THE IMPACT OF OBESITY AND WEIGHT LOSS TREATMENT ON METABOLIC PARAMETERS, CARDIOVASCULAR AUTONOMIC AND SENSORY NERVE FUNCTIONS, AS WELL AS THE SUCCESS OF *IN VITRO* FERTILIZATION TREATMENT IN INFERTILE WOMEN WITH OBESITY

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Ph.D. Thesis

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Relevant publications

Full papers

I. Keller N, Zádori J, Lippai B, Szöllősi D, Márton V, Wellinger K, Lada S, Szűcs M, Menyhárt A, Kempler P, Baczkó I, Várkonyi T, Lengyel C, Vágvölgyi A. Cardiovascular autonomic and peripheral sensory neuropathy in women with obesity. Front Endocrinol (Lausanne). (2024) 15:1386147. doi: 10.3389/fendo.2024.1386147. **D/Q rank: Q1**; **Impact factor: 4.6**

II. Vágvölgyi A, Vedelek V, **Keller N**, Szöllősi D, Lada S, Nemes A, Kempler P, Menyhárt A, Baczkó I, Várkonyi T, Lengyel C, Zádori J. The impact of obesity and weight loss treatment on metabolic parameters, cardiovascular autonomic and sensory nerve function and *in vitro* fertilization outcomes in infertile women: a pilot study. Front Endocrinol (Lausanne). (2025) 16:1548587. doi: 10.3389/fendo.2025.1548587. **D/Q rank: Q1**; **Impakt factor: 4.6**

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Other publications

Vamos M, Zsigmond EJ, Biffi M, Gausz FD, **Keller N**, Kupo P, Szili-Torok T, Ziacchi M, Benz AP, Spittler R, Vagvolgyi A. Efficacy and safety of the subcutaneous implantable cardioverter-defibrillator in patients with and without obesity: A meta-analysis. Heart Rhythm. (2025) 22(2):375-387. doi: 10.1016/j.hrthm.2024.07.021. . **D/Q rank: Q1**; **Impact factor: 5.7**

Keller N, Viola R, Várkonyi T, Lengyel Cs, Vágvölgyi A, Vámos M. A liraglutid szerepe az obezitással társult pitvarfibrilláció kezelésében. Diabetologia Hungarica. (2025) 33(2):117-127. doi: 10.24121/dh.2025.15.

Impact factor of papers related to the subject of the thesis: 9.2

Impact factor of other papers: 5.7

Total impact factor: 14.9

Abbreviations

ACE: angiotensin-converting enzyme

ACR: albumin/creatinine ratio

AF: atrial fibrillation

AMH: anti-Müllerian hormone

anti-TPO: anti-thyroid peroxidase

ART: Assisted Reproductive Technology

ALAT/GPT: alanine aminotransferase

ASAT/GOT: aspartate aminotransferase

ALP: alkaline phosphatase

BMC: bone mineral content

BMR: basal metabolic rate

BMI: body mass index

BP: blood pressure

CPT: current perception threshold

CRP: C-reactive protein

DPP-4: dipeptidyl peptidase-4

eGFR: estimated glomerular filtration rate

ESHRE: European Society of Human Reproduction and Embryology

FFMI: fat-free mass index

fT3: free triiodothyronine

fT4: free thyroxine

HDL-cholesterol: high-density lipoprotein cholesterol

LDL-cholesterol: low-density lipoprotein cholesterol

HRRDB: the heart rate response to deep breathing

HRRSU (30/15 ratio): the heart rate responses to standing up

GGT: γ-glutamyl transferase

GLP-1: glucagon-like peptide-1

GLP-1R: glucagon-like peptide-1 receptor

GLP-1 receptor agonists: glucagon-like peptide-1 receptor agonists

HbA1c: hemoglobin A1c

HOMA-IR: homeostatic model assessment

IBS: InBody score

ICSI: intracytoplasmic sperm injection

IGT: impaired glucose tolerance

IL-6: interleukin-6

IVF: *in vitro* fertilization

LH: left hallux

LR: left radius

n: frequencies

NHANES: National Health and Nutrition Examination Survey

NM2000: current perception threshold value of the median nerve at a stimulation frequency of 2000 Hz

NM250: current perception threshold value of the median nerve at a stimulation frequency of 250 Hz

NM5: current perception threshold value of the median nerve at a stimulation frequency of 5 Hz

NP2000: current perception threshold value of the peroneal nerve at a stimulation frequency of 2000 Hz

NP250: current perception threshold value of the peroneal nerve at a stimulation frequency of 250 Hz

NP5: current perception threshold value of the peroneal nerve at a stimulation frequency of 5 Hz

PBF: body fat percentage

PCOS: polycystic ovarian syndrome

R: resistance

RH: right hallux

RR: right radius

SBPRSU: Systolic blood pressure response to positional change from lying to standing up

SHBG: sex hormone binding globulin

DHEAS: dehydroepiandrosterone sulfate

SD: standard deviation

SMM: skeletal muscle mass

T2DM: type 2 diabetes

Tmax: time to maximum plasma concentration

TSH: thyroid-stimulating hormone

VR: Valsalva-ratio

VFA: visceral fat area

WBPA: whole body phase angle

WHO: World Health Organization

WHR: waist-to-hip ratio

Xc: reactance

25OHD3/D2-vitamin: 25-hydroxy-D3- and D2-vitamin

1. Introduction and aims of the studies

Obesity has become a global health crisis, with its prevalence increasing dramatically over the past few decades[1,2]. Nutritional status is most commonly assessed using the body mass index (BMI), in accordance with the classifications of the World Health Organization (WHO), which define individuals with a BMI between 25 and 29.9 kg/m² as overweight and those with a BMI of 30 kg/m² or higher as obese[3]. According to data from the 2017–2018 National Health and Nutrition Examination Survey (NHANES) conducted in the United States, 30.7% of individuals were classified as overweight, 42.4% as obese, and 9.2% as severely obese, indicating a very high prevalence of overweight and obesity[2]. This trend is not unique to the United States; in fact, similar patterns are observed in Hungary. Data from the World Obesity Federation's Global Obesity Observatory[1], published in 2022, show that 36.2% of Hungary's population aged 18 years and older is classified as overweight (40.7% of men and 32.1% of women), while 22.2% is considered obese (24.6% of men and 20.0% of women).

Obesity alone, even in the absence of overt diabetes mellitus, is now recognized as a major factor contributing to the increased prevalence of both central and peripheral neuropathies[4]. Accordingly, obesity is currently regarded as the second most important metabolic risk factor for neuropathy, second only to diabetes[5]. In addition, dyslipidemia has also been implicated as a contributing factor in non-diabetic individuals[6,7]. In a study by Callaghan et al. (2020)[8], normoglycemic individuals with a BMI over 35 kg/m² had a higher prevalence of neuropathy compared to normal-weight control subjects. Obesity may lead to the development of neuropathy through various pathophysiological mechanisms, including chronic low-grade inflammation[9], insulin resistance[10], oxidative stress[11], dyslipidemia[12], and selective injury to small nerve fibers[13]. Additionally, neuropathy was significantly associated with waist circumference, but not with general obesity. Research conducted in the United States, Denmark, the Netherlands, and Germany has demonstrated an independent relationship between waist circumference and neuropathy, while other anthropometric parameters were not examined[13–16]. According to Lieb et al. (2012)[17], abdominal obesity may not be a primary driving factor of distal symmetric polyneuropathy; rather, the true mediators could be inflammatory markers originating from adipose tissue, such as interleukin-6 (IL-6) concentrations or the adiponectin-to-leptin ratio. Several studies have confirmed the important role of cardiovascular risk factors in the development of cardiovascular autonomic neuropathy, including elevated systolic blood pressure (BP), triglyceride levels, high BMI, and smoking[18–20]. A study examining the

relationship between comprehensive anthropometric measurements and neuropathy found a significant association between neuropathy and female sex[21].

Research suggests that newer glucose-lowering medications, such as glucagon-like peptide-1 (GLP-1) receptor agonists, may offer beneficial effects in the treatment of autonomic neuropathy[22]. Liraglutide, an acylated glucagon-like peptide-1 (GLP-1) analog, was the first-choice medication for individuals with a BMI over 30 kg/m² or those with a BMI over 27 kg/m² in the presence of obesity-related comorbidities (such as diabetes, prediabetes, hypertension, obstructive sleep apnea syndrome, or polycystic ovarian syndrome (PCOS)) in accordance with the recommendations of the Hungarian guidelines during the study period[23,24]. Endogenous glucagon-like peptide-1 (GLP-1) is a 30–amino acid peptide hormone predominantly secreted from three tissues in the human body: enteroendocrine L-cells in the distal intestine, pancreatic α-cells, and the central nervous system. Its secretion is stimulated primarily after oral glucose ingestion - but not following intravenous glucose administration - and it exerts glucose-dependent insulinotropic effects, among other roles in glucose homeostasis. However, native GLP-1 has an exceptionally short plasma half-life of less than two minutes in vivo, as it is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4)[25,26]. Liraglutide is produced by replacing lysine at position 34 with arginine and attaching a 16-carbon saturated fatty acid (palmitic acid) to position 26 via a γ-glutamic acid spacer[26]. Thanks to its structural modifications, liraglutide has an extended half-life of approximately 13 hours, allowing for once-daily administration as a subcutaneous injection. Its prolonged presence in the circulation is attributed to a combination of albumin binding, aggregation at the injection site, and decreased susceptibility to degradation by the enzyme DPP-4, resulting in a Tmax (time to maximum plasma concentration) of 9 to 13 hours[26]. Liraglutide binds to the GLP-1 receptor (GLP-1R), enhancing satiety signals while reducing hunger signals, thereby promoting a decrease in food intake. Furthermore, liraglutide stimulates insulin secretion in a glucose-dependent manner and reduces glucagon release, thereby enhancing glucose uptake in peripheral tissues and lowering both fasting and postprandial glucose levels[24].

In addition to reducing blood glucose levels, GLP-1 receptor agonists (GLP-1RAs) influence the function of multiple organs, including the heart, gastrointestinal tract, and liver[27]. GLP-1RAs also delay gastric emptying and act on the hypothalamus to enhance the feeling of satiety[28]. Several mechanisms of GLP-1 related to the immune system have also been identified. Overall, GLP-1 receptor signaling exerts anti-inflammatory effects and modulates cardiovascular function across various tissues, in both physiological and pathological

conditions[27,29]. Despite the fact that GLP-1 receptor agonists enhance insulin secretion, GLP-1 receptor agonists do not cause β-cell exhaustion, because, besides promoting insulin secretion in a glucose-dependent manner, they reduce apoptotic cell death of β-cells and stimulate their proliferation[30]. However, chronic hyperglycemic exposure leads to a decrease in GLP-1 receptor expression in β-cells, weakening the protective effect of GLP-1 and GLP-1RAs against glucotoxicity. This highlights the importance of initiating treatment early, even before the development of type 2 diabetes (T2DM)[30].

Obesity, a significant public health concern, is associated with numerous comorbidities, including cardiovascular and metabolic diseases, various cancers, obstructive sleep apnea, dementia, reduced quality of life, and life expectancy, as well as reproductive disorders[31]. Obesity gradually increases the risk of atrial fibrillation (AF) in a BMI-dependent manner[32–34]. A meta-analysis by Wong et al., including data from 13 studies with 157,518 patients, reported that the risk of developing AF increases by 10–29% for every 5-unit increase in BMI[32]. In the Framingham Heart Study, each 1-unit increase in BMI was associated with a 4% higher risk of developing AF[34]. Additionally, excess body weight may increase the risk of ischemic stroke, thromboembolism, and mortality in patients with AF[33]. Consequently, the obesity pandemic is a major contributor to the increasing global prevalence of AF[35].

As previously mentioned, obesity plays a role in the development of numerous comorbidities, including reproductive dysfunctions[31] such as prolonged time to conception[36] and reduced fertility[37–39]. In Hungary, approximately 3% of newborns are born following pregnancies conceived through assisted reproductive technologies (ART)[40]. In 2024, the total number of live births was 77,500[41] in Hungary. According to NICE recommendations, a BMI between 19 and 30 kg/m² is advised for women prior to initiating ART, as values outside this range may reduce treatment success rates[42]. Literature data regarding female obesity, preconception weight reduction, and IVF outcomes remain contradictory. Koatz and colleagues, in their recent review, reported that while two studies found no association between female obesity and *in vitro* fertilization (IVF) outcomes, another 13 studies identified negative effects on certain parameters. However, there was no clear consensus on whether weight reduction had an impact on these outcomes[43]. A recent study involving 537 patients undergoing IVF, with or without intracytoplasmic sperm injection (ICSI) cycles and successful oocyte retrieval, found no strong correlation between endometrial thickness, BMI, and clinical pregnancy outcomes[31]. Beyond these findings, obesity represents a major risk factor for complications during pregnancy[44]. Multiple studies have demonstrated that losing weight

before undergoing IVF can substantially enhance pregnancy and live birth rates[45–48]. Additionally, research has shown that weight loss interventions can decrease the number of IVF cycles needed to achieve pregnancy[49]. For infertile women with obesity and PCOS, lifestyle modifications are advised as the primary treatment approach[50]. Increasing evidence from randomized clinical trials indicates that physical activity alone may improve pregnancy rates in women with reproductive health issues[51].

The aim of the first study (referred to as the "obesity study") was to assess cardiovascular autonomic and peripheral sensory nervous system function, anthropometric measurements, and laboratory parameters in obese, nondiabetic female patients, and to compare these parameters with those of age-matched healthy controls with a normal BMI (18.5–24.99 kg/m²) and without severe comorbidities. The second study (referred to as the "preconceptional weight reduction study") focused on infertile obese women who presented for preconceptional weight optimization prior to planned assisted reproductive procedures. This study investigated the effects of weight reduction on peripheral sensory and cardiovascular autonomic nervous system function, metabolic parameters, and IVF success (clinical pregnancy rates). In addition, potential correlations between these factors were explored, and the study assessed whether weight loss could beneficially influence any identified abnormalities, thereby contributing to improved risk-reduction strategies.

2. Study populations

2.1 Study populations of the obesity study

In our first cross-sectional observational study, we recruited 71 female patients with obesity (mean ± SD; age: 36.1 ± 8.34 years; BMI: 40.2 ± 8.47 kg/m²) and without diabetes, prior to initiating obesity treatment. Additionally, a control group of 36 age-matched female volunteers with normal BMI (age: 36.4 ± 13.25 years; BMI: 21.6 ± 2.13 kg/m²) was included. Patients were enrolled at the Endocrinology and Diabetology Outpatient Clinic, Department of Medicine, Albert Szent-Györgyi Medical School, University of Szeged, Hungary. Data collection and measurements were conducted between March 2021 and May 2023. Exclusion criteria included individuals with a previously or newly diagnosed case of diabetes, chronic heart failure, planned invasive cardiovascular interventions (e.g., percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, valve repair or replacement), uncontrolled hypertension (blood pressure > 160/100 mmHg), chronic renal failure, oncological diseases, chemical exposure, significant cognitive dysfunction, or lack of cooperation. All participants were of Caucasian descent. During the study period, a total of 71 female and only

11 male patients sought treatment for obesity at our clinic. Given the considerable gender imbalance and to avoid potential bias, male patients were excluded from the analysis, and the study focuses exclusively on female participants.

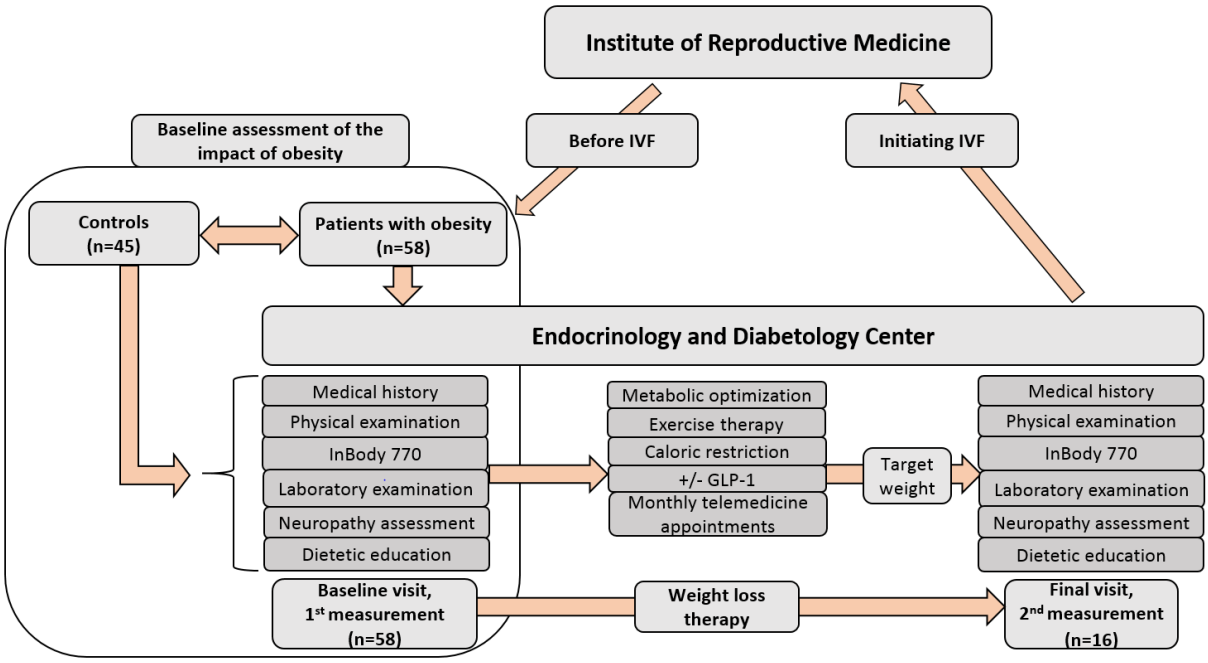
2.2 Study populations of the preconceptional weight reduction study

This second study was designed as a single-center cohort study and took place at the Endocrinology and Diabetology Outpatient Clinic, Department of Medicine, Albert Szent-Györgyi Medical School, University of Szeged, in partnership with the University's Institute of Reproductive Medicine. The study was conducted among women with obesity (BMI > 30 kg/m²) who were referred from the Institute of Reproductive Medicine and sought obesity management prior to IVF treatment, with the aim of optimizing their body weight. Preconception weight loss therapy was supervised by an endocrinologist. Between March 1, 2021, and March 31, 2024, clinical and anthropometric data were systematically gathered from women seeking to improve their body weight prior to undergoing ART. For baseline comparisons, we also enrolled age-matched healthy women with a BMI in the normal range (18.5–24.99 kg/m²) and without any recorded fertility disorders. The baseline parameters of the patient group were compared with those of the control group. The comparison group for baseline parameter analysis was drawn from the QT Registry[52], a continuously maintained database established in 2019. This registry prospectively documents participants' medical history, anthropometric measurements, laboratory and diagnostic findings, as well as results from standardized neuropathy assessments, and includes both healthy individuals and those with various health conditions. For the present study, eligible controls were identified from entries recorded between March 1, 2021, and March 31, 2024. All participants were of Caucasian origin. Recruitment into the QT Registry is ongoing and does not involve a separate enrollment campaign. Potential volunteers without significant health problems are often identified through informal channels - such as personal recommendations, accompanying relatives or friends attending clinic visits, or professional networks including medical students, colleagues, and acquaintances - and are offered the opportunity to participate in neuropathy-related research. This approach has allowed the registry to build a diverse and accessible control pool. The present work is a retrospective evaluation of observational, real-world data collected prospectively within this registry.

The management of obesity, including pharmacological treatment, was carried out in alignment with the latest Hungarian guidelines[23]. Following the baseline medical assessment, which included laboratory tests, body composition analysis, and complex neuropathic

examination, participants received individualized dietary counseling from a dietitian as a component of their lifestyle modification. The lifestyle modification plan also included an individually tailored physical activity program. Patients with obesity contacted the attending physician via email on a monthly basis or whenever necessary for immediate consultation regarding any concerns. There was no pre-set time limit for the weight loss or metabolic optimization program. The target weight was determined collaboratively by the endocrinologist and the IVF specialist together with the patient, taking into account the patient's age, comorbidities, and fertility status. The weight loss therapy was overseen by an endocrinologist. Preconception endocrine evaluation focused not only on obesity but also on detecting and optimizing additional endocrine and metabolic abnormalities. The second and final consultation was conducted after the patient had reached the target weight, just before the initiation of IVF treatment. At this visit, a reassessment of clinical, laboratory, body composition, and neuropathic parameters was performed, and renewed dietary counseling was provided. Patients with obesity who successfully reached their target weight before undergoing IVF and returned for this final evaluation were classified as "*finishers*". The study design, including the number of participants, is illustrated and summarized in **Figure 1**. The primary outcome recorded was the occurrence or absence of clinical pregnancy, which was defined at seven weeks of gestational age, following the guidelines of the International Committee for Monitoring Assisted Reproductive Technology[53].

Figure 1. Design of the preconceptional weight reduction study with the number of participants.



IVF: in vitro fertilization; GLP-1: glucagon like peptide analogue therapy.

3. Methods

3.1 Ewing's five standard cardiovascular reflex tests and autonomic score

In the studies, autonomic nervous system function was characterized using Ewing's five standard cardiovascular reflex tests[21]. This well-established methodology is regarded as the gold standard for the clinical evaluation of autonomic dysfunction, offering reproducible, clinically relevant, standardized, and non-invasive measurements. The performance of cardiovascular reflex tests is recommended by several national societies (e.g., in Italy and the United States) through their clinical practice guidelines[54,55]. Of the five reflex tests, three assess heart rate variability in response to specific physiological maneuvers, providing insight into parasympathetic regulation, while two evaluate blood pressure responses, which predominantly reflect sympathetic activity[56]. Measurements included continuous recording of six-lead electrocardiogram signals alongside blood pressure monitoring. Electrocardiogram data were digitized with a multichannel acquisition system (Cardiosys-A01, MDE Heidelberg GMBH, Heidelberg, Germany) at a sampling frequency of 2 kHz and stored for subsequent processing. Heart rate changes were examined during controlled deep breathing, the transition from a supine to a standing position (30/15 ratio), and the Valsalva maneuver[55]. Systolic blood pressure responses were assessed after standing up from the supine position, while diastolic responses were recorded during a three-minute sustained handgrip. Each cardiovascular reflex test was

evaluated separately and scored as 0 (normal), 1 (borderline), or 2 (abnormal). The total autonomic score was calculated as the sum of the individual test scores, providing a composite measure of the severity of autonomic neuropathy.

3.1.1 Heart rate response to deep breathing

Under normal physiological conditions, heart rate accelerates during inspiration and decelerates during expiration. This phenomenon, known as respiratory arrhythmia, has been well recognized for a long time and is predominantly mediated by parasympathetic control. Participants were instructed to breathe at a fixed rate of six cycles per minute (five seconds inhalation, five seconds exhalation). The difference between the highest and lowest heart rate (beats per minute) was calculated over six cycles[21].

3.1.2 Heart rate response to standing (30/15 ratio)

A typical healthy response to standing involves a rapid heart rate increase, peaking near the 15th beat after standing, followed by a transient slowing around the 30th beat. Participants began in a supine position, then stood up while electrocardiogram data were continuously recorded. The 30/15 ratio was calculated by dividing the longest R–R interval (around the 30th beat) by the shortest R–R interval (around the 15th beat), and this index is used to assess the autonomic innervation of the cardiovascular system[56].

3.1.3 Heart rate response to the Valsalva maneuver (Valsalva-ratio [VR])

The Valsalva maneuver normally causes a temporary drop in blood pressure accompanied by a rise in heart rate, followed by blood pressure overshoot and heart rate slowing after release. Participants exhaled into a manometer through a mouthpiece, maintaining 40 mmHg for 15 seconds. The Valsalva-ratio was obtained by dividing the longest post-maneuver R–R interval by the shortest interval during the maneuver[56,57].

3.1.4 Systolic blood pressure response to postural change (from lying to standing up, SBPRSU)

When moving from lying to standing, blood is transiently redistributed to the lower limbs. In healthy individuals, this shift is rapidly compensated by peripheral vasoconstriction, which maintains blood pressure and prevents orthostatic hypotension. In contrast, orthostatic hypotension is a hallmark of cardiovascular autonomic neuropathy, reflecting impaired compensatory mechanisms, and its presence indicates disturbed sympathetic innervation. In addition to the loss of vasoconstriction in the peripheral vessels, dysfunction of the splanchnic

vasculature also plays a major role in the blood pressure fall, as the vasoconstriction that would normally occur in this region is absent. The exact threshold distinguishing normal from pathological blood pressure changes, however, remains a matter of debate[58]. To assess this mechanism, systolic blood pressure was recorded after 10 minutes supine, and at 1, 5, and 10 minutes after standing. The largest drop from the supine measurement was documented as the orthostatic response.

3.1.5 Diastolic blood pressure response during sustained handgrip

During the sustained handgrip test, diastolic blood pressure responses were evaluated. Maximal grasping force was determined using a hand-held dynamometer in the dominant hand. Participants then maintained 30% of their maximal force for 3 minutes. Blood pressure was measured once per minute on the contralateral arm, and the maximum increase from baseline was recorded as the handgrip response. The most recent recommendations, including the 2011 Toronto Consensus, propose the orthostatic hypotension test for the assessment of sympathetic autonomic function, while the measurement of diastolic blood pressure rise during sustained handgrip has been omitted from the guidelines[55].

3.2 Peripheral sensory nerve testing

3.2.1 Neurometer

The sensory function of different types of peripheral nerve fibers was assessed in a simple, non-invasive, and reproducible manner using the Neurometer device (NM-01/CPT Neurometer, MDE Heidelberg GmbH, Heidelberg, Germany)[59]. Low-voltage transcutaneous electrical stimulation (0.01–9.99 mA) was applied to determine the current perception threshold (CPT), providing a sensitive and specific measure of nerve fiber integrity[60]. Both the median and peroneal nerves were tested. Two pairs of 1-cm-diameter surface electrodes were placed on the distal phalanx of the index finger for the median nerve and on the hallux for the peroneal nerve. CPT values were recorded at three distinct stimulation frequencies (2000 Hz, 250 Hz, and 5 Hz), allowing selective assessment of large myelinated, small myelinated, and unmyelinated pain-sensing fibers in all four limbs: 2000 Hz for large myelinated fibers mediating vibration sensation, 250 Hz for small myelinated fibers responsible for thermal perception, and 5 Hz for thin unmyelinated fibers involved in pain sensation. Participants reported the minimal current intensity at which they first sensed the stimulus, allowing precise quantification of sensory thresholds in both upper and lower limbs. Comprehensive, non-invasive assessment of sensory function using the Neurometer takes approximately 12–18 minutes per patient[61].

3.2.2 128-Hz Rydel-Seiffer graduated tuning fork

The calibrated tuning fork provides information on vibration sense, thereby reflecting the function of large nerve fibers. In clinical practice, the 128-Hz Rydel–Seiffer calibrated tuning fork has proven to be the most useful[62]. A 128-Hz Rydel-Seiffer graduated tuning fork was utilized to assess vibration sense at the distal end of the radius and at the level of the hallux. The obtained results were compared with age-dependent normal values published by Martina et al. in 1998[63]. On a scale of 1-8, the normal range was 7-8, borderline was 6, and abnormal was 1-5, indicating an impaired sense of vibration.

3.2.3 Semmes-Weinstein monofilament test®

For the assessment of protective sensation, the use of the 5.07/10 g Semmes–Weinstein monofilament is most commonly recommended. The Semmes-Weinstein Monofilament Test® was performed using a 10 g monofilament to directly screen for loss of protective sensation[64]. The filament was applied perpendicularly to the tested site with sufficient pressure to bend the filament, maintained for approximately 1.5 seconds[65]. Participants were blinded to the location and application of the filament, and the test was conducted under calm and quiet conditions. Traditionally examined sites included the hallux, the dorsum of the foot between the first and second metatarsals, and the plantar surface corresponding to the first and fifth metatarsal heads[66]. In this study, five plantar regions were tested: hallux, first and second metatarsal heads, and third and fifth metatarsal heads. Detection in at least four sites was considered normal, three or fewer sites indicated abnormal sensation.

3.2.4 Tiptherm®

The Tiptherm® device (Tip-Therm GmbH, Düsseldorf, FRG) is designed for the early detection of polyneuropathy of a symmetrical pattern by assessing temperature sensitivity of the skin[67]. This pen-shaped instrument features flat sides and consists of a 14 mm diameter plastic cylinder and a 14 mm diameter metal cylinder on each end separately. During the test, the examiner randomly touches the skin of the patient for one second on both hands and feet. The patient is then asked to identify which touch feels colder[68]. Individuals with normal temperature perception (<10°C) can differentiate between the two subjective sensations elicited by the flat surfaces of the Tiptherm®, whereas those with impaired temperature sensitivity are unable to differentiate between them.

3.2.5 Sudomotor function testing by Neuropad®

Sudomotor dysfunction, a typical manifestation of autonomic neuropathy, was evaluated in all participants, including both patients and controls, using the Neuropad® screening test, which is an adhesive indicator patch designed to measure sweat production on the plantar surface of the foot[69,70]. This method has a high sensitivity for detecting neuropathy[70], as nerve fiber impairment in the distal extremities affects both sensation and perspiration. The blue indicator patch turns pink after absorbing an adequate amount of sweat. This color change is based on a simple chemical reaction. The test kit contains an adhesive pad infused with blue anhydrous cobalt (II) chloride; each molecule of this salt can absorb six molecules of water and turns pink upon hydration. Since sweat contains water, inadequate sweating inhibits this chemical reaction[70]. Testing was conducted at room temperature (23°C), following a 10-minute resting period after patients removed their footwear (shoes and socks). The pads were applied to the soles, on both sides between the heads of the first and second metatarsi. The color change was evaluated 10 minutes post-application, with total pink decoloration indicating normal function, a mix of pink and blue ("spotted") classified as pending, and a total blue result regarded as abnormal[70].

3.3 Questionnaire for the evaluation of neuropathic complaints

Neuropathic symptoms were evaluated using a questionnaire. Each participant was asked to report whether they experienced sensations such as burning, pinpricks, numbness, tingling, hypoesthesia, or hyperesthesia. Additionally, they were required to specify the intensity of these symptoms and how frequently they occurred.

3.4 Body composition analysis

Body composition was assessed by a qualified dietitian using segmental bioelectrical impedance analysis with an InBody 770 device (InBodyUSA, Cerritos, CA). The device perform 30 impedance measurements at 6 different frequencies (1, 5, 50, 250, 500, and 1000 kHz)[71]. Before the measurement, the participant's age, sex, and height were entered into the software. During the assessment, the participant stood with their feet centered on the electrodes and held the hand electrodes so that the arms were sufficiently away from the torso and did not touch it. The participant maintained this position for the entire duration of the test, which took approximately 60 seconds. The evaluation encompassed several metrics: skeletal muscle mass (SMM, kg); fat-free mass index (FFMI), calculated by dividing fat-free mass by height squared (m^2); and body fat percentage (PBF, %), obtained by expressing total body fat mass as a

proportion of total body weight and multiplying by 100. The whole body phase angle (WBPA, °) was determined, reflecting the ratio between electrical resistance (R) and reactance (Xc) in the body, derived as WBPA = arctan{Xc/R} in degrees. Additional parameters included bone mineral content (BMC, kg) and visceral fat area (VFA, cm^2), indicating the volume of visceral adipose tissue in the abdominal region. Basal metabolic rate (BMR, kcal) was also documented. Finally, an InBody score (IBS) between 0 and 100 was assigned, where higher values correspond to more favorable body composition and overall physiological status.

3.5 Laboratory data

Laboratory results conducted within one month of the appointment date at the obesity clinic were considered valid. If available, the following parameters were recorded in both studies: white blood cell count (G/L), red blood cell count (T/L), hemoglobin (g/L), hematocrit (%), mean corpuscular volume of red blood cells (MCV; fL), thrombocyte (G/L), sodium (mmol/L), potassium (mmol/L), adjusted calcium (mmol/L), magnesium (mmol/L), phosphate (mmol/L), glucose (mmol/L), hemoglobin A1c% (HbA1c; %), insulin (mIU/L), blood urea nitrogen (mmol/L), creatinine (µmol/L), estimated glomerular filtration rate (eGFR; ml/min/1.73 m2), uric acid (µmol/L), total protein (g/l), albumin (g/l), total cholesterol (mmol/L), triglyceride (mmol/L), HDL-cholesterol (mmol/L), LDL-cholesterol (mmol/L), aspartate aminotransferase (ASAT/GOT; U/l), alanine aminotransferase (ALAT/GPT; U/L), γ-glutamyl transferase (GGT; U/L), total bilirubin (µmol/L), direct/conjugated bilirubin (µmol/L), alkaline phosphatase (ALP; U/L), amylase (U/L), lipase (U/L), C-reactive protein (CRP; mg/L), ferritin (ng/mL), serum iron (µmol/L), thyroid-stimulating hormone (TSH; mIU/L), parathormone (pmol/L), 25-hydroxyvitamin D3 and D2 (25OHD3/D2; nmol/L).

The following urinary parameters were collected and documented: urine creatinine (µmol/L), urine total protein (mg/dL), urine albumin (mg/L), albumin/creatinine ratio (ACR; mg/mmol), urine nitrite, urine pH, urine protein, urine glucose, urine ketone bodies, urine urobilinogen, urine bilirubin, urine white blood cells, urine red blood cells. Insulin resistance was assessed using the homeostatic model assessment (HOMA-IR), calculated with the formula: fasting glucose (mmol/L) × fasting insulin (mIU/L) / 22.5.

In the second study, the laboratory parameters were supplemented with the following: free triiodothyronine (fT3; pmol/L), free thyroxine (fT4; pmol/L), anti-thyroglobulin (anti-TG; IU/mL), anti-thyroid peroxidase (anti-TPO; IU/mL), anti-Müllerian hormone (AMH; ng/ml), testosterone (nmol/L), sex hormone binding globulin (SHBG; nmol/L), dehydroepiandrosterone sulfate (DHEAS; µmol/L), cortisol (nmol/L), prolactin (mIU/L).

3.6 Weight loss therapy

The management of obesity, including dietary, lifestyle, and pharmacological strategies, was conducted in accordance with the latest Hungarian clinical guidelines[23]. The duration of the metabolism-optimizing/weight-reduction therapy was not restricted.

3.6.1 Pharmacological therapy

The limitations of conventional lifestyle modification and risk factor modification programs are well known[72], so modern anti-obesity pharmacotherapy is rightly expected to make a meaningful contribution to the more effective treatment of this patient group. In Hungary, the most recent guidelines, which have been in effect since December 2023, provide guidance on the pharmacological treatment options for obesity[23]. At the time of the study, three pharmaceutical active substances were available in Hungary for weight reduction[23]. Orlistat is a pancreatic lipase inhibitor that prevents the absorption of approximately 30% of the ingested fat[73]. The naltrexone/bupropion combination includes the opioid receptor blocker naltrexone, which helps reduce the reward sensation associated with eating. Bupropion is used for major depression, affective disorders, and smoking cessation support, although it is not approved for the latter purpose in Hungary[74]. Orlistat and the naltrexone–bupropion combination therapy were not employed in this study population. Liraglutide, offering recognized cardiovascular benefits[75,76] and a relatively short washout period prior to IVF, was administered to a significant proportion of participants consenting to drug therapy. Pharmacotherapy was not obligatory; instead, decisions were reached collaboratively with patients, aiming to tailor therapy to the most suitable choice for each case. Preconception endocrine evaluation focused not only on obesity but also on detecting and optimizing additional endocrine and metabolic abnormalities under endocrinologist supervision.

3.6.2 Physical activity

After the baseline evaluation - which encompassed clinical and laboratory assessments, body composition analysis, and a comprehensive neuropathy examination - participants were provided with individualized dietary counseling delivered by a dietitian as an integral component of lifestyle intervention. In parallel, recommendations for physical activity were customized jointly by the physician and the dietitian, with careful consideration of each patient’s habitual lifestyle and baseline fitness status. These exercise prescriptions emphasized the gradual incorporation of feasible and patient-appropriate modalities of movement, along with structured advice on both frequency and duration. The overarching objective was to

achieve at least the minimum activity thresholds recommended by the World Health Organization, namely 150–300 minutes of moderate-intensity activity per week, 75–150 minutes of vigorous-intensity activity per week, or an equivalent combination of both, with additional health benefits attainable at higher volumes of exercise. Furthermore, muscle-strengthening activities involving major muscle groups on at least two days per week were recommended as part of the regimen[77]. Participants were encouraged to systematically track their physical activity, with particular attention to combining aerobic (cardio-type) training with resistance-based exercise, facilitated through the use of freely accessible online applications. Patients with obesity were advised to perform self-weighing on a weekly basis, as frequent self-weighing is associated with favorable weight change regardless of specific weight loss interventions[78], and to maintain contact with the supervising physician via email on a monthly basis or whenever necessary.

3.6.3 Dietary intervention

The nutritional management of patients with obesity followed the national guideline for adult obesity treatment[23] and was implemented within the framework of the Nutrition Care Process[79]. An individualized daily energy deficit of approximately 500–1000 kcal was prescribed, with careful attention to preventing micronutrient deficiencies. To support adherence to the prescribed deficit, determined using InBody 770 body composition analysis, patients were encouraged to monitor their energy intake through calorie tracking. For this purpose, they were advised to use freely accessible mobile applications throughout the course of weight reduction therapy. Meal timing and structure were emphasized, with plans generally built around three main meals and two snacks per day, tailored to individual needs and consistent with national nutritional standards[80]. In line with the Hungarian "OKOSTÁNYÉR®" dietary guidelines[80], patients were encouraged to complement this structure by consuming at least five daily portions of vegetables and fruits (with a predominance of vegetables, ideally fresh and partly raw), prioritizing whole-grain cereals, selecting lean protein sources such as poultry, fish, and legumes, and limiting salt, sugar, and processed food intake. Macronutrient distribution adhered to these recommendations, with total daily intake composed of 45–60% carbohydrates (mainly from low glycemic index sources), 15–20% protein, and 25–35% fat (predominantly from unsaturated fatty acids). Follow-up consultations with dietitians were available in case of any nutritional questions. Before IVF procedures, a final dietary consultation was conducted to review progress and reinforce individual goals.

4. Statistical analyses

In the first study, the data were presented as mean ± standard deviation (SD) or, when appropriate, as frequencies (n) and percentages (%). To analyze categorical variables, either Pearson's chi-square test or Fisher's exact test was applied, while continuous data were assessed using an independent samples t-test. Relationships between continuous or ordinal variables were explored using Pearson's or Spearman's correlation analysis. For the power analysis for the transition study, the calculation of the sample size was carried out using the G*Power software (Version 3.1.9.7, University of Düsseldorf, Germany). The calculated sample sizes for the control and patient groups were determined to be 33 and 69, respectively, assuming an effect size of d = 0.7, a Type I error (alpha) of 0.05, and a power value of 0.9. All statistical analyses were conducted with the R software environment (version 3.6.1; https://www.r-project.org/), and results with p-values below 0.05 were regarded as statistically significant.

In the second study, we conducted a retrospective analysis of data that had been collected prospectively. To determine statistical significance, Welch's two-tailed significance t-test was applied. Cohen's effect size (d) was calculated for each dimension, and statistical power was assessed. Correlation analysis was performed using the Pearson coefficient, with corresponding two-sided p-values also computed. All statistical analyses, including the Kaplan-Meier survival curve, were implemented in Python 3.6 using statsmodels, NumPy, SciPy, Pandas, and Lifelines libraries. The primary power calculation analyses focused on the decrease in BMI. Based on initial estimates, the mean value of BMI in the obese group was 33.8 ± 3.3, with an anticipated 10% decrease, and a significance level of $p<0.05$ with a statistical power of 80% were set. According to these calculations, a total of 30 measurements - 15 at baseline and 15 at second measurements - would be required to determine whether the weight reduction was statistically significant. However, since a low number of participants (n=16) returned for the second measurement, post hoc power calculations were conducted to estimate the required sample sizes to reach at least $p<0.05$ significance, taking Cohen's effect size (d) into account for each individual dimension. This approach provided a reliable assessment of the trustworthiness of the calculated significance individually for each parameter. If the sample size was close to or smaller than the number of available measurements, the chance of a significant difference was high. For a more critical evaluation of the sample sizes and corresponding p-values, post hoc statistical power (expressed as %) was calculated for each significant change using the online tool available at https://clincalc.com/stats/Power.aspx. All calculations were performed with an alpha level of 0.05.

5. Ethics statements

Both studies were conducted in accordance with the principles of the World Medical Association's Declaration of Helsinki (2000). The neuropathic investigations were approved by the Hungarian Medical Research Council (approval no. 219 31891-5/2019/EÜIG) and, based on this approval, also by the Regional and Institutional Review Board of Human Investigations at the University of Szeged (October 21, 2019). The retrospective analysis of preconceptional weight reduction was likewise approved by the Hungarian Medical Research Council (approval no. BM/18153-1/2023) and subsequently by the Regional and Institutional Review Board of Human Investigations at the University of Szeged (April 18, 2024). The research was carried out in accordance with applicable local legislation and institutional requirements. All participants provided written informed consent to participate.

6. Results

6.1 Results of the obesity study

6.1.1 Clinical data of female patients with obesity and control subjects

Table 1 presents the relevant clinical data of female patients with obesity (referred to as "patients") and control subjects ("controls"). In our study, a total of 71 female patients with obesity and 36 age-matched female volunteers with normal BMI were enrolled. None of the patients with obesity had diabetes. There were no significant differences between the two groups in terms of age, smoking habits, alcohol consumption history, or previously known hypercholesterolemia. However, the patients exhibited significantly higher resting mean systolic blood pressure (137.5 ± 16.87 vs. 114.6 ± 14.81 mmHg; $p<0.001$) and diastolic blood pressure (83.0 ± 11.71 vs. 69.8 ± 11.17 mmHg; $p<0.001$) compared to the controls. Additionally, hypertension was significantly more prevalent among the patients than the controls (23 vs. 0; $p<0.001$), and the prevalence of polycystic ovarian syndrome (36 vs. 2; $p<0.001$), hirsutism (30 vs. 0; $p<0.001$), and hypothyroidism (17 vs. 0; $p<0.001$) was also significantly higher. The occurrence of impaired glucose tolerance was also notably higher in patients with obesity than in the controls (13 vs. 0; $p=0.006$). A significantly greater number of patients were treated with metformin (40 vs. 1; $p<0.001$) and β-blockers (8 vs. 0; $p=0.036$). In contrast, there were no significant differences in the administration of other antihypertensive medications (ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, imidazoline derivatives, or α2-adrenergic receptor agonists), nor in the use of statins or diuretics between the groups.

Table 1. Relevant clinical data in the two study groups.

	Controls (n=36)	Patients with obesity (n=71)	P-value
Clinical Data			
Age (year)	36.4±13.25	36.1±8.34	0.872
Body Mass Index (kg/m^2)	21.6±2.13	40.2±8.47	***<0.001***
Waist-hip-ratio	0.74±0.05	0.85±0.12	0.017
Resting systolic BP (mmHg)	114.6±14.81	137.5±16.87	***<0.001***
Resting diastolic BP (mmHg)	69.8±11.17	83.0±11.71	***<0.001***
Smoking history (%)	6 (17)	17 (24)	0.387
Alcohol consumption (%)	10 (28)	10 (14)	0.086
Impaired glucose tolerance (%)	0 (0)	13 (18)	***0.006***
Hypertension (%)	0 (0)	23 (32)	***<0.001***
Polycystic ovarian syndrome (%)	2 (6)	36 (51)	***<0.001***
Hirsutism (%)	0 (0)	30 (42)	***<0.001***
Hypothyroidism (%)	0 (0)	17 (24)	***<0.001***
Hypercholesterolemia (%)	2 (6)	2 (3)	0.498
Medication			
Metformin (%)	1 (3)	40 (56)	***<0.001***
β-blocker (%)	0 (0)	8 (11)	***0.036***
ACE inhibitor or ARB (%)	0 (0)	9 (13)	0.097
Ca-antagonist (%)	0 (0)	4 (6)	0.147
Imidazoline receptor agonist (%)	0 (0)	3 (4)	0.211
Alpha-2 adrenergic receptor agonist	0 (0)	7 (10)	0.051
Statin (%)	2 (6)	2 (3)	0.480
Diuretics (%)	0 (0)	5 (7)	0.103

BP: blood pressure; ACE: angiotensin-converting enzyme; ARB: angiotensin-receptor blocker; Ca: calcium; ASA: acetylsalicylic acid; PAI: platelet aggregation inhibitor. P-values below the 0.05 level are marked bold and italic.

6.1.2 Laboratory data of female patients with obesity and control subjects

Table 2 presents the relevant data of both patients and control subjects. Compared to controls with a normal BMI range, patients with obesity exhibited significantly higher levels of white blood cell count, hematocrit, thrombocyte, potassium, glucose, uric acid, triglycerides,

ALAT/GPT, GGT, ALP, and CRP. Conversely, serum phosphate, albumin, creatinine, HDL-cholesterol, amylase, lipase, and 25OHD3/D2-vitamin levels were significantly lower in the patient group. No significant differences were observed between the two groups in terms of sodium, adjusted calcium, magnesium, insulin, HOMA-IR, HbA1c%, urea nitrogen, eGFR, total protein, total cholesterol, LDL-cholesterol, ASAT/GOT, total bilirubin, ferritin, iron, TSH, parathormone, urine total protein, urine albumin, urine creatinine, ACR, and routine urine test results. Because outpatient care is individualized, not all parameters were determined for every participant.

Table 2. Relevant laboratory data in the two study groups.

Laboratory Data	**Controls (n=36)**	**Patients with obesity (n=71)**	**P-value**
White blood cell count (G/L)	6.6±1.34 (n=34)	8.3±1.95 (n=65)	***<0.001***
Hemoglobin (g/L)	130.4±10.07 (n=34)	134.6±18.25 (n=66)	0.220
Hematocrit (L/L)	0.39±0.03 (n=34)	0.41±0.03 (n=66)	***<0.001***
Mean cellular volume (fL)	86.7±3.84 (n=34)	85.9±4.15 (n=66)	0.364
Thrombocyte (G/L)	277.67±71.47 (n=33)	313.6±62.95 (n=66)	*0.012*
Sodium (mmol/L)	138.6±2.36 (n=34)	139.1±2.25 (n=66)	0.346
Potassium (mmol/L)	4.3±0.34 (n=34)	4.6±0.39 (n=66)	***<0.001***
Adjusted calcium (mmol/L)	2.3±0.07 (n=31)	2.3±0.09 (n=62)	0.309
Magnesium (mmol/L)	0.9±0.09 (n=27)	0.9±0.09 (n=61)	0.854
Phosphate (mmol/L)	1.2±0.27 (n=29)	1.0±0.17 (n=57)	***<0.001***
Glucose (mmol/L)	4.7±0.62 (n=33)	5.1±0.61 (n=68)	***<0.001***
Insulin (mIU/L)	11.2±14.91 (n=8)	17.1±18.97 (n=48)	0.400
HOMA-IR	2.5±3.44 (n=8)	4.0±4.79 (n=46)	0.383
HbA1c (%)	5.4±0.33 (n=32)	5.4±0.39 (n=67)	0.786
Blood urea nitrogen (mmol/L)	4.3±1.92 (n=35)	5.2±6.73 (n=65)	0.397
Creatinine (µmol/L)	69.0±9.37 (n=35)	65.2±9.09 (n=64)	***0.050***
eGFR (mL/min/1.73m^2)	90.0±17.21 (n=33)	96.9±28.72 (n=62)	0.210
Uric acid (µmol/L)	219.5±40.81 (n=34)	308.4±76.75 (n=60)	***<0.001***

Total protein (g/L)	72.9±4.06 (n=29)	72.7±5.48 (n=57)	0.831
Albumin (g/L)	48.5±2.57 (n=35)	47.13±2.69 (n=62)	***0.017***
Total cholesterol (mmol/L)	4.9±0.96 (n=35)	5.0±0.72 (n=64)	0.736
Triglyceride (mmol/L)	1.0±0.43 (n=35)	1.5±0.75 (n=64)	***<0.001***
HDL-cholesterol (mmol/L)	1.8±0.44 (n=34)	1.3±0.34 (n=63)	***<0.001***
LDL-cholesterol (mmol/L)	2.6±0.84 (n=35)	2.9±0.62 (n=59)	0.074
ASAT/GOT (U/L)	20.9±7.70 (n=34)	23.4±16.69 (n=63)	0.414
ALAT/GPT (U/L)	19.5±11.5 (n=34)	28.3±14.69 (n=63)	***0.003***
Gamma GT (U/L)	13.1±5.73 (n=34)	27.9±20.88 (n=63)	***<0.001***
Total bilirubin (µmol/L)	8.8±5.67 (n=33)	7.9±3.49 (n=61)	0.360
Alkaline phosphatase (U/L)	59.7±17.20 (n=34)	75.2±20.81 (n=62)	***<0.001***
Amylase (U/L)	72.6±24.98 (n=20)	46.6±16.85 (n=46)	***<0.001***
Lipase (U/L)	50.4±13.90 (n=8)	30.5±8.69 (n=42)	***<0.001***
C-reactive protein (mg/L)	1.2±3.28 (n=31)	8.9±8.84 (n=57)	***<0.001***
Ferritin (ng/mL)	42.9±23.04 (n=7)	77.9±55.72 (n=50)	0.109
Iron (µmol/L)	17.0±7.49 (n=20)	14.2±5.36 (n=60)	0.083
TSH (mIU/L)	2.3±1.61 (n=21)	2.2± 1.27 (n=64)	0.849
Parathormone (pmol/L)	3.2±1.79 (n=14)	4.5±5.48 (n=60)	0.390
25OHD3/D2-vitamin (nmol/L)	80.9±28.07 (n=17)	63.3±23.51 (n=60)	***0.011***
Urine creatinine (µmol/L)	10229.8±5572.89 (n=13)	13521.3±16938.03 (n=32)	0.499
Urine total protein (mg/dL)	7.9±4.99 (n=22)	8.4±6.29 (n=36)	0.732
Urine albumin (mg/L)	9.5±16.54 (n=18)	12.1±19.0) (n=35)	0.620
ACR (mg/mmol)	1.4±1.99 (n=10)	2.0±2.95 (n=25)	0.607
Urine pH	6.0±0.59	6.0±0.71	0.912
Urine nitrite, negative (n/%)	25 (100.0%)	42 (100.0%)	0.999
Urine protein, negative (n/%)	22 (78.6%)	35 (81.4%)	0.770
Urine glucose, negative (n/%)	28 (100.0%)	43 (100.0%)	0.075

Urine ketone, negative (n/%)	27 (96.4%)	41 (97.6%)	0.770
Urine urobilinogen, negative (n/%)	27 (96.4%)	43 (100.0%)	0.212
Urine bilirubin, negative (n/%)	28 (100.0%)	43 (100.0%)	0.075
Urine white blood cell, negative (n/%)	21 (75.0%)	35 (81.4%)	0.519
Urine red blood cell, negative (n/%)	24 (85.7%)	38 (90.5%)	0.540

The data are presented as mean ± SD. HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: hemoglobin A1c; eGFR: estimated glomerular filtration rate; HDL-cholesterol: high-density lipoprotein, LDL-cholesterol; low-density lipoprotein cholesterol; ASAT/GOT: aspartate aminotransferase; ALAT/GPT: alanine aminotransferase; GGT: γ-glutamyl transferase; TSH: thyroid-stimulating hormone; 25OHD3/D2-vitamin: 25-hydroxy-D3- and D2-vitamin; ACR: albumin/creatinine ratio; n: number of the negative results; %: percentage of the negative results compared to the tested total sample. P-values below the 0.05 level are marked bold and italic.

6.1.3 Cardiovascular autonomic function tests of female patients with obesity and control subjects

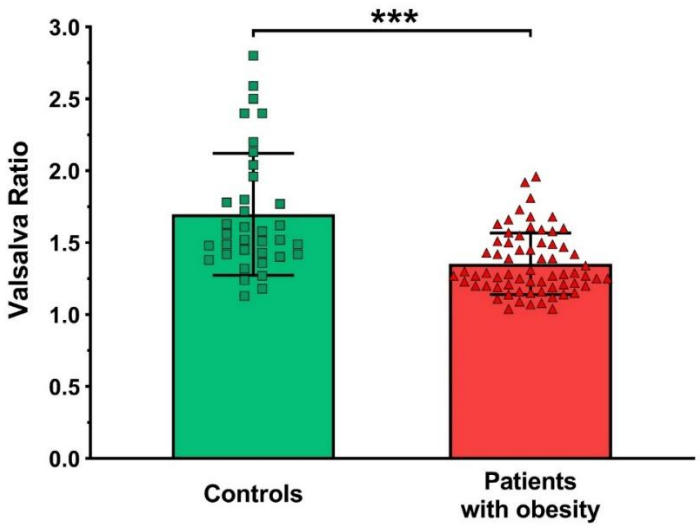
The outcomes of cardiovascular autonomic function testing are summarized in **Table 3**. Patients exhibited a significantly reduced Valsalva-ratio (**Figure 2**) compared with controls (1.4 ± 0.21 vs. 1.7 ± 0.42; p<0.001). No additional significant group differences were observed in the other autonomic test parameters.

Table 3. Results of the cardiovascular autonomic function tests in female patients with obesity and controls.

Reflex tests	**Controls (n=36)**	**Patients with obesity (n=69)**	**P-value**
HRRDB (1/min)	24.0±7.86	24.6±8.21	0.709
HRRSU (30/15 ratio)	1.1±0.12	1.2±1.22	0.596
Valsalva-ratio	1.7±0.42	1.4±0.21	***<0.001***
SBPRSU (mm Hg)	3.6±4.39	3.7±8.32	0.925
Handgrip (mmHg)	11.2±10.43	12.1±9.55	0.670
Autonomic score	1.9±1.30	2.25±1.59	0.248

HRRDB: the heart rate response to deep breathing; HRRSU (30/15 ratio): the heart rate responses to standing up; Valsalva-ratio: the heart rate responses to Valsalva maneuver; SBPRSU: the systolic blood pressure response to standing up. P-values below the 0.05 level are marked bold and italic.

Figure 2. Heart rate responses to Valsalva maneuver (Valsalva-ratio) in the study groups.



*** *p<0.001*

6.1.4 Peripheral sensory function in female patients with obesity and control subjects

Peripheral sensory function of the median nerve was impaired at all three tested frequencies as assessed by the Neurometer®. Female patients with obesity exhibited significantly higher current perception thresholds than controls (2000 Hz: 204.6 ± 70.90 vs. 168.1 ± 66.87 CPT, p=0.013; 250 Hz: 84.4 ± 38.91 vs. 56.5 ± 34.82 CPT, p<0.001; 5 Hz: 58.5 ± 31.22 vs. 36.9 ± 29.07 CPT, p<0.001; **Table 4**). Thresholds were also elevated in the peroneal nerve, although these differences did not reach statistical significance.

Table 4. Peripheral sensory function testing by Neurometer® assessing the threshold of the current sensations at the median and peroneal nerves at three different stimulating frequencies (2 kHz, 250 Hz, 5 Hz).

	Controls (n=36)	**Patients with obesity (n=66)**	**P-value**
NM2000	168.1±66.87	204.6±70.90	***0.013***
NM250	56.5±34.82	84.4±38.91	***<0.001***
NM5	36.9±29.07	58.5±31.22	***<0.001***
NP2000	320.9±114.97	341.6±113.76	0.385
NP250	158.8±63.06	165.5±89.07	0.689
NP5	98.6±47.88	101.5±58.85	0.799

NM2000: CPT (current perception threshold) value of the median nerve at stimulating frequency of 2000 Hz; NM250: CPT value of the median nerve at stimulating frequency of 250 Hz; NM5: CPT value of the median nerve at stimulating frequency of 5 Hz; NP2000: CPT value of the peroneal nerve at stimulating frequency of 2000 Hz; NP250: CPT value of the peroneal nerve at stimulating frequency of 250 Hz; NP5: CPT value of the peroneal nerve at stimulating frequency of 5. P-values below the 0.05 level are marked bold and italic.

Assessment of peripheral sensory function with a 128-Hz Rydel-Seiffer graduated tuning fork (**Table 5**) demonstrated significant impairment of lower limb vibration perception in patients, with bilaterally reduced perception thresholds observed among women with obesity. However, evaluations using the Semmes-Weinstein Monofilament Test® and Tiptherm® detected no significant differences between patients and controls.

Table 5. Peripheral sensory function testing in the study groups by 128-Hz Rydel-Seiffer graduated tuning fork on the distal end of right and left radius and the right and left hallux.

	Controls (n=36)	**Patients with obesity (n=67)**	**P-value**
Right radius	7.5±0.78	7.4±0.63	0.636
Left radius	7.6±0.50	7.4±0.69	0.087
Right hallux	7.4±0.80	6.8±0.94	***0.030***
Left hallux	7.3±0.94	6.9±0.84	***0.029***

P-values below the 0.05 level are marked bold and italic.

Evaluation of sudomotor function using Neuropad® (**Table 6**) revealed a significant impairment of autonomic function in patients, reflected by decreased perspiration on both the right and left soles compared with controls.

Table 6. Sudomotor function testing with Neuropad® in the study groups.

	Controls (n=27)	Patients with obesity (n=62)	P-value
Left side			***0.008***
Blue	1 (3.7%)	0 (0.0%)	
Mixed	1 (3.7%)	19 (30.6%)	
Rose	25 (92.6%)	43 (69.4%)	
Right side			***0.035***
Blue	1 (3.7%)	1 (1.6%)	
Mixed	1 (3.7%)	17 (27.4%)	
Rose	25 (92.6%)	44 (71.0%)	

P-values below the 0.05 level are marked bold and italic.

6.1.5 Body composition analysis

Significant differences were observed in all InBody® parameters (**Table 7**) between the groups, in line with our preliminary assumptions.

Table 7. Body composition analysis with InBody® in the study groups.

InBody® parameters	Controls (n=23)	Patients with obesity (n=51)	P-value
SMM (kg)	25.0±3.12	31.9±4.68	***<0.001***
FFMI (kg/m^2)	16.0±1.27	20.4±2.22	***<0.001***
PBF (%)	23.5±4.73	46.5±5.12	***<0.001***
WBPA (°)	5.3±0.46	5.7±0.52	***<0.001***
BMC (kg)	2.7±0.33	3.2±0.48	***<0.001***
VFA (cm^2)	60.9±16.77	220.4±50.44	***<0.001***
IBS	77.2±4.22	58.4±8.79	***<0.001***

SMM: skeletal muscle mass; FFMI: free fat muscle index; PBF%: percent body fat; WBPA: whole body phase angle; BMC: bone mineral content; VFA: visceral fat area; IBS: in body score. P-values below the 0.05 level are marked bold and italic.

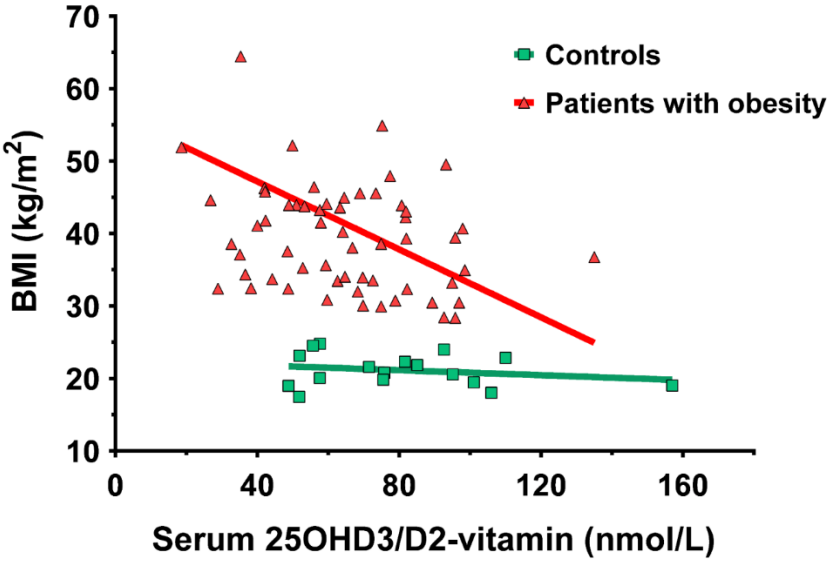
6.1.6 Evaluation of neuropathic symptoms with a questionnaire

There was no significant difference in the prevalence of neuropathic symptoms between patients and controls (12 [17.6%] vs. 2 [5.6%], p=0.086).

6.1.7 Correlations between studied parameters

In female patients with obesity, BMI demonstrated a negative correlation with 25OHD3/D2-vitamin levels (r=-0.41, p=0.00126) and a positive correlation with resting systolic blood pressure (r=0.26, p=0.0325). Additionally, in the patient group, 25OHD3/D2-vitamin levels showed a significant negative correlation with waist circumference (r=-0.26, p=0.0493), a correlation that was not observed among controls. In patients with obesity, both waist circumference (r=0.28, p=0.0214) and hip circumference (r=0.39, p=0.00124) correlated positively with resting systolic blood pressure. This correlation was also present in controls (r=0.38, p=0.045). Furthermore, in the patient group, skeletal muscle mass (r=0.41, p=0.00355), fat-free mass index (r=0.5, p=0.000263), body fat percentage (r=0.29, p=0.0403), and basal metabolic rate (r=0.42, p=0.00265) all exhibited a positive correlation with resting systolic blood pressure. Among controls, a similar correlation was found only between body fat percentage and both resting systolic (r=0.43, p=0.0381) and diastolic blood pressure (r=0.46, p=0.0279). Additionally, in patients with obesity, bone mineral content displayed a slight positive correlation with the Valsalva-ratio (r=0.27000, p=0.0498), which was not observed in the control group (**Figure 3**). A prominent positive correlation (r=0.56, p=0.00468) was identified between the CPT value of the median nerve at a stimulation frequency of 2000 Hz (NM2000) and the albumin/creatinine ratio (ACR) in patients with obesity. Moreover, the CPT value of the peroneal nerve at the stimulation frequency of 2000 Hz (NP2000) showed a negative correlation with the results of the sustained handgrip test (r=-0.30000, p=0.0234) in this group. Among controls, the CPT value of the median nerve at the stimulating frequency of 2000 Hz (NM2000) was negatively correlated with hip circumference (r=-0.43, p=0.0229), while at the stimulation frequency of 5 Hz (NM5), it exhibited a positive correlation with CRP levels (r=0.36, p=0.0461).

Figure 3. Negative correlation between BMI and the 25-hydroxy-D3- and D2-vitamin level in the female patients with obesity.



BMI: body mass index; 25OHD3/D2-vitamin: 25-hydroxy-D3- and D2-vitamin

6.2 Results of the preconceptional weight reduction study

6.2.1 Baseline clinical data in infertile women with obesity and in controls

Our study involved 58 infertile female patients with obesity (mean ± SD; age: 33.1 ± 5.42 years; BMI: 39.3 ± 6.90 kg/m²) before undergoing lifestyle changes aimed at weight loss, with or without medical treatment. Additionally, a control group of 45 age-matched female volunteers with a normal BMI (age: 32.1 ± 7.67 years; BMI: 21.1 ± 2.02 kg/m²) was included. A detailed overview of the baseline clinical characteristics of the participants is presented in **Table 8**.

Table 8. Relevant clinical data in the two study groups at baseline.

Clinical Data	Controls (n=45)	Infertile Patients with Obesity (n=58)	P-value
Age (year)	32.1 ± 7.67	33.1 ± 5.42	n.s.
Height (cm)	168.0 ± 6.16	166.7 ± 6.19	n.s.
Weight (kg)	59.6 ± 6.20	109.5 ± 21.59	***<0.001***
Body mass index (kg/m^2)	21.1 ± 2.02	39.3 ± 6.90	***<0.001***
Waist circumference (cm)	69.0 ± 5.46	108.0 ± 15.12	***<0.001***
Hip circumference (cm)	94.7 ± 5.09	127.1 ± 15.94	***<0.001***
Waist-hip-ratio	0.73 ± 0.05	0.86 ± 0.13	***<0.001***
Resting systolic BP (mmHg)	113.4 ± 13.87	136.8 ± 14.55	***<0.001***
Resting diastolic BP (mmHg)	69.4 ± 11.48	84.7 ± 11.98	***<0.001***
Smoking history	7 (16 %)	18 (31 %)	n.s.
Alcohol consumption	10 (22 %)	9 (16 %)	n.s.
Impaired glucose tolerance	0 (0%)	16 (28%)	***<0.001***
Type 2 diabetes mellitus	0 (0 %)	5 (9%)	***<0.05***
Type 1 diabetes mellitus	0 (0 %)	1 (2%)	n.s.
Hypertension	0 (0 %)	17 (29%)	***<0.001***
Polycystic ovarian syndrome	5 (11%)	32 (55%)	***<0.001***
Hirsutism	1 (2%)	32 (55%)	***<0.001***
Hypothyroidism	0 (0 %)	17 (29%)	***<0.001***
Endometriosis	2 (4%)	3 (5%)	n.s.
Medication			
Metformin	2 (4%)	41 (71%)	***<0.001***
β-blocker	0 (0 %)	8 (14%)	***<0.01***
ACE inhibitor or ARB	1 (2%)	3 (5%)	n.s.
Ca-antagonist	0 (0 %)	2 (3%)	n.s.
Imidazoline receptor agonist	0 (0 %)	1 (2%)	n.s.
α_2 adrenergic receptor agonist	0 (0 %)	9 (16%)	***<0.01***
Statin	1 (2%)	0 (0 %)	n.s.
ASA	0 (0 %)	4 (7%)	***<0.05***
Diuretics	0 (0 %)	1 (2%)	n.s.
Dopamine agonist	1 (2%)	2 (3%)	n.s.

Levothyroxine	0 (0 %)	13 (22%)	***<0.001***

BP: blood pressure; ACE: angiotensin-converting enzyme; ARB: angiotensin-receptor blocker; Ca: calcium; ASA: acetylsalicylic acid; PAI: platelet aggregation inhibitor; n.s.: non-significant. P-values below the 0.05 level are marked bold and italic.

No significant differences were observed between the infertile women with obesity and the control group regarding age, height, prior pregnancies, miscarriages, abortions, previous unsuccessful IVF attempts, number of children, cesarean sections, endometriosis, smoking, or alcohol consumption.

Compared to the control group, patients with obesity exhibited significantly higher mean resting systolic blood pressure (113.4 ± 13.87 vs. 136.8 ± 14.55 mmHg; $p<0.001$) and diastolic blood pressure (69.4 ± 11.48 vs. 84.7 ± 11.98 mmHg; $p<0.001$). Infertile patients with obesity had significantly higher rates of hypertension (0 vs. 17, $p<0.001$), impaired glucose tolerance (IGT; 0 vs. 16, $p<0.001$), T2DM (0 vs. 5, $p<0.05$), PCOS (5 vs. 32, $p<0.001$), hirsutism (1 vs. 32, $p<0.001$), and hypothyroidism (0 vs. 17, $p<0.001$).

A significantly greater number of patients with obesity in the infertile group were prescribed metformin, β-blockers, α2-adrenergic receptor agonists, acetylsalicylic acid, and levothyroxine. However, no significant differences were observed between the two groups regarding the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, Ca^{2+}-channel blockers, imidazolidines, statins, or diuretics (**Table 8**).

6.2.2 Laboratory data of infertile women with obesity and control subjects

Relevant clinical data for patients with obesity and the control group are summarized in **Table 9**. Compared to individuals with a normal BMI, patients with obesity exhibited significantly higher values for white and red blood cell count, thrombocyte, sodium, glucose, insulin, HbA1c%, uric acid, triglycerides, LDL-cholesterol, ASAT/GOT, ALAT/GPT, GGT, ALP, ferritin, and CRP. Conversely, mean cellular volume, serum phosphate, albumin, creatinine, HDL-cholesterol, amylase, lipase, iron, and SHBG levels were significantly lower in the group of patients with obesity.

No significant differences were observed in other laboratory parameters between the two groups. Due to the individualized nature of ambulatory patient care, not all participants had all collected parameters assessed. Additionally, because blood samples from patients with obesity were not consistently collected on days 3–5 of the menstrual cycle, an objective comparison of FSH, LH, and estradiol hormone levels was not feasible due to their cycle sensitivity.

For infertile female patients with obesity (n=36), the baseline AMH value was recorded as 3.6 ± 3.80 ng/ml. However, only two of the finishers had their AMH values measured at baseline, with a mean value of 10.0 ± 6.91 ng/ml.

Table 9. Relevant laboratory data in the two study groups at baseline.

Laboratory Data	**Controls**	**Infertile Patients with Obesity**	**P-value**
White blood cell count (G/L)	6.3± 1.36 (n=44)	8.4 ± 2.02 (n=54)	***<0.001***
Red blood cell count (T/L)	4.5 ± 0.28 (n=44)	4.8 ± 0.38 (n=55)	***<0.001***
Hemoglobin (g/L)	133.0 ± 9.22 (n=44)	134.2 ± 19.11 (n=55)	n.s.
Hematocrit (L/L)	0.39 ± 0.02 (n=44)	0.41 ± 0.03 (n=55)	n.s.
Mean cellular volume (fL)	87.2 ± 3.50 (n=44)	84.1 ± 8.56 (n=55)	***<0.05***
Thrombocyte (G/L)	256.1 ± 59.93 (n=44)	318.4 ± 62.46 (n=55)	***<0.001***
Sodium (mmol/L)	138.2 ± 2.05 (n=43)	139.2 ± 2.37 (n=55)	***<0.05***
Potassium (mmol/L)	4.3 ± 0.34 (n=43)	4.6 ± 1.46 (n=55)	n.s.
Adjusted calcium (mmol/L)	2.3 ± 0.06 (n=40)	2.3 ± 0.09 (n=50)	n.s.
Magnesium (mmol/L)	0.9 ± 0.06 (n=39)	0.9 ± 0.07 (n=49)	n.s.
Glucose (mmol/L)	4.7 ± 0.50 (n=45)	5.3 ± 0.98 (n=58)	***<0.001***
Insulin (mIU/L)	5.8 ± 4.49 (n=21)	16.3 ± 19.67 (n=42)	***<0.01***
HOMA-IR	1.3 ± 1.00 (n=21)	4.0 ± 4.96 (n=40)	***<0.05***
HbA1c (%)	5.2 ± 0.26 (n=40)	5.5 ± 0.67 (n=55)	***<0.01***
eGFR (mL/min/1.73m^2)	91.1 ± 12.91 (n=43)	95.3 ± 14.20 (n=53)	n.s.
Uric acid (µmol/L)	219.1 ± 45.79 (n=40)	328.59 ± 71.46 (n=50)	***<0.001***
Total protein (g/L)	73.4 ± 3.80 (n=42)	70.9 ± 11.22 (n=45)	n.s.
Albumin (g/L)	48.2 ± 2.13 (n=43)	46.7 ± 2.66 (n=49)	***<0.01***
Total cholesterol (mmol/L)	4.9 ± 0.87 (n=42)	4.9 ± 0.74 (n=51)	n.s.
Triglyceride (mmol/L)	0.9 ± 0.37 (n=42)	1.6 ± 0.86 (n=51)	***<0.001***
HDL-cholesterol (mmol/L)	1.8 ± 0.42 (n=37)	1.2 ± 0.28 (n=51)	***<0.001***

LDL-cholesterol (mmol/L)	2.5 ± 0.74 (n=36)	2.9 ± 0.64 (n=50)	***<0.01***
ASAT/GOT (U/L)	19.0 ± 6.54 (n=45)	24.4 ± 18.27 (n=53)	***<0.05***
ALAT/GPT (U/L)	18.1 ± 8.78 (n=45)	30.4 ± 16.53 (n=53)	***<0.001***
Gamma GT (U/L)	11.5 ± 4.77 (n=45)	27.9 ± 22.19 (n=52)	***<0.001***
Total bilirubin (µmol/L)	9.6 ± 5.91 (n=45)	8.0 ± 3.51 (n=50)	n.s.
Alkaline phosphatase (U/L)	56.2 ± 13.51 (n=45)	77.2 ± 22.08 (n=50)	***<0.001***
C-reactive protein (mg/L)	1.5 ± 1.06 (n=39)	9.1 ± 8.34 (n=42)	***<0.001***
Ferritin (ng/mL)	38.4 ± 21.62 (n=17)	76.71 ± 56.45 (n=38)	***<0.001***
Iron (µmol/L)	16.8 ± 7.94 (n=39)	13.6 ± 5.62 (n=50)	***<0.05***
TSH (mIU/L)	2.6 ± 1.52 (n=42)	2.1 ± 1.32 (n=50)	n.s.
fT3 (pmol/L)	5.1 ± 0.79 (n=17)	4.9 ± 0.73 (n=15)	n.s.
fT4 (pmol/L)	16.2 ± 2.12 (n=19)	17.1 ± 5.00 (n=18)	n.s.
Anti-TG (IU/mL)	49.8 ± 77.37 (n=19)	27.0 ± 58.17 (n=40)	n.s.
Anti-TPO (IU/mL)	19.0 ± 40.45 (n=17)	39.8 ± 75.69 (n=42)	n.s.
Parathormone (pmol/L)	3.2 ± 1.36 (n=34)	4.7 ± 6.13 (n=48)	n.s.
25OHD3/D2-vitamin (nmol/L)	81.0 ± 42.04 (n=36)	65.8 ± 22.47 (n=47)	n.s.
Testosterone (nmol/L)	1.1 ± 0.65 (n=24)	1.3 ± 0.74 (n=44)	n.s.
SHBG (nmol/L)	77.0 ± 40.34 (n=25)	33.7 ± 24.33 (n=44)	***<0.001***
DHEAS (umol/L)	6.2 ± 3.18 (n=22)	7.1 ± 3.11 (n=42)	n.s.
Cortisol (nmol/L)	345.9 ± 102.33 (n=17)	354.8 ± 149.95 (n=34)	n.s.
Prolactin (mIU/L)	370.6 ± 190.31 (n=23)	401.4 ± 229.51 (n=43)	n.s.

The data are presented as mean ± SD. HOMA-IR: homeostasis model assessment of insulin resistance; HbA1c: hemoglobin A1c; eGFR: estimated glomerular filtration rate; HDL-cholesterol: high-density lipoprotein, LDL-cholesterol; low-density lipoprotein cholesterol; ASAT/GOT: aspartate aminotransferase; ALAT/GPT: alanine aminotransferase; GGT: γ-glutamyl transferase; TSH: thyroid-stimulating hormone; fT3: free triiodothyronine; fT4: free thyroxine; Anti-TG: anti-thyroglobulin; Anti-TPO: anti-thyroid peroxidase; 25OHD3/D2-vitamin: 25-hydroxy-D3- and D2-vitamin; SHBG: sex hormone binding globulin; DHEAS: dehydroepiandrosterone sulfate; n.s.: non-significant. P-values below the 0.05 level are marked bold and italic.

6.2.3 Cardiovascular autonomic function tests of infertile women with obesity and control subjects

Patients with obesity exhibited a significant impairment in the Valsalva-ratio (1.5 ± 0.23 vs. 1.4 ± 0.22; p<0.001) and the 30/15 ratio (1.12 ± 0.13 vs. 1.07 ± 0.12; p<0.05) compared to the control group. Although reduced, both parameters remained within the normal physiological range. No other significant differences were observed in the autonomic test results between the two groups. In addition, the autonomic score, which reflects cardiovascular autonomic neuropathy by summarizing the results of the Ewing tests, was significantly higher in patients with obesity compared to controls (1.2 ± 1.33 vs. 2.3 ± 1.83; p<0.001), indicating a greater burden of autonomic dysfunction in the obese group.

6.2.4 Peripheral sensory function in infertile female patients with obesity and control subjects

The 128-Hz Rydel-Seiffer graduated tuning fork test (**Table 10**) demonstrated an impaired sense of vibration in infertile patients with obesity compared to controls, with symmetrically reduced perception detected in all four limbs at the levels of the radius and great toes.

Using the Neurometer, a significantly higher CPT value was detected in the median nerve at the 2000 Hz stimulation frequency in patients with obesity (198.365 ± 62.183 vs. 172.133 ± 39.9; p<0.05). However, at rest, CPT values at other tested frequencies for the median and peroneal nerves did not show statistically significant differences between patients with obesity and controls.

Table 10. Peripheral sensory function testing in the study groups by 128-Hz Rydel-Seiffer graduated tuning fork on the distal end of right and left radius and the right and left hallux in infertile women with obesity and in controls.

Rydel-Seiffer graduated tuning fork tests	**Controls (n=45)**	**Infertile Patients with Obesity (n=57)**	**P-value**
Right radius	7.71 ± 0.46	7.37 ± 0.58	***<0.01***
Left radius	7.62 ± 0.49	7.30 ± 0.72	***<0.01***
Right hallux	7.58 ± 0.54	6.97 ± 0.89	***<0.001***
Left hallux	7.47 ± 0.63	6.98 ± 0.74	***<0.001***

P-values below the 0.05 level are marked bold and italic.

6.2.5 Body composition analysis

Significant differences in all InBody® parameters (**Table 11**) were observed between the two groups, consistent with our initial assumptions.

Table 11. Body composition analysis with InBody at baseline in infertile women with obesity and in controls.

InBody Parameters	Controls	Infertile Patients with Obesity	P-value
SMM (kg)	24.4 ± 2.53 (n=45)	32.2 ± 4.98 (n=42)	***<0.001***
FFMI (kg/m²)	15.8 ± 1.16 (n=43)	20.5 ± 2.43 (n=42)	***<0.001***
PBF (%)	24.7 ± 5.16 (n=45)	46.667 ± 5.19 (n=43)	***<0.001***
WBPA (°)	5.2 ± 0.61 (n=40)	5.6 ± 0.44 (n=41)	***<0.01***
BMC (kg)	2.658 ± 0.275 (n=45)	3.2 ± 0.48 (n=43)	***<0.001***
BMR (kcal)	1332.0 ± 91.24 (n=45)	1606.3 ± 186.21 (n=43)	***<0.001***
VFA (cm²)	63.2 ± 18.54 (n=42)	216.9 ± 59.28 (n=43)	***<0.001***
IBS	75.6 ± 3.96 (n=45)	57.2 ± 9.21 (n=43)	***<0.001***

SMM: skeletal muscle mass; FFMI: free fat muscle index; PBF (%): percent body fat; WBPA: whole body phase angle; BMC: bone mineral content; VFA: visceral fat area; BMR: basal metabolic rate; IBS: in body score. P-values below the 0.05 level are marked bold and italic.

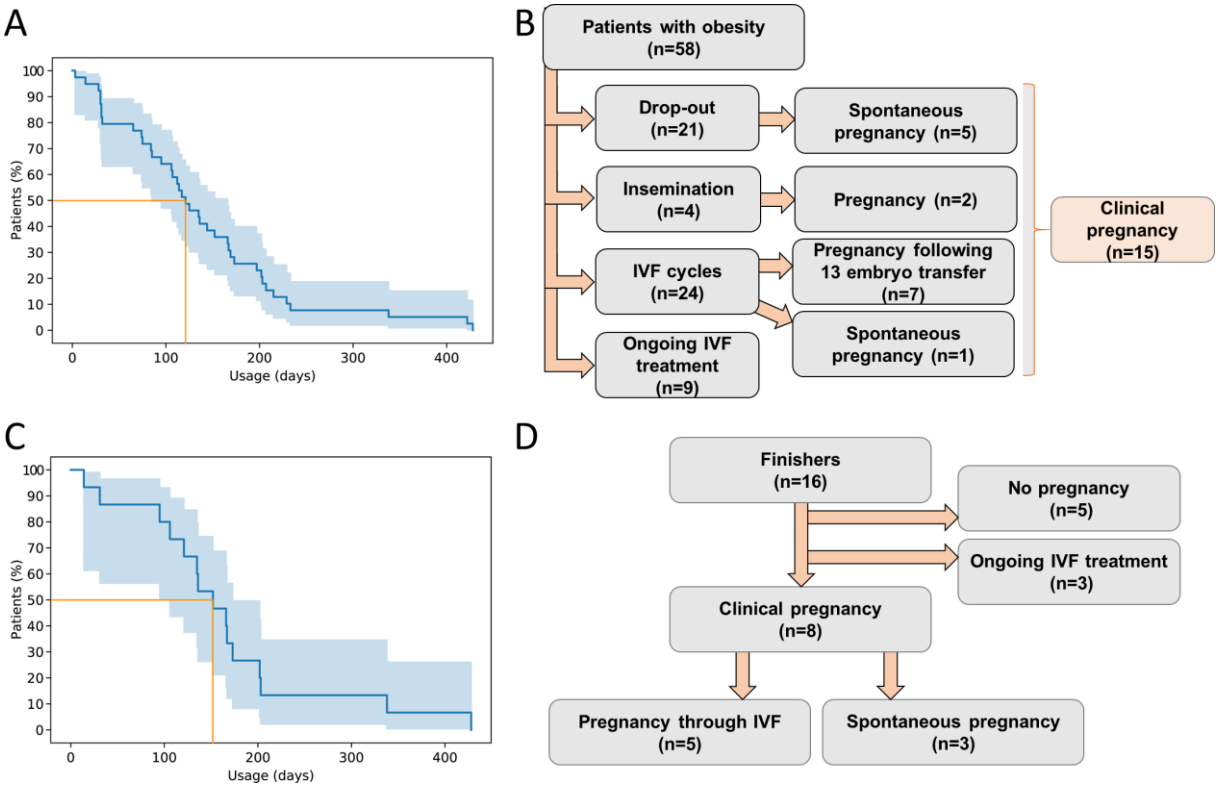
6.2.6 Results at baseline (1st measurement) and after weight loss therapy (2nd measurement) in infertile women with obesity; clinical pregnancy outcome

Among the initial cohort of 58 patients with obesity, 48 initiated liraglutide therapy in addition to implementing lifestyle and dietary changes. In this subgroup, liraglutide was administered for a mean period of 156.3 ± 129.9 days (n=42), with an average dose of 2.04 ± 0.57 mg (n=43). **Figure 4A** shows the gradual reduction in treatment adherence among obese patients on liraglutide, starting from 41 individuals at baseline. At the initial assessment, prior to any weight-loss intervention, 15 out of 58 obese infertile women achieved pregnancy, as shown in **Figure 4B**. Out of the 58 patients with obesity, 21 dropped out (including 5 spontaneous pregnancies), 9 are still undergoing IVF treatment, 4 underwent insemination (resulting in 2 pregnancies), and 24 embarked on IVF cycles (with 1 spontaneous conception following a failed IVF attempt). Furthermore, 13 embryo transfers were performed, which resulted in 7 successful pregnancies. Among the 58 women, 10 did not receive liraglutide

treatment. Of the 15 pregnancies, only one was achieved by a woman who had not been treated with liraglutide before fertility therapy, whereas all remaining pregnancies occurred in women who had received liraglutide as part of their weight-loss regimen.

Out of the initial 58 patients, only 16 women with obesity - referred to as "finishers" - completed the final assessments after achieving their target weight reduction before undergoing IVF. This resulted in a retention rate of 27.6% within this highly motivated cohort. As a result, the second measurement was carried out exclusively on these 16 individuals, during which they underwent a medical visit with an endocrinologist specializing in obesity, a reassessment of neuropathic status, body composition analysis, relevant laboratory tests, and additional dietary education. **Figure 4C** illustrates the decline in the number of patients with obesity who continued liraglutide treatment over time, beginning with an initial sample size of 16 of the finishers. All finishers received liraglutide therapy alongside lifestyle and dietary changes, with an average treatment duration of 159.7 ± 103.4 days (n=16) and an average liraglutide dose of 1.99 ± 0.52 mg (n=16). Among the finishers, 8 became pregnant - 5 through IVF and 3 spontaneously - while 3 remain in ongoing IVF treatment, and 5 either did not conceive or discontinued IVF therapy. Importantly, 3 of the 16 women conceived spontaneously, indicating that IVF was unnecessary for 18.75% of this group. Excluding these 3 patients, 5 out of the remaining 13 patients did not proceed with IVF treatment. Of the remaining 8 patients, 5 achieved pregnancy via IVF, corresponding to a success rate of 62.5%. **Figure 4D** provides a flowchart summarizing the clinical pregnancy outcomes for the 16 finishers.

Figure 4 (A) Decrease in the number of patients continuing liraglutide treatment over time, starting with an initial sample size of 41. Blue line represents Kaplan-Meier estimator; the light blue shading represents 95% confidence interval. Orange line represents where 50% percent of the patients stop using the substance. **(B)** Flow chart represents the pregnancy outcome of 58 infertile women with obesity. **(C)** The decrease in the number of patients continuing liraglutide treatment over time, starting with the finisher sample size of 16. Blue line represents Kaplan-Meier estimator; the light blue shading represents 95% confidence interval. Orange line represents where 50% percent of the patients stop using the substance. **(D)** Flowchart represents the pregnancy outcome of the 16 finishers.



IVF: in vitro fertilization

The effectiveness of obesity therapy among the finishers was demonstrated by a significant reduction in both body weight (from 104.3 ± 16.64 kg to 89.1 ± 15.74 kg; $p<0.05$) and BMI (from 38.5 ± 5.02 kg/m² to 32.9 ± 5.20 kg/m²; $p<0.01$). The treatment lasted an average of 232.9 ± 170.86 days, starting with an initial weight of 104.3 ± 16.11 kg and concluding at 89.09 ± 15.24 kg, corresponding to an average weight loss of 15.2 ± 6.96 kg. Overall, the finishers achieved an average weight reduction of 14.6 ± 6.06% of their initial body weight. Significant decreases in HbA1c% levels and body fat percentage were also observed, while vitamin D levels increased significantly, partly due to supplementation in 7 of the 16 finishers. Significant changes following weight-loss therapy were limited to the summarized parameters presented in **Table 12**. No statistically significant differences were detected in cardiovascular autonomic function or peripheral sensory test results before and after the intervention.

Table 12. Clinical, laboratory, and test data showing significant change from the baseline (1st measurement) to after weight loss therapy (2nd measurement) in infertile women with obesity.

Clinical and Laboratory Data	Patients at 1st Measurement	Patients at 2nd Measurement	P-value
Weight (kg)	104.3 ± 16.64 (n=16)	89.1 ± 15.74 (n=16)	***<0.05***
BMI (kg/m²)	38.5 ± 5.02 (n=16)	32.9 ± 5.20 (n=16)	***<0.01***
Waist circumference (cm)	108.7 ± 20.05 (n=15)	94.0 ± 13.58 (n=16)	***<0.05***
Hip circumference (cm)	120.1 ± 15.21 (n=15)	115.6 ± 12.54 (n=16)	n.s.
Waist-hip-ratio	0.92 ± 0.21 (n=15)	0.81 ± 0.08 (n=15)	n.s.
Resting systolic BP (mmHg)	133.3 ± 12.91 (n=16)	127.5 ± 15.76 (n=16)	n.s.
Resting diastolic BP (mmHg)	85.0 ± 10.82 (n=16)	80.44 ± 10.46 (n=16)	n.s.
HbA1c (%)	5.4 ± 0.38 (n=15)	5.1 ± 0.18 (n=11)	***<0.05***
25OHD3/D2-vitamin (nmol/L)	66.9 ± 24.55 (n=15)	103.9 ± 44.09 (n=11)	***<0.05***
PBF%	47.0 ± 3.81 (n=13)	42.5 ± 6.50 (n=13)	***<0.05***

BP: blood pressure; BMI: body mass index; HbA1c: hemoglobin A1c; 25OHD3/D2-vitamin: 25-hydroxy-D3- and D2-vitamin; PBF%: percent body fat; n.s.: non-significant. P-values below the 0.05 level are marked bold and italic.

Throughout the weight-loss therapy, changes were made to antihypertensive and thyroid hormone replacement medications between the first and second assessments. Although TSH levels did not significantly differ between the two measurements (1st vs. 2nd measurement; 2.2 ± 1.51 vs. 2.4 ± 1.30; n.s.) for the 16 patients with obesity who were retested, considering the latest European Society of Human Reproduction and Embryology (ESHRE) guidelines[81], which recommend specific TSH target levels for planned IVF. As a result, levothyroxine therapy was initiated in three patients, dosage adjustments were made in two additional cases, and one patient discontinued thyroid hormone replacement.

Regarding antihypertensive treatment, new therapy was introduced and adjusted in two cases, and prenatal multivitamins were prescribed for four patients. Based on baseline laboratory results indicating low 25OHD3/D2 vitamin levels, vitamin D3 supplementation - including an initial loading dose - was started in seven out of 16 patients. Prior to beginning weight-loss therapy, 11 of the 16 patients were already on metformin for conditions such as PCOS, impaired fasting glucose (IFG), IGT, or T2DM. Following further medical evaluation,

metformin was initiated in two more patients, leaving only three who did not receive metformin alongside liraglutide therapy. No additional pharmacological interventions were introduced by the endocrinologist overseeing the weight-loss therapy.

6.2.7 Correlations in the whole population

The initial body weight exhibited a significant positive correlation with insulin resistance ($r=0.550$, $p<0.001$), IGT ($r=0.299$, $p=0.002$), hypertension ($r=0.433$, $p<0.001$), PCOS ($r=0.377$, $p<0.001$), hirsutism ($r=0.404$, $p<0.001$), and hypothyroidism ($r=0.312$, $p=0.001$).

Additionally, fasting glucose ($r=0.269$, $p=0.006$), fasting insulin ($r=0.381$, $p=0.002$), correspondingly with the HOMA-IR index ($r=0.380$, $p=0.003$) all showed a positive correlation with initial body weight, as did HbA1c% levels ($r=0.250$, $p=0.015$), triglyceride levels ($r=0.393$, $p<0.001$), and inflammatory markers such as CRP ($r=0.527$, $p<0.001$) and ferritin ($r=0.336$, $p=0.012$). Similarly, blood count elements - including white blood cell count ($r=0.503$, $p<0.001$), red blood cell count ($r=0.460$, $p<0.001$), and platelet count ($r=0.322$, $p=0.001$) - were positively associated with initial body weight. Liver enzyme levels (GOT: $r=0.245$, $p=0.015$; GPT: $r=0.479$, $p<0.001$; ALP: $r=0.451$, $p<0.001$; GGT: $r=0.463$, $p<0.001$) were also positively correlated, as were uric acid levels ($r=0.659$, $p<0.001$). Conversely, initial body weight was negatively correlated with AMH levels ($r=-0.363$, $p=0.030$), HDL cholesterol ($r=-0.534$, $p<0.001$), amylase ($r=-0.496$, $p<0.001$), iron levels ($r=-0.240$, $p=0.023$), and 25OHD3/D2 vitamin levels ($r=-0.270$, $p=0.014$).

A significant positive correlation was observed between initial body weight and resting systolic ($r=0.629$, $p<0.001$) and diastolic blood pressure ($r=0.527$, $p<0.001$). In terms of neuropathic test results, the initial body weight positively correlated with the autonomic score ($r=0.328$, $p<0.001$), but showed a negative correlation with the Valsalva-ratio, the 30/15 ratio, and all tuning fork test results (RR: $r=-0.280$, $p=0.004$; RH: $r=-0.331$, $p<0.001$; LR: $r=-0.217$, $p=0.029$; LH: $r=-0.348$, $p<0.001$). As expected, BMI displayed similar correlations to those of initial body weight. Regarding autonomic function, the autonomic score negatively correlated with three out of the four tuning fork tests (RR: $r=-0.200$, $p=0.045$; LR: $r=-0.277$, $p=0.005$; LH: $r=-0.231$, $p=0.020$) and with the InBody score ($r=-0.262$, $p=0.014$). However, it was positively associated with BMR ($r=0.357$, $p<0.001$), FFMI ($r=0.320$, $p=0.003$), and PBF ($r=0.273$, $p=0.010$).

7. Discussion

7.1 Discussion of the obesity study

7.1.1 Autonomic function in female patients with obesity

Obese female patients showed significantly poorer results on the Valsalva test compared to the control group. Although the mean Valsalva-ratio in both cohorts remained within the normal range (≥ 1.21), the observed difference points toward the onset of parasympathetic impairment in women with obesity. This alteration could also be attributed to increased sympathetic nervous system activity. Interestingly, in the patient group, a slight positive correlation was detected between bone mineral content and the Valsalva-ratio, suggesting that better bone health may be linked to more favorable outcomes in this cardiovascular reflex test. The role of the autonomic nervous system in bone remodeling and maintenance of bone mass has been supported by several studies, although the most of the evidence originates from rodent models. Some earlier human studies have reported an association between autonomic dysfunction and reduced bone mineral density[82–85], yet the findings remain inconsistent.

In our study, sudomotor function - a common early marker of autonomic neuropathy - was assessed using the Neuropad® screening method. This test revealed a decreased sweat secretion on both the right and left plantar surfaces in the patient group with obesity compared with healthy controls, providing clear evidence of compromised autonomic. According to our review of the available literature, no earlier studies have documented small fiber dysfunction in women with obesity using this method. Thus, our findings represent the first evidence confirming such impairment in this population. Supporting our findings, a recent study involving a small cohort of non-diabetic individuals with obesity (n=26) prior to bariatric surgery also detected both small and large fiber neuropathy when compared to controls without obesity (n=20), although sex distribution was not specified in that sample[86].

7.1.2 Sympathetic nervous system overdrive in obesity and biometric parameters

Obesity has been linked to increased sympathetic nervous system activity, which is thought to act as a compensatory adaptation counterbalancing elevated energy intake, thereby facilitating the restoration of energy equilibrium[87,88]. Consistent with this concept, prior studies have reported an association between sympathetic activity and the extent of visceral adiposity[89]. In our study population, both waist and hip circumferences showed a positive correlation with resting systolic blood pressure, a pattern that was also evident among the controls. Furthermore, in obese participants, free fat mass index and percentage body fat exhibited positive correlations

with systolic blood pressure at rest, further reinforcing the link between increased sympathetic drive and visceral adiposity in this group. Excessive sympathetic overactivation may contribute to several metabolic disturbances, including the development of insulin resistance with compensatory hyperinsulinemia, IGT, T2DM, and dyslipidemia[90,91].

7.1.3 Additional factors influencing autonomic function

In our cohort, obese patients were free of diabetes and did not differ significantly from the control group in terms of age, smoking habits, alcohol intake, or history of hypercholesterolemia. Nevertheless, the patient group displayed markedly higher resting systolic and diastolic blood pressure values, along with a greater prevalence of hypertension, polycystic ovary syndrome, and hypothyroidism compared to controls.

A significantly larger proportion of patients were treated with metformin and β-blockers; however, no notable differences were observed between the groups regarding the use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, imidazoline or α2-adrenergic receptor agonists, statins, or diuretics. Considering that β-adrenergic blockers decrease sympathetic activity, they may partly obscure or diminish the extent of altered responses observed in reflex evaluations[92,93]. Similarly, metformin may exert a comparable effect by optimizing carbohydrate metabolism, thereby partially masking underlying autonomic dysfunction[94].

7.1.4 Peripheral sensory function in female patients with obesity

Neurometer® assessments revealed significant changes in the peripheral sensory function of the median nerve across all three stimulation frequencies. In obese female patients, testing of the median nerve demonstrated elevated thresholds at every examined frequency, reflecting impaired function of large myelinated, small myelinated, and unmyelinated fibers. In the peroneal nerve, threshold values also tended to be elevated, though these differences did not reach statistical significance. A larger sample size could potentially substantiate the trend toward higher CPT values observed in the peroneal nerve compared with controls in the normal BMI range.

Evaluation with the 128-Hz Rydel–Seiffer graduated tuning fork demonstrated a marked reduction in vibratory sensation in the lower limbs of obese patients. To our knowledge, this is the first study to report vibratory sensory impairment specifically in women with obesity. In contrast, the Semmes–Weinstein Monofilament® and Tiptherm® tests did not show significant distinctions between the study groups.

7.1.5 Evaluation of laboratory data of female patients with obesity

We observed significantly elevated CRP levels in patients with obesity compared to controls with a normal BMI range. These results are consistent with earlier studies highlighting the strong association between CRP elevation and obesity as a component of the metabolic syndrome[95–98]. According to Fröhlich et al.[95], CRP values were significantly correlated with BMI in a large community sample. Similarly, Choi et al.[96], in a systematic review, confirmed a strong association between BMI and CRP, which appeared to be more pronounced in women than in men. In a recent cross-sectional investigation, Cohen et al.[97] examined 7,526 male and 3,219 female participants, assessing white blood cell and platelet counts, erythrocyte sedimentation rate, and CRP across normal weight, overweight, obese, and morbidly obese BMI categories. Individuals with abnormal BMI values showed significantly elevated inflammatory markers compared with those in the normal BMI range, and this effect was more evident in women than in men. The observed sex-related differences in inflammatory profiles may be explained by gender-specific fat distribution patterns (visceral vs. subcutaneous) and the modulatory effects of sex hormones[96,97].

Excess adipose tissue may contribute to elevated CRP levels in individuals with obesity, as fat is not only a storage organ but also a key regulator of inflammation, coagulation, and fibrinolysis[98]. Functioning as an active endocrine organ, adipose tissue secretes proinflammatory mediators such as tumor necrosis factor-α and IL-6, and IL-6 is the main driver of hepatic CRP synthesis[96,98–100]. Evidence suggests that weight reduction, whether achieved through medication or lifestyle modifications, reduces CRP and cytokine levels[98]. Overall, increased white blood cell counts and CRP levels are indicators of chronic, nonspecific inflammation and point toward systemic metabolic dysregulation in our patient cohort.

In our cohort, hematocrit, white blood cell, and platelet counts were significantly elevated in patients with obesity compared with controls with normal BMI. Epidemiological evidence indicates that, while individuals with obesity tend to have higher leukocyte counts, the absolute values usually remain within the normal physiological range, which is consistent with our findings. Herishanu et al. specifically investigated the impact of leukocytosis in populations consisting solely of obese individuals[101].

According to Samocha-Bonet et al.[102], platelet counts were markedly increased in obese women compared with women of normal weight, a difference potentially explained by higher body fat mass. In contrast, no significant rise in platelet counts was observed among men. These results suggest that obesity may be associated with platelet elevation primarily in females with

underlying chronic inflammation[102]. More recently, Christakoudi et al.[103] confirmed in a large cohort that BMI correlates positively with platelet count in women, whereas associations in men were weak or even inverse, highlighting a clear sex-specific pattern.

According to our investigation, hematocrit was significantly higher in obese female patients than in normal-weight controls, a result consistent with earlier evidence[104]. Prior research has demonstrated that hemoglobin and hematocrit levels correlate positively with anthropometric measures such as body weight, stature, BMI, skinfold thickness, and lean body mass[105]. Consequently, the adjustment of reference intervals for blood count components according to overweight and obesity status has become a topic of growing scientific discussion[103,106].

In our study, both serum amylase and lipase levels were significantly lower in patients than in controls. Similarly, Kondo et al.[107] reported that individuals with obesity exhibited significantly reduced serum amylase and trypsin levels, although lipase levels were not affected, when compared with subjects of ideal body weight. Additionally, serum amylase showed a significant inverse correlation with body weight, suggesting that lower pancreatic enzyme levels in obese individuals may be related to dietary intake[107]. Evidence increasingly indicates that decreased serum amylase is associated with prevalent conditions such as obesity, diabetes, and metabolic syndrome[108]. A recent systematic review confirmed that diabetes mellitus, excessive adiposity, and metabolic syndrome are characterized by reduced serum levels of amylase, lipase, and trypsin, highlighting their potential role as biomarkers for these metabolic disorders[109].

As observed in our research, plasma 25OHD3/D2 vitamin levels were significantly lower in patients than in controls. Consistent evidence from meta-analyses[110–112], cross-sectional[113], and cohort[114] studies also supports an inverse relationship between vitamin D levels and body weight. Several mechanisms may explain the reduced vitamin D in obesity, including volumetric dilution, sequestration into adipose tissue, decreased sun exposure, impaired vitamin D synthesis in adipose tissue and liver[110], low physical activity, and insufficient dietary intake of vitamin D[115]. In the present study, bone mineral content showed no correlation with 25OHD3/D2 vitamin levels. Notably, vitamin D status has also been linked to the presence of painful neuropathy[116,117].

Analysis revealed that serum uric acid levels were significantly higher in patients compared with controls, a finding consistent with previous literature[118,119]. Based on current evidence, elevated uric acid levels in obesity can be explained by several mechanisms, including increased hepatic synthesis, insulin resistance, the endocrine role of adipokines, genetic factors, and

dietary influences such as high fructose intake[118]. Elevated uric acid is recognized as a strong predictor of comorbidities such as diabetes, obesity, and hypertension[120,121], and has also been associated with nonalcoholic steatohepatitis[122]. Obesity can impair liver function through multiple mechanisms[123]. Serum levels of the liver enzyme ASAT/GOT were not significantly elevated in obese female patients compared with controls, whereas ALAT/GPT, GGT, and ALP were all significantly higher. These findings are in line with previous studies reporting positive correlations between abdominal obesity and elevated ALAT/GPT and GGT levels[124,125].

Dyslipidemia is frequently present in individuals with obesity, typically characterized by elevated triglycerides, very-low-density lipoprotein, apolipoprotein B, and LDL-cholesterol, along with reduced HDL-cholesterol and apolipoprotein A-I levels[126–129]. In the present analysis, patients exhibited significantly higher serum triglyceride concentrations and significantly lower HDL-cholesterol levels compared with the control group.

Obesity is widely acknowledged as a major risk factor for disturbances in carbohydrate metabolism[130–132]. In line with this, our cohort of patients showed a significantly higher prevalence of IGT and elevated fasting blood glucose compared with controls. While HbA1c% values were nearly identical in both groups, fasting insulin levels and HOMA-IR indices exhibited a non-significant upward trend in the group with obesity. Moreover, the observation of markedly increased fasting plasma glucose in patients with obesity further underscores the contribution of obesity to the development of carbohydrate metabolism disorders.

7.1.6 Limitations of the obesity study

When interpreting the findings of patients versus controls, it is important to recognize that a substantially higher proportion of patients were treated with metformin and β-blockers. Since β-blockers reduce sympathetic nerve activity[92,93], they may partly diminish the extent of abnormalities detected in reflex testing within the patient cohort. Metformin treatment, by improving carbohydrate metabolism[94], may also exert a similar masking influence.

To strengthen the statistical robustness of the study and to enable a more detailed evaluation of how obesity and other factors relate to neuropathic status and its possible alterations, a larger sample size would be beneficial.

7.2 Discussion of the preconceptional weight reduction study

7.2.1 Baseline clinical data in infertile women with obesity and controls

Before initiating lifestyle interventions for weight loss, infertile female patients with obesity exhibited significantly higher mean resting systolic and diastolic blood pressure. In agreement with the literature, these patients were more frequently affected by hypertension [133], IGT[134], T2DM[135], PCOS[136], hirsutism[137], and hypothyroidism[138] compared to the control group. Furthermore, the use of medications such as metformin, β-blockers, the α2-adrenergic receptor agonist methyldopa, acetylsalicylic acid, and levothyroxine was significantly higher among obese patients. However, no statistically significant difference was observed between the two groups in the use of ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, imidazolines, α2-adrenergic receptor agonists, statins, and diuretics. The potential influence of β-blockers and methyldopa on cardiovascular reflex test outcomes is further addressed in the limitations section. Overall, infertile women with obesity were characterized by a higher prevalence of metabolic disorders and an increased need for pharmacological treatment compared with controls.

7.2.2 Laboratory data of infertile women with obesity and control subjects

Vitamin D

Unexpectedly, baseline plasma concentrations of 25OHD3/D2 vitamin did not show a significant reduction in female patients with obesity compared to the control group, although a decreasing trend was apparent (**Table 9**). In contrast, evidence from meta-analyses consistently reports an inverse relationship between vitamin D status and body weight[110,111]. Based on initial laboratory tests indicating vitamin D deficiency, D3 vitamin loading and supplementation were initiated in 7 of 16 patients with obesity, resulting in a marked improvement in 25OHD3/D2 levels at the second assessment. Previous randomized clinical trials[139] have shown that weight reduction is associated with a more pronounced rise in serum 25(OH)D compared with weight maintenance. Similarly, in the placebo-controlled study by Holt et al.[140], dietary weight loss led to a significant increase in serum 25(OH)D levels. In our study, however, because vitamin D insufficiency was corrected by oral supplementation, the isolated effect of weight loss on serum 25OHD3/D2 levels could not be determined. Nevertheless, follow-up measurements demonstrated a statistically significant increase compared with baseline values.

Inflammatory markers

Numerous preclinical and clinical studies have demonstrated that chronic low-grade inflammation in adipose tissue is closely associated with metabolic disorders and complications in organ tissues among individuals with obesity[141]. In line with our findings, CRP levels were significantly higher in obese patients compared to those with a normal BMI. Prior studies[96,97], including our own work[142], have consistently reported a strong link between increased CRP levels and obesity, with the association being more pronounced in women than in men[97]. Beyond CRP, other inflammatory markers - such as white blood cell and platelet counts - were also elevated in individuals with abnormal BMI, especially among females[97]. In our cohort of infertile patients with obesity, white blood cell, platelet, and red blood cell counts were all significantly higher compared to controls with normal BMI. Epidemiological data suggest that although obese individuals tend to have higher white blood cell counts, these values usually remain within the reference range, which was also observed in our study. Red blood cell count was significantly higher in our patients with obesity, whereas hemoglobin and hematocrit levels were also elevated but did not reach statistical significance. The difference in red blood cell counts aligns with existing literature[104]. Furthermore, serum iron levels were significantly lower, while ferritin levels were elevated, and mean corpuscular volume of red blood cells was reduced in infertile patients with obesity compared to controls. This finding aligns with existing literature, as obesity is linked to iron deficiency and increased serum ferritin levels[143].

Dyslipidaemia, liver enzymes, carbohydrate metabolism, hyperuricemia

Obesity contributes to liver dysfunction through multiple mechanisms [123,144]. In this study, markers of liver function, including ASAT/GOT, ALAT/GPT, GGT, and ALP, were significantly higher in female patients with obesity compared to the control group. Additionally, dyslipidemia was evident in the obese cohort, with elevated triglycerides and LDL-cholesterol and reduced HDL-cholesterol, consistent with previous findings in individuals with obesity [126]. Given that obesity is a well-established risk factor for carbohydrate metabolism disorders [130–132], our study found that IGT, T2DM prevalence, HbA1c%, fasting serum glucose, insulin levels, and insulin resistance (HOMA-IR) were significantly increased in patients with obesity compared to controls. Notably, HbA1c% levels improved significantly following weight loss therapy, as observed at the second measurement among patients who completed treatment. Patients with obesity also had significantly higher serum uric acid levels than controls, a result supported by previous research [118,119].

Sex hormone levels, thyroid function

Due to the inconsistent timing of blood sample collection (not uniformly obtained on days 3-5 of the menstrual cycle), a direct comparison of FSH, LH, and estradiol hormone levels was not feasible, as these values fluctuate throughout the cycle. No significant differences in thyroid function were detected between the groups, suggesting that hypothyroidism in the patient group was well-managed, leading to a medically appropriate euthyroid state. Furthermore, no notable variations were observed in androgen, cortisol, or prolactin levels between the groups. However, SHBG levels were significantly lower in female patients with obesity, a well-documented finding in prior research[145,146]. This reduction is expected due to the influence of obesity itself[147] and is further reinforced by the higher prevalence of PCOS in this population[148].

7.2.3 Cardiovascular autonomic function tests of infertile women with obesity and control subjects at baseline, and following weight loss therapy

Valsalva-ratio values were reduced in infertile female patients with obesity compared to the control group, although both cohorts still demonstrated mean ratios within the normal threshold (≥ 1.21). In addition, the 30/15 ratio showed a marked decrease compared with female patients with normal BMI, suggesting the presence of early parasympathetic impairment. This alteration may also be indicative of an upregulated sympathetic tone. Taken together, these deviations in the two autonomic function tests resulted in a significant difference in the composite autonomic score, which quantifies the degree of cardiovascular autonomic neuropathy. A recent investigation in individuals with obesity but preserved glucose tolerance demonstrated that a higher waist-to-hip ratio (WHR), reflecting greater visceral fat accumulation, was linked to disturbances in cardiac autonomic regulation involving both parasympathetic and sympathetic branches[149]. In line with these findings, our first study of 72 non-diabetic women with obesity also identified evidence of parasympathetic dysfunction when compared with healthy controls[142]. In contrast, within the present analysis we did not observe any association between WHR and the applied neuropathy tests. Moreover, cardiovascular autonomic function parameters showed no significant changes following anti-obesity treatment.

7.2.4 Peripheral sensory function in infertile female patients with obesity and control subjects at baseline, and following weight loss therapy

The 128-Hz Rydel-Seiffer graduated tuning fork test demonstrated a symmetric reduction in vibration perception in infertile women with obesity compared to controls, measured at the

radius in the upper limbs and the hallux in the lower limbs. As previously presented, our earlier obesity study was the first to report impaired vibrational sensation in women with obesity[142].

Using the Neurometer at 2000 Hz, a significant difference in peripheral sensory function was detected exclusively in the median nerve, suggesting compromised function of large myelinated sensory fibers. A larger sample size could potentially confirm additional deficits in small myelinated and unmyelinated fibers, as reflected by elevated CPT values at 250 and 5 Hz relative to individuals with normal BMI. No statistically significant changes were observed in peripheral sensory parameters following weight loss therapy.

7.2.5 Correlations

The associations identified between BMI and baseline body weight, along with findings from the tuning fork and cardiovascular reflex assessments across the study population, highlight the important contribution of elevated body weight as a pathogenic factor in neuropathy.

7.2.6 Results at baseline (1st measurement) and after weight loss therapy (2nd measurement) in infertile women with obesity

Of the initial participants, only 16 female patients with obesity completed the second follow-up measurement, representing 27.6% of this highly motivated cohort engaged in weight reduction. The duration of therapy among these completers ("finishers") averaged 232.9 ± 170.86 days (approximately 7.6 months), with a substantial standard deviation. According to published data, the retention rate for weight loss programs at six months (26 weeks) is typically around 22%[150].

In our cohort, participants experienced an average weight reduction of 15.2 kg, equivalent to a 14.6% loss of their initial body weight, accompanied by a mean BMI decrease of 5.6 kg/m² (**Table 5**). These outcomes substantially surpass the average reductions reported by Barboza et al.[151], who documented decreases of only 3.35 kg and 1.45 kg/m², respectively. Furthermore, we observed notable improvements in HbA1c%, a finding not captured in their meta-analysis[151]. While differences in study populations, baseline characteristics, and treatment protocols - such as higher initial body weight and differing mean age in our sample - limit direct comparison, these results underscore the pronounced effectiveness of our comprehensive weight loss program. Similarly, a recent systematic review and meta-analysis including 1,627 women with overweight or obesity reported much more modest reductions, with an overall mean weight loss of only −4.62 kg, −2.49 kg with pharmacological intervention and −5.49 kg

with lifestyle modification, prior to IVF treatment[152]. We also observed significant improvements in body composition, with mean body fat percentage decreasing from 47.0 ± 3.81% to 42.5 ± 6.50% ($n = 13$; $p < 0.05$) and waist circumference declining from 108.7 ± 20.05 cm to 94.0 ± 13.58 cm ($p < 0.05$). These changes were consistent with the findings of Elkind-Hirsch et al.[153], who, in a randomized placebo-controlled phase 3 trial evaluating liraglutide 3.0 mg in women with obesity and PCOS, reported similar reductions in waist circumference (111 ± 2.2 cm to 101 ± 2.0 cm; $p = 0.011$) and body fat percentage (47.6 ± 0.8% to 46.0 ± 0.9%; $p = 0.028$). It should be noted that their body composition assessments relied on dual-energy X-ray absorptiometry, which may influence comparability. Recent literature suggests that body composition metrics are particularly informative for predicting reproductive potential[154,155], and a recent meta-analysis of 12 randomized clinical trials ($n = 1921$) found that pre-IVF weight loss increased overall and unassisted pregnancy rates[156]. Although reductions in systolic and diastolic blood pressure showed a downward trend similar to previous reports[151,157], these changes did not reach statistical significance in our study. In contrast, cardiovascular autonomic and peripheral sensory function measures showed no significant post-intervention changes. To our knowledge, no prior research has specifically addressed these functions in infertile female patients with obesity. We anticipate that, with larger sample sizes, the baseline deficits identified in our cross-sectional analysis - such as reduced Valsalva and 30/15 ratios compared to normal-BMI controls, decreased vibration perception across all limbs, and elevated current perception thresholds of large myelinated sensory fibers in the median nerve - may also improve as metabolic health is enhanced through weight loss therapy.

IVF success rates per oocyte retrieval vary across European clinics, primarily influenced by patient age, clinic protocols, and other health-related factors. According to the ESHRE, typical pregnancy rates per retrieval generally fall within the 20–30% range[158].

Taking these factors into account, our results are encouraging, although the small sample size necessitates cautious interpretation. Three of the 16 participants conceived naturally, representing 18.75% of the cohort who did not require IVF. Excluding these three patients, five of the remaining 13 chose not to proceed with IVF treatment. Of the eight patients who underwent IVF, five achieved a clinical pregnancy, corresponding to a success rate of 62.5% in this subgroup.

7.2.7 Limitations of the preconceptional weight reduction study

We conducted a retrospective analysis of the collected data, recognizing that the intervention represented a real-world program. It should be noted that a significantly higher

proportion of patients with obesity were taking metformin, β-blockers, and α2 adrenergic receptor agonists compared to the control group. Beta-blockers reduce sympathetic nervous system activity[92,93], which could potentially diminish the observed abnormalities in reflex test results among the obese participants. Similarly, metformin may exert a masking effect by improving carbohydrate metabolism[94]. Among the α2-agrenerg receptor agonists, only methyldopa was administered to patients with obesity; this drug lowers sympathetic tone[159]. Experimental studies in rats have shown that systemic α-methyldopa administration decreases mean arterial pressure, initially causing a transient rise in heart rate followed by a sustained reduction[160]. Importantly, these studies did not indicate an increased risk of orthostatic hypertension[161]. Therefore, methyldopa use may obscure sympathetic overactivity in patients with obesity, and its rare potential for orthostatic hypotension could also affect performance on sympathetic function tests.

The study initially enrolled 58 participants, but only 16 completed the intervention. This discrepancy in sample size has two main implications: subtle but potentially meaningful differences may fail to reach statistical significance, while small yet statistically significant findings may carry greater uncertainty. To address the limitations imposed by the small sample size, we reported the statistical power for each significant outcome. A power greater than 80% suggests that these findings are likely to represent true effects. Nonetheless, increasing the sample size would enhance statistical power across multiple study parameters and allow for a more thorough assessment of how obesity and other variables relate to neural function. Given these limitations, the analysis focused more intensively on comparisons between infertile patients with obesity and normal-weight controls without fertility issues. The small number of patients completing the therapy also restricted analyses related to fertility outcomes, as the sample was insufficient for definitive statistical evaluation, and conclusions are therefore primarily descriptive regarding fertility rates. Future studies are needed to further explore the impact of lifestyle interventions, including dietary guidance and structured exercise, on neuropathic outcomes.

8. Conclusions and new findings

8.1 Conclusions and new findings of the obesity study

1. In female patients with obesity without diabetes, impaired parasympathetic cardiovascular autonomic function was reflected in reduced Valsalva-ratios compared to controls.
2. In female patients with obesity without diabetes, peripheral sensory dysfunction were observed by multiple assessments:
   - Neurometer® testing showed elevated sensory thresholds across all three fiber types in the median nerve, indicating early impairment of large myelinated, small myelinated, and small unmyelinated sensory fibers.
   - Rydel-Seiffer tuning fork testing confirmed involvement of large myelinated fibers.
3. Neuropad® testing demonstrated sudomotor dysfunction in women with obesity without diabetes. To our knowledge, this is the first study to suggest small fiber dysfunction in non-diabetic women with obesity using the Neuropad® testing method.
4. A significant negative association was found between BMI and 25OHD3/D2 vitamin levels, highlighting the potential need for vitamin D3 supplementation in this population.

8.2 Conclusions and new findings of the preconceptional weight reduction study

1. Impaired peripheral sensory nerve function was observed in female patients with obesity and infertility compared to normal-BMI controls:
   - Reduced vibration perception was detected in all four limbs using the tuning fork test.
   - Neurometer® assessments confirmed elevated thresholds in the median nerve at 2000 Hz.
2. Cardiovascular autonomic testing, including the 30/15 and Valsalva-ratio, indicated parasympathetic dysfunction in female patients with obesity and infertility.
3. In infertile obese women, preconceptional weight loss therapy led to significant reductions in body weight, BMI, HbA1c%, and body fat percentage, along with a significant increase in 25OHD3/D2-vitamin levels, while no statistically significant changes were observed in cardiovascular autonomic or peripheral sensory function following the intervention within the present sample size.
4. Among the patients who achieved their preconception target weight:
   - Three out of sixteen (18.75%) conceived naturally, thereby avoiding IVF.
   - Of the eight women who proceeded with IVF treatment, five (62.5%) achieved a clinical pregnancy, exceeding the average success rates commonly reported.

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