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

Nóra Keller, Pharm.D.

Summary of Ph.D. Thesis

Supervisor:

Anna Vágvölgyi, M.D., Ph.D.

University of Szeged

Doctoral School of Clinical Medicine

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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**; **Impact 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.

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1. Introduction and aims of the studies

Obesity has become a global health crisis, with its prevalence increasing dramatically over recent decades. Nutritional status is most commonly assessed using the body mass index (BMI), according to World Health Organization (WHO) classifications: individuals with a BMI between 25 and 29.9 kg/m² are considered overweight, while those with a BMI ≥30 kg/m² are classified as obese. According to data from the World Obesity Federation's Global Obesity Observatory (2022), 36.2% of Hungary's adult population were reported to be overweight (40.7% of men, 32.1% of women), while 22.2% are obese (24.6% of men, 20.0% of women).

Even in the absence of diabetes, obesity is recognized as a major risk factor for both central and peripheral neuropathies. It is currently regarded as the second most important metabolic risk factor for neuropathy after diabetes. Dyslipidemia, insulin resistance, chronic low-grade inflammation, oxidative stress, and selective injury to small nerve fibers have all been implicated in the pathogenesis of obesity-related neuropathy. Evidence indicates that waist circumference is significantly associated with neuropathic alterations, especially in women. Several studies have confirmed the association between central obesity and the development of neuropathy, which may be mediated by inflammatory markers derived from adipose tissue, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-1 beta (IL-1β), and resistin, as well as by an altered adiponectin-to-leptin ratio. It is well established that, in addition to the classical cardiovascular risk factors (elevated systolic blood pressure, increased triglyceride levels, and smoking), a high body mass index (BMI) — the main focus of our study — further contributes to the risk of autonomic neuropathy.

Thanks to recent therapeutic advancements, liraglutide, a glucagon-like peptide-1 receptor agonist, has become available for use in the treatment of obesity. During the study period, Hungarian guidelines recommended liraglutide for individuals with a BMI ≥30 kg/m² or ≥27 kg/m² in the presence of obesity-related comorbidities (e.g., prediabetes, hypertension, dyslipidaemia, or obstructive sleep apnea).

Obesity adversely effects reproductive function in both women and men, contributing to reduced fertility. The most advanced and specialized approach to infertility treatment today is represented by assisted reproductive technologies (ART), which offer support for conception when natural methods are unsuccessful. In Hungary, approximately 3.5% of newborns in 2024 were born as a result of pregnancies conceived through ART. According to the National Institute for Health and Care Excellence 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. Data in the literature regarding female obesity, preconception weight reduction, and *in vitro* fertilization (IVF) outcomes remain contradictory. While some investigations reported no association between BMI and IVF outcomes, others identified negative impacts on oocyte quality, fertilization rates, and embryo development. Emerging evidence suggests that preconception weight reduction can improve clinical pregnancy and live birth rates, reduce the number of IVF cycles needed, and potentially optimize endocrine and metabolic status in women with polycystic ovarian syndrome (PCOS). Lifestyle interventions, including dietary modification and physical activity, are concidered first-line strategies in infertile women with obesity.

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 nondiabetic female patients with obesity, 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 women with obesity who presented for preconceptional weight optimization prior to planned ART. 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.

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, 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.

2.2 Study populations of the preconceptional weight reduction study

This single-center cohort study was conducted at the same institution, in collaboration with the University's Institute of Reproductive Medicine. Participants were women with obesity (BMI > 30 kg/m²) referred for preconception weight management prior to IVF treatment, supervised by an endocrinologist. Between March 2021 and March 2024, clinical and anthropometric data were collected from these patients. For baseline comparison, age-matched healthy women with normal BMI (18.5–24.9 kg/m²) and no fertility disorders were selected from the QT Registry, a prospectively maintained database established in 2019. This registry prospectively collects data on participants' medical history, anthropometric measurements, laboratory, electrocardiographic (ECG) parameters and diagnostic findings, as well as results from standardized neuropathy assessments, and includes both healthy individuals and patients with various health conditions. All participants were Caucasian. The present analysis represents a retrospective evaluation of prospectively collected registry data.

3. Methods

Both studies were conducted in accordance with the principles of the World Medical Association's Declaration of Helsinki (2000). The "obesity study" was approved by the Hungarian Medical Research Council (approval no. 219 31891-5/2019/EÜIG) and subsequently by the Regional and Institutional Review Board of Human Investigations at the University of Szeged (approval no. 170/2019-SZTE). The "preconceptional weight reduction study" received, in addition to the above approval, ethical clearance from the Hungarian Medical Research Council (approval no. BM/18153-1/2023) followed by approval from the Regional and Institutional Review Board of Human Investigations at the University of Szeged (approval no. 113/2023-SZTE). The research was conducted in accordance with applicable local laws and institutional regulations. All participants provided written informed consent to participate.

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

Autonomic function was characterized using Ewing's five standard cardiovascular reflex tests, the gold standard for assessing autonomic dysfunction through reproducible and non-invasive measures. Three tests evaluated heart rate variability (parasympathetic activity), while

two assessed blood pressure responses (sympathetic function). 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. 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 physiological conditions, heart rate increases during inspiration and decreases during expiration. 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.

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 15^{th} beat after standing, followed by a transient slowing around the 30^{th} beat. From a supine position, subjects stood up while ECG was recorded continuously. The 30/15 ratio was calculated by dividing the longest R–R interval (around the 30^{th} beat) by the shortest R–R interval (around the 15^{th} beat), and this index is used to assess the autonomic innervation of the cardiovascular system.

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

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.

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

Systolic blood pressure was recorded after 10 minutes supine, and at 1, 5, and 10 minutes after standing. This measurement reflects sympathetic vasoconstrictor capacity and helps identify orthostatic hypotension.

3.1.5 Diastolic blood pressure response during sustained handgrip

Maximal handgrip strength was determined using a handheld dynamometer on the dominant hand. Participants then maintained 30% of their maximal strength 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.

3.2 Peripheral sensory nerve testing

3.2.1 Neurometer®

Current perception thresholds (CPTs) were determined by low-voltage transcutaneous stimulation (0.01–9.99 mA) using the Neurometer device (NM-01/CPT Neurometer, MDE Heidelberg GmbH, Heidelberg, Germany), providing a sensitive and specific measure of nerve fiber integrity. Both the median and peroneal nerves were tested. Measurements were performed on the distal phalanx of the index finger (median nerve) and the hallux (peroneal nerve) at 2000, 250, and 5 Hz: 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. At the start of the assessment, the current intensity was gradually increased until the participant first reported perceiving the stimulus. Subsequently, brief stimulations lasting 2–5 seconds were delivered at progressively lower intensities to identify the lowest level of current consistently perceived by the subject.

3.2.2 128-Hz Rydel-Seiffer graduated tuning fork

Vibration sense was tested on the distal radius and hallux using a calibrated 128-Hz tuning fork. 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®

The Semmes-Weinstein Monofilament Test® was performed using a 10 g monofilament to directly screen for loss of protective sensation. Participants were blinded to the location and application of the filament. The assessment was performed on all four limbs, and 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. 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. 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. 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.

3.3 Body composition analysis

Body composition was assessed by segmental bioelectrical impedance analysis with an InBody 770 device (InBodyUSA, Cerritos, CA). 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 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.

3.4 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 m^2), uric acid (μmol/L), total protein (g/l), albumin (g/l), total cholesterol (mmol/L), triglyceride (mmol/L), HDL-cholesterol (high-density lipoprotein- cholesterol; mmol/L), LDL-cholesterol (low-density lipoprotein-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). Urinary parameters (protein, albumin, albumin-to-creatinine ratio, pH, nitrite, etc.) were documented to evaluate renal status. 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).

3.5 Weight loss therapy

The management of obesity, including dietary, lifestyle, and pharmacological strategies, was conducted in accordance with the latest Hungarian clinical guidelines, dated December 2023. The duration of the weight-reduction therapy was not restricted. After initial clinical and laboratory assessment, body composition analysis, and neuropathic testing, participants received individualized dietary counseling and tailored physical activity plans. Patients with obesity contacted the attending physician via email on a monthly basis or whenever necessary for immediate consultation regarding any concerns. The program duration was individualized, and target weight was determined collaboratively by the endocrinologist, IVF specialist, and

patient, considering 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. Upon achieving the target weight, a final reassessment was performed before IVF initiation, including updated clinical, laboratory, and neuropathic evaluations. Patients with obesity who successfully reached their target weight before undergoing IVF and returned for this final evaluation were classified as "*finishers*". 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.

4. Results

4.1 Results of the obesity study

4.1.1 Clinical and laboratory data

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. The patients with obesity 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 (IGT) was also notably higher in patients with obesity than in the controls (13 vs. 0; $p=0.006$). Patients received metformin (40 vs. 1; $p<0.001$) and β-blockers (8 vs. 0; $p=0.036$) more frequently, while use of other antihypertensives or statins did not differ significantly.

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 group differences were observed in other biochemical markers including insulin, HOMA-IR, HbA1c%, total cholesterol, LDL-cholesterol, ferritin, TSH, or renal function indices.

4.1.2 Cardiovascular autonomic function tests

Patients exhibited a significantly reduced Valsalva-ratio 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.

4.1.3 Peripheral sensory function

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). Thresholds were also elevated in the peroneal nerve, although these differences did not reach statistical significance. The 128-Hz Rydel-Seiffer graduated tuning fork demonstrated significant impairment of lower limb vibration perception in patients, with bilaterally reduced perception thresholds observed among women with obesity. In contrast, the Semmes-Weinstein Monofilament Test® and Tiptherm® detected no significant differences between patients and controls. Neuropad® testing indicated reduced plantar sweating in patients, confirming sudomotor dysfunction.

4.1.4 Correlations between studied parameters

In female patients with obesity, BMI showed a negative correlation with serum 25OHD3/D2-vitamin levels (r = –0.41, p = 0.001) and a positive correlation with resting systolic blood pressure (r = 0.26, p = 0.033). In the patient group, 25OHD3/D2-vitamin concentrations were significantly and negatively correlated with waist circumference (r = –0.26, p = 0.049), a relationship that was not observed in the control group. Among patients with obesity, both waist circumference (r = 0.28, p = 0.021) and hip circumference (r = 0.39, p = 0.001) were positively associated with resting systolic blood pressure. This association was also evident among controls (r = 0.38, p = 0.045).

4.2 Results of the preconceptional weight reduction study

4.2.1 Baseline clinical and laboratory data

Our study involved 58 infertile female patients with obesity (mean ± SD; age: 33.1 ± 5.42 years; BMI: 39.3 ± 6.90 kg/m²) and 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) were included. No significant differences were observed between the infertile women with obesity and the control group regarding age, height, 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), IGT (0 vs. 16, p<0.001), type 2 diabetes (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. The use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, statins, and diuretics did not differ significantly between the two groups.

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. Due to the individualized nature of ambulatory patient care, not all participants had all collected parameters assessed. For infertile female patients with obesity (n=36), the baseline AMH value was recorded as 3.6 ± 3.80 ng/ml.

4.2.2 Cardiovascular autonomic function tests

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. The autonomic score 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.

4.2.3 Peripheral sensory function

The 128-Hz Rydel-Seiffer graduated tuning fork test 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 (198.4 ± 62.18 vs. 172.1 ± 39.9; p<0.05). CPT values at other tested frequencies for the median and peroneal nerves did not show statistically significant differences between patients with obesity and controls.

4.2.4 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). 15 out of 58 obese infertile women achieved pregnancy.

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. 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. Importantly, 3 of the 16 women conceived spontaneously, indicating that IVF was unnecessary for 18.75% of this group.

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 serum 25OHD3/D2-vitamin levels increased significantly. No statistically significant differences were detected in cardiovascular autonomic function or peripheral sensory test results before and after the intervention.

5. Discussion

5.1 Discussion of the obesity study

Obese female patients without diabetes 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. Sudomotor function 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 function. Our findings represent the first evidence confirming such impairment in this population.

Neurometer® testing showed elevated thresholds in the median nerve across all frequencies, reflecting impaired large, small, and unmyelinated fiber function, while the peroneal nerve showed a non-significant upward trend. Vibratory sensation assessed by the 128-Hz Rydel–Seiffer tuning fork was markedly reduced in the lower limbs, representing the first such observation in women with obesity. Other sensory tests (Semmes–Weinstein Monofilament®, Tiptherm®) showed no group differences.

Although the patient cohort was free of diabetes and similar to controls in age, smoking, alcohol use, and hypercholesterolemia history, they displayed higher systolic and diastolic blood pressure, more hypertension, PCOS, and hypothyroidism, and a higher proportion used metformin and β-blockers**.** No significant differences were found for other cardiovascular medications.

Inflammatory markers, including CRP, white blood cell, and platelet counts, were significantly higher in obese patients. This aligns with previous findings highlighting chronic low-grade inflammation in obesity, particularly among women, and the endocrine role of adipose tissue in secreting proinflammatory mediators. Hematocrit was elevated in obese patients, consistent with prior evidence linking hemoglobin and hematocrit to anthropometric measures, such as body weight, height, BMI, skinfold thickness, and lean body mass. Serum amylase and lipase levels were lower, confirming the association between reduced pancreatic enzyme activity and obesity as well as metabolic syndrome. Increasing evidence suggests that these enzymes show an inverse correlation with body weight, and their decreased levels may be linked to metabolic disturbances, indicating their potential as biomarkers for such conditions. Plasma 25OHD3/D2-vitamin levels were significantly lower, consistent with known inverse relationships between vitamin D3 and body weight. Metabolic markers showed higher uric acid, liver enzymes (ALAT/GPT, GGT, ALP), triglycerides, LDL-cholesterol, and lower HDL-cholesterol in obese patients, reflecting common obesity-associated metabolic dysregulation, such as dyslipidemia and hepatic steatosis. Obesity is widely acknowledged as a major risk factor for disturbances in carbohydrate metabolism. In line with this, our cohort of patients showed a significantly higher prevalence of IGT and elevated fasting blood glucose compared with controls.

In our cohort, waist and hip circumferences correlated positively with resting systolic blood pressure, reinforcing the link between visceral adiposity and sympathetic overactivation. This sympathetic overactivation may contribute to insulin resistance, IGT, T2DM, and dyslipidemia.

Treatment with metformin and β-blockers may partially mask abnormalities in autonomic tests, highlighting the need for cautious interpretation. Overall, these results demonstrate that obesity in women is associated with early autonomic dysfunction, peripheral sensory deficits, chronic inflammation, and metabolic disturbances.

5.2 Discussion of the preconceptional weight reduction study

Before the complex anti-obesity treatment, infertile women with obesity exhibited higher resting systolic and diastolic blood pressure and a greater prevalence of hypertension, IGT, T2DM, PCOS, hirsutism, and hypothyroidism compared with controls. Pharmacological treatment was also more common, including metformin, β-blockers, methyldopa, acetylsalicylic acid, and levothyroxine. The potential effects of β-blockers and methyldopa on cardiovascular reflex tests are considered a study limitation, as both drugs can influence autonomic function.

Baseline 25OHD3/D2-vitamin levels in infertile patients with obesity were lower compared to controls, although the difference did not reach statistical significance. Following the comprehensive anti-obesity treatment, a significant increase in plasma 25OHD3/D2-vitamin levels was observed among the “finisher” group. This improvement was likely influenced by vitamin D3 loading and subsequent supplementation in seven out of sixteen patients, confirming the effectiveness of targeted replacement therapy. However, due to this confounding factor, the independent effect of weight loss on plasma 25OHD3/D2-vitamins level could not be reliably determined. Consistent with prior research, chronic low-grade inflammation was evident, with elevated CRP levels, white blood cell counts, and platelet counts in obese participants. Serum iron levels were reduced in patients compared with controls, while the non-specific inflammatory marker ferritin was increased, and mean corpuscular volume decreased, reflecting the association between obesity and iron deficiency. However, the relationship between obesity and iron deficiency remains unclear. Markers of liver dysfunction (ASAT/GOT, ALAT/GPT, GGT, ALP) were higher in obese patients, and dyslipidemia was observed, characterized by elevated triglycerides and LDL-cholesterol and reduced HDL-cholesterol. Women with obesity also exhibited a significantly higher prevalence of IGT and T2DM, elevated fasting glucose, insulin levels, and HOMA-IR, confirming obesity’s role in carbohydrate metabolism disturbances.

Autonomic testing revealed reduced Valsalva- and 30/15-ratios, indicating early parasympathetic dysfunction and potential sympathetic overactivity. These changes contributed to a higher composite autonomic score, quantifying cardiovascular autonomic neuropathy. Vibratory sensation, assessed by the 128-Hz Rydel-Seiffer tuning fork, was symmetrically reduced in upper and lower limbs, and median nerve CPT at 2000 Hz indicated large myelinated fiber impairment, while smaller fibers showed non-significant trends. Weight loss did not produce significant changes in autonomic or sensory measures. Thus, with the current sample size, we were unable to demonstrate a statistically significant change.

Of the initial 58 participants, only 16 individuals (~28%) completed the final assessment; these participants were defined as "finishers." In this subgroup, the average duration of the complex anti-obesity therapy was approximately 7.6 months. By comparison, according to published data, the six-month (26-week) retention rate for weight loss programs is typically around 22%, highlighting the above-average compliance and retention in this highly motivated patient population. Therapy led to a mean weight loss of 15.2 kg (14.6% of initial body weight), BMI reduction of 5.6 kg/m², body fat decrease from 47.0% to 42.5%, and waist circumference reduction from 108.7 cm to 94.0 cm. Significant HbA1c% improvements were also observed, confirming the effectiveness of the comprehensive weight loss program. Regarding fertility outcomes, three of sixteen patients conceived naturally, while among the eight undergoing IVF, five achieved clinical pregnancy, yielding a 62.5% success rate.

Interpretation is limited by small sample size and the influence of pharmacological treatments such as metformin, β-blockers, and methyldopa. Beta-blockers reduce sympathetic nervous system activity, which could potentially diminish the observed abnormalities in cardiovascular reflex test results. Similarly, metformin may exert a masking effect by improving carbohydrate metabolism. Methyldopa, administered in a few cases, lowers sympathetic tone and may also influence reflex testing outcomes. Overall, the findings indicate that lifestyle interventions in infertile women with obesity result in substantial weight loss, metabolic improvements, and potential fertility benefits, although autonomic and sensory neuropathic functions were unchanged in this small cohort. Larger studies are needed to further explore the effects of weight reduction on neural function and reproductive outcomes.

6. Conclusions and new findings

6.1 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 was 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.

6.2 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.