BIOMEDICAL RESEARCH PROTOCOL (VMU)

1. Title of Biomedical Research

„Ambient air and noise pollution effect on cardiovascular health risk and lifestyle intervention to attenuate it in 45–64-year population“ (METSGREEN) is a part of the international study "*Noise and/or ultrafine particulate matter induced cerebral and cardiovascular damage: novel insights from experimental and epidemiological studies and computational models*"

Protocol Number: 1
Version: 3
Date: 2025-03-19

2. Hypothesis of the METSGREEN Biomedical Research

Ambient ultrafine particles in the living environment increases the risk of cardiovascular diseases (CVD) among residents aged 45–64, however, this risk can be reduced by increasing physical activity in green spaces.

3. Aim of the Biomedical Research

Primary Objective (Observational Study):

To determine the association between ambient exposure to ultrafine (PM0.1) particulate, as well as noise, and the risk of cardiovascular diseases (CVD) among 45–64-year-old residents of Kaunas.

Secondary Objectives (Experimental Clinical Trial):

- ✓ To determine the association between ultrafine particulate concentrations and key indicators of metabolic syndrome.
- ✓ To assess the impact of physical activity in green spaces and healthy nutrition on individuals with central obesity and elevated blood pressure.
- ✓ To provide recommendations for reducing the risk of metabolic disorders.

4. Objectives

Objectives of the Observational Study involving 1,000 participants:
To measure and model the levels of air pollution and noise exposure in Kaunas, and to determine the relationship between individual exposure and CVD risk, depending on the participants' social and economic status.
To assess the long-term impact of road traffic intensity, PM0.1 and noise exposure on key indicators of metabolic disorders.
Objectives of the Experimental Clinical Trial (180 Participants)

To determine the impact of a healthy lifestyle (physical activity in green spaces and the Mediterranean diet) on indicators of metabolic disorder risk and to provide recommendations for reducing the risk of metabolic disorders.

5. Detailed Description of the Biomedical Research Outcomes (Primary and Secondary Endpoints)

Primary Endpoints:
Measured concentrations of PM0.1 and noise levels in 20 locations across the city of Kaunas. Using a land-use regression (LUR) model, exposure levels for 1,000 participants will be modelled and linked to self-reported health outcomes: prevalence of CVD, blood pressure, waist circumference, and anthropometric data.
Secondary Endpoints:
During the experimental clinical trial (7-day healthy lifestyle intervention), involving 180 participants, key indicators of metabolic disorders will be assessed, including waist circumference and blood pressure measurements, high-density lipoprotein (HDL) levels, triglycerides, plasma glucose concentration, as well as biological markers from metabolomics, proteomics, and DNA methylation. These will be analyzed in relation to environmental exposure, socioeconomic status, and healthy lifestyle factors. Recommendations will be developed for reducing the risk of metabolic disorders.

6. Justification for the Biomedical Research

The international research's "Noise and/or ultrafine particulate matter induced cerebral and cardiovascular damage: novel insights from experimental and epidemiological studies and computational models" (MARKOPOLO) part conducted at Vytautas Magnus University (VMU), title: "Ambient air and noise pollution effect on cardiovascular health risk and lifestyle intervention to attenuate it in 45–64-year population" (METSGREEN), is being conducted to the order of the European Commission, which provides the necessary funding for its implementation. The MARKOPOLO is coordinated by Johannes Gutenberg University in Germany.
Environmental exposures increase health risk factors and account for up to two-thirds of all chronic non-communicable diseases[1]. Environmental-related risk factors, among them air pollution, and personal lifestyle habits can contribute to the increase the global burden of CVD such metabolic syndrome, arterial hypertension, and diabetes mellitus. In Europe, approximately 500,000 premature deaths occur annually due to air pollution. However, particulate matter and noise thresholds are not currently incorporated into clinical guidelines. Moreover, the official European environmental pollution limits significantly exceed the standards set by the World Health Organization (WHO). This issue is especially critical for the most vulnerable populations—those affected by social exclusion, chronic illnesses, or advanced age.
METSGREEN study will use clinical and mathematical models, environmental epidemiology methods, and exposure-response modelling to generate new scientific data on the relationship between PM0.1 exposure and changes in biological markers of metabolic disorders, particularly

in the context of healthy lifestyle interventions. The findings will support the development of preventive programs and measures aimed at reducing the risk of metabolic disorders.

The role of PM0.1 exposure in the pathogenesis of metabolic disorders that promote the development of CVD and metabolic syndrome remains unclear. When comparing the health effects of fine and coarse particulate matter, the damage caused by PM0.1 is greater due to its ability to directly penetrate lung tissue and enter the bloodstream. This leads to systemic inflammation, endothelial dysfunction, coagulation abnormalities, and oxidative stress—processes that contribute to the development of metabolic disorders and CVD.[1] In Lithuania, cardiovascular diseases affect approximately 40% of the population aged 45–64 and have a significant impact on public health and the healthcare system[2]. Central obesity is the most important indicator of metabolic disorder; however, data on its association with PM0.1 exposure levels and individual health behaviours are lacking. According to the joint recommendation of international scientific societies on the diagnosis of metabolic syndrome, the key diagnostic criteria include central obesity, elevated blood pressure, increased plasma glucose concentration, elevated triglyceride levels, and low high-density lipoprotein (HDL) cholesterol concentration.[3] Early identification and management of metabolic syndrome can significantly reduce the risk of CVD and fatal outcomes[4]. Physical activity in green spaces can improve health by reducing stress responses and through the regulation of brain-derived neurotrophic factor (BDNF), enhancing cardiac function and lowering blood pressure. Regular 30-minute walks combined with a healthy dietary intervention can significantly improve heart function, reduce blood pressure, and lower body mass index (BMI)[5,6]. There is a link between central obesity, body mass index, and biological markers associated with cardiometabolic disorders, as well as blood pressure and the prevalence of hypertension[7]. There are some evidences that physical activity in green space (Nature therapy) produces an opportunity to decrease the risk for obesity, other chronic diseases and can mitigate the negative effects of environmental exposure[8]. However, evidence and frameworks to implement nature-based therapies for metabolic syndrome are scarce. Thus, the investigation of metabolic disorder pathophysiology and the reduction of these disorders through physical activity in green spaces represent one of the modern and promising approaches of nature therapy. Based on the METSGREEN study results, we will provide recommendations for reducing the risk of metabolic disorders, both for public health professionals involved in population health monitoring and for the public.

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[6] Chin, S.-H., Kahathuduwa, C. N., & Binks, M. (2016). Physical activity and obesity: What we know and what we need to know. *Obesity Reviews, 17*(12), 1226–1244. https://doi.org/10.1111/obr.12460

[7] Giontella, A., et al. (2021). Causal effect of adiposity measures on blood pressure traits in 2 urban Swedish cohorts: A Mendelian randomization study. *Journal of the American Heart Association, 10*(e020405). https://doi.org/10.1161/JAHA.120.020405

[8] Münzel, T., Hahad, O., & Daiber, A. (2021). Running in polluted air is a two-edged sword – Physical exercise in low air pollution areas is cardioprotective but detrimental for the heart in high air pollution areas. *European Heart Journal, 42*(25), 2498–2500. https://doi.org/10.1093/eurheartj/ehab227

7. Characteristics of the study population

Observational Study Participants: The study will include men and women aged 45–64 who are residents of Kaunas City and live in private apartments. Approximately 1,000 individuals will be randomly selected. Individuals living in institutions, rented accommodations, or nursing homes will be excluded from the study.

Experimental Clinical Trial Participants – Inclusion Criteria: Participants must be aged 45–64, male or female, residents of Kaunas City, and randomly selected from the observational study cohort. They must agree to wear a smart wristband sensor for 7 days and sign an informed consent form to participate in the healthy lifestyle intervention. A total of 180 participants will be enrolled.

Exclusion Criteria for Clinical Trial: Participants will be excluded if they have unstable angina, cardiomyopathy, hypertension with blood pressure greater than 160/110 mmHg, cardiac pacemakers, neurological diseases, alcohol dependence, limited mobility, or if they are pregnant.

Justification for Sample Size: The experimental clinical trial involving 180 participants will be conducted by researchers at VMU. The trial will assess the impact of a healthy lifestyle on early metabolic responses (biomarkers and health risks), considering individual PM0.1 exposure levels. To enhance data reliability and reducing the risk of Type I error (false positive) the confidence level is set up at 99.9%. The sample size was calculated using the formula:

$N=2\times(Z1-\alpha/2+Z1-\beta/\delta 0)2\times p\times(1-p)$,

Where:

- N = required sample size
- $Z_1-\alpha/2 = 3.29$ (for a 0.1% significance level)
- $Z_1-\beta = 0.842$ (for 80% statistical power)
- δ_0 = effect size = 0.1
- p = expected proportion = 0.3

This gives:

$N \approx 2 \times (3.29 + 0.842 / 0.1)^2 \times 0.3 \times 0.7 \approx 58$ participants

To account for potential dropouts (e.g., due to acute infections), a sample size of 60 per group is proposed. Therefore, a total of 180 participants will be enrolled in the clinical trial.

8. Biomedical Research Methodology

Measures to Reduce Subjective Bias: Participants will be randomly assigned to the three clinical trial groups. Only individuals without the listed health exclusions and who sign informed consent will be included. Assignment to the one of three groups will follow a 1:1:1 ratio. The researcher will handle group allocation and coding. Objective environmental pollution measurements and individual residential exposure will be taken. Seven-day monitoring health outcomes include physical activity, sleep disturbances, heart function. Two health checks will include measurements of blood pressure, body composition, waist circumference, body mass index (BMI) and biomarkers. Blood samples (up to 20 ml) will be collected to analyse metabolic biomarkers.

Mathematical modelling will be used during data analysis. It will control for confounding factors to assess the strength of the exposure–health outcome relationship.

Biomedical Study Plan: The study plan and phases are shown in Figure 1.
Observational Study: A representative sample survey of Kaunas residents will be conducted by a certified survey company that complies with strict personal data security, privacy principles, and legal requirements. In accordance with the General Data Protection Regulation (GDPR), data will be securely stored, lawfully processed, and participants will be able to exercise their rights easily. The internet survey will be conducted using the Google Forms platform, which provides data encryption and access control (limited to authorized personnel). Google services comply with EU data protection regulations, including two-factor authentication, ensuring the confidentiality and security of personal data. Approximately 1,000 individuals aged 45–64 will be included based on their responses to an anonymous online survey, which collects self-reported health indicators (physician-diagnosed chronic diseases), socioeconomic status, and basic residential information.
Experimental Clinical Trial: Participants who completed the online survey and conform to the study inclusion criteria will be eligible. Those interested in participating in the healthy lifestyle clinical trial can contact the researchers via the details provided in the internet survey form. Upon contact, participants will be asked to updated information on waist circumference, blood pressure, and address. Eligible participants will be invited to the Family Medicine Clinic of Kaunas Clinics at the Lithuanian University of Health Sciences (LSMU).
Inclusion criteria: Age 45–64, residence in Kaunas City on streets with identified high air pollution levels, and self-reported metabolic disorder indicators such as increased waist circumference and/or elevated blood pressure. Following contact, appointment times will be coordinated. At the initial visit, researchers will review the participant's health status. Participants will be informed about the experimental clinical trial and will sign an informed consent form. They will then be randomly assigned to one of three healthy lifestyle groups and instructed on their 7-day health behaviour study. Groups: 1. Control group (usual routine); 2. Physical activity in green space (daily 30-minute brisk walk); 3. Mediterranean diet intervention.
All groups' participants health assessments will be conducted by family doctor on Day 1 and Day 8 using certified medical equipment. Participants will be asked to arrive fasting or having not eaten for at least 2 hours before their visit.
Procedures will include:

- Blood sampling (up to 20 ml) for analysis of metabolic biomarkers
- Blood pressure measurement
- Waist circumference measurement
- Body composition analysis

All participants will wear Fitbit Alta wristband sensors for 7 days, which will collect digital data on physical activity (METs), step count, heart rate, sleep quality. Collected data will be transferred to a secure digital platform and coded to assess the results.

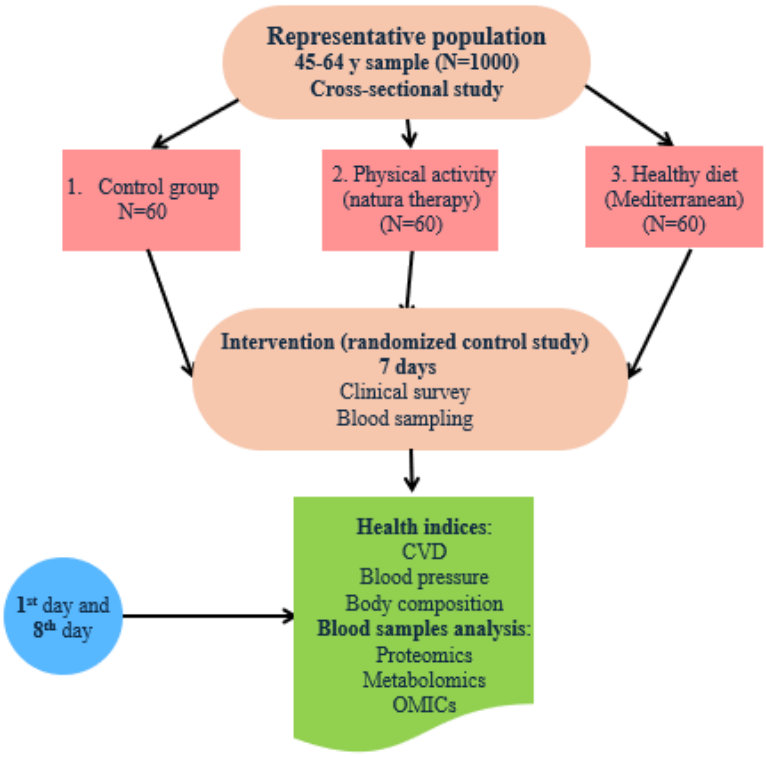

Fig. 1 Scheme of the Kaunas Clinical study

Each group's clinical health indices and metabolic responses will be assessed by measurements blood pressure, waist circumference, high-density lipoproteins (HDL), triglycerides, plasma glucose levels, also metabolomic, proteomic, and DNA methylation biomarkers before and after the 7-day intervention. Primary proteomics screening comprises of 4–6 oxidative stress and inflammation markers via immunoblotting. In a second phase, based on the initial results, advanced OMICs analysis will be conducted using high-resolution techniques, such as redox/phospho-proteomics, metabolomics, DNA methylation via EPIC chip (for genome-wide methylation analysis). All data will be analysed using mathematical modelling to account for confounding variables. Results will substantiate recommendations for reducing metabolic disorder risks, targeted to healthcare professionals and the public.

Data Analysis and Evaluation: Both the observational and experimental clinical studies will use a standardized exposure–health response analysis approach. Key methods: multivariate logistic regression analysis, adjustment for confounding factors using mathematical modelling to estimate the strengths of the relationship and 95% confidence intervals between the variables.

Biological samples for metabolic response analysis will be sent to accredited partner laboratories:

- DNA methylation analysis: National Center of Pathology (Lithuania), Laboratoire National de Santé (Luxembourg)
- Proteomic analysis: University of Southern Denmark (Odense, Denmark)

- Metabolomic analysis: Institute for Molecular Sciences, University of Eastern Finland (Finland)

9. Description of Study Sites and Institutions Involved in Biomedical Research Activities

The METSGREEN biomedical research is being conducted together with 15 international scientific MARKOPOLO project centres, as part of an international collaboration. The participating institutions are:

1. Universitätsmedizin der Johannes Gutenberg-Universität Mainz (Germany) – *Coordinator*
2. Università degli Studi di Padova (Italy)
3. Medical University of South Carolina (United States)
4. Vytautas Magnus University (Lithuania)
5. University of Belgrade, Faculty of Medicine (Serbia)
6. The Cyprus Institute (Cyprus)
7. Concentris research management gmbh (Germany)
8. Max Planck Society for the Advancement of Science (Germany)
9. Laboratoire National de Santé (Luxembourg)
10. Luxembourg Institute of Health (LIH) (Luxembourg)
11. University of Eastern Finland (Finland)
12. Julius-Maximilians-Universität Würzburg (Germany)
13. University of Southern Denmark (Denmark)
14. Danish Cancer Society (Kræftens Bekæmpelse) (Denmark)
15. Swiss Tropical and Public Health Institute (Switzerland) – *Associated partner*

These institutions are responsible for various aspects of the MARKOPOLO study, including coordination, exposure measurements, modelling and health risk assessments at the country-level, experimental studies using laboratory animals, biomarkers analysis, data interpretation and results dissemination. Vytautas Magnus University (Lithuania) is leading the implementation of the epidemiological human studies and clinical trial component.

10. Planned Duration of the Biomedical Research

The total planned duration of the biomedical research is 48 months.

11. Duration of Participant Involvement in the Biomedical Research

The METSGREEN study majority of participants—approximately 1,000 individuals—will participate only once in an anonymous online questionnaire survey, which will take up to 30 minutes to complete. A smaller group of 180 individuals enrolled in the clinical trial—those without the specified health contraindications and who consent to participate in the 7-day healthy lifestyle intervention—will have two visits to the clinic with 8-day interval. Each visit will last up to 60 minutes.

12. Description of Criteria for Termination of the Biomedical Research

Participants will be informed that they may withdraw from the biomedical research at any time. A participant has the right to revoke their consent to participate if they are declared legally incapable or partially incapable, or if their health condition renders them unable to reasonably assess their own interests. Participation in the study is entirely voluntary; therefore, participants have the right to refuse or withdraw at any point without providing any reason or justification. Biomedical research data collected prior to the receipt of a participant's written request to withdraw consent will not be deleted and may still be used in the analysis.

13. Procedure for Inviting Individuals to Participate in the Biomedical Research

A representative sample survey of Kaunas residents will be conducted by a professional survey company that adheres to strict principles and legal requirements regarding personal data security and privacy. Approximately 1,000 participants will be included in the observational study.
An anonymous online questionnaire will be used for the survey. At the end of the questionnaire, information will be provided about the planned clinical trial, including details about the study process and contact information for the research team. Individuals who are interested in participating in the healthy lifestyle clinical trial will be able to reach out to the researchers directly. Upon initial contact, the research team will request clarification on several indicators, including the participant's waist circumference, blood pressure, and residential address. If the individual meets the inclusion criteria, the research team will invite them to participate in the clinical trial.
Inclusion criteria for the clinical trial:

- Age between 45 and 64 years
- Residence in Kaunas City, specifically in areas with documented high levels of air pollution
- Reported health data indicating signs of metabolic disorders, such as increased waist circumference and/or elevated blood pressure.

14. Procedure and Specifics of Informing About the Biomedical Research and Obtaining Informed Consent

When a respondent contacts the research team and expresses interest to participate in the healthy lifestyle clinical trial, the researcher will verify whether the individual meets the inclusion criteria. If eligible, the respondent will be provided with detailed information about the study and asked to share contact information for further communication. An appointment will then be arranged at the Family Medicine Clinic of Kaunas Clinics. Participants will be asked to arrive having fasted for at least two hours, as a blood sample (up to 20 ml) will be collected from a vein at the start of the clinical study.
During the first visit, the participant will be thoroughly informed about:

- The purpose and design of the biomedical research
- The methods applied in the study

- The decisions of the ethics committee
- The potential benefits of participation
- The participant's rights
- Possible risks and inconveniences
- The right to withdraw written consent to participate at any time
- The consequences of withdrawing from the study
- Confidentiality and data protection assurances.

After reviewing this information, the participant will be asked to sign the Informed Consent Form. The participant will also be reminded that a second blood sample will be collected after 8 days. For this second collection, fasting for at least two hours will again be required.

15. Potential Benefits of the Biomedical Research to the Participants

Participants in the biomedical research will not receive direct personal benefit. However, those taking part in the experimental clinical trial may experience indirect benefits. The participant groups will be analyzed and described in detail, and everyone's body composition will be assessed—providing insights into their metabolic condition and cardiovascular disease (CVD) risk. Additionally, anonymized data collected from the study will contribute to the development of preventive strategies aimed at improving public health and reducing CVD risk at the population level.

16. Assessment of Possible Risks and Inconveniences to the Participants

Participation in the experimental biomedical study is expected to involve only minimal inconvenience. Daily routines will not be significantly disrupted, as the interventions involve 30-minute walks in a park or following a Mediterranean diet—both of which are middle in intensity and easy to integrate into daily life. The time commitment will be relatively small, not exceedingly approximately 2 hours in total. Venous blood sampling (approximately 20 ml) may cause minor discomfort. All data will be processed anonymized, using participant codes in accordance with personal data protection regulations. The electronic database linking participants' names to their assigned codes will be securely stored by the principal investigator on a password-protected computer. Only the principal investigator and an authorized researcher will have access to this information.

17. Procedure for Documentation and Evaluation of Adverse Events Observed During the Biomedical Research

The anonymous online questionnaire survey involving 1,000 individuals is not expected to cause any adverse events. In the experimental clinical trial involving 180 participants, venous blood sampling (up to 20 ml) is considered safe and non-harmful to health; therefore, no adverse events related to the procedure are anticipated. In the unexpected reaction, the physician-investigator will

provide immediate medical assistance. A report form documenting the incident will be completed, and the participant's family physician will be informed accordingly.

18. Processing of Personal Data, Assurance of Participant Confidentiality, and Personal Data Protection (this section must address all the questions listed below):

Types of Personal Data Collected: In the observational environmental-epidemiological study, data will be collected via an anonymous online questionnaire completed by approximately 1,000 individuals. Collected data will include age, gender, marital status, education level, employment status, monthly income, height, weight, waist circumference, body composition, blood pressure, chronic diseases diagnosed by a physician, smoking status, physical activity, perception of the residential environment, feedback and concerns about the study, and willingness to participate in the 7-day clinical trial. These data will be used to characterize individuals with signs of metabolic disorders for potential inclusion in the experimental clinical study.

For participants of the clinical trial, in addition to the self-reported questionnaire data, the following data will be collected on Days 1 and 8: blood pressure, body composition, heart function, sleep disturbances, physical activity, HDL cholesterol, triglycerides, plasma glucose concentration, and biomarkers from metabolomics, proteomics, and DNA methylation analysis—dependent on particulate matter exposure.

Source of Personal Data: In the observational study, all data are provided voluntarily by participants via the anonymous online questionnaire. In the experimental clinical trial, additional data will be obtained during health assessments and laboratory analysis of blood samples.

Data Coding and Anonymization: All collected data will be coded numerically. Each participant will be assigned a numerical code. Separate databases will be created for coded data and personal-identification data. These databases will be stored on electronic media.

Access to Identifiable Data (Uncoded): Access to identifiable data from the anonymous online survey will be limited to the principal investigator and her designated research colleague, Dr. Sandra Andrušaitytė, as well as the biomedical centre staff conducting health checks, supervisory bodies (e.g., bioethics committees), and authorized oversight personnel.

Access to Coded Health Data: Coded health data will be accessible only to researchers and scientists preparing scientific publications, reports, and materials for academic and public dissemination, including conferences.

Data Processing Methods: Data from the anonymous survey and clinical trial will be entered into an electronic system. Anonymized databases with assigned participant codes will be stored on the principal investigator’s password-protected computer at the VMU Research Centre. Clinical data will be transferred via USB every 10 days to the principal investigator’s device and coded accordingly.

Data Storage at the Research Centre: The principal investigator will be responsible for securely storing the electronic data for 10 years following the end of the study. The database containing links between identifying information and codes, as well as the anonymized data, will be stored at

the VMU Research Centre, which is accessible only with a coded key. Data access on the principal investigator's computer is password-protected.

Data Transfer to Sponsor or Third Parties: In accordance with the international research protocol, anonymized coded data will be shared with consortium partners for integrated data analysis. Grouped data will also be used in joint publications.

Data Controller and Contact Information: Data Controller: Vytautas Magnus University (VMU) – Faculty of Natural Sciences, Department of Environmental Sciences Address: Universiteto g. 10-152, Akademija, 53361 Kaunas District, Lithuania, Responsible person: Prof. Regina Gražulevičienė, Authorized representative: Dr. Sandra Andrušaitytė. Data Protection Officer (DPO): Ingrida Bukantaitė, email: **dap@vdu.lt**

Participants have the right to complaining to the State Data Protection Inspectorate:
Address: L. Sapiegos g. 17, 10312 Vilnius, Lithuania Tel: (+370 5) 212 7532 Email: **ada@ada.lt**
Or online via their e-service platform: /go.php/lit/Prisijungti/37L.

Right to Access and Correct Personal Data: Upon request, participants will be granted access to their personal data and may request corrections of incorrect, incomplete, or inaccurate information. This may be done in person at the VMU Research Centre by contacting the principal investigator or the authorized representative, Assoc. Prof. Dr. Sandra Andrušaitytė. Questions about data processing may also be directed to Prof. Regina Gražulevičienė at **regina.grazuleviciene@vdu.lt**, who will forward inquiries to the responsible DPO.

Data Storage Period and Responsible Parties: Personal data will be stored for 10 years following the completion of the study, as required by the international agreement, to support long-term European Commission initiatives aimed at improving urban environmental quality and public health. Data storage is the responsibility of the Principal investigator and her authorized representative, Dr. Sandra Andrušaitytė.

Right to Withdraw Informed Consent: Participants may withdraw from the online survey at any point by ceasing to complete the questionnaire. The right to withdraw informed consent from the clinical trial is clearly stated in the Informed Consent Form. Participants may revoke consent in writing at any time without providing justification. Upon withdrawal, data collection will cease; however, previously collected data will not be deleted.

19. Description of Criteria for Modifying the Biomedical Research

Modifications to the biomedical research protocol may only be made with the approval of the International Study Supervisory Committee and upon receiving consent from the Kaunas Regional Biomedical Research Ethics Committee. Minor modifications may be implemented in response to unforeseen circumstances or upon identifying new findings of scientific value.

20. Person Responsible for Compensating Expenses and Time Lost Due to Participation in the Biomedical Research, and Procedure and Conditions for Calculating and Paying Compensation

No compensation or payment will be provided for participation in the biomedical research. Participation is voluntary and short-term, requiring minimal time and financial effort, and does not significantly alter the participants' usual daily routines.

21. Funding of the Biomedical Research

The MARKOPOLO research is funded by the European Commission's Horizon Europe programme, which enables researchers in Lithuania to conduct internationally recognized studies, strengthen the national scientific infrastructure, and contribute to the advancement of the European Union’s scientific and technological base. The METSGREEN clinical trial component is financed through the MARKOPOLO project budget. This funding will cover the costs associated with180 participants two times blood collection and laboratory analysis of lipid metabolism indicators (total cholesterol, HDL, LDL, triglycerides) and plasma glucose concentration.

22. Procedure for Compensation in Case of Harm Resulting from Participation in the Biomedical Research (Insurance)

Insurance coverage is not applied to this study, as no harm to participants is anticipated.

23. Procedure for Publishing the Results of the Biomedical Research

The summarized and anonymized results of the study will be published in scientific journals and presented at conferences and expert meetings. The findings will also be used to develop recommendations for the European Environment Agency aimed at improving environmental quality. Additionally, guidance will be prepared for both patients and healthcare professionals to help reduce the risk of cardiovascular diseases.

24. Description of Ethical Issues Related to the Biomedical Research, If Not Addressed Elsewhere

Ethical considerations related to the biomedical research have been addressed in Section 14, which describes the procedures for informing participants and obtaining informed consent. Participants have the right to file a complaint regarding the processing of their personal data with the State Data Protection Inspectorate. Complaints may be submitted by mail (L. Sapiegos g. 17, 10312 Vilnius, Lithuania) or via the Inspectorate’s electronic services platform: /go.php/lit/Prisijungti/37L.Contact phone: (+370 5) 212 7532 Email: ada@ada.lt

25. Confirmation that a Medicinal Product Reimbursed from the Compulsory Health Insurance Fund Budget Will Be Prescribed in Accordance with the Approved Diagnostic and Treatment Guidelines in the Context of a Non-Interventional Biomedical Study

A non-interventional biomedical study involving a medicinal product is not planned. Therefore, no use of reimbursed medicinal products is foreseen within the scope of this research.

26. Confirmation that the Biomedical Research Will Be Conducted in Compliance with the Biomedical Research Protocol and Applicable Legal Requirements:

We confirm that we are familiar with the legal acts of the Republic of Lithuania regulating the conduct of biomedical research. We will strictly adhere to the conditions outlined in the approved biomedical research protocol. We also commit to notifying the Kaunas Regional Biomedical Research Ethics Committee of any changes to the biomedical research protocol, as well as of the research results.

27. Explanation of Which Institutional Documents Grant the Right to Conduct the Biomedical Research and Confirmation that the Investigator (Institution) Will Facilitate Oversight, Auditing, Ethical Review, and Inspection, Including Access to Source Documents

The biomedical research will be conducted only after obtaining formal approval from the Kaunas Regional Biomedical Research Ethics Committee. Conditions will be established to allow for full oversight of the study, including auditing, ethical supervision, and inspection, with direct access to original data and source documents. As part of the biomedical research application, official written consent has been obtained from the head of the Family Medicine Clinic of the Lithuanian University of Health Sciences (LSMU Kaunas), confirming authorization to carry out the planned research at the designated site.

Signatures of the Biomedical Research Protocol Author(s), Principal Investigator, and Sponsor's Authorized Representative

Protocol Author: Prof. habil. Dr. Regina Gražulevičienė

Principal Investigator: Prof. habil. Dr. Regina Gražulevičienė

Sponsor/Authorized Representative: Prof. Dr. Julija Kiršienė
Vice-Rector for Research, Vytautas Magnus University (VMU)