=34)*
III ст.	1 (2,9)	2 (5,9)
IV ст.	8 (23,5)	16 (47,1)
V ст.	25 (73,5)	16 (47,1)

Results are presented as number of participants (percentage).

2. Perinatal history and family burden of hyperandrogenic and reproductive disorders

During the structured interview, several risk factors were assessed for all participants, including birth weight, birth length, and gestational age at birth, as well as the presence of a family history of hyperandrogenic conditions and/or reproductive problems among first-degree relatives (**Table 3**).

Table 3. Perinatal characteristics and family history of participants by study groups

PARAMETER	***NCCAH** (N=34)*	***CONTOLS** (N=34)*	***P-VALUE***
birth weight (kg)	3115,0±427,4	3197,0±544,7	ns
birth length (cm)	49,8±2,0	49,5±2,6	ns
gestational age (weeks)	38,7±1,0	38,3±1,0	ns
family history of hyperandrogenism/menstrual disorders (%)	17,6 (n=6)	0 (n=0)	***0,011***
maternal age at menarche (years)	12,2±1,2	11,9±1,2	ns

Results are presented as mean ± SD or percentage; ns – non-significant difference. $p < 0.05$ was considered statistically significant.

Preterm birth was reported in **5.8%** of participants with NCCAH. No significant differences were observed between the two groups with respect to auxological parameters or morphological maturity at birth ($p > 0.05$). Partial correlation analysis between birth weight/length and current anthropometric parameters revealed that only in the control group did current body weight correlate significantly with birth weight (**r = 0.402, *p* = 0.018**).

Directed interviews regarding the age at menarche of the participants mothers revealed no significant differences between the two groups for this parameter (Table 3). However, a significantly higher frequency of family history of hyperandrogenic or menstrual disorders was reported among families of NCCAH patients compared with controls ($p = 0.011$). Importantly, none of the participants reported a family history of reproductive failure among first- or second-degree relatives.

3. Auxological characteristics of NCCAH participants and controls, and prevalence of overweight and obesity

In accordance with the study protocol, all adolescent girls and young women underwent auxological measurements, followed by detailed clinical evaluation.

No statistically significant differences were identified between the two groups with respect to the assessed auxological parameters, nor in the indices of generalized and abdominal obesity (BMI and WC) (Table 4). Likewise, no significant differences in these parameters were observed when comparing NCCAH participants receiving versus not receiving pharmacological therapy ($p > 0.05$).

Table 4. Anthropometric data of study participants

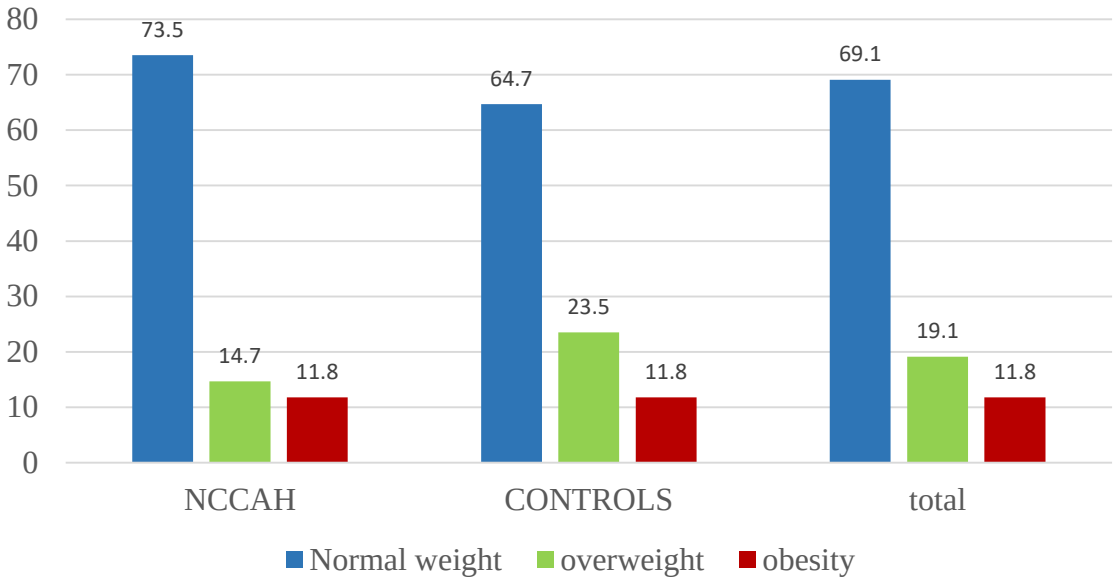
PARAMETER	***NCCAH*** *(N=34)*	***CONTROLS*** *(N=34)*	***P-VALUE***
weight (kg)	57,3±12,0	58,9±9.2	ns
height (cm)	159,1±6,6	161,4±6,0	ns
BMI (kg/m²)	22,6±4,3	22,4±3,2	ns
Waist circumference (cm)	74,9±9,7	72,7±7,4	ns

Data are presented as mean ± SD; ns – non-significant difference.

Using partial correlation analysis, while controlling for age, pubertal stage, and ongoing therapy, the expected positive correlation between BMI and WC was confirmed both in the NCCAH group (**$r = 0.723$, $p < 0.0001$**) and among the adolescent girls and young women in the control group (**$r = 0.650$, $p < 0.0001$**).

Applying the international age- and sex-specific BMI reference values proposed by **Cole et al.** for the 85th and 95th percentiles, together with the corresponding diagnostic cut-offs for adults, the prevalence of generalized overweight (OW) and obesity (OB) was determined for the study population, including subgroup analysis by study group (**Figure 1**).

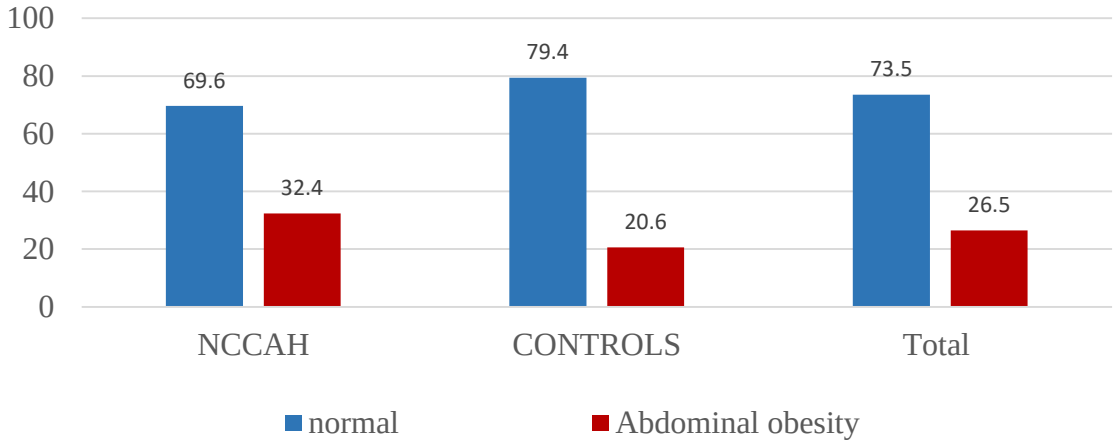
Figure 1. Prevalence of generalized overweight and obesity



It was established that slightly over **30%** of all participants were classified as overweight or obese, with no statistically significant difference in the distribution of categories between the two groups (p = 0.643).

Applying the national reference values for waist circumference (WC) for Bulgarian girls, and the IDF criteria for participants aged ≥18 years, the relative prevalence of abdominal obesity was calculated (**Figure 2**). Overall, **26.5%** of the adolescent girls and young women enrolled in the study were found to have abdominal obesity at the time of assessment.

Figure 2. Prevalence of abdominal obesity in NCCAH patients and controls %



Although approximately one-third of the NCCAH patients included in the analysis and only one-fifth of the corresponding controls were classified as having central obesity, the difference in the distribution between the two groups did not reach statistical significance ($p = 0.275$).

4. Clinical symptomatology and assessment of hyperandrogenism among study participants

Based on the structured interview and clinical examination, several leading subjective complaints and clinical symptoms associated with hyperandrogenism were evaluated.

A history of premature pubarche (PP) was reported by **27.9%** of the participants (n = 19). The proportion of adolescent girls and young women with NCCAH who reported early pubarche was approximately twice as high as that of the controls with a similar medical history **(38.2% vs. 17.6%, $p = 0.06$).**

The clinical profile of girls and adolescents from both study groups is presented in **Table 5**. Although the prevalence of androgen-related symptoms was significantly higher among NCCAH patients, no cases of clitoromegaly were identified during physical examination in either group.

Table 5. Prevalence of clinical manifestations of hyperandrogenism by group

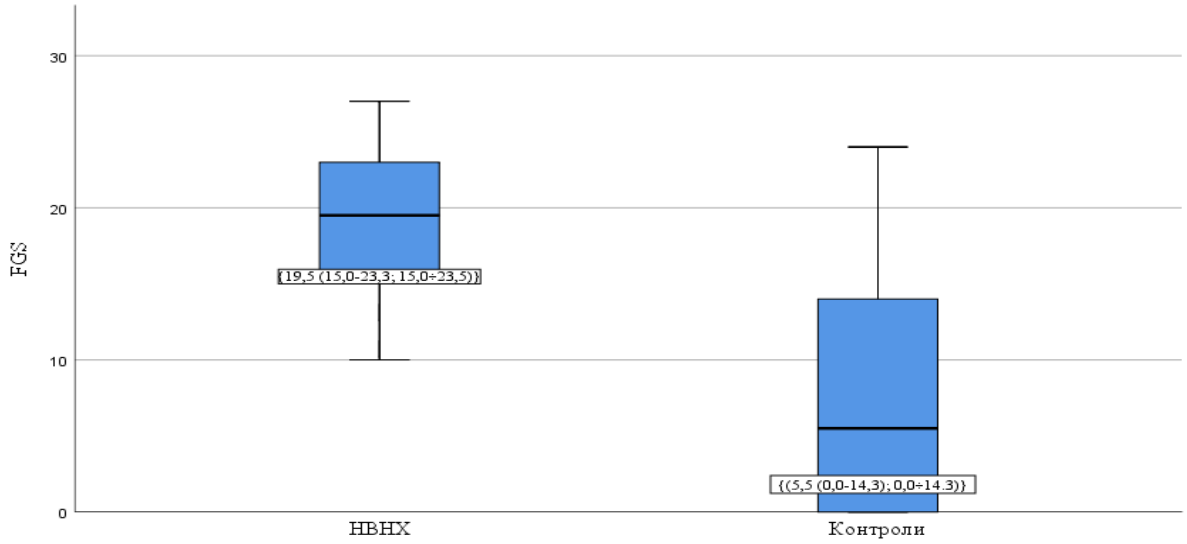
CLINICAL MANIFESTATION	***NCCAH** (N=34)*	***CONTROLS** (N=34)*	***P-VALUE***
hirsutism	97,1	23,5	***<0,0001***
acne	94,1	23,5	***<0,0001***
alopecia	64,7	11,8	***<0,0001***
Oily hair	79,4	11,8	***<0,0001***

Results are presented as percentages; ns – non-significant difference.

The presence of hirsutism was assessed using a modified version of the Ferriman–Gallwey scale (FGS), evaluating hair growth in nine androgen-dependent areas, each scored from 0 to 4. The analysis demonstrated significantly higher calculated FGS values in women with NCCAH compared to the corresponding control group ($p<0.0001$) (**Figure 3**).

Additional comparison within the NCCAH group, stratified by treatment status, revealed that participants “on therapy” exhibited significantly higher clinical androgen scores compared to those without pharmacological treatment {22 (19–25; 10–27) vs. 15 (13.5–20; 10–25)}, p=0.017.

Figure 3. Clinical hirsutism score according to group affiliation.
Data are presented as median (IQR 25–75; min–max) (Mann–Whitney U test, p<0.0001).



The comparison of auxological parameters, age at menarche, and calculated FGS in participants diagnosed with NCCAH, stratified according to their history of premature pubarche (PP), is presented in Table 6. It was demonstrated that girls with a history of PP (n=13) had significantly lower body weight and BMI, with only one participant classified as overweight according to generalized criteria (p<0.05).

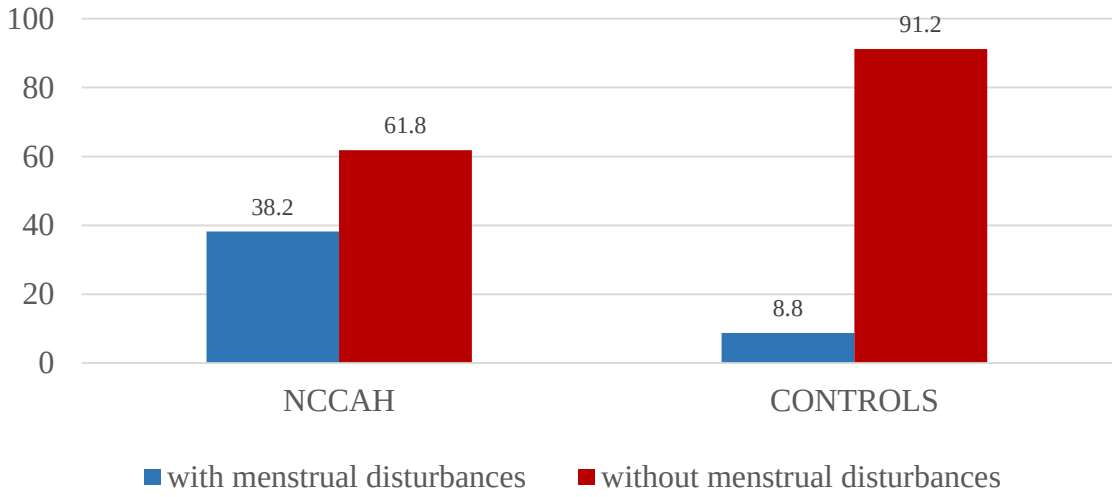
Table 6. Comparison of NCCAH participants according to history of premature pubarche (PP).

PARAMETER	***PP-POSITIVE (N=13)***	***PP-NEGATIVE (N=21)***	***P-VALUE***
Age (years)	16,8±3,0	15,8±3,7	ns
Weight (kg)	52,4±8,3	60,3±13,1	***0,038***
Height (cm)	158,7±5,0	159,4±7,6	ns
BMI (kg/m²)	20,9±3,2	23,7±4,6	***0,039***
Waist circumference (cm)	71,6±6,0	77,0±11,1	ns
Age at menarche (years)	11,2±1,6	11,8±1,1	ns
FGS	17,6±5,6	19,3±5,2	ns
Overweight/Obesity (%)	7,7/0	19/19	***0,016***
Abdominal obesity (%)	15,4	42,9	ns

Data are presented as mean ± SD or %; ns – non-significant difference.

Among the adolescent girls and young women examined in our study, a significant difference in the prevalence of menstrual disturbances was observed between patients with NCCAH and the corresponding controls ($p = 0.005$) (**Figure 4**).

Figure 4. Menstrual disturbances among participants by group (%).



** p=0,005

Table 7 presents the comparison of NCCAH participants with and without menstrual disturbances in relation to selected auxological and clinical characteristics. Although adolescent girls and young women with NCCAH and menstrual disturbances were approximately three years older than those without menstrual problems (p = 0.007), the comparison between these two subgroups did not demonstrate statistical significance for the other analyzed characteristics.

Table 7. Auxological and clinical characteristics of NCCAH patients according to the presence of menstrual disturbances.

PARAMETER	***WITH MENSTRUAL DISTURBANCES (N=13)***	***WITHOUT MENSTRUAL DISTURBANCES (N=21)***	***P-VALUE***
Age (years)	18,2±3,8	15,0±2,5	***0,007***
Weight (kg)	60,2±16,6	55,5±8,0	ns
Height (cm)	157,5±8,2	160,1±5,5	ns
BMI (kg/m²)	24,2±5,6	21,7±2,9	ns
Waist circumference (cm)	75,7±13,7	74,5±6,6	ns
Age at menarche (years)	11,9±1,2	11,4±1,4	ns
FGS	17,7±4,8	19,3±5,7	ns
Overweight/Obesity (%)	15,4/23,1	14,3/5,8	ns
Abdominal obesity (%)	30,8	33,3	ns
Hirsutism (%)	92,3	100	ns
Acne (%)	84,6	100	ns
Alopecia (%)	76,9	57,1	ns
Oily hair (%)	92,3	71,4	ns

Data are presented as mean ± SD or %; ns – non-significant difference.

A significant positive correlation was demonstrated between certain anthropometric parameters, FGS, and the clinical symptoms of hyperandrogenism after adjusting for the effects of age, pubertal stage of development, and group affiliation of the participants (**Table 8**).

Partial correlation analysis revealed a moderate to strong association between the absolute FGS value and indices of general/abdominal obesity ($p<0.01$), as well as clinical signs of hyperandrogenism ($p<0.001$). These findings confirm the significant influence of androgens on both the scalp (manifesting as seborrhea and androgenic alopecia) and on hair follicles and sebaceous glands of the skin (manifesting as hirsutism and acne) (**Table 8**).

Table 8. Correlation coefficients (*r*) between selected anthropometric parameters and manifestations of hyperandrogenism.

MD: menstrual disturbances

	FGS	***HIRSUTISM***	***ACNE***	***ALOPECIA***	***OILY HAIR***	***MD***
WC	***0,369****	***0,335****	0,157	0,131	0,204	0,152
BMI	0,207	***0,281****	0,059	0,108	0,228	0,201
hirsutism	***0,543****	-	***0,388****	0,106	0,562	0,238
acne	***0,344****	***0,388****	-	***0,268****	0,104	0,092
alopecia	***0,302****	0,106	***0,268****	-	***0,696****	***0,277****
Oily hair	***0,329****	0,562	0,104	***0,696****	-	***0,456****
MD	0,079	0,238	0,092	***0,277****	***0,456****	-

- Correlation is significant at the 0.05 level (2-tailed).

  ** Correlation is significant at the 0.01 level (2-tailed).

The ultrasonographic examination demonstrated normal ovarian structure in all adolescent girls and young women included in the study (both NCCAH patients and controls), with the presence of single ovarian cysts observed only in two NCCAH participants ($p>0.05$).

5. Hormonal profile of the study participants

Following the initial evaluation and diagnosis of patients with NCCAH, laboratory analyses were performed in all participants to assess the following hormonal parameters: LH, FSH, estradiol, testosterone, DHEA-S, androstenedione, 17OHP, SHBG, AMH, prolactin, and cortisol (**Table 9**).

Table 9. Hormonal parameters of the participants according to group affiliation

PARAMETER	***NCCAH*** *(N=34)*	***CONTROLS*** *(N=34)*	***P-VALUE***
LH (mUI/mL)	5,5±3,2	5,4±3,8	ns
FSH (mUI/mL)	5,5±2,5	4,7±1,9	ns
Estradiol (pmol/l)	270,3±152,3	399,5±303,5	***0,031***
Testosterone (nmol/l)	1,6±0,7	1,3±0,6	***0,025***
Androstendione (nmol/l)	15,4±6,7	9,8±5,3	***<0,0001***
DHEA-s (umol/l)	8,0±5,5	6,8±3,7	ns
17OHP (nmol/l)	2,2±1,3	0,8±0,6	***<0,0001***
SHBG (nmol/l)	34,5±17,6	39,8±24,6	ns
AMH (pmol/l)	29,7±25,6	33,3±27,3	ns
Prolactin (ng/ml)	275,7±185,3	242,4±126,2	ns
Cortisol (nmol/l)	444,4±196,8	424,0±152,1	ns

Data are presented as mean ± SD or %; ns – non-significant difference.

Significantly higher serum levels of testosterone, androstenedione, and 17OHP were observed in NCCAH patients compared to participants in the control group ($p<0.05$), whereas the latter demonstrated higher serum estradiol concentrations ($p = 0.031$). Additional comparison of hormonal parameters within the NCCAH group, stratified according to the presence or absence of ongoing pharmacological therapy, revealed higher mean values among those receiving treatment (T, 1.8 ± 0.7 vs. 1.5 ± 0.7 nmol/L, $p = 0.273$; A, 18.4 ± 5.2 vs. 13.6 ± 6.8 nmol/L, $p = 0.038$; DHEA-S, 8.2 ± 4.3 vs. 8.2 ± 1.7 μmol/L, $p = 0.987$; 17OHP, 2.2 ± 1.1 vs. 2.1 ± 1.4 nmol/L, $p = 0.759$).

Table 10 presents the correlation, adjusted for age, pubertal stage of development, and group affiliation, between the clinical hirsutism score (FGS) and selected hormonal parameters.

Table 10. Partial correlation coefficient (*r*)

	FGS	***T***	***17OHP***	***DHEA-S***	***A***	***SHBG***
FGS	-	***0,260****	***0,390****	0,029	***0,528****	-0,181
T	-	-	-0,010	***0,418****	***0,512****	-0,165
17OHP	-	-	-	-0,055	0,100	-0,073
DHEA-S	-	-	-	-	0,179	-0,193
A	-	-	-	-	-	-0,105
SHBG	-	-	-	-	-	-

Abbreviations: 17OHP – 17-hydroxyprogesterone; T – testosterone; DHEA-S – dehydroepiandrosterone sulfate; SHBG – sex hormone-binding globulin; A – androstenedione.

- Correlation is significant at the 0.05 level (2-tailed).
  ** Correlation is significant at the 0.01 level (2-tailed).

It was demonstrated that serum concentrations of androstenedione and 17OHP represent predictive hormonal markers with significant relevance for the development of hirsutism, acne, alopecia, and menstrual disturbances among the studied participants, independent of age, pubertal stage of development, and group affiliation (Tables 11–15).

Table 11. Multiple linear regression analysis with "FGS"

	Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
Model	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	-1.762	2.492		-.707	.483	-6.792	3.267
Androstendione	.711	.163	.510	4.352	***.000***	.381	1.040
17OHP	3.523	.971	.425	3.629	***.001***	1.564	5.483

dependent variable: FGS

Table 12. Multiple linear regression analysis with “clinical hirsutism”

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	(Constant)	-.149	.143		-1.043	.303	-.436	.139
	Androstendione	.034	.009	.427	3.615	***.001***	.015	.053
	17OHP	.235	.056	.499	4.223	***.000***	.123	.347

dependent variable: hirsutism

Table 13. Multiple linear regression analysis with “acne”

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	(Constant)	-1.280	.469		-2.731	.009	-2.228	-.333
	Testosterone	.501	.099	.621	5.084	***.000***	.302	.700
	17OHP	.260	.053	.551	4.874	***.000***	.152	.367
	Age	-.043	.019	-.298	-2.251	***.030***	-.082	-.004
	Tanner	.311	.111	.336	2.797	***.008***	.086	.536

Table 14. Multiple linear regression analysis with “alopecia”

Model	Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	-.190	.152		-1.255	.216	-.496	.116
Androstendione	.029	.010	.386	2.902	***.006***	.009	.049
17OHP	.154	.059	.347	2.607	***.013***	.035	.273

Dependent variable: alopecia

Table 15. Multiple linear regression analysis with “menstrual disturbances”

Model	Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	-.307	.122		-2.509	.016	-.553	-.060
Androstendione	.029	.008	.461	3.649	***.001***	.013	.045
17OHP	.137	.048	.364	2.879	***.006***	.041	.233

Dependent variable: menstrual disturbances

6. Cardiometabolic parameters in participants according to group affiliation

To address some of the study objectives, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured in all participants, along with an assessment of glucose and lipid metabolism parameters.

6.1. Blood pressure and heart rate

The analysis of the data revealed no significant differences in SBP, DBP, and HR values between patients with NCCAH and healthy controls ($p>0.05$) (**Table 16**).

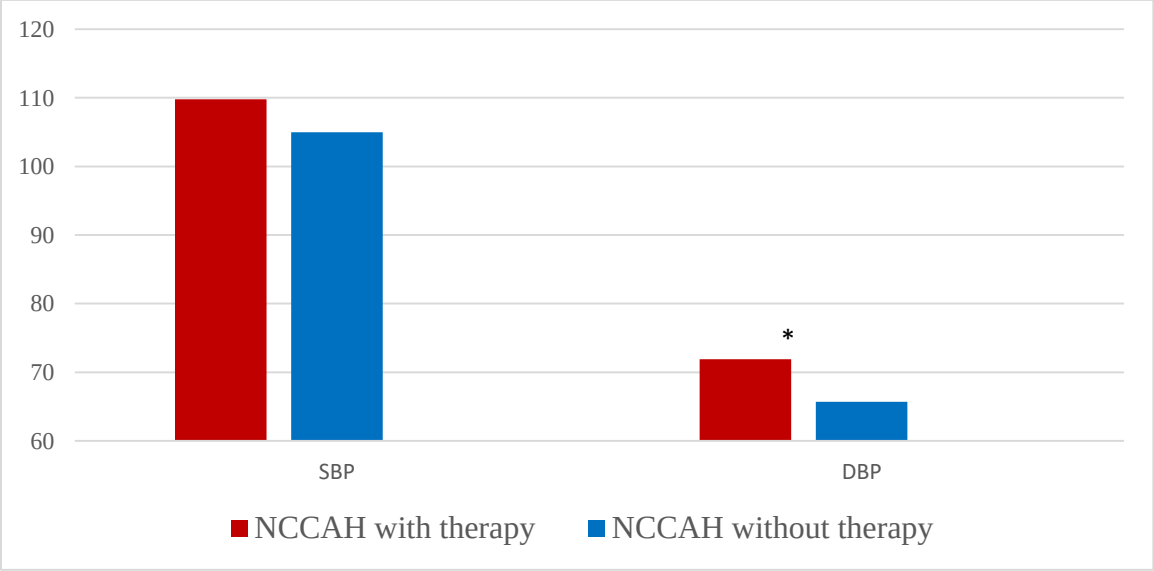
Table 16. Blood pressure and heart rate (beats/min) in participants according to group affiliation

Parameter	***NCCAH*** *(n=34)*	***Controls*** *(n=34)*	***P-value***
SBP (mmHg)	106,6±8,6	108,4±12,8	ns
DBP (mmHg)	68,4±*7,9*	71,3±*11,7*	ns
HR (bpm)	79,1±8,8	80,3±9,6	ns

The data are presented as mean ± SD; ns – non-significant difference.

When comparing these parameters among NCCAH participants according to treatment status, it was found that girls and young women under therapy had higher mean values of SBP and DBP compared to untreated patients, with statistical significance reached only for DBP (p=0.026) (**Fig. 5**).

Fig. 5. Blood pressure values in NCCAH patients according to treatment status (mmHg).



Applying the criteria of The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (242), systolic hypertension was identified only in **2.9%** of the control group, whereas diastolic hypertension was observed in **2.9%** of participants with androgen excess disorder and in **5.9%** of the corresponding controls.

A strong association was found between SBP and DBP (r=0.901, **p<0.0001**), with BMI emerging as the most significant independent predictor of their variation. Specifically, with each unit increase in BMI, SBP increased by 0.87 mmHg (95% CI 0.12–1.61, **p=0.023**), while DBP rose by 0.78 mmHg (95% CI 0.04–1.52, **p=0.04**).

The correlation coefficients between adiposity measures, SBP, DBP, HR, FGS, and clinical manifestations of hyperandrogenism, adjusted for the effects of age and pubertal stage of the participants in both groups, are presented in **Table 17**.

Table 17. Correlation coefficients between adiposity indices, hyperandrogenism, blood pressure and heart rate by groups

	SBP	**DBP**	**HR**
WC	***0,249****	0,203	0,044
BMI	***0,339****	***0,268****	0,028
FGS	***0,354****	***0,319****	-0,008
hirsutism	0,231	0,132	0,044
acne	0,038	0,134	0,072
alopecia	0,008	0,001	0,005
oily hair	0,002	0,040	-0,181
MD	0,064	0,109	-0,131
Testosteron	0,158	0,227	***-0,247****
Androstendion	0,205	***0,263****	0,051
17OHP	0,063	0,047	0,236

MD: menstrual disturbances

Correlation is significant at the 0.01 level (2-tailed).
Correlation is significant at the 0.05 level (2-tailed).

Analysis of the associations between the examined parameters in the group of adolescent girls and young women with NCCAH demonstrated that DBP significantly correlated with serum androstenedione concentrations (r=0.263, p=0.012), whereas HR showed an inverse correlation with serum testosterone levels (r=−0.247, p=0.027).

6.2. Glucose Metabolism

Alterations in glucose homeostasis, including fasting plasma glucose (FPG) and insulin levels, were assessed in all study participants. The HOMA-IR index (homeostatic model assessment of insulin resistance) was calculated using the standard formula. A comparison of these parameters between groups is presented in **Table 18**, with no significant differences detected between the profiles of NCCAH patients and their healthy controls.

Table 18. Glucose metabolism parameters by group

parameter	***NCCAH** (n=34)*	***CONTROLS** (n=34)*	***P-value***
glucose (mmol/l)	4,56±0,39	4,45±0,52	ns
insulin (mIU/ml)	13,3±4,1	13,1±8,2	ns
HOMA-IR	2,71±0,84	2,65±1,76	ns

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

Applying the IDF criteria for metabolic syndrome and impaired glucose homeostasis, two participants (2.9%) were identified with fasting hyperglycemia (FPG ≥5.6 mmol/l) in the entire study cohort – one from the NCCAH group and one from the control group.

Partial correlation analysis, adjusted for age, pubertal stage, and NCCAH status, revealed significant associations between glucose homeostasis parameters, adiposity, and clinical/biochemical hyperandrogenism (**Table 19**). A weak to moderate positive correlation was demonstrated between serum insulin concentration and indices of total/abdominal adiposity, as well as a significant correlation with serum testosterone levels and the absolute value of the clinical androgen score (**FGS**).

Table 19. Correlation coefficients (r) between anthropometric and hormonal parameters and glucose homeostasis indices

	Glucose	***Insulin***	***HOMA-IR***
WC	0,128	***0,255****	0,195
BMI	0,058	***0,308****	***0,295****
SBP	0,123	0,201	***0,267****
DBP	-0,124	0,024	0,074
FGS	0,130	***0,249****	0,241
Hirsutism	0,178	0,190	0,198
Acne	0,210	0,011	0,021
Alopecia	-0,042	0,134	0,145
Oily hair	-0,097	0,188	0,189
MD	-0,014	0,093	0,134
Testosterone	0,079	***0,239****	0,187
Androstendione	0,116	0,191	0,184
17OHP	0,132	-0,041	-0,029

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

The selected analysis of correlation coefficients between glucose homeostasis parameters, adiposity, and clinical/biochemical hyperandrogenism within the NCCAH group demonstrated an inverse correlation between glucose concentration and clinical hirsutism (r = –0.497, **p = 0.004**) as well as scalp/hair seborrhea (r = –0.404, **p = 0.022**), while insulin levels showed a positive association with BMI values (r = 0.368, **p = 0.039**).

The multivariate linear regression analysis identified those variables exerting a significant influence on a given dependent outcome. Linear regression was examined while controlling for factors such as age, pubertal stage, WC, BMI, FGS, serum testosterone, and other androgenic hormones in relation to fasting glucose, insulin, and HOMA-IR values. It was established that, exclusively within the NCCAH group, an increase in serum 17OHP concentration predicted a significant rise in glucose levels **(p = 0.033**) (**Table 20**). In contrast, higher body weight and younger age with incomplete pubertal development significantly predicted increased insulin concentrations and elevated HOMA-IR across participants, irrespective of group allocation (p < 0.05) (**Tables 21, 22**).

Table 20. Multiple linear regression analysis with dependent variable "glucose"

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
	(Constant)	4.914	.309		15.905	.000	4.284	5.544
	17-OHP	.110	.049	.364	2.229	***.033***	.009	.211

dependent variable:glucose[a]; group = NCCAH[b]

Table. 21. Multiple linear regression analysis with dependent variable "insulin"

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
	(Constant)	6.473	4.717		1.372	.175	-2.951	15.897
	Testosterone	2.617	1.153	.265	2.270	***.027***	.314	4.921
	Age	-.725	.234	-.367	-3.093	***.003***	-1.193	-.257
	Weight	.246	.067	.406	3.691	***.000***	.113	.379

Dependent variable: insulin

Table. 22. Multiple linear regression analysis with dependent variable "HOMA-IR"

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	(Constant)	1.479	1.042		1.419	.161	-.603	3.562
	Age	-.136	.052	-.324	-2.630	***.011***	-.240	-.033
	Weight	.046	.015	.354	3.098	***.003***	.016	.075

*dependent variable:*HOMA-IR

6.3 Lipid profile

No significant differences were observed in the levels of total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol between participants from the two subgroups ($p > 0.05$) (**Fig. 6, Fig. 7**).

Fig. 6. Lipid metabolism parameters (total cholesterol and LDL-cholesterol) compared between groups. *Data are presented as median (IQR 25–75; min–max) (Mann-Whitney U test, $p > 0.05$).*

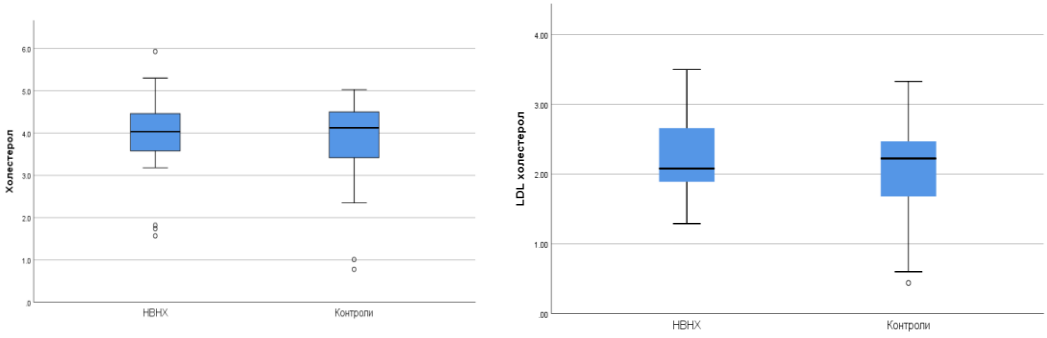
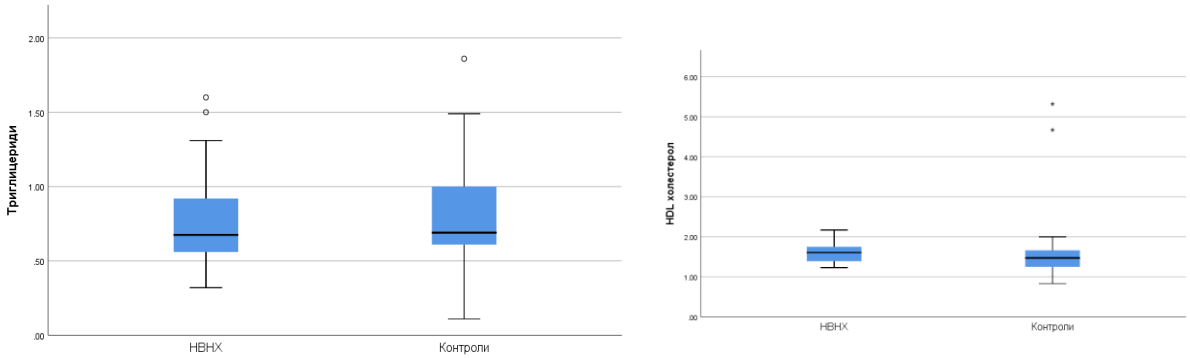


Fig. 7. Lipid metabolism parameters (triglycerides and HDL-cholesterol) compared between groups. *Data are presented as median (IQR 25–75; min–max) (Mann-Whitney U test, p > 0.05).*



Applying the IDF criteria for dyslipidemia, only one participant (1.5%) from the control group was identified with fasting triglycerides ≥1.7 mmol/l across the entire study cohort. A total of four participants (5.9%)—again exclusively from the group of girls and young women without evidence of hyperandrogenism—had HDL-cholesterol levels below 1.03/1.1 mmol/l (according to age).

The correlations between lipid metabolism parameters and other investigated variables (auxological parameters, glucose metabolism indices, and the presence of hyperandrogenism), after adjustment for age, group allocation, and pubertal stage, are presented in **Table 23**. A significant inverse correlation was also observed between triglyceride levels and AMH concentrations (r = –0.358, p = 0.016).

Table 23. Correlation coefficients (r) between lipid metabolism parameters and selected clinical, auxological, and hormonal variables

	CHOLESTEROL	***TG***	***HDL-C***	***LDL-C***
Weight	***0,277****	***0,443****	-0,231	0,021
WC	0,212	0,158	***-0,233****	0,047
BMI	0,172	***0,302****	-0,015	0,135
SBP	0,132	-0,038	0,197	0,169
DBP	0,156	-0,021	0,229	0,196
Glucose	-0,029	0,075	-0,128	0,124
Insulin	0,161	***0,256****	***-0,270****	-0,036
HOMA-IR	0,099	***0,273****	-0,168	-0,002
FGS	0,181	-0,053	-0,242	0,083
Hirsutism	0,206	0,035	-0,109	0,009
Acne	0,005	-0,167	-0,093	0,003
Alopecia	0,010	-0,195	0,032	0,018
Oily hair	0,025	***0,321****	-0,024	0,056
MD	-0,018	0,154	0,024	0,025
Testosterone	0,293	-0,234	-0,168	0,203
Androstendione	0,110	0,005	-0,248	0,014
17OHP	0,154	0,079	-0,048	0,019
Cholesterol	-	0,205	***-0,626****	***0,403****
TG	0,205	-	-0,131	0,076
HDL-C	***-0,626****	-0,131	-	-0,057
LDL-C	***0,403****	0,076	-0,057	-

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

When the analysis was restricted to the group of girls and young women with NCCAH, significant associations were observed. Serum total cholesterol showed an inverse correlation with BMI (r = –0.416, ***p = 0.018***) and with the presence of hirsutism (r = –0.421, ***p = 0.016***).

Triglyceride levels correlated inversely with total testosterone concentration (r = –0.416, p = 0.018) and DBP (r = –0.376, ***p = 0.034***), while LDL-cholesterol concentrations were positively associated with serum glucose levels (r = 0.456, ***p = 0.009***).

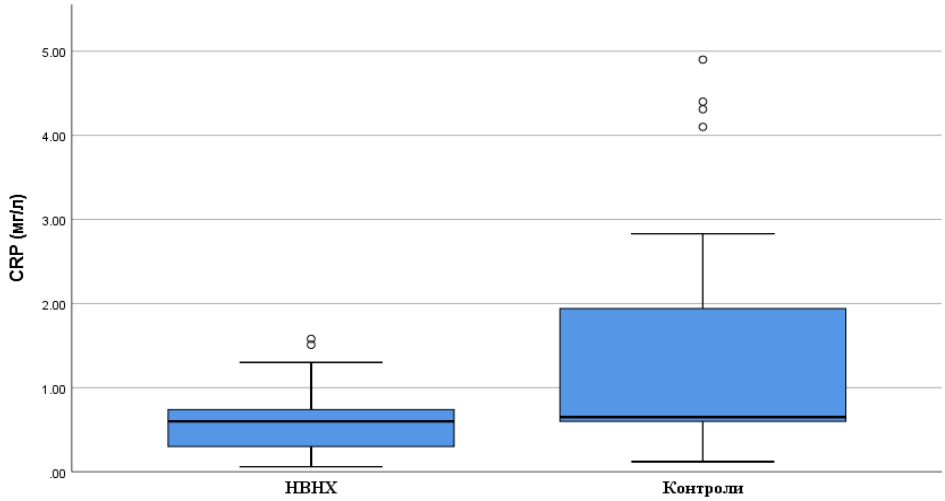
Multivariate linear regression analysis examining the dependence of serum triglyceride concentration on factors such as age, pubertal stage, body weight, BMI, and WC demonstrated that body weight was the principal predictor of triglyceride levels (B-coefficient 0.012; 95% CI 0.004–0.020, ***p < 0.0001***).

Similarly, multiple regression analysis with HDL-cholesterol as the dependent variable and age, pubertal stage, body weight, BMI, and WC as predictors revealed a significant association with body weight (B-coefficient –0.058; 95% CI –0.080 to –0.031, ***p < 0.0001***) and with FGS values (B-coefficient –0.020; 95% CI –0.037 to –0.003, p = 0.019).

7. Levels of adipokines and CRP in participants according to group allocation

Low-grade inflammatory activity was assessed by measuring serum CRP concentration, which demonstrated significantly lower median and interquartile range values in NCCAH patients compared with controls {0.60 (0.3–0.7) vs. 0.7 (0.6–2.0) mg/L} (p = 0.002) (Fig. 8).

Fig. 8. Serum CRP concentration by group. Data are presented as median (IQR 25–75; min–max) (Mann–Whitney U test, p = 0.002).



The correlation relationships between CRP and other examined variables, after adjusting for age and pubertal stage, according to the participants' group allocation, are presented in **Table 24.**

Table 24. Correlation coefficients (r)

	НВНХ *(N=34) CRP*	***КОНТРОЛИ*** *(N=34) CRP*
Weight	0,314	0,244
WC	***0,435****	0,058
BMI	0,261	0,015
SBP	0,243	0,118
DBP	0,164	0,043
Glucose	-0,268	-0,124
Insulin	0,003	0,217
HOMA-IR	-0,109	0,171
Cholesterol	-0,105	0,242
TG	-0,061	***0,356****
HDL-C	-0,241	-0,201
LDL-C	-0,114	-0,059
FGS	***0,363****	-0,072
Hirsutism	0,099	0,008
Acne	-0,038	-0,107
Alopecia	0,253	-0,197
Oil hair	0,147	-0,043
MD	0,104	-0,038
Testosterone	-0,145	-0,015
Androstendione	0,089	0,162
17OHP	0,315	0,353
DHEA-S	0,177	0,008

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

The significant correlation identified between the inflammatory marker CRP and WC ($p = 0.013$) as well as FGS ($p = 0.041$) in adolescent girls and young women with NCCAH was further evaluated using multiple linear regression analysis. The model demonstrated that, among all independent variables (age, pubertal stage, WC, BMI, FGS, testosterone, androstenedione, and 17OHP), WC emerged as the most significant predictor of CRP levels in the NCCAH group (**Table 25**).

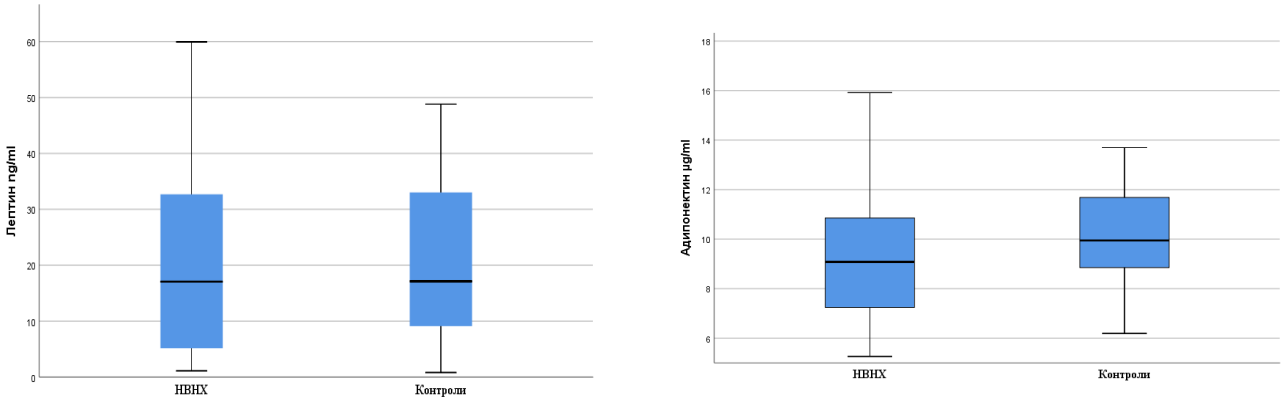
Table 25. Multiple linear regression analysis with dependent variable "CRP" in NCCAH patients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	.695	1.078		.645	.524	-1.506	2.896
WC (sm)	.016	.006	.385	2.519	***.017***	.003	.029
FGS	.020	.012	.262	1.714	.097	-.004	.044
17OHP	.096	.048	.299	2.003	.054	-.002	.193

Dependent variable : CRP; *selecting only cases for which group =* $NCCAH_a$

The analysis of the adipokines leptin and adiponectin among participants from both groups revealed lower serum concentrations in adolescent girls and young women with NCCAH compared to controls, although the differences did not reach statistical significance ($p > 0.05$) (**Fig. 9**). Comparison of serum adipokine levels within the NCCAH group further demonstrated that glucocorticoid therapy was associated with lower leptin concentrations {14.7 (3.6–19.7) vs. 21.7 (9.9–33.4) ng/ml}, although the difference did not reach statistical significance ($p = 0.329$).

Fig. 9. Serum concentrations of leptin and adiponectin by group. Data are presented as median (IQR 25–75) (Mann–Whitney U test).



When examining the association between adiponectin and leptin with auxological and cardiometabolic parameters across all participants, while controlling for age, pubertal stage, group allocation, and the presence of abdominal obesity, a statistically significant inverse correlation was observed between adiponectin and leptin ($r = –0.379$; $p = 0.002$), as well as between adiponectin and LDL-cholesterol ($r = –0.301$; $p = 0.016$). These significant correlations remained evident when the analysis was restricted to NCCAH participants ($r = –0.412$ and $r = –0.401$, respectively; $p < 0.05$), while an additional inverse but non-significant association was demonstrated between adiponectin and serum glucose levels ($r = –0.316$; $p = 0.07$).

Partial correlation analysis did not reveal significant associations between adipokines and the clinical or hormonal parameters of androgen excess, either in the overall cohort or within the subgroups analyzed separately (**Table 26**).

Table 26. Correlation coefficients (r)

	***NCCAH** (N=34)*		***CONTROLS** (N=34)*	
	LEPTIN	*ADIPONECTIN*	*LEPTIN*	*ADIPONECTIN*
FGS	-0,072	0,249	-0,034	-0,025
Hirsutism	-0,082	0,269	-0,025	0,081
Acne	0,055	0,190	-0,277	0,131
Alopecia	-0,075	0,319	-0,098	-0,055
Oil hair	-0,110	***-0,358****	-0,072	-0,067
MD	-0,230	0,291	-0,156	-0,034
Testosterone	0,097	-0,075	-0,124	-0,106
Androstendione	-0,037	0,131	-0,004	-0,293
17OHP	-0,071	0,090	-0,261	0,179
DHEA-S	-0,226	0,036	0,102	-0,166

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

The multiple linear regression analysis of leptin and adiponectin as dependent variables, with independent predictors including age, pubertal stage, BMI, WC, insulin, HOMA-IR, testosterone, androstenedione, 17OHP, DHEA-S, and SHBG in NCCAH patients, is presented in **Tables 27 and 28**. The analysis demonstrated that leptin concentration showed a significant positive association with WC (B-coefficient 0.669; 95% CI 0.006–1.331, ***p = 0.048***), while adiponectin was most strongly associated with pubertal stage, followed by DHEA-S, HOMA-IR, and testosterone levels (***p < 0.05***).

Table 27. Multiple linear regression analysis with dependent variable "leptin" in NCCAH patients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% CI for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
Constant	-19.815	20.824		-.952	.355	-63.749	24.120
WC (sm)	.669	.314	.543	2.129	***.048***	.006	1.331
DHEA-S	-1.852	1.003	-.471	-1.846	.082	-3.968	.264

Dependent Variable: leptin ng/ml[a]; Selecting only cases for which group = NCCAH[b]

Table 28.Multiple linear regression analysis with dependent variable "adiponectin" in NCAH patients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% CI for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	42.710	9.065		4.712	.000	23.127	62.293
Tanner	-7.438	1.805	-1.185	-4.121	***.001***	-11.338	-3.538
Testosterone	-3.020	1.382	-.657	-2.186	***.048***	-6.005	-.035
DHEA-S	.641	.193	.806	3.313	***.006***	.223	1.059
HOMA IR	5.071	2.307	1.465	2.198	***.047***	.088	10.054

Dependent Variable: adiponectin µg/ml[a]; Selecting only cases for which group = NCCAH[b]

8. Body composition of participants according to group affiliation

In all participants, body composition, fat mass (FM)/lean mass (LM) distribution, and bone mineral density (BMD) were assessed using whole-body DXA scanning. The data, stratified by group affiliation of the studied adolescent girls and young women, are presented in **Table 29**.

Table 29. Body composition of participants by group

Parameter	***NCCAH** (n=34)*	***CONTROLS**(n=34)*	***p***
BMC g/cm	0,94±0,10	1,01±0,21	ns
BMC z-score	0,69±0,42	0,94±0,78	ns
LM (кg)	32,435±3,971	34,568±6,592	ns
Total body fat mass (kg)	34,0±5,4	36,1±8,8	ns
Fatt mass (%)	33,4±6,2	36,2±9,5	ns
Android/Gynoid FM (%)	0,85±0,25	0,90±0,21	ns
BMD	1550±216	1625±289	ns

Results are presented as mean ± SD; ns – non-significant difference. Abbreviations: **BMD** – bone mineral density; **LM** – lean mass; **FM** – fat mass; **BMC** – bone mineral content.

Although the differences in body composition parameters between the NCCAH group and the controls did not reach statistical significance, patients with the hyperandrogenic disorder demonstrated lower mean bone mineral density (BMD), bone mineral content (BMC), lean mass (LM), and total body fat mass (FM), respectively ($p>0.05$), with no differences observed in relation to treatment status.

The partial correlation analysis (adjusted for age, pubertal stage, and treatment status) between DXA-derived parameters and auxological, cardiometabolic, clinical, and hormonal hyperandrogenism indicators in the NCCAH patient group is presented in **Table 30**.

Table 30. Correlation coefficients (r) in NCCAH patients

	BMD	**BMC**	**LM**	**FM**	***A/G FM (%)***
WC	0,242	***0,551****	***0,413****	0,211	0,244
BMI	0,145	***0,465****	***0,479****	0,304	0,005
SBP	0,036	0,290	0,290	0,190	0,292
DBP	0,029	0,159	0,152	0,073	0,271
Glucose	-0,201	-0,062	-0,130	0,074	0,125
Insulin	0,192	0,044	0,0025	0,039	-0,144
HOMA-IR	0,103	-0,068	-0,144	0,113	-0,204
FGS	-0,138	0,231	0,170	-0,316	0,153
Hirsutism	0,286	0,193	0,275	0,018	0,048
Acne	-0,299	0,248	0,113	0,155	0,160
Alopecia	-0,337	-0,054	-0,127	-0,089	-0,300
Oily hair	-0,210	0,108	0,173	0,053	-0,313
MD	0,102	-0,196	-0,208	0,086	-0,179
Testosterone	-0,105	0,061	-0,074	-0,032	0,117
Androstendione	-0,090	0,052	-0,208	-0,165	0,125
17OHP	0,066	0,241	0,302	-0,029	0,261
TG	-0,077	0,270	0,082	0,004	-0,201
HDL-C	-0,210	-0,136	-0,186	0,230	-0,195
Leptin	0,176	-0,025	0,121	0,117	-0,122
Adiponectin	-0,030	0,012	0,063	0,089	-0,129
LM	0,332	***0,770****	-	***0,359****	***0,381****
FM	***0,361****	***0,467****	***0,359****	-	***0,507****
A/G FM (%)	***0,381****	***0,588****	0,300	***0,507****	-

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Applying the same correlation analysis in the control group, a significant positive association was observed between bone mineral content (BMC) and the android/gynoid fat mass ratio (A/G FM, %) ($r=0.528$; $p=0.002$), as well as with lean body mass (LBM) ($r=0.917$; $p<0.001$).

Furthermore, LBM showed a statistically significant correlation with A/G FM (%) (r=0.575; p=0.001) and with total fat mass (FM) (r=0.743; p<0.0001). FM was positively correlated with body mass index (BMI) (r=0.474; p=0.006), systolic blood pressure (SBP) (r=0.375; p=0.034), and serum triglyceride (TG) levels (r=0.454; p=0.009).

9. Psychological Assessment and Quality of Life

All participants in the study underwent an evaluation of psychological characteristics and personality constructs using the standardized **EDI-3 self-report questionnaire**. The data were processed through an electronic platform, enabling graphical representation and point-based scoring across the **12 individual scales** (including psychological domains and body image-related constructs), as well as **3 to 5 composite indices**, in accordance with age-specific normative data. The obtained results are presented graphically by radar charts (**Fig. 10, Fig. 11**).

Fig. 10. Radar chart of the psychological assessment results (EDI-3).

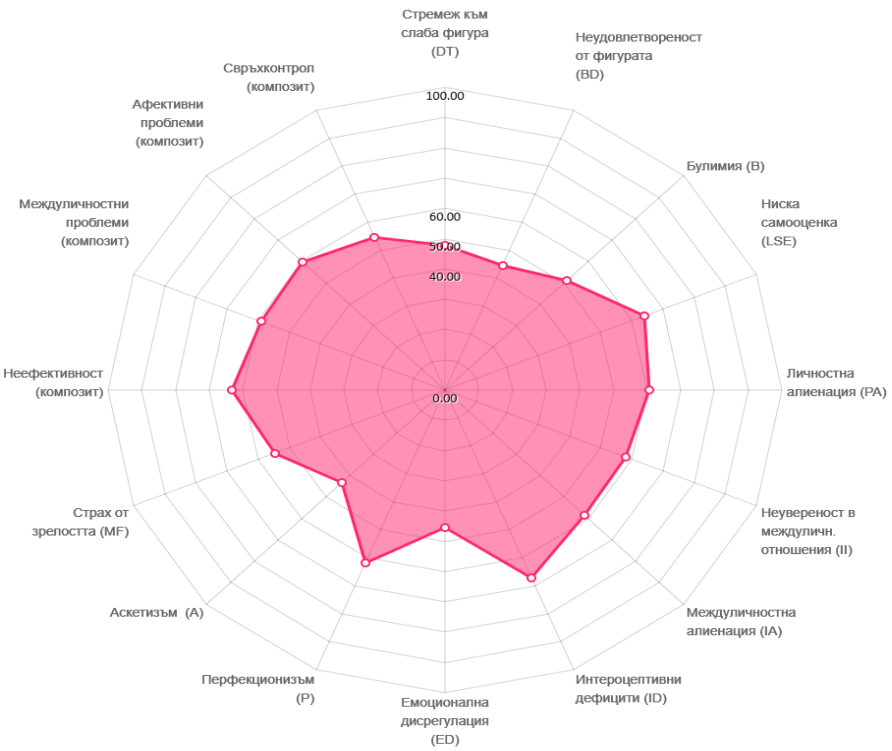


Fig. 11

The comparison of the mean values of the individual indicators assessed by the EDI-3 instrument between the adolescent girls and young women from the two groups is presented in **Table 31.**

Table 31. Psychological assessment by groups

parameter	***NCCAH*** *(n=34)*	***Controls*** *(n=34)*	***p***
Interpersonal Alienation	56,1±9,5	50,9±7,9	***0,017***
Interoceptive Deficits	57,4±13,5	52,0±8,9	***0,05***
Emotional Dysregulation	63,0±14,8	53,8±10,6	***0,004***
Low Self-Esteem	58,7±9,1	52,7±7,8	***0,005***
Affective Problems	61,0±15,3	53,5±10,2	***0,02***
General Psychological Maladjustment	60,0±11,9	53,7±8,1	***0,013***
Overcontrol	54,0±11,2	49,6±6,6	***0,05***
Personal Alienation	58,8±11,0	54,9±7,7	ns
Interpersonal Insecurity	54,9±9,0	53,3±9,7	ns
Perfectionism	50,7±12,2	47,8±9,0	ns
Asceticism	56,3±11,9	52,7±10,9	ns
Maturity Fears	53,9±10,2	52,5±9,1	ns
Ineffectiveness	59,6±9,4	54,3±7,3	***0,011***
Interpersonal Problems	56,0±9,1	52,7±9,1	ns

The results are presented as mean value ± SD, ns – non-significant difference.

The multiple regression analysis of the dependent variables from the psychological EDI-3 assessment, with independent predictors including age, pubertal stage, group, BMI, WC, insulin, HOMA-IR, testosterone, androstenedione, 17OHP, DHEA-S, SHBG, clinical manifestations of hyperandrogenism, and body composition, is presented in **Tables 32, 33, 34, and 35**.

Table 32. Multiple linear regression with dependent variable "Interpersonal Problems"

aModel		Unstandardized Coefficients		Standardized Coefficients			95.0% CI for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	Constant	38.468	9.109		4.223	.000	20.265	56.672
	WC (sm)	.350	.124	.329	2.832	***.006***	.103	.598
	17OHP	-2.476	.887	.321	2.792	***.007***	.704	4.248
	%FM	-.330	.132	-.289	-2.494	***.015***	-.595	-.066
	Alopecia	-5.632	2.236	-.299	-2.519	***.014***	-10.100	-1.164

Dependent Variable: interpersonal problems

Table 33. Multiple linear regression with dependent variable "Maturity Fears"

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	(Constant)	52.411	1.715		30.562	.000	48.985	55.837
	Acne	5.069	2.632	.261	1.926	.059	-.189	10.327
	Alopecia	-13.999	3.769	-.711	-3.715	***.000***	-21.528	-6.470
	Oil hair	6.865	3.652	.357	1.880	.065	-.430	14.160

Dependent Variable: "maturity fears"

Table 34. Multiple linear regression with dependent variable "Interpersonal Insecurity"

Model		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
	(Constant)	38.733	9.532		4.063	.000	19.672	57.794
	WC (sm)	-.247	.139	.230	1.775	.081	-.031	.526
	Oil hair	-5.421	2.476	-.292	-2.190	***.032***	-10.372	-.471

Dependent Variable: interpersonal insecurity

Table 35. Multiple linear regression with dependent variable "Low Self-Esteem"

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
	(Constant)	57.030	4.912		11.611	.000	47.215	66.846
	Insulin	.315	.152	.227	2.069	***.043***	.011	.619
	Fat %	-.212	.123	-.192	-1.728	.089	-.458	.033
	Alopecia	-6.132	2.383	-.336	-2.573	**.012**	-10.895	-1.370

Dependent Variable:"Low Self-Esteem"

Comparison of the individual domains of quality of life between patients with NCCAH and healthy controls is presented in **Table 36**. It was demonstrated that adolescent girls and young women with NCCAH exhibited lower scores across all assessed domains of quality of life compared to non-androgenic controls, with these differences reaching statistical significance for domains D1 (physical health) and D2 (psychological health) (***p<0.001***).

Table 36. Results from the WHOQOL-BREF questionnaire in NCCAH patients and healthy controls

parameter	***NCCAH*** *(n=34)*	***CONTROLS(****n=34)*	***p***
D1 Physical health	50,5±11,0	62,9±11,5	***<0,001***
D2 Psychological health	57,6±11,5	68,7±10,5	***<0,001***
D3 Social relationships	63,4±14,6	67,8±14,0	ns
D4 Environment	67,2±11,5	71,6±10,6	ns

Results are presented as mean ± SD; ns – non-significant difference.

Partial correlation analysis revealed that the D1 score was significantly associated with the presence of low self-esteem (r=0.258, p=0.038), interpersonal alienation (r=0.277, p=0.026), and overall psychological maladaptation (r=0.298, p=0.016), as well as with the clinical manifestations of hirsutism (r=-0.259, p=0.037), acne (r=-0.275, p=0.027**)**, and the serum concentration of 17OHP (r=-0.291, p=0.019). The D2 score showed a significant positive correlation with the score assessing physical health (r=0.243, p=0.05). A higher score in D4

(environmental quality of life), encompassing aspects such as services, acquisition of skills and knowledge, leisure, environment, and transport, was significantly but inversely correlated with body weight ($r=-0.244$, $p=0.05$) and waist circumference ($r=-0.326$, $p=0.008$).

PART V. DISCUSSION

1. Participants in the study

A total of 68 adolescent girls and young women with comparable age and weight profiles participated in the present study. They were divided into two groups—patients with non-classic congenital adrenal hyperplasia (NCCAH) and healthy controls. The median age of participants (15.5 years) indicates that the majority of the study population falls within adolescence—a critical developmental period for the manifestation and clinical evolution of hyperandrogenic conditions.

Several authors emphasize the key importance of age homogeneity between study groups in research on NCCAH, due to the specific physiological maturation processes of the hypothalamic–pituitary–adrenal and gonadal axes during puberty and early adulthood. According to *Speiser et al.* (1), maintaining a uniform age distribution is an essential methodological prerequisite to minimize the impact of physiological variations in steroidogenesis. *Loli et al*. highlight that adolescence is the period when NCCAH most frequently becomes clinically apparent, with manifestations such as hirsutism, acne, and/or menstrual irregularities—symptoms that are often misinterpreted as physiological pubertal phenomena or attributed to polycystic ovary syndrome (PCOS) (218).

The absence of an age difference between the NCCAH and control groups in our study is of methodological importance for data analysis and subsequent interpretation. It allows the exclusion of age as a potential confounding factor, ensuring statistically valid comparability between the groups and enabling accurate discussion of the results and conclusions regarding the effects of NCCAH on metabolic, hormonal, and psychophysical characteristics in adolescent and young adult females.

In our cohort, the mean age at NCCAH diagnosis was slightly lower than that reported in other studies. This likely reflects earlier referral to specialized endocrine care in the presence of overt clinical symptoms. It should be noted, however, that the diagnostic age in our NCCAH group aligns with multiple published cohorts—namely, adolescence, when the first clinical signs of hyperandrogenism typically appear. For instance, *Azziz et al*. report a mean diagnostic age of 16.8 years (219), while *Bidet et al.* found that most patients are diagnosed in mid-adolescence (35), following the onset of androgenic features. Nonetheless, the literature also describes cases in which NCCAH is confirmed during childhood due to premature pubarche and/or accelerated growth (29, 30).

At the time of the study, approximately half of the NCCAH patients were receiving pharmacological treatment with hydrocortisone—a proportion lower than the globally reported prevalence, where up to ~80% of NCCAH patients are treated with glucocorticoids (1, 193). This finding may partially reflect the fact that nearly three-quarters of our NCCAH participants had completed pubertal development, in whom non-glucocorticoid therapeutic approaches (e.g., antiandrogens) are preferred after completion of linear growth. This is consistent with current expert recommendations advocating individualized therapy based on age, phenotypic presentation, and treatment goals (growth, fertility, and symptom control) (1, 193). Another factor limiting hydrocortisone use among younger girls in our study is its restricted availability in the country, with administration permitted only after obtaining authorization from the national medicines agency.

The higher proportion of participants with completed pubertal development in the NCCAH group may be associated with chronically elevated androgen levels, which contribute to accelerated bone maturation, premature adrenarche (in prepubertal age), and an increased risk of central precocious puberty (220, 221) and hyperandrogenic symptoms during adolescence (222). This observation demonstrates that in NCCAH, not only the hormonal profile but also the tempo of pubertal maturation differs from that of healthy peers. Such deviations may lead to misdiagnosis or diagnostic delay if early maturation is interpreted as a physiological variant of normal development, including in relation to earlier menarche onset (223).

2. Risk factors and family history

The analysis of potential perinatal risk factors (birth weight, birth length, and gestational age) revealed no association between the examined auxological and morphological parameters at birth and the later development of hyperandrogenic conditions. This finding underscores the predominant and significant influence of hormonal factors on growth patterns in girls with non-classic congenital adrenal hyperplasia (NCCAH).

The presence of a family history of hyperandrogenic conditions or menstrual irregularities in approximately one-fifth of NCCAH patients highlights the contribution of genetic factors to disease development and supports the need for targeted screening among relatives of affected individuals. Several comprehensive reviews in the international literature have explored in detail the role and potential benefits of systematic family screening and the establishment of genetic counseling strategies in NCCAH, emphasizing both the phenotypic heterogeneity of the

disorder and the diagnostic challenges in differentiating it from other forms of hyperandrogenism (29, 193).

3.Auxological characteristics of the participants, including prevalence of overweight and obesity

When comparing auxological indicators of generalized and abdominal obesity, no statistically significant differences were observed between participants with non-classic congenital adrenal hyperplasia (NCCAH) and healthy non-androgenic controls. Approximately one-third of all participants presented with overweight or generalized obesity.

Globally, most studies investigating the prevalence and characteristics of obesity have focused primarily on individuals with the classic form of congenital adrenal hyperplasia (CAH). Several international studies have reported frequencies of generalized obesity among adolescents and adults with CAH similar to or higher than those found in our study. For example, the U.S. **National Institutes of Health** reported that approximately 35% of children with CAH are obese, with no significant difference between the classic and non-classic forms of the disease (91). In a cohort of NCCAH girls diagnosed during childhood, *de Vries et al.* found no significant difference in the prevalence of overweight and/or obesity compared to the general population (100), while *Delai et al*. reported a 31% obesity rate among NCCAH patients—consistent with our findings (102).

Conversely, other studies have demonstrated a markedly higher prevalence of obesity among NCCAH patients compared with the general population (85, 91, 92). For instance, in 2021, *Yoon et al.* reported that 56% of Korean children and young adults with CAH were either overweight or obese (101).

The heterogeneity in reported prevalence rates of generalized overweight and obesity across studies underscores the importance of population-specific characteristics, including age, sex distribution, and ethnicity, as well as differences in the diagnostic criteria used to define obesity. These discrepancies highlight the need for larger-scale, longitudinal studies that address a broader spectrum of potential contributing factors—such as dietary habits, physical activity, treatment regimens, and socioeconomic status—to better identify those determinants that exert the most significant influence on the development of overweight and obesity in NCCAH patients. Such insights would be essential for designing effective, individualized preventive strategies.

The relatively higher proportion of participants with abdominal (central) obesity among NCCAH patients in our study cannot be fully interpreted due to the limited sample size. Nevertheless, the well-established association between abdominal obesity and the development of insulin resistance, dyslipidemia, and arterial hypertension underscores the importance of active cardiometabolic screening in NCCAH patients—particularly during progressive pubertal development or while receiving glucocorticoid therapy.

4. Clinical manifestations and assessment of hyperandrogenism

The clinical polymorphism characteristic of patients with non-classic congenital adrenal hyperplasia (NCCAH) was also evident in our study cohort. One of the most frequent and earliest clinical signs—premature pubarche—was identified in more than one-third of NCCAH participants. This finding is consistent with global data reporting premature pubarche in 5–30% of NCCAH patients (193, 222). For instance, a study of 238 French children with NCCAH reported a 4.2% prevalence of premature pubarche (29), whereas a multicenter study involving 220 patients divided into three age groups (<10 years, 10–19 years, and 20–29 years) found that 92%, 8%, and 4%, respectively, had a history of premature adrenarche (41). Collectively, these findings underscore the importance of considering premature pubarche as part of the differential diagnostic evaluation in patients presenting with hyperandrogenism (223, 224).

An interesting observation in our cohort was that girls and young women with NCCAH and a history of premature pubarche exhibited lower body weight and BMI, in line with previous reports in children and adolescents with premature adrenarche, who tend to display a more favorable auxological profile during early development (228, 229). This finding suggests that in NCCAH, where the phenotype is highly heterogeneous and shaped by the interaction of genetic and environmental factors (18, 225, 226), a history of premature pubarche may serve as an important predictive marker for auxological characteristics during adolescence and early adulthood.

Consistent with data from numerous international studies (29, 41, 45, 46), the most common clinical manifestation of androgen excess in our NCCAH cohort was hirsutism, observed in nearly all patients. The second most prevalent hyperandrogenic feature was acne, a leading dermatological manifestation of androgen excess, whose severity and frequency may vary with age (18, 218, 225, 226). Notably, acne may occasionally represent the sole clinical manifestation of NCCAH, underscoring the need for heightened clinical vigilance in adolescent girls presenting with severe or treatment-resistant acne (60).

Androgenic alopecia or hair loss—although a recognized symptom of hyperandrogenism—is often underreported in the context of NCCAH due to limited available data. Similar to our findings, other studies have reported that approximately 50% of women with NCCAH experience alopecia or hair thinning as a manifestation of androgen excess (63, 64), with a significantly higher frequency among female NCCAH patients compared to the general population (227).

Seborrhea, manifested as excessive scalp oiliness, is not systematically studied as an isolated androgen-dependent clinical feature and is rarely cited independently in the literature concerning adolescent and adult females with hyperandrogenism. Consequently, data on its global prevalence are lacking. Seborrhea is most commonly discussed as part of the so-called seborrheic syndrome, encompassing acne and androgenic alopecia/hair loss—dermatologic signs within the spectrum of androgen-dependent manifestations. In our study, seborrhea affected nearly four-fifths of NCCAH participants, suggesting the need for further investigation and potential inclusion of this parameter in the clinical evaluation of hyperandrogenic conditions.

In clinical practice, the Ferriman–Gallwey Score (FGS) is commonly used to assess hirsutism severity. In our study, NCCAH participants demonstrated significantly higher FGS values compared to the control group, a finding consistent with previous reports validating this semi-subjective instrument as a reliable tool for phenotypic assessment of hirsutism (225, 226). The FGS thus represents an essential clinical measure for both diagnosis and follow-up of hyperandrogenism in NCCAH and related disorders. Furthermore, we observed significantly higher FGS values among treated participants, a methodologically expected and plausible outcome, given that therapeutic intervention is typically initiated in patients with more severe androgenic symptoms. Therefore, higher FGS values in treated individuals likely reflect a more pronounced baseline clinical phenotype rather than a treatment effect.

Androgen excess, dysregulation of the hypothalamic–pituitary–gonadal axis, insufficient or suboptimal disease control, various metabolic and psycho-emotional factors, as well as age-related characteristics (e.g., the physiologically higher prevalence of irregular menses during adolescence), all contribute to menstrual dysfunction in NCCAH. In our cohort, menstrual irregularities were significantly more common among NCCAH patients than in controls, consistent with data from the international literature. For instance, *Loli et al.* (2025) reported menstrual irregularities in 56% of NCCAH patients, highlighting their potential for misdiagnosis as polycystic ovary syndrome (PCOS) (218). Similarly, *Engberg et al*. found that

menstrual disorders affected 30–60% of untreated NCCAH patients (230), and *Wan et al.* (2023) reported that menstrual irregularities and/or infertility may represent the sole clinical manifestations of NCCAH (231). Several analyses have also confirmed a direct age-related effect on the occurrence of menstrual dysfunction (30, 193, 224).

Another noteworthy finding from our study is the role of abdominal adiposity, as measured by waist circumference, as a key modulator of the clinical androgenic phenotype. We observed a significant correlation between waist circumference and both the modified FGS and hirsutism severity. These results are consistent with previous studies showing that central obesity in NCCAH is associated not only with increased cardiometabolic risk but may also predict more pronounced androgen-dependent clinical features. In one of the largest cross-sectional studies, Costa et al. identified waist circumference as an independent risk factor for metabolic syndrome in NCCAH, while also demonstrating higher FGS values in the same cohort (122)—thereby highlighting the clinical interplay between abdominal obesity and hirsutism severity. Similarly, *Adriaansen et al*., in their comprehensive review on therapeutic challenges in NCCAH, emphasized that central obesity exacerbates both metabolic risk and hyperandrogenism severity, particularly in the presence of menstrual irregularities (225). Although our data did not confirm a significant influence of waist circumference on all androgen-dependent clinical features, this parameter should nevertheless be integrated into both metabolic risk assessment and prediction models for hirsutism severity in NCCAH.

The observed strong correlation between clinical manifestations of hyperandrogenism and FGS values further validates the reliability of this instrument in objectifying androgen-dependent features and demonstrates consistency between subjective and objective findings in NCCAH (53, 63). Additionally, the significant interrelation among individual hyperandrogenic symptoms highlights shared pathophysiological mechanisms through which androgen excess exerts effects on hair follicles and pilosebaceous units—resulting in hirsutism, acne, seborrhea, and hair loss. Elevated adrenal androgens (particularly androstenedione and testosterone) in NCCAH locally activate 5α-reductase, enhancing sebum production and promoting follicular inflammation. This mechanism explains the frequent co-occurrence of hirsutism and acne within the hyperandrogenic spectrum (122), as also observed in our cohort.

In contrast, the association between acne, hair loss, and seborrhea—arising from shared androgen-dependent pathogenesis involving increased androgen activity and/or heightened local follicular sensitivity to androgens—has been less extensively studied (193, 229). In the largest clinical cohort of women with NCCAH described to date, *Livadas et al.* (193) reported

the prevalence of hirsutism, acne, alopecia, and menstrual disorders, though without analyzing interrelations among these features. Similarly, *Costa et al.* (122) characterized individual androgenic manifestations in NCCAH but did not assess their mutual correlations.

Therefore, our findings not only expand current understanding but also, for the first time, demonstrate specific correlation patterns between cutaneous and systemic manifestations of androgen excess in NCCAH. These results underscore the clinical relevance of additional androgen-dependent features (such as seborrhea and androgenic alopecia/hair loss), which should be regarded not merely as concomitant symptoms but also as potential phenotypic markers predictive of hyperandrogenism severity.

5.Hormonal profile

The present study clearly demonstrates distinct differences in the hormonal profile of patients with non-classic congenital adrenal hyperplasia (NCCAH) compared with healthy controls, showing significantly elevated serum levels of testosterone, androstenedione, and 17-hydroxyprogesterone (17OHP), which were strongly correlated with the clinical severity of hyperandrogenic manifestations. Elevated 17OHP levels in adolescent girls and young women with NCCAH confirm its established role as a principal biochemical marker for the diagnosis and monitoring of the disease, while concomitant increases in androstenedione and testosterone enhance their prognostic value as independent predictors of clinical hyperandrogenism. These findings are consistent with data from international literature emphasizing the relationship between chronic hyperandrogenemia and the phenotypic expression of the disorder (232). It has been shown that chronic androgen excess in NCCAH patients disrupts the normal luteinizing hormone (LH) surge, preventing ovulation even when estradiol levels remain within normal limits. This mechanism explains the presence of irregular or absent menstrual bleeding in some patients with NCCAH.

In contrast to androgens, other examined hormones did not show significant intergroup differences (237). These results are in line with previous reports suggesting that, unlike polycystic ovary syndrome (PCOS), NCCAH does not typically exhibit the characteristic “LH dominance”; the LH:FSH ratio generally remains within or below the reference range, and variations in anti-Müllerian hormone (AMH), sex hormone-binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEA-S) lack independent diagnostic significance for this hyperandrogenic disorder (232, 234, 235). However, some studies in untreated NCCAH women have shown approximately 28% higher mean DHEA-S levels compared to healthy controls

(235), reflecting variability in androgen synthesis, while SHBG is generally reduced in conditions associated with hyperandrogenism and/or insulin resistance (236). In our cohort, no significant differences were found in SHBG or DHEA-S levels between groups, likely due to the age characteristics of participants and the relatively low prevalence of obesity.

Stratification of NCCAH patients based on pharmacological treatment revealed higher mean concentrations of androstenedione, testosterone, 17OHP, and DHEA-S in treated participants. This result is expected, as treatment initiation is usually prompted by more severe clinical manifestations of hyperandrogenism, leading to higher baseline serum androgen levels in treated patients. The significantly elevated androstenedione observed in treated NCCAH participants is biologically plausible, as androstenedione is a sensitive biomarker of adrenal androgen excess, while 17OHP, characterized by greater analytical and biological variability, is more effectively suppressed by glucocorticoid therapy.

Performing a comprehensive hormonal panel—including 17OHP, testosterone, and androstenedione—alongside clinical evaluation, is essential not only for diagnosis but also for therapeutic monitoring in NCCAH. Adherence to standardized sampling conditions (morning collection during the early follicular phase) and consideration of treatment type, dosage, and duration are strongly recommended, as these factors allow distinction between treatment effects and baseline hyperandrogenism severity, thus improving the interpretability of hormonal data.

A controlled analysis of the relationship between hirsutism severity, assessed via the modified **Ferriman–Gallwey Score (FGS)**, and serum androgen concentrations revealed the strongest and most significant correlation with androstenedione. As an intermediate steroid of both ovarian and adrenal origin, androstenedione often correlates better with clinical phenotype, supporting its value as a biochemical correlate of hyperandrogenism severity. Furthermore, several studies have confirmed its high diagnostic and prognostic utility not only for assessing hyperandrogenism severity in NCCAH but also for identifying patients at risk of metabolic disturbances (236).

The weaker correlation between testosterone levels and hirsutism severity can be explained by the influence of SHBG and individual variability in tissue androgen sensitivity and local 5α-reductase activity (7, 225). Therefore, measuring free testosterone or calculating the free androgen index is recommended, as these parameters demonstrate stronger correlations with the clinical phenotype.

The moderate correlation observed between FGS and 17OHP aligns with published data indicating that 17OHP primarily serves as a diagnostic marker of NCCAH rather than a direct indicator of hyperandrogenic symptom severity (232). Moreover, several authors have described a phenotype–biochemical dissociation in NCCAH, showing variable hirsutism severity among women with similar androgen levels. This phenomenon may be explained by differences in follicular enzyme activity, ethnic and genetic variability, and the influence of concomitant metabolic conditions (225, 236).

The predictive role of androstenedione and 17OHP in the development of hirsutism, as assessed by FGS in our cohort, was further confirmed through multiple linear regression analysis, which demonstrated that both hormones independently contribute to the clinical presentation of hirsutism. These findings reinforce their importance as principal biochemical determinants and reliable tools for evaluating and monitoring hyperandrogenism severity in NCCAH, consistent with international studies (232, 234, 238).

The multiple regression model using “acne” as a dependent variable revealed that both specific androgenic hormones (testosterone and 17OHP) and demographic factors (age and Tanner stage of pubertal development) exert significant independent effects on acne presence and severity. Serum testosterone concentration showed the strongest predictive value for acne development, consistent with its established role in stimulating sebaceous gland activity and follicular keratinization (234). Similarly, 17OHP emerged as an independent predictor, confirming its role as a biochemical substrate of androgen-related dermatologic manifestations in NCCAH, as reported in other studies (232). Younger age and ongoing pubertal progression were also associated with higher acne prevalence, consistent with clinical improvement observed with advancing age and stabilization of hormonal profiles (238).

Androgenic alopecia in NCCAH was also significantly associated with elevated androstenedione and 17OHP concentrations, in agreement with published evidence highlighting the role of these intermediate steroid metabolites in the pathogenesis of androgen-dependent dermatological manifestations in women with NCCAH (232, 234, 239, 240).

Similarly, the presence and severity of menstrual irregularities in NCCAH were significantly influenced by increased androstenedione and 17OHP levels, consistent with global literature (234, 239). Elevated concentrations of these intermediate metabolites disrupt normal ovulatory mechanisms, leading to anovulatory cycles and menstrual dysfunction (232, 238). This finding

underscores the clinical relevance of these hormonal markers for predicting and monitoring reproductive function in NCCAH patients.

6. Cardio-metabolic parameters

The analysis of cardiovascular indices in participants with non-classic congenital adrenal hyperplasia (NCCAH) versus controls revealed no statistically significant differences in systolic blood pressure (SBP), diastolic blood pressure (DBP), or heart rate (HR). Stratification of the NCCAH group by treatment status showed higher mean SBP and DBP among treated adolescents and young women. This pattern is clinically plausible and methodologically expected, as therapy is typically initiated in those with more pronounced hyperandrogenism and an unfavorable metabolic profile, conditions that predispose to higher blood pressure values. Nevertheless, NCCAH per se was not associated with an increased risk of arterial hypertension compared with healthy controls.

Published data on blood pressure and heart rate in NCCAH are limited and heterogeneous. For example, Costa et al. observed neither adverse differences in BP and HR nor an elevated cardiovascular risk in NCCAH compared with controls (122). Moreover, the increased prevalence of cardiometabolic disease reported for classic CAH has not been consistently demonstrated in NCCAH (225, 241). Isolated case reports describe malignant hypertension requiring antihypertensive therapy in adults with comorbidities and NCCAH (242). Persistently elevated BP and HR in NCCAH warrant regular monitoring, as multiple factors—including androgen excess, metabolic profile, age, and pharmacotherapy—may adversely affect these cardiovascular parameters. In our study, we confirmed significant correlations between adiposity indices, aggregate androgen score, and BP values, consistent with previous findings (25, 193, 239). The positive association between androstenedione and DBP supports the hypothesis that chronic androgen exposure alters vascular tone and arterial stiffness; recent data indicate that women with NCCAH or other hyperandrogenic states have a higher risk of BP dysregulation due to androgen-induced vascular remodeling and activation of the renin–angiotensin system (236).

It is important to emphasize that relationships between androgens and cardiovascular indices are multifactorial and depend on age, body weight, insulin resistance (IR), corticosteroid therapy, and other variables. Several reviews conclude that hyperandrogenemia alone does not

fully account for the elevated cardiovascular risk in NCCAH, which arises from complex hormonal effects combined with additional metabolic and hemodynamic factors (7, 218).

No significant differences were observed between NCCAH patients and controls for fasting glucose, insulin, or HOMA-IR, aligning with contemporary studies showing that an NCCAH diagnosis per se is not a universal risk factor for impaired glucose homeostasis, particularly in normal-weight patients (30). For instance, *Macut et al*. reported that IR and elevated HOMA-IR occur primarily in overweight and obese patients, whereas normal-weight women show no significant differences versus controls (95).

Recent work highlights that the development of metabolic abnormalities in NCCAH is modified by body composition and lifestyle. Costa et al. found that normal-weight women with NCCAH have a metabolic profile similar to controls, whereas central obesity markedly increases the risk of IR and metabolic syndrome in hyperandrogenic patients (122). In this context, the absence of major anthropometric differences between groups in our study may explain the preservation of normal glucose metabolism among non-obese NCCAH participants. These findings underscore the importance of early prevention of overweight/obesity and individualized follow-up to mitigate metabolic risk.

Overweight/obesity is a key risk factor for IR and metabolic disturbances in women with NCCAH (30). Consistent with prior studies, our cohort exhibited a positive association between SBP and HOMA-IR, likely reflecting the role of IR in the pathogenesis of hypertension through interactions with hyperandrogenism, adiposity, prolonged androgen exposure, and corticosteroid treatment (95, 122, 225). Our results corroborate those of Piróg et al., who reported associations of clinical hirsutism (FGS) and serum testosterone with less favorable glucose metabolism and higher IR prevalence (235).

Correlation analyses among glucose homeostasis, adiposity, and clinical/biochemical hyperandrogenism in NCCAH participants revealed differential dynamics between metabolic indices and the hyperandrogenic phenotype, with adiposity emerging as the principal determinant of insulin sensitivity. The inverse relationships between glycemia and cutaneous hyperandrogenism (hirsutism, seborrhea) were more unexpected, indicating that more pronounced clinical hyperandrogenism often occurs in women with lower glucose levels. This illustrates the phenotypic heterogeneity of the condition: leaner patients may display more marked clinical features of hyperandrogenism, whereas metabolic abnormalities cluster in overweight women (122, 245). A similar dissociation is described in PCOS, where hirsutism

severity does not reliably correlate with IR (243, 244). Possible mechanisms include local dermal 5α-reductase activity, variability in androgen receptor expression, and local steroid metabolism that modulate cutaneous signs independently of systemic glycemic indices.

The positive correlation between insulin and BMI, and the significant predictive role of body weight for insulin and HOMA-IR, are consistent with evidence that overweight/obesity are major risk factors for IR and metabolic syndrome in CAH across phenotypes. Meta-analyses and cohort studies confirm a substantially increased metabolic risk in CAH in the presence of marked adiposity, irrespective of circulating androgen levels (122, 235, 241, 245). These observations support current recommendations for routine metabolic screening and early lifestyle intervention in women with hyperandrogenism, including NCCAH, as a key strategy to prevent cardiometabolic complications.

Regression analyses in our cohort also demonstrated an independent predictive role of serum testosterone for insulin concentration, though not for HOMA-IR. Similar associations have been reported by *Macut et al.,* who proposed that chronic androgen exposure in NCCAH impairs cellular insulin signaling, leading to compensatory hyperinsulinemia (95). Subsequently, *Piróg et al.* confirmed higher testosterone and HOMA-IR levels in women with NCCAH versus controls, consistent with a more adverse metabolic profile (235).

In summary, body weight is the principal predictor of metabolic risk in women with NCCAH, and its adverse effect is amplified by pronounced androgen excess. These findings are highly relevant to clinical practice, highlighting the need to control both body weight and hormonal imbalance to prevent cardiometabolic sequelae.

No statistically significant differences were detected between NCCAH patients and controls for total cholesterol, LDL-cholesterol, HDL-cholesterol, or triglycerides. The literature remains contradictory on this topic, with divergent results often explained by heterogeneity in age, body weight and obesity severity, pharmacotherapy and its duration, and other factors. For example, Piróg et al. reported a less favorable metabolic profile—including selected lipid indices—in NCCAH compared with controls (235), whereas Costa et al. found no significant lipid differences in young women with NCCAH (122). Reviews across CAH phenotypes, including NCCAH, emphasize that dyslipidemia is generally associated with obesity or prolonged high-dose glucocorticoid therapy, while maintenance of normal weight together with adequate therapeutic control tends to preserve lipid parameters within reference ranges (245, 246).

Within our study, complex interrelationships emerged among anthropometric measures, hyperandrogenism, and glucose and lipid homeostasis. Indices of weight status and adiposity were significantly associated with lipid parameters, consistent with the classic atherogenic profile seen in overweight/obesity, in which IR plays a central pathogenic role. The observed positive correlations between whole-body adiposity, clinical hirsutism, and total cholesterol are expected and concordant with prior NCCAH studies (225). Conversely, the inverse association between testosterone and triglycerides may reflect androgen-induced increases in lipolysis and visceral lipogenesis, leading to lower circulating triglycerides (95). Similar associations have been reported elsewhere, though their clinical significance remains debated (7, 232). Notably, an elevated cardiovascular risk may persist in NCCAH even with low triglyceride levels, driven by severe hyperandrogenemia and alternative mechanisms such as endothelial dysfunction, pronounced IR, and pro-atherogenic alterations in the LDL fraction (236).

Overall, the lipid profile in NCCAH is substantially influenced by body weight and IR, with hyperandrogenism and adiposity-related inflammation acting as additional modulators. Together, these factors shape a specific metabolic and cardiovascular risk profile in adolescent girls and young women with NCCAH, underscoring the need for regular follow-up to enable early identification and prevention of potential complications, and advocating for an individualized yet integrated care strategy targeting both hyperandrogenism control and early prevention of metabolic and cardiovascular disorders.

7. Adipokines and Inflammation

In our study, we demonstrated a significantly lower serum concentration of C-reactive protein (CRP) a marker of low-grade systemic inflammation among girls and young women with non-classic congenital adrenal hyperplasia (NCCAH) compared with healthy controls. This result does not conform to the expected inflammatory profile in NCCAH, since most publications to date report that hyperandrogenic conditions (classic CAH and polycystic ovary syndrome, PCOS) are associated with heightened inflammatory activity and, accordingly, elevated CRP levels likely related to coexisting obesity and chronic glucocorticoid therapy (241, 245). Studies specifically investigating systemic inflammation in NCCAH are very scarce and notably heterogeneous. The lower inflammatory activity observed in our cohort may be attributable to the small sample size, the younger mean age, inclusion of participants with incomplete pubertal development, shorter disease duration, restriction to female patients, the absence of severe obesity, and differences in exposure to or absence of pharmacotherapy. In a 2025 study of women with NCCAH, *Costa et al*. showed that metabolically healthy patients without

significant adiposity had CRP levels comparable to or lower than those of healthy controls (122). Other research groups have considered a potential immunomodulatory i.e., anti-inflammatory effect of moderately elevated androgen levels in young women (7), as well as the role of chronic, prolonged glucocorticoid treatment in the development of metabolic disturbances and associated inflammatory activity. These conflicting findings underscore that low-grade inflammatory activity is not an obligatory feature of NCCAH; rather, it reflects the complex interaction between adiposity, the androgen profile, and treatment status. For this reason, CRP cannot be used as a standalone biomarker for metabolic risk stratification in NCCAH; its interpretation must be integrated with other clinical and biochemical parameters, accounting for each patient's individual phenotype.

Of clinical relevance, we identified a significant correlation between CRP and indices of abdominal adiposity (waist circumference, WC), as well as clinical assessment of hirsutism (Ferriman–Gallwey score, FGS). Regression analysis confirmed the leading predictive role of abdominal fat mass for the presence of chronic systemic inflammation. Numerous studies in the general population have established the key involvement of central obesity in the synthesis and secretion of pro-inflammatory adipocytokines, including IL-6–induced hepatic overproduction of CRP. Investigations in classic CAH and PCOS likewise demonstrate the dominant role of abdominal obesity in driving inflammatory activity (241, 245). Collectively, this supports the thesis that in NCCAH, low-grade inflammation is phenotype-dependent and most pronounced in patients with central adiposity.

Leptin and adiponectin are key adipokines with opposing metabolic functions. Leptin participates in the regulation of energy homeostasis and appetite, whereas adiponectin exerts insulin-sensitizing, anti-inflammatory, and anti-atherogenic effects. In the present study, we observed a trend toward non-significantly lower serum concentrations of both adipokines in girls and young women with NCCAH compared with controls. These findings are not fully consistent with reports in patients with classic CAH (245), warranting cautious interpretation of the role of adipokines in metabolic regulation in NCCAH and their utility as sensitive biomarkers of metabolic risk in this hyperandrogenic condition. Factors that may influence serum leptin and adiponectin levels and partially explain discrepancies between our cohort and other reports include differences in sample size, participants' sex and age, body composition (amount and distribution of adipose tissue), the presence of concomitant cardiometabolic abnormalities, and heterogeneity in hormonal regimens and their duration among treated patients (245). These determinants can modulate adipokine secretion and action. In 2025,

Bacila et al. reported no significant differences in leptin and adiponectin concentrations in female CAH participants versus controls, while hyperandrogenic males exhibited significantly higher leptin than male controls in the same multicenter study (247). A characteristic inverse relationship between administered glucocorticoid dose and leptin secretion has also been observed, suggesting that more intensive, high-dose therapy suppresses leptin production. A negative association between adiponectin and androgenic steroids (17OHP, androstenedione, testosterone, 11-hydroxyandrostenedione, and 11-ketotestosterone) has likewise been demonstrated, consistent with an impact of androgen status on metabolic regulation. These findings delineate the complex interrelationships among adipokine secretion and action, metabolic indices, and hormonal therapy in CAH. Thus, lower adiponectin levels in patients with poorly controlled androgen excess may represent a potential mechanism linking hormonal imbalance with the development of insulin resistance (IR) and an unfavorable cardiometabolic profile. Concordant results were reported by *Apsan et al*. in children and young people with CAH aged 7–22 years, where higher androgen levels were associated with lower adiponectin, without a significant relationship between leptin and the androgens studied (123). In the same study, overweight patients displayed elevated leptin with low adiponectin—a characteristic adipokine constellation in metabolic syndrome. Overall, leptin emerges as a sensitive indicator of excess body fat and its distribution, whereas adiponectin serves as an indicator of concomitant metabolic and androgenic dysregulation. Again, it must be emphasized that contradictory findings across studies may stem from smaller cohort sizes and differences in sex, age, pubertal stage, hormonal and body composition, and pharmacologic disease control.

Of practical clinical importance in our cohort is the inverse correlation between adiponectin concentration and LDL-cholesterol, suggesting an anti-atherogenic role for adiponectin in the early stages of atherogenesis. Although inverse associations between adiponectin and atherogenic lipids have been reported in endocrine and metabolic conditions such as PCOS and metabolic syndrome, systematic data in NCCAH are currently lacking (248).

Despite the lack of statistical significance, the inverse relationship between adiponectin and glucose in NCCAH patients is biologically plausible. A very large body of evidence supports adiponectin's role in improving insulin sensitivity and peripheral glucose clearance, regulating hepatic gluconeogenesis, and promoting fatty-acid oxidation. Accordingly, reduced adiponectin is associated with increased risk of hyperglycemia, IR, impaired glucose tolerance, and diabetes (248), including in cohorts with various forms of CAH (123). It should be noted that therapeutic control of androgen excess also modulates the adipokine and glycemic profile in this disease:

very high glucocorticoid doses and prolonged treatment may worsen insulin sensitivity irrespective of adiponectin concentration, positioning therapeutic “balance” as a key moderator of metabolic effects (247).

In our cohort, we found no significant correlations between the adipokine profile (adiponectin and leptin) and indices of clinical or biochemical hyperandrogenism. Although androgens can pathophysiologically modulate the amount and distribution of adipose tissue and adipokine secretion, published data are inconsistent. For example, *Apsan et al*. reported an inverse association between adiponectin and androstenedione but no relationship with clinical manifestations of hyperandrogenism, whereas *Bacila et al.* emphasized that therapeutic control of the disease, rather than androgen levels per se, exerts greater influence on serum adipokine dynamics (123, 247). It should be stressed that data on the relationships among adipokines, metabolic status, and androgen excess in NCCAH are extremely limited and substantially influenced by determinants such as sample size, sex and age, weight and pubertal status, type and duration of pharmacotherapy, etc., underscoring the need for larger, well-designed future studies.

In our NCCAH cohort, linear regression confirmed the role of abdominal adiposity—assessed by WC as the only independent predictor of serum leptin concentration, whereas pubertal maturation stage and DHEA-S concentration exerted the strongest influence on adiponectin levels. WC correlates strongly with both the amount and abdominal distribution of adipose tissue well-established risk factors for IR and cardiometabolic complications—and is associated with increased leptin production (245). The dependence of adiponectin on pubertal stage corroborates the well-known physiological decline in adiponectin during puberty, a period characterized by physiological insulin resistance and, correspondingly, an increased HOMA-IR index (247). At the same time, the significant predictive role of DHEA-S and testosterone for lower adiponectin levels in NCAH supports the hypothesis of a negative impact of hyperandrogenemia on adiponectin secretion, as shown in other CAH cohorts (123).

A synthesis of these results allows several principal conclusions. First, NCCAH is not characterized by a significantly elevated systemic inflammatory activity overall, yet CRP remains a sensitive indicator in the presence of abdominal obesity. Second, leptin concentration associates with the degree of adiposity and does not correlate with the severity of hyperandrogenism. Third, adiponectin supports its role as a marker of early metabolic derangements. Accordingly, in the clinical follow-up of girls and young women with NCCAH, monitoring should encompass not only the hyperandrogenic state but also the dynamics of

adipokine and inflammatory profiles, given their prognostic potential for future cardiometabolic complications.

8. Body Composition

In the present study, no significant differences were observed in the analyzed DXA parameters between girls and young women with non-classic congenital adrenal hyperplasia (NCCAH) and healthy controls. Nevertheless, the presence of hyperandrogenism was found to be associated with lower bone mineral density (BMD), bone mineral content (BMC), lean body mass (LBM), and total body fat mass (FM). These early alterations in body composition among NCCAH patients may be regarded as an initial manifestation related to the endocrine–metabolic characteristics of the disorder.

A review of the literature concerning bone metabolism in patients with congenital adrenal hyperplasia (CAH) reveals inconsistent findings, likely resulting from the complex interplay between androgen exposure, glucocorticoid dose and duration, muscle mass, and mechanical skeletal loading during physical activity all of which influence bone mineralization (249, 251). Some studies in classic forms of CAH have reported significantly reduced bone mineral density due to suppressed bone anabolism under high-dose glucocorticoid therapy (253). In contrast, patients with mild classic forms or NCCAH, characterized by moderate androgen excess, have been shown to exhibit normal or slightly elevated BMD and BMC, presumably reflecting the anabolic and stimulatory effects of androgens on muscle mass and bone metabolism (250, 254).

In our study, a strong positive correlation was demonstrated between bone mineral content and lean body mass, confirming the physiological interrelationship between muscle and skeletal mass and the mechanical load exerted on bone an association previously described by *Halper et al.* in children with classic CAH (253).

No significant correlations were observed between bone parameters and metabolic indices in our cohort, a finding that may be explained by the participants' young age, absence of severe obesity, adequate metabolic control, and relatively short disease duration. These results are consistent with those reported *by Espinosa Reyes et al.,* who likewise found no significant association between insulin resistance indices and bone mineral density in young patients with NCCAH (250). Conversely, *Ben Simon et al.* reported higher fat mass and a greater predisposition to metabolic syndrome in children and adolescents with NCCAH who were overweight (252).

Despite these discrepancies, the overall evidence supports the conclusion that monitoring body composition parameters in NCCAH patients facilitates the early identification of risks for osteopenia and related metabolic disturbances during youth and early adulthood.

9. Psychological Assessment and Quality of Life

Psychological assessment of the participants using the standardized instrument Eating Disorder Inventory-3 (EDI-3) revealed clear differences across several psychological domains between patients with non-classic congenital adrenal hyperplasia (NCCAH) and healthy controls. These findings reflect the complex interaction between hormonal status, somatic changes, and psycho-emotional adaptation within this patient population.

Girls and young women with NCCAH demonstrated significantly higher risk scores for interpersonal alienation, interoceptive deficits, emotional dysregulation, low self-esteem, ineffectiveness, overcontrol, and overall composite psychological maladjustment. These results are consistent with previous studies reporting that patients with CAH particularly women with clinical manifestations of hyperandrogenism are at increased risk for emotional and interpersonal difficulties and reduced body image acceptance (194, 256).

Among the principal predictors of interpersonal difficulties in the NCCAH group were higher waist circumference (WC), elevated 17OHP levels, and presence of alopecia. These associations suggest that the clinical and hormonal aspects of hyperandrogenism affect not only physical health but also social and emotional functioning. Similar associations between the phenotypic expression of hyperandrogenism and social insecurity, low self-esteem, and heightened body image self-criticism have been documented in women with polycystic ovary syndrome (PCOS) (262) and in patients with chronic dermatological manifestations such as acne and androgenic alopecia (257, 258, 261).

The “fear of maturity” subscale score demonstrated a significant association with the androgen-dependent manifestation of alopecia and a non-significant relationship with acne and seborrhea. This may represent a defensive psychological mechanism against the perceived discrepancy between biological sex characteristics and social expectations of femininity. The heightened fear of maturity and overcontrol observed in these patients likely reflect a compensatory adaptive response aimed at emotional restraint and self-regulation in the context of an altered body image a phenomenon described in young women with chronic endocrine conditions (259, 260).

A higher serum insulin concentration emerged as an independent significant predictor of low self-esteem, which may be interpreted in light of the established association between insulin resistance (IR) and central effects on mood and cognitive regulation, as described in psychoneuroendocrine models of stress and metabolism (90). Concurrently, the presence of alopecia remained an important determinant of self-esteem, once again confirming the influence of body image on psychological well-being in women with NCCAH.

The lower scores across all quality-of-life domains among patients with NCCAH in this study are consistent with previously published reports (215, 255), which also indicate reduced quality of life in women with various forms of CAH, particularly within physical and psychosocial domains. Significant correlations were identified between lower scores in the physical domain (D1) and low self-esteem, interpersonal alienation, psychological maladjustment, and more pronounced hyperandrogenic features (hirsutism, acne, elevated 17OHP levels). These findings emphasize the integrated influence of endocrine and psychological factors on the subjective well-being of patients with NCCAH.

Of particular social relevance is the inverse relationship between environmental quality-of-life indicators (D4 domain) and anthropometric parameters such as body weight and WC, supporting global evidence that environmental factors (e.g., limited physical activity, sedentary lifestyle, urbanization) negatively impact weight status, especially among individuals with chronic health conditions (194).

In summary, the results of this study confirm that the psychological characteristics and quality-of-life outcomes of patients with NCCAH are closely associated with their body image, hormonal status, and metabolic profile. These interrelations underscore the importance of a multidisciplinary approach to the management and follow-up of women with NCAH one that integrates endocrinological, psychological, and psychosocial assessment and care. Early identification of at-risk psychological profiles and timely, targeted intervention may substantially improve long-term health, emotional, and social outcomes in this patient population.

PART VI. MAIN CONCLUSIONS

Task 1:

1.2 The mean age at diagnosis of non-classic congenital adrenal hyperplasia (NCCAH) was 14.4 years, with more than one-third of girls and young women reporting a history of premature pubarche.

1.3 Patients with NCCAH exhibited marked clinical hyperandrogenism, manifested by hirsutism and acne in nearly all participants, whereas seborrhea and alopecia were observed less frequently.

1.4 The Ferriman–Gallwey score (FGS) showed a significant correlation with clinical hirsutism in NCCAH patients and represents a reliable tool for its assessment and monitoring during therapy.

1.5 NCCAH patients demonstrated significantly higher serum levels of testosterone, androstenedione, and 17-hydroxyprogesterone (17OHP) compared with healthy controls, and these correlated with FGS values.

1.6 The most significant predictors of hirsutism, acne, alopecia, and menstrual disorders in NCCAH were the serum concentrations of androstenedione and 17OHP. Among them, androstenedione was the strongest biochemical correlate of FGS, while 17OHP remained the key diagnostic laboratory marker for confirming the disease.

Task 2:

2.1 The prevalence of general overweight among NCCAH patients was 14.7%, and of obesity was 11.8%, with no significant difference compared with the healthy population. However, abdominal obesity was present in 32.4% of NCAH patients—approximately one-third higher than in controls.

Task 3:

3.1 Patients with NCCAH showed mean values of systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate (PR) comparable to those of the control group, with diastolic hypertension identified in only 2.9% of hyperandrogenic women.

3.2 The indices of glucose–insulin homeostasis were similar between the two groups, with fasting hyperglycemia found in only 1.5% of hyperandrogenic women.

3.3 NCCAH patients displayed similar lipid profiles to healthy controls, with no elevated triglycerides (TG) or low HDL cholesterol according to IDF criteria.

Task 4:

4.1 The presence of hirsutism, quantitatively assessed by the FGS, correlated significantly with total and abdominal adiposity indices.

4.2 The most significant predictor of SBP and DBP in women with NCCAH was body mass index (BMI), with blood pressure values also correlating proportionally with FGS—demonstrating the influence of adiposity and hyperandrogenism on cardiovascular parameters in these patients.

4.3 Indices of total and abdominal adiposity in NCCAH correlated with insulin and HOMA-IR concentrations, confirming the key role of adiposity as a predictor of metabolic risk in these patients.

4.4 Body mass was the main independent predictor of triglyceride and HDL-cholesterol concentrations, supporting the role of adiposity as a primary metabolic determinant in hyperandrogenic girls and young women.

Task 5:

5.1 NCCAH patients demonstrated lower CRP concentrations compared with controls, with abdominal adipose tissue, clinically assessed by waist circumference (WC), serving as the most significant predictor of CRP levels.

5.2 Serum leptin and adiponectin levels showed a significant inverse correlation in girls and young women with NCCAH but did not differ significantly from controls. In hyperandrogenic girls, leptin concentration was predicted by waist circumference, whereas adiponectin was most strongly influenced by pubertal stage and DHEA-S, HOMA-IR, and testosterone levels.

Task 6:

6.1 Body composition analysis revealed a trend toward lower bone mineral density (BMD), bone mineral content (BMC), lean mass, and total body fat mass in NCCAH patients, though without statistically significant differences compared to controls.

6.2 A strong positive correlation was established between bone mineralization and lean body mass, both dependent on adiposity indices, confirming the key role of muscle mass in maintaining bone health in NCCAH patients.

Task 7:

7.1 Results from the standardized EDI-3 questionnaire indicated poorer psychological and psychosocial health in NCAH patients compared with controls.

7.2 The main independent determinants of impaired psychological well-being in NCCAH girls were higher adiposity indices (WC, BMI, fat mass) and the presence of clinical hyperandrogenism (alopecia, seborrhea).

Task 8:

8.1 Girls and young women with NCCAH exhibited reduced quality of life compared with controls, with the greatest impairments observed in physical and psychological health domains.

8.2 Among NCCAH patients, poorer quality of life was associated with worse psychological well-being and was negatively influenced by the presence of hyperandrogenism and increased adiposity.

PART VII. CONTRIBUTIONS OF THE DISSERTATION

1. This is the first study in Bulgaria and one of the few worldwide to provide a comprehensive evaluation of non-classic congenital adrenal hyperplasia (NCCAH) in girls and young women, significantly enhancing current knowledge about this disorder, which clinically overlaps with other hyperandrogenic conditions.

2. The research is unique on a national level and among the few internationally that examine the association between NCCAH and the development of cardiometabolic abnormalities in adolescent and young adult females.

3. This dissertation represents the first national and one of the few global studies to investigate serum adiponectin and leptin levels in NCCAH patients and to explore their relationship with cardiometabolic risk and hyperandrogenism.

4. For the first time, this study evaluates body composition in girls and young women with NCCAH and examines its association with cardiometabolic risk markers and clinical/laboratory hyperandrogenism, making it unique on a global scale.

5. This is the first study in Bulgaria and one of the few worldwide specifically focused on the psychological health of girls and young women with NCCAH.

6. The project is unique nationally and internationally, as it provides the first assessment of quality of life among girls and young women with NCCAH.

7. The findings highlight the need for early screening, timely treatment, and long-term follow-up of patients with NCCAH in routine clinical practice, with the aim of reducing complication risk and improving quality of life. The results support the development of a structured algorithm for the early clinical, biochemical, and psychological evaluation of young women with NCCAH to facilitate the prevention of disease-related complications.

PART VIII. CONCLUSION

The present dissertation provides an in-depth analysis of the clinical, laboratory, and metabolic profile of girls and young women with non-classic congenital adrenal hyperplasia (NCCAH), as well as the impact of these factors on their psychological well-being and quality of life. The findings and conclusions derived from this research contribute to improving the multidisciplinary management of the disease and to ensuring a comprehensive understanding of its effects on multiple dimensions of health in affected individuals.

Beyond confirming several well-established facts, the results of this study reveal novel and previously underexplored aspects of NCCAH particularly the interaction between chronic androgen excess and adiposity and their combined influence on the hormonal, metabolic, and body composition characteristics of affected young individuals. These interactions may represent early determinants of long-term cardiometabolic complications as the disease progresses.

The dissertation also explores, for the first time, the psychological profile of NCCAH patients, demonstrating elevated anxiety levels, low self-esteem, and impaired personality functioning, particularly in women exhibiting more pronounced hyperandrogenism and body image disturbance. These findings underscore the need for timely psycho-emotional monitoring and the integration of psychological support into standard care protocols for this population.

Moreover, the study examines the quality of life of girls and young women with NCCAH an aspect that has remained largely unexplored to date. The results highlight the multifactorial nature of quality-of-life impairment, which is influenced not only by the severity and chronicity of symptoms but also by social and psycho-emotional factors. Consequently, incorporating early psychological counseling and support into patient management emerges as a crucial component for optimizing disease outcomes and improving long-term prognosis.

The findings of this research emphasize the need for a comprehensive yet individualized approach to the assessment, treatment, and follow-up of NCCAH patients one that encompasses their physical, metabolic, and psychosocial health. Such an approach is essential to improving overall quality of life and minimizing associated health risks in this patient group.

This dissertation lays the foundation for future longitudinal studies aimed at evaluating the long-term cardiometabolic and psychological outcomes of NCCAH, assessing the impact of therapeutic interventions, validating specific biomarkers of metabolic risk, and exploring the potential role of genetic and epigenetic determinants underlying individual variability in disease expression and treatment response.

In conclusion, the results of this study both in their original contributions and within the framework of existing scientific evidence clearly delineate the necessity for systematic and comprehensive evaluation of the clinical and functional status of NCCAH patients. Specifically, the integrated assessment of anthropometric parameters, physical condition, metabolic and hormonal profiles, body composition, psychological state, and quality of life should become an essential component of routine clinical practice in the management of individuals with this condition.

PART IX. SCIENTIFIC PUBLICATIONS AND PRESENTATIONS RELATED TO THE DISSERTATION

Scientific Publications

1.T. Karamfilova, S. Galcheva, V. Yotova. Non-classic congenital adrenal hyperplasia in adolescent girls and women – clinical and paraclinical characteristics. Science Endocrinology 2022; 1: 4–9.

2.T. Karamfilova, S. Galcheva. Clinical and laboratory characteristics, psychological assessment, and quality of life in patients with non-classic congenital adrenal hyperplasia. Varna Medical Forum 2023; 12, Online First. ISSN 2367-5519.

Scientific Contributions and Conference Participation

1. Karamfilova T, Galcheva S, Mladenov V, Boyadzhiev V, Bazdarska Y, Yordanova N, Iotova V. Clinical and metabolic characteristics of hyperandrogenic girls with non-classic congenital adrenal hyperplasia and polycystic ovary syndrome. 59th ESPE Annual Meeting 2021, 22 – 26 September (Online) 94:P2-15

2. Karamfilova T, Galcheva S, Bocheva Y, Ivanova D, Bazdarska Y, Iotova V. Clinical, laboratory and body composition profile of young female patients with non-classic congenital adrenal hyperplasia. 60 st Annual ESPE Meeting 2022, 15-19 September 2022, Rome, Italy, P1-409.

3. Galcheva S, **Karamfilova T**, Yordanova N, Bocheva Y, Mladenov V, Iotova V. Precocious pubarche in girls - a clinical sign for underlying hyperandrogenic disease. 60 st Annual ESPE Meeting 2022, 15-19 September 2022, Rome, Italy

4. Karamfilova T, Galcheva S, Bocheva Y, Iotova V. Effects of hyperandrogenism on psychological perception and quality of life in patients with non-classical congenital adrenal hyperplasia. 61st Annual ESPE Meeting 2023, 21-23 September 2023, Hague, Netherlands P1-205

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