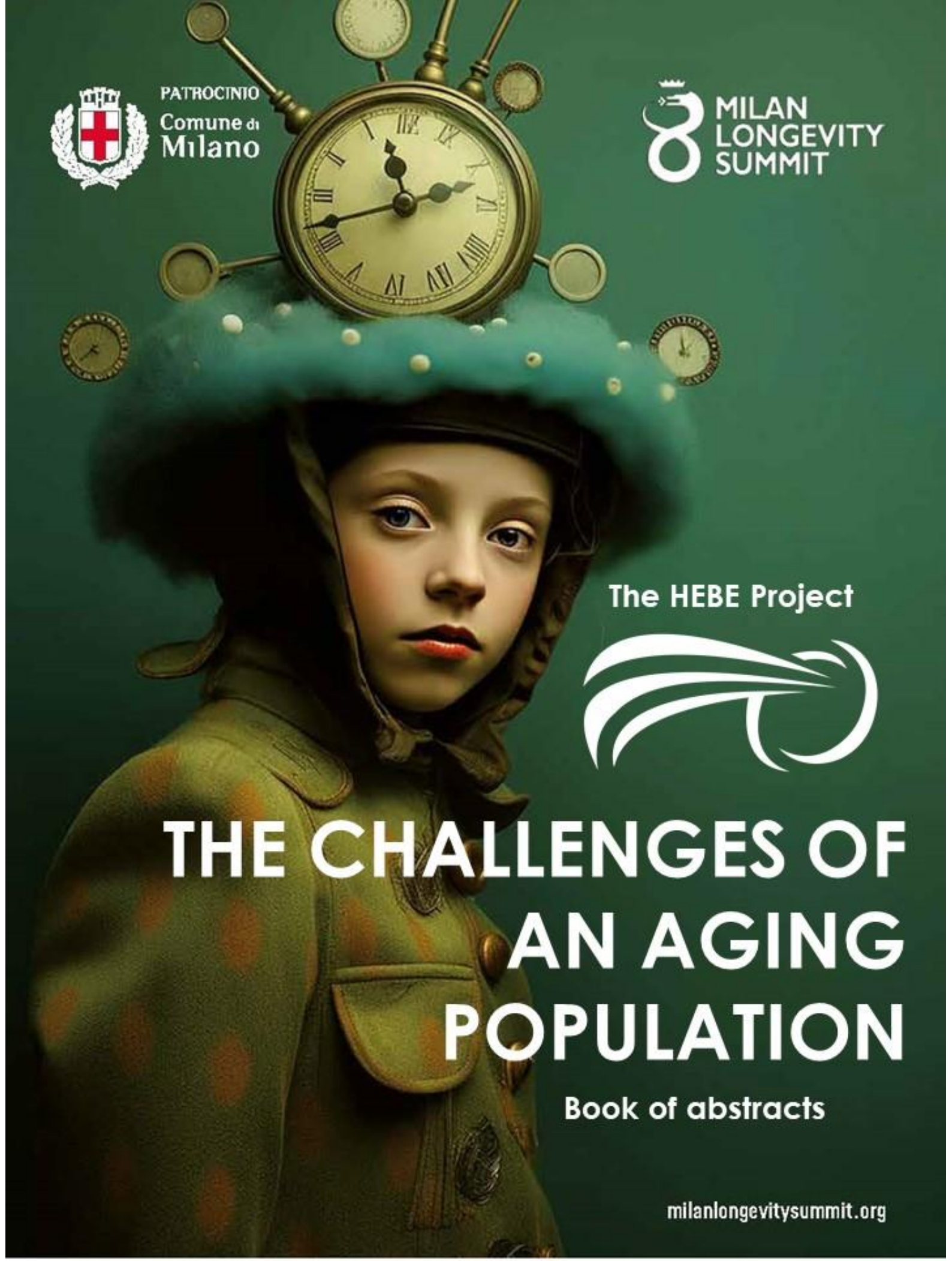


CON IL CONTRIBUTO DI
Regione Lombardia

UN PROGETTO DI
BRAINCIRCLE ITALIA

HEBE
SALUTE IN MOVIMENTO

Welcome to the **Milan Longevity Summit**, an event dedicated to exploring the challenges and opportunities linked with an aging population. In this context, we dedicated one day to focus on Protective Medicine, spanning the spectrum from the link between Health and scientific humanism, to the profound exploration of Healthy aging beginning in the womb, touching upon Epigenetics and the role of the brain in long-term health, culminating in discussions encompassing Health, Beauty, and Brain. Throughout this day, the guiding thread will be the **project HEBE**, driving our exploration and understanding. Together, we embark on a journey to closely examine the ethical, scientific, and social intricacies surrounding the extension of human life and the promotion of healthy aging.

HEBE, an acronym for "**H**ealthy aging versus inflamm-aging: the role of physical **E**xercise in modulating the **B**iomarkers of age-associated and **E**nvironmentally determined chronic diseases", represents a cross-disciplinary and multidisciplinary project involving **94 researchers from the University of Milan**, spanning 40 Scientific-Disciplinary Sectors, structured into 13 departmental units and 10 research lines.

Born from the desire to unite and coordinate multidisciplinary expertise to address the challenges of healthy aging and prevention, the HEBE project responded to the University's Grand Challenges (GSA "Grandi Sfide di Ateneo" call, published in 2021 for the activation of Special Projects within the framework of the National Recovery and Resilience Plan (PNRR). Winner of this call, HEBE aims to reduce inflammation (**"Inflammaging"**) through an integrated and personalized approach. Non-communicable diseases represent one of the leading causes of premature aging and death globally, and HEBE aims to understand and mitigate such pathologies through the exploration of personalized interventions based on physical exercise and lifestyle improvement.

HEBE's mission is to **promote a culture of health and prevention**, highlighting the importance of a healthy and personalized lifestyle to foster active and healthy aging.

Sharing the vision and commitment of HEBE consortium, we introduce the abstracts of the sessions, hoping to gain inspiration and knowledge from this multidisciplinary debate on healthy aging and human well-being.

Mario Clerici and Elia Biganzoli
Principal Investigators of the HEBE Project
on Behalf of the HEBE Consortium

Abstract 1

HEBE Project: Healthy aging versus inflamm-aging: the role of physical exercise in modulating the biomarkers of age-associated and environmentally determined chronic diseases – Study description

Francesca Bianchi, Elia Mario Biganzoli, Valentina Bollati, Mario Clerici, Daniela Lucini, Chiara Mandò, Federica Rota, on behalf of the HEBE Consortium.

Inflamm-aging refers to the chronic low-grade inflammation that occurs with aging and cellular senescence, and it is linked to various diseases. Understanding the markers involved in inflammation and aging, as well as their interaction with environmental factors and bodily control mechanisms, can provide crucial tools for assessing the resilience (i.e. the ability to adapt and improve) of the human body, particularly in the presence of chronic degenerative conditions or vulnerable life stages, that place the individual and the community to which he belongs in a state of potential fragility.

HEBE focuses on physical exercise, along with nutritional and lifestyle recommendations, to reduce systemic inflammation and promote healthy aging.

HEBE encompasses multiple research lines (LR). In the ongoing LR1 ("proof of concept"), healthy lifestyle recommendations were provided to University of Milan employees, and changes in quality of life and well-being were assessed using a specialized questionnaire. The first 100 eligible subjects, who expressed their willingness to participate, underwent a personalized physical exercise protocol based on clinical and objective assessments. Biomedical samples were collected at baseline (T0) and follow-up (T1) to establish a shared biobank and identify non-invasive biomarkers that monitor the impact of physical exercise on individual characteristics such as cardiovascular and metabolic health. Subsequently (LR2-LR10), the proof of concept findings will be expanded to include various conditions of vulnerability such as obesity, cancer, endocrine disorders, cardiovascular diseases, infertility, functional syndromes, respiratory disorders, neurodegenerative diseases, and autoimmune conditions.

The research lines will leverage the expertise of the 94 participating investigators to form a collaborative network that maximizes the potential for investigation and knowledge exchange. This approach fosters a culture of health promotion and disease prevention.

Abstract 2

Dissemination of the HEBE Project: Promoting a Culture of Health and Well-being

Chiara Mandò*, Federica Rota*, Daniela Lucini, Valentina Bollati, Francesca Bianchi, Mario Clerici, Elia Mario Biganzoli, on behalf of the HEBE Consortium.

The HEBE Project's mission is to promote a culture of health and prevent diseases by emphasizing the positive impact of lifestyle changes on personal well-being, quality of life, and health. It is well-known that certain actions, such as regular physical activity, a balanced diet, not smoking, stress management, and good sleep hygiene, have beneficial effects on health. However, it is essential to seek specific scientific evidence to understand why these actions are beneficial, allowing us to understand better how to promote targeted and personalized adoption of healthy lifestyles. HEBE's website, regularly updated at https://hebe.unimi.it, provides information to the general public at different life stages, offering simple and effective guidelines for well-being through a healthy lifestyle, including physical activity, nutrition, sleep, and more.

The main goal of the website is to engage the public and promote a culture of health, which has a significant positive impact on the community. The website is structured to inform citizens and patients about the project, its researchers, and evidence-based recommendations related to physical activity, nutrition, and psychophysical well-being. It also aims to keep users updated on the latest scientific discoveries and medical society guidelines related to health and lifestyle.

Furthermore, HEBE disseminates information through its official Instagram profile (@hebe_unimi), sharing weekly updates, posts, and stories to create a social community that enhances citizen engagement and

awareness. Both the website and social media platforms support a comprehensive approach to health literacy, emphasizing that lifestyle changes can positively impact individual well-being and quality of life in a personalized context.

HEBE's outreach activities include cultural and social events, such as guided tours of Ca' Granda, Milano's historic hospital building. These events aim to foster a sense of community belonging and raise awareness of the project's goals. Additionally, HEBE collaborates with schools to educate younger generations about the importance of a healthy lifestyle. An example is Giosuè, a student who interviewed the HEBE team for his middle school project, highlighting the project's role in promoting well-being among youth.

The project also celebrates its achievements, such as the completion of the first phase of research involving 100 participants who followed personalized lifestyle improvement programs. These milestones are commemorated through events like the Meda Urban Race, promoting social cohesion and showcasing the remarkable progress made by HEBE participants.

HEBE's impact extends to national television, with appearances on Rai1 programs like "Unomattina Estate" and "SuperQuark." These opportunities allow HEBE to share its scientific expertise, promoting the idea that physical activity is a "longevity elixir" that improves well-being and prevents diseases.

In conclusion, the dissemination efforts of the HEBE Project are multifaceted and encompass various activities, from cultural events to school collaborations and media appearances. These initiatives collectively promote a culture of health and well-being, emphasizing the importance of personalized lifestyle changes for better individual and community health.

Abstract 3

Public sentiments towards health research broadcasted on social media: the experience of the HEBE team in Italy

Giuseppe Marano, Ester Luconi, Daniela Lucini, Chiara Mandò, Federica Rota, Elia M. Biganzoli, on behalf of the HEBE Consortium.

Background: The general aim of this work was to evaluate people's reactions to the HEBE promoting program concerning health and correct lifestyle through the dissemination of evidence-based research. To such end, sentiment analysis was performed on 195 comments published on YouTube in reply to the episode of Superquark (24 August 2022) dedicated to the benefits of daily walking.

Methods: A pertinence score was calculated to separate the comments related to the topics in the video clip from out-of-context ones (i.e., spam). People's reactions were evaluated using VADER sentiment scores. The main topics in the comments were then extracted independently by two authors.

Results: from the 195 comments, 128 in-context comments were analyzed. The proportions of positively oriented, negative, and neutral comments were 59.4%, 26.6%, and 14.0%, respectively. Five main topics were identified, concerning 1) earned benefits of walking, 2) unearned benefits, issues, and obstacles, 3) fitness practice, 4) questions, and 5) remarks. Many commenters claimed beneficial effects in physical and/or psychological domains or sketched out their personal experience of a consistent practice of walking and physical activity in general. However, specific conditions, including age-related factors, can hinder the enjoyment of such positive outcomes.

Conclusion: This event allowed us to obtain valuable insights into people's reactions to the central topics in HEBE program and to investigate the propensity toward a healthy lifestyle using text mining and natural language processing (NLP) methods.

Abstract 4

Sustainability and feasibility of an exercise training program to reduce mortality risk in clinical practice: preliminary data from HEBE study.

Daniela Lucini, Luca Giovanelli, Giuseppina Bernardelli, Gianluigi Oggionni, Mara Malacarne, Ester Luconi, Giuseppe Marano, Federica Rota, Francesca Bianchi, Chiara Mandò, Mario Clerici, Valentina Bollati, Elia Biganzoli, on behalf of the HEBE Consortium.

Exercise training represents a pivotal strategy to reduce any cause of mortality, preventing and managing many diseases, such as cardiometabolic, oncological, and functional ones. Notably, nowadays, it is also considered a fundamental approach to fostering well-being and improving physical/psychological/social performances, hence bettering quality of life. These advantages of exercise render it a cornerstone of healthy aging programs. Nevertheless, an intervention program should be considered "efficacious" only if its application improves the underlying pathogenetic mechanisms. The advantages range from improving immunological, hormonal, and autonomic profiles to improving hemodynamics. Moreover, a successful exercise intervention in clinical practice must be feasible and sustainable from the economic and organizational points of view.

HEBE Project (Healthy aging versus inflamm-aging: the role of physical Exercise in modulating the Biomarkers of age-associated and Environmentally determined chronic diseases, registered in ClinicalTrials.gov, Identifier: NCT05815732) was designed to study how exercise could modify mechanisms involved in the aging process, with a particular focus on inflammation and its relationship with other vital mechanisms such endocrine and autonomic controls. HEBE received Ethics Approval from the University of Milan Ethical Committee (approval 62/22, date 30/06/2022).

In its initial proof-of-concept phase, HEBE focused on assessing the impact of an unsupervised personalized exercise program integrated with nutrition and general healthy lifestyle recommendations. This study involved a cohort of 100 healthy volunteer employees from the University of Milan, comprising 50 males

and 50 females, with 50 being within the normal weight range and 50 classified as overweight. These participants sought evaluation and received personalized exercise programs at the Exercise Medicine Unit, Istituto Auxologico Italiano, Milan. The exercise programs were designed based on cognitive-behavioral strategies and tailored prescriptions of home-based aerobic endurance and strength exercises, both at the beginning of the program and after six months. Participants were provided with video tutorials on strength training. An ad hoc questionnaire was administered to gather information on self-perception and program satisfaction.

A cardiopulmonary exercise test (CPX) was conducted both at the beginning of the program and after six months, at the "Centro Sanitario Polifunzionale di Milano" under the Department of Public Safety. The goal was to assess cardiorespiratory fitness (CRF), measured by VO2max and achieved peak of Metabolic equivalents (METS), in addition to Maximal Heart Rate. Measurements of heart rates during **p**ersonalized training were determined using the Heart Rate Reserve formula. According to the current literature, improvements in CRF of ≥1.0 MET are relevant and have been associated with reduced mortality risk, regardless of the participant's baseline CRF status.

Personalized training heart rates were determined using the Heart Rate Reserve formula, and participants were provided with video tutorials on strength training. An ad hoc questionnaire was administered to gather information on self-perception and program satisfaction. According to the current literature, improvements in CRF of ≥1.0 MET are relevant and have been associated with reduced mortality risk, regardless of the participant's baseline CRF status.

In the Proof-of-concept of HEBE, CRF was improved in 82% of 84 subjects with complete measurements (mean value: from 8.54 to 9.45 peak METS at the beginning and six months, respectively). Forty-point eight percent of them achieved an improvement of CRF ≥1.0 MET. Ninety-two percent of subjects reported perceiving a betterment in personal well-being and overall satisfaction was, on average, 8.4 points (score from 0 to 10).

This proof of concept phase of HEBE project (considering only data regarding CRF) showed that the applied protocol was capable of reducing CRF. Therefore, a reduction in mortality risk could be expected in 40.8% of the participants' cohort. The protocol was cost- and time-effective (only three medical encounters to

define and optimize the intervention close to two phone/mail contacts) and well appreciated by the participants. Further analysis, considering all the measurements collected under the protocol, will be necessary to study the relationship between exercise and the multifarious mechanisms implied in the aging process.

Abstract 5

Physical exercise as a sustainability tool in men affected with metabolic syndrome-related late-onset central hypogonadism: role of endocrine-metabolic and neurovegetative outcomes

Luca Giovanelli, Biagio Cangiano, Silvia Federici, Marco Bonomi, Daniela Lucini

Background: Late-onset central hypogonadism (LOH), whose prevalence is high among dysmetabolic males, impairs quality of life and increases cardiovascular risk. Although lifestyle modification is the first-line therapeutic strategy, it often fails in clinical practice, probably due to socio-cultural, economic and organizational barriers, as well as the lack of effective and sustainable intervention programs.

Aim: To delineate sustainable physical exercise programs and to assess the effects of such programs mainly on endocrine-metabolic and neurovegetative outcomes in a cohort of men with metabolic syndrome-related central LOH.

Methods: 18-80-year-old men, consecutively referred to IRCCS Istituto Auxologico Italiano due to dysmetabolic central LOH, will be enrolled in this prospective study. Participants will undergo a structured and personalized exercise program (accompanied by an adequate nutrition program). After 6 months they will be subdivided into two groups, according to the weekly physical activity volume actually performed (above or below 600 MET·minutes/week). Changes in endocrine-metabolic and neurovegetative outcomes (e.g., gonadal axis function, glucose and lipid profile, body composition, cardiac autonomic regulation (CAR)) will be compared between the two groups. In particular, Autonomic Nervous System Index (ANSI),

being extracted from the autoregressive spectral analysis of heart rate variability by combining the three most informative variables, will be used for non-invasive assessment of CAR. Besides, genetic investigations will be performed to explore the potential role of genetic predisposition in the development of dysmetabolic LOH.

Preliminary results and discussion: Seven patients have been hitherto enrolled. As expected, they presented low percentages of fat free mass in the face of increased fat mass, and low SHBG levels. An impairment in both autonomic function (ANSI) was observed in comparison with reference populations from other studies. This is the first study assessing neurovegetative control by means of ANSI in hypogonadal men. Indeed, obesity/insulin resistance is associated with neurovegetative dysfunction, and lifestyle interventions have been shown to improve CAR in dysmetabolic patients. In this context, it will be interesting to explore the possible crosstalk between metabolic syndrome-related hypogonadism, autonomic dysfunction, and physical exercise. Notably, exercise prescription requires the clear definition of modality, intensity, frequency, duration and progression of exercise, tailored on patient's clinical conditions and goals. Intervention programs should be sustainable in economic and organisational terms, with a view to embedding behavioural changes in patient's everyday life.

Abstract 6

Crosstalk between neurovegetative control, bone status, and physical exercise: clinical use of a unitary autonomic nervous system index in postmenopausal women with osteoporosis

Luca Giovanelli, Vittoria Favero, Mara Malacarne, Sabrina Corbetta, Daniela Lucini

Background: In the multifarious etiopathogenesis of skeletal fragility, an intriguing novel element is the possible existence of a crosstalk between autonomic nervous system (ANS) function and bone health. Indeed, sympathetic overactivity might stimulate osteoclastogenesis and inhibit osteoblastic proliferation, whereas opposite effects would be exerted by a prevalent parasympathetic activation. Autonomic Nervous

System Index (ANSI) is a percent ranked (0–100) unitary proxy of cardiac autonomic regulation (CAR), derived from the autoregressive spectral analysis of heart rate variability by combining the three most informative indexes. It is a simple and non-invasive method for evaluating ANS function, being by design free of age and gender bias. Notably, this index has also proved to highlight neurovegetative improvements induced by therapeutic strategies such as aerobic endurance exercise. In this respect, physical activity itself is well-known to play a key part in reducing (re)fracture risk.

Aim: To investigate CAR as well as the effects of a structured and personalized exercise program on CAR, skeletal turnover, bone mass and body composition, in postmenopausal women affected with osteopenia/osteoporosis.

Methods: 17 osteopenic/osteoporotic women aged between 50 and 70, referred to the Exercise Medicine Clinic of Istituto Auxologico IRCCS, were enrolled. Several evaluations, including bone turnover markers, CAR (by means of ANSI), body composition, and lifestyle (with ad hoc questionnaires) were performed, and a structured and personalized exercise program was prescribed.

Preliminary results and future perspectives: At the baseline assessment, bone turnover biomarkers were significantly impaired in comparison with reference populations from other studies. High variability in the ANSI score (32.5-79.5%) was observed. It will be interesting to explore CAR in a wider sample, as well as the effects of a customized exercise program both on bone metabolism and ANS function (as evaluated by non-invasive techniques), and their potential mutual correlation/interaction.

Abstract 7

Comparative analysis of metabolic and transcriptomic features of Nothobranchius furzeri

Caterina AM La Porta, Maria Rita Fumagalli, Francesc Font Clos, Simone Milan, Stefano Zapperi

Some species have a longer lifespan than others, but usually lifespan is correlated with typical body weight. Here, we study the lifetime evolution of the metabolic behaviour of Nothobranchius furzeri, a killifish with an extremely short lifespan with respect to other fishes, even when taking into account rescaling by body weight. Comparison of the gene expression patterns of N. furzeri with those of zebrafish Danio rerio and mouse (Mus musculus) shows that a broad set of metabolic genes and pathways are affected in N. furzeri during ageing in a way that is consistent with a global deregulation of chromatin.

Computational analysis of the glycolysis pathway for the three species highlights a rapid increase in the metabolic activity during the lifetime of N. furzeri with respect to the other species. Our results highlight that the unusually short lifespan of N. furzeri is associated with peculiar patterns in the metabolic activities and in chromatin dynamics.

Abstract 8

Biomarkers of plasma Vesicles in HEBE Project by quantitative Proteomics

Alfonsina D'Amato, Natalia Platonova, Gilda Aiello, Mirjam Hoxha, Laura Cantone, Lavinia Casati, Stefano Centanni, Valentina Bollati, Raffaella Chiaramonte, on behalf of the HEBE Consortium.

HEBE Project - Healthy aging versus inflamm-aging: the role of physical Exercise in modulating the Biomarkers of age-associated and Environmentally determined chronic diseases is focused on physical

exercise (possibly assisted by other interventions of a metabolic and/or pharmacological), to promote the reduction of inflammation and promote healthy aging. Inflamm-aging, a state of chronic low-grade inflammation that accompanies the process of cellular aging and senescence has been associated with the onset of numerous pathologies. HEBE is divided into different lines of research and our specific aim was to identify markers and their relationship and interaction with environmental aspects in plasma-secreted vesicles. This may offer fundamental tools for monitoring the Resilience of the "human being" system in its complexity, to overcome inflamm-aging. Extracellular vesicles (EVs) encapsulating bioactive cargo such as proteins, lipids, and nucleic acids in the circulation can transfer their cargo to elicit functional effects in the recipient cell. Extracellular vesicles (EVs) are membranous vesicles released by a variety of cells into their microenvironment. Recent studies have elucidated the role of EVs in intercellular communication, pathogenesis, drug, vaccine and gene-vector delivery, and as possible reservoirs of biomarkers (Thery C, Ostrowski M, Segura E (2009) Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 9: 581–593). Here, we used a label-free quantitative proteomic approach to examine the changes in plasma EV proteome. 100 human volunteers were selected from the cohort of UniMi workers by an online questionnaire. They followed a prescribed protocol of physical exercise and diet for 6 months. Plasma samples were withdrawn at the beginning and at the end of the program. The EVs were isolated from blood plasma samples by ultracentrifugation at 110.000xg (4°C), followed by washing of the pellets by PBS. Then, the EVs were lysated in Urea buffer (6M), and the proteins were digested by trypsin after reduction and alkylation. The resulting peptides were overloaded on 5 ug C18 ziptips to normalize the peptide content and to concentrate the entire sample. The eluted peptides were then analyzed by nLC MSMS (Exploris 240, UnitechOmics, University of Milan). More than 1000 proteins were identified by bioinformatic analyses, of which 70% overlapped with Vesiclepedia database (Kalra H, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol. 2012;10(12):e1001450. doi: 10.1371/journal.pbio.1001450) attesting the specific enrichment of plasma EVs. In addition, we found about 50% of most abundant proteins discovered in EVs (Fonseka et al. (2021) FunRich enables enrichment analysis of OMICs datasets. Journal of Molecular Biology. 166747), such as 14-3-3 isoforms, Talin 1, Transferrin receptor protein 1, T-complex protein 1, Ras-related protein Rab-7a and 10, Profilin-1 and Integrin beta-1. In addition, the EV proteins were enriched in Gene ontology terms related to specific

biological process, such as immune response, cell communication and signal transduction.The entirety of raw data will undergo analysis utilizing quantitative software and a statistical approach. This analytical process aims to establish correlations in extracellular vesicle (EV) protein profiling before and after the treatment. The objective will be the identification of potential biomarkers that may indicate and contribute to an improvement in the overall health state of the individuals involved.

Abstract 9

Epigenetics and the power of the Arts: A great potential for health and well-being

Marta Pizzolante, Marta Gallazzi, Elia Mario Biganzoli, Valentina Bollati

Epigenetics, the study of heritable changes in gene expression without alterations to DNA sequences, has drawn increasing attention due to its implications for gene regulation and chromatin stability. Epigenetic mechanisms are pivotal in shaping gene-environment interactions, influencing individual development and adaptation. Key epigenetic regulators include DNA methylation, histone modifications, and non-coding RNAs. Environmental factors such as stress, toxins, and social interactions can trigger epigenetic changes with profound impacts on health and well-being.

While much research has focused on the effects of detrimental factors on epigenetics, the influence of positive experiences, especially through engagement with the Arts, remains an intriguing and underexplored area. Visual art and music, in particular, have been linked to emotional responses and the release of neurotransmitters, offering potential transformative benefits. Recent investigations have begun to unveil the links between positive experiences and epigenetic modifications. Initial findings suggest that encounters with visual art and music may induce dynamic and enduring changes in gene expression profiles.

However, this field is still in its infancy, necessitating further research to establish clear connections. Collaborative efforts across disciplines including genetics, epigenetics, neuroscience, psychology, and the Arts are essential for comprehensive understanding. Future research directions may encompass longitudinal studies that track sustained exposure to positive experiences and delve into the role of childhood artistic education in shaping the biological basis of art and music's therapeutic effects. Ultimately, unraveling how positive experiences influence epigenetics could provide novel insights into enhancing human well-being over the long term.

Abstract 10

Interleukin-8 is a potential senescence inducer in lung fibroblasts

Clara Bernardelli, Piera Selvaggio, Silvia Rosa, Melania Lazzari, Lavinia Casati, Raffaella Chiaramonte, Elena Lesma

In aging, lung parenchyma remodeling leads to a progressive deterioration of lung function, with the impairment of gas exchange and immunologic changes that prompt infections. Even if it is well established that the risk of primary lung cancer, respiratory infections, obstructive and fibrotic lung diseases increase with age, the drivers of lung aging remain largely unknown. Being the interface with the external environment, lung cells must respond to increasing chemical, mechanical, biological, immunological and xenobiotic solicitations, so they must strongly rely on stress response pathways over the lifetime. Cellular senescence is a stress-response process characterized by the irreversible inhibition of cell proliferation and by the acquisition of a senescence-associated secretory phenotype (SASP). SASP consists in the secretion of a complex mixture of factors, including proinflammatory cytokines, chemokines, growth factors and proteases, as matrix metalloproteases (MMPs). SASP is evolutionally conserved and reinforces senescence in a cell-autonomous manner. However, it can boost a proinflammatory microenvironment with a systemically defective immunosurveillance in many age-related diseases. Moreover, the upregulation of the

cell cycle inhibitors p16 and p21 in senescent progenitor cells limits tissue regeneration, further damaging tissue architecture through MMPs.

As a paradigm of the lung impairment driven by senescence, we developed an in vitro model of human pulmonary microenvironment by using healthy primary lung fibroblasts (PLFs) and primary LAM/TSC cells, isolated from a chylothorax, characterized by the constitutive activation of mTOR due to the lack of its regulatory protein tuberin. Knowing that the activation of mTOR contributes to cellular senescence, we firstly demonstrated that LAM/TSC cells have senescent features depending on mTOR hyperactivation, since their high positivity to SA-β galactosidase and to phospho-histone H2A.X are reduced by inducing tuberin expression and by inhibiting mTOR with rapamycin. Furthermore, the LAM/TSC cells have the capability to induce senescence in PLFs through factors contained in their conditioned medium (CM). To explore the communication in lung microenvironment, we assessed that LAM/TSC cells secrete extracellular vesicles (EVs), which can be carriers of senescence-inducing insoluble mediators. Both tuberin expression and and rapamycin treatment reduced LAM/TSC cell-mediated EVs secretion, implying a modulation of EVs release by mTOR pathway. Intriguingly, senescent-induced PLFs enhance the expression and secretion of IL-8, a potent neutrophil chemotactic factor that also mediates the spreading of the SASP response in the microenvironment.

This evidence suggests that senescence spreading in lung might reduce the proliferation of lung resident cells and, at the same time, might promote a pro-inflammatory microenvironment that ultimately has detrimental effects on the lung tissue.

To deepen this, the CM of PLFs was supplemented with IL-8, observing a significant increase of senescence, while blocking IL-8 receptor modulated the senescent features of cells. This suggests that, through SASP, senescent cells might establish an effective communication within lung microenvironment with a possible direct involvement of IL-8. Indeed, the high levels of this chemokine might induce senescence and promote a pro-inflammatory/senescent milieu, ultimately causing the disruption of lung parenchyma. Taken together, these results make senescence, and its modulation, an intriguing field of study to understand the mechanisms underlying lung disruption in pulmonary chronic diseases. Moreover, dissecting the

pathological communication in the senescent lung microenvironment might allow to identify targets for early diagnosis and novel therapies.

Abstract 11

Impact of cigarette smoke on vascular smooth muscle cell senescence

Clara Rossi, Marco Venturin, Alberto Corsini, Cristina Battaglia, Stefano Bellosta

Aging and smoking are major risk factors for cardiovascular diseases. One characteristic of aging is the accumulation of senescent cells (e.g. cells that permanently lose the ability to divide). Senescent vascular smooth muscle cells (VSMCs) are present in atherosclerotic plaques, inducing an inflammatory environment and contributing to plaque instability and atherosclerotic complications.

Traditional cigarette (TC) smoke is a known exacerbator of age-related diseases and the combustion of tobacco releases thousands of toxic chemicals. To reduce the harm associated with TC, alternative products, such as tobacco heating-not-burning products (THPs), have been developed.

In our study, we compared, in the context of aging, the effects of aqueous extracts (AEs) from TC and THP on senescent VSMCs. Doxorubicin-induced senescent (DIS) VSMCs have been used as a positive control. VSMCs passaged 5 to 7 times (non-senescent cells) were incubated for 48h with 10% TC and THP AEs or doxorubicin 100 nM. Then, we measured several typical senescence markers, such as senescence associated-β-galactosidase (SA-β-gal) activity, cell proliferation, cell cycle, senescence-associated genes and proteins expression, reactive oxygen species (ROS) production, and morphological changes.

TC increased SA-β-gal positive cells, slowed down cell proliferation, and down-regulated proliferation cell nuclear antigen (PCNA) expression, similar to what we observed in the DIS VSMCs. Instead, THP did not affect cell proliferation or PCNA expression. Expression of cell cycle inhibitors was upregulated in both TC and THP, but only TC arrested cells at the G2/M phase. Moreover, only TC significantly induced the expression of inflammatory markers (IL1β, IL6, IL8, and MMP3) and increased mitochondrial and

intracellular ROS production. Finally, TC, but not THP, increased the number of large and regular nuclei, a typical feature of senescent cells.

In conclusion, traditional cigarette smoke induces senescence-associated markers in VSMCs similar to what we observed in doxorubicin-treated VSMCs. On the contrary, THP lacked most TC and doxorubicin-related functional, structural, and molecular effects on senescence, demonstrating a significantly reduced impact on VSMC senescence.

Abstract 12

Gut Inflammation and Cardiac Health: an Ultrastructural Morphological Study to Probe Dysbiosis-Induced Changes and Dietary Intervention Effects

Francesca Bianchi, Francesca Arnaboldi, Martina Di Modica, Alessandra Stacchiotti, Tiziana Triulzi, Marilena Iorio, Lucia Sfondrini, Valentino Mario Le Noci, Elda Tagliabue, Serenella M. Pupa, Nicoletta Gagliano

Background: The development of a chronic low-grade pro-inflammatory state, termed "inflamm-aging," is closely associated with various unhealthy habits, contributing to age-related non-communicable diseases (NCDs). Among these habits, such as a low-fiber diet, excessive antibiotic use, obesity, and a sedentary lifestyle, dysbiosis—alterations in the intestinal microbiota—emerges. Recent clinical and experimental evidence underscores the significance of gut microbiota dysbiosis in the onset and progression of cardiovascular diseases (CVDs), but also suggests diet intervention as a potential strategy to mitigate dysbiosis-induced cardiotoxicity.

Aim: Within this framework, our study delves into an in vivo experimental model to examine the repercussions of altered gut microbiota homeostasis induced by antibiotic administration in drinking water

on cardiac muscle tissue. Additionally, we explore the potential protective effects of a high-fiber diet against cardiac damages.

Material and Methods: Groups: untreated (CT), treated with vancomycin followed after by administration of normal (VAN) or high fiber diet (HFiber) Friend leukemia virus B (FVB) mice. Heart tissue structure and ultrastructure have been analyzed by light and transmission-electron microscopy (TEM), respectively; mitochondria dynamics have been assessed by Real-time PCR and proteomic approaches (FT-Orbitrap); systemic inflammation and markers of cardiotoxicity has been analysed by Luminex.

Results: Light microscopy analysis did not reveal any morphological alterations induced by antibiotics. TEM image analysis of cardiomyocytes indicated preserved myofibril arrangement, although mitochondria in the VAN group were significantly larger and more elongated compared to CT. The VAN group exhibited a lower number of preserved mitochondria and a higher count of partially and totally damaged mitochondria compared to CT. Notably, the HFiber group showed no significant alterations. Molecular characterization of mitochondrial dynamics revealed upregulated expression of genes governing mitochondrial fusion (OPA1, DNM1, MFS1) in the oral antibiotics group, consistent with the observed mitochondrial size increase. Moreover, elevated SOD2 expression confirmed ongoing oxidative damage.

Conclusion: Our findings indicate that vancomycin treatment induces early ultrastructural alterations in intermyofibrillar mitochondria of cardiomyocytes. TEM analysis emerges as a valuable tool for detecting early ultrastructural alterations in the absence of evident histological tissue damage. Importantly, a high-fiber diet demonstrated the potential to counteract the detrimental effects triggered by dysbiosis induced by antibiotic administration. Ongoing analyses include the profiling of immune cell populations.

Abstract 13

Oxidative damage and microRNA profile of obese and overweight women at third trimester of pregnancy

Chiara Novielli, Gaia Maria Anelli, Anna Maria Nuzzo, Chiara Lubrano, Alice Genovese, Laura Moretti, Alessandro Rolfo, Irene Cetin, Chiara Mandò

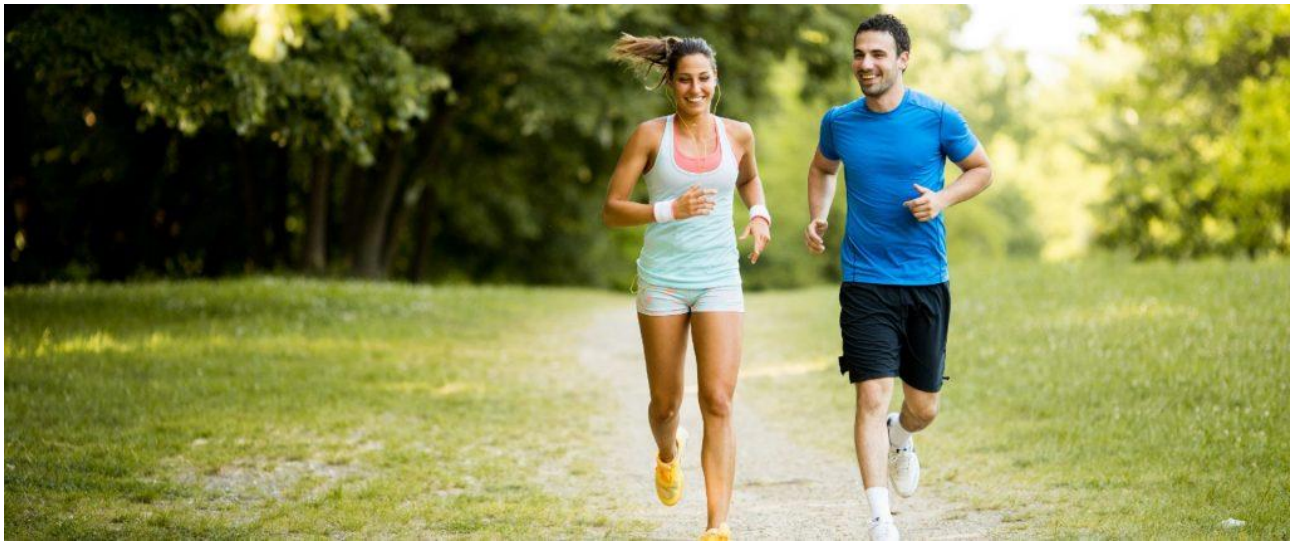
Introduction: Maternal health is a key determinant of offspring health. Maternal obesity is expanding worldwide and is associated with adverse maternal and fetal outcomes, with short- and long-term complications. Growing evidence suggests that obesity is characterized by oxidative stress and epigenetic alterations contributing to the pathogenesis of metabolic diseases. Here we analyzed DNA/RNA oxidative damage and microRNA profile (small non-coding RNA with biological roles) at the 3rd trimester of pregnancy in healthy women with different pregestational BMI.

Methods: Healthy Caucasian women with single spontaneous pregnancy were enrolled at the 3rd trimester of pregnancy (30-36 weeks) and grouped based on pregestational BMI: 17 obese (OB), 32 overweight (OW) and 76 normal weight (NW). Any maternal/fetal pathology, maternal-specific diet, smoke, drug or alcohol abuse was excluded. Maternal plasma from fasting venous blood (centrifugation: 4°C, 1500g, 10') was stored at -80°C until analyses. DNA/RNA oxidative damage was assessed by ELISA *(Cayman Chemical)*; in a subset of 24 samples RNA was isolated and levels of 179 microRNA (the most expressed in human plasma) were quantified by Realtime PCR *(Qiagen)*. Fold regulation |2| and $p<0.05$ were set for comparison analysis (*GeneGlobe* software). Associated pathways were identified by *DIANA-miRPath* and *miTALOS* tools. Oxidative and clinical data were statistically analyzed by *SPSS* v.28 *(IBM)*.

Results: Maternal age and gestational age at birth were similar in the three groups. At 3rd trimester, weight gain was lower in OB *versus* both OW and NW women ($p=0.000$). However, considering the Institute Of Medicine recommendations for weight gain during pregnancy (which suggest a smaller increase in weight with increasing pre-pregnancy BMI) about 31% OB and 60% OW exceeded recommendations, while NW were mostly within/below recommendations. Fasting glycemia progressively increased with BMI, being

significantly higher in OW and OB compared to NW (p=0.000). DNA/RNA oxidative damage was higher in OB and OW mothers when compared to NW (p= 0.004; p=0.035). Interestingly, there was a significant correlation between maternal DNA/RNA oxidative damage at 3rd trimester and pre-gestational BMI (p=0.001; r=0.284). After excluding low-quality data/samples, 6 differentially expressed microRNA were found in OB plasma (hsa-miR 362-3p, 93-3p, 375, 99b-5p, 1-3p, 30e-3p) and 3 in OW plasma (hsa-miR 132-3p, 362-3p, 99b-5p) compared to NW. These miRNAs resulted associated with specific pathways, notably: lysine degradation, fatty acids biosynthesis, ECM interaction, stress, signaling of FoxO, Hippo and p53 (proliferation / cell cycle arrest), mTOR (lipid metabolism, protein synthesis), TGFβ (anti-inflammatory, metabolism), insulin, leptin pathways.

Conclusion: Both OB and OW mothers displayed oxidative damage and epigenetic alterations of pivotal mechanisms, such as cell and energy metabolism (e.g. lipid metabolism, protein synthesis, anti-inflammatory responses). These alterations likely characterize and impact the intrauterine environment of women with higher BMI, thus possibly affecting the fetal development and/or fetal programming, thus predisposing to disorders later in life. Overall, these results confirm the riskiness of pregestational high BMI, pinpointing the urgency for women to be aware of the importance of a correct lifestyle (particularly, nutrition and physical activity) before and during pregnancy to limit BMI and weight gain.



Abstract 14

Unraveling the enigma of the complex relationship between aging, senescence, molecular biomarkers, and sex/gender influences: a roadmap of open questions.

Cristina Battaglia, Maria Grazia Cattaneo, Claudia Germiniani, Marco Venturin

The intricate network among senescence, molecular biomarkers and sex/gender influences captivates the scientific community and offers profound insights into the complexities of the aging process. We navigate through the current state of knowledge in this intriguing field, focusing on the various questions still open.

Aging, as a holistic process, involves a myriad of molecular changes at the cellular, tissue, and systemic levels. The identification of reliable molecular biomarkers holds promise for tracking and understanding the aging process. However, challenges persist in the establishment of universally applicable biomarkers that accurately reflect biological age.

Senescence, a hallmark of aging, represents a multifaceted process marked by permanent cell cycle arrest. Despite significant advances in knowledge of molecular mechanisms underlying senescence, several outstanding questions persist. What are the molecular triggers priming senescence? How do they vary across different cell types and tissues? Understanding the origin of heterogeneity in senescent cells and its effect on tissue dysfunction is a pressing question that needs to be fully addressed.

The impact of biological sex on aging introduces a layer of complexity that demands further exploration. While it is recognized that sex-specific factors influence aging trajectories, the underlying molecular mechanisms remain elusive. How do sex-specific factors modulate senescence, and to what extent do they influence the molecular signatures associated with aging? Unraveling these mysteries will help to develop targeted interventions that account for sex/gender-specific distinctions in aging.

The integration of senescence, sex/gender, aging, and molecular biomarkers raises critical questions about the interplay between all these elements. The attempt of addressing these questions will allow to advance

our understanding of the aging process, paving the way toward tailored preventive treatments and a more personalized approach to healthy aging in men and women.

Abstract 15

HEBE project and inflammation: DNA methylation as a potential biomarker for lifestyle improvement

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The term "Inflammaging," denoting inflammation associated with aging, defines a chronic, acute or subclinical systemic inflammatory state common to various age-related diseases.

In this context, Project HEBE (Healthy aging versus inflamm-aging: the role of physical Exercise in modulating the Biomarkers of age-associated and Environmentally determined chronic diseases) aims to clarify the role of chronic low-grade inflammation in non-communicable disease risk, also identifying new potential biological markers. In the present study, we explored the effects of an intervention aimed at improving lifestyle habits, specifically through physical activity and proper nutrition, on inflammation by assessing DNA methylation levels. In the initial phase, 100 volunteer healthy workers of the University of Milan were enrolled (T0) and underwent lifestyle modifications, including physical activity and a targeted diet for six months. Various biological samples, including peripheral blood, anthropometric, and clinical parameters, were collected both at T0 and after the intervention protocol (T1). After DNA extraction from peripheral blood mononuclear cells, we measured the methylation of the inflammatory genes iNOS TNFα, LINE-1, and HERV-W1 by Pyrosequencing analysis and are investigating their association with the anthropometric, biochemical, and clinical parameters collected at both T0 and T1 timepoints.

The results obtained will provide information on the link between inflammaging, physical activity, and DNA methylation. The multidisciplinary approach of Project HEBE offers a platform for further research on the relationship between long-term health chronic inflammation and epigenetics.

Abstract 16

The effect of lifestyle changes on inflamm-aging biomarkers: a focus on telomere length

Rachele Matsagani, Mirjam Hoxha, Chiara Favero, Simona Iodice, Laura Dioni, Daniela Lucini, Federica Rota, Valentina Bollati, Elia Biganzoli on behalf of the HEBE Consortium.

Non-communicable diseases (NCDs), also known as chronic diseases, are responsible for 74% of all deaths globally. They result from the combination of genetic, physiological, environmental, and behavioral factors and are anticipated by phenomena of premature aging linked to chronic inflammation (early inflamm-aging), which are preventable on a personalized basis.

Telomere length of peripheral blood mononuclear cells (PBMCs) is a biomarker of cell senescence, oxidative stress, and inflammation on which physical exercise has been shown to have a protective effect.

In the context of the project HEBE, telomere length was measured before (T0) and after (T1) the 6-month-long personalized physical exercise program to which each participant was subjected. PBMCs were isolated from blood samples collected at T0 and T1, and DNA was extracted for analysis through quantitative PCR (Real-Time PCR 7900 Detection System, Applied Biosystems) of the telomere sequence and β globin (HbG), the endogenous single-copy gene used for normalization.

The assessment of telomere length modulation by the 6-month-long personalized program is aimed at investigating to which extent physical exercise can reduce cell senescence, especially when put about other individual interacting factors (e.g. BMI, age, etc.). This will enable us to get insights on the mechanisms through which lifestyle change can improve health and promote healthy aging, as well as to validate the use of telomere length as an inflamm-aging biomarker for monitoring it.

Abstract 17

Healthy aging vs inflamm-aging: how miRNAs expression changes in response to a personalized physical activity intervention

Eva Dariol, Laura Dioni, Mirjam Hoxha, Simona Iodice, Luca Ferrari, Federica Rota, Elia Biganzoli, Valentina Bollati, on behalf of the HEBE Consortium.

Non-communicable diseases are the cause of early aging and death of 41 million people every year. They are strictly connected to a context of growing urbanization and globalization, which encourage a lifestyle wanting in physical activity.

Biological aging is known to be associated with a chronic low-grade inflammation state, which has led to the designation of the term “inflamm-aging”. Since this latter is at the basis of non-communicable diseases, identifying new potential biomarkers involved in this process is crucial.

In this context, microRNAs (miRNAs) are promising biomarkers of effect. Being post-transcriptional regulators of gene expression, they represent an epigenetic mechanism whose action also depends on external influencing factors, such as physical activity. miRNAs can be stored in and delivered by Extracellular Vesicles (EVs), allowing cell-to-cell long-distance communication, therefore contributing to the whole body homeostasis.

The present study aims to evaluate inflammation-related miRNA EV content (EV-miRNAs) expression changes resulting from the implementation of a personalized protocol of physical activity, in order to find potential biomarkers of inflamm-aging or healthy aging.

HEBE project enrolled 100 subjects, from who were collected peripheral blood samples at two time points: at the beginning (T0) and at the end (T1) of the study, with in between 6 months of personalized physical activity protocol to be followed. Plasmatic EVs were isolated, and the EV-miRNAs were extracted. The miRNome (755 miRNAs) was profiled through high-throughput quantitative Real-Time PCR. The

differentially expressed miRNAs for each subject between T0 and T1 were evaluated through statistical analysis.

The identification of EV-miRNAs differentially expressed between the 2 timepoints, will highlight the targeted molecular pathway influenced by the protocol of physical activity intervention. This trend may allow for the discovery of new potential biomarkers associated with a healthy aging process.

Abstract 18

The effect of a personalized lifestyle program on DNA/RNA oxidative damage: The HEBE Study

Gaia Maria Anelli, Chiara Novielli, Martina Pisati, Benedetta Petrone, Chiara Favero, Daniela Lucini, Chiara Mandò on behalf of the HEBE Consortium.

Physical exercise is widely recognized as a main component of a healthy lifestyle, with numerous benefits for overall well-being. Regular exercise has been associated with improved cardiovascular health, enhanced mood, and reduced risk of chronic diseases such as obesity, diabetes, and hypertension. Oxidative stress is the result of the unbalance between the production of reactive oxygen species and antioxidant defenses which can lead to damage to various cellular components, including lipids, proteins, and DNA, ultimately contributing to the pathogenesis of numerous diseases and the aging process.

The relationship between physical exercise, health, and oxidative stress is complex and multifaceted. While moderate regular exercise is generally beneficial and can enhance antioxidant defenses, excessive or sporadic physical activity may overwhelm these defenses, leading to an accumulation of oxidative damage. Furthermore, individual factors such as age, fitness level, and genetic predisposition can influence susceptibility to oxidative stress induced by exercise.

Understanding the interplay between physical exercise, health outcomes, and oxidative stress is of paramount importance for optimizing exercise prescription and maximizing the benefits of physical activity

while minimizing potential risks. In the HEBE (Healthy aging versus inflamm-aging: the role of physical Exercise in modulating the Biomarkers of age-associated and Environmentally determined chronic diseases) study, we investigate the potential impact of a personalized exercise program on modifying oxidative stress and consequent nucleic acid damage. To assess DNA/RNA oxidative damage we focused on the oxidation of guanosine nucleosides in DNA to 8-Hydroxy-2'-deoxyguanosine (8-OHdG). This biomarker, quantifiable in plasma using ELISA assays, serves as a reliable indicator of an individual's overall oxidative state.

In the context of the project HEBE, 8-OHdG was measured in 100 healthy workers of the University of Milan before (T0) and after (T1) the 6-month-long personalized physical exercise program to which each participant was subjected. Plasma was separated by centrifugation from EDTA-blood samples collected at T0 and T1, and DNA/RNA oxidative damage was assessed by ELISA *(Cayman Chemical)*.

The findings from the HEBE study hold significant implications for public health and personalized medicine. Demonstrating the efficacy of a tailored exercise program in modulating oxidative stress could support the development of targeted interventions for promoting healthy aging and preventing chronic diseases. Moreover, understanding the mechanistic link between exercise, oxidative stress, and health outcomes provides valuable insights into the optimal prescription of physical activity for individuals across different age groups and fitness levels.

Abstract 19

Perception of the effectiveness of a lifestyle change promoting strategy and of adherence to exercise in an adult population: HEBE project

Giuseppina Bernardelli, Mara Malacarne, Luca Giovanelli and Daniela Lucini, on behalf of the HEBE Consortium.

Introduction: The predominant barriers hindering the regular performance of physical activity are represented by sociodemographic, physical, physiological, behavioral but also environmental factors (lack of time), as well as distance (home-gym) and motivation. Home-based programs could represent a solution, such as those available online having increased in popularity during Covid-19 pandemic. Nevertheless, such

programs are not always safe since they are carried out with no supervision or prescription. The HEBE project promoted lifestyle changes through the personalized prescription of physical exercise to be performed at home.

Aim: To evaluate the perception regarding adherence to exercise and lifestyle change among HEBE project participants.

Method: 100 UNIMI employees were enrolled on a voluntary basis, and assessed at T0 and T1 (6 months). After 2 and 4 months of intervention they were contacted for evaluating adherence to exercise, and after 3 months they underwent a clinical evaluation aimed at monitoring the progress of the prescribed exercise program. Concerning clinical and instrumental evaluations, at T0 and T1 Six minute walking test (6MWT) was employed to measure the distance covered by walking quickly on a linear trajectory in 6 minutes, while at T1 a semi-structured interview was performed to investigate the perception of both adherence to the program, fatigue and satisfaction. Physical exercise was promoted by means of videos (also encompassing walking), which were specifically created, made available on a platform, and individually prescribed according to subject's characteristics and needs.

Results: 85 participants completed the program. We found an average score of 6.58 (scale 1-10) for perception of adherence to the program, and 8.39 for satisfaction. Among those who felt "much" or "somewhat improved" (92% of participants), the improvement in the distance covered during 6MWT was significantly greater as compared to the rest of the group (Mean Δ = 33 m vs -22 m, p=0.001).

Discussion: These findings indicate that those who felt improved actually trained and followed the exercise prescription, thus improving their performance.

Conclusion: Albeit HEBE project lasted only 6 months, it was sustainable for both researchers and participants, who were satisfied to be part of the protocol and who obtained significant improvement of their performance.