

**"G. d'Annunzio" University
of Chieti-Pescara**



SCUOLA SUPERIORE "G. D'Annunzio"

**PhD Course in
Medical Biotechnologies**

**Mediterranean Diet Impact on Longevity in Abruzzo:
A Study of Nonagenarians and Centenarians**

*Department of Neurosciences, Imaging, and Clinical Sciences
Scientific Disciplinary Sector BIO/09*

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Academic Years 2021/2024

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ABSTRACT

This dissertation explores the potential longevity benefits associated with the Mediterranean diet in the mountainous regions of Abruzzo, focusing on nonagenarians and centenarians. Funded under the National Operational Program 'Research and Innovation' 2014-2020, this research aligns with strategic educational and environmental sustainability objectives, emphasizing innovation in health and social care systems. The core investigation centers around the formulation of an "Apennine Diet," adapted from the traditional Mediterranean diet to meet the unique climatic and nutritional demands of Abruzzo's mountainous landscapes. This diet reflects the consumption patterns of the region's oldest populations, characterized by high intakes of local, minimally processed foods rich in micronutrients beneficial for aging populations. Research methods included comprehensive demographic analysis across four provinces in Abruzzo, leveraging both quantitative and qualitative data collection. Statistical methods were employed to isolate environmental from socio-economic longevity influences, supported by a rigorous review of epidemiological literature. Traditional Abruzzese recipes were collected and analyzed for their nutritional content and potential health impacts, particularly focusing on their role in promoting longevity. The findings suggest a significant correlation between the local dietary habits and the high incidence of nonagenarians and centenarians in these areas. Notably, the diet's rich antioxidant and anti-inflammatory properties are hypothesized to contribute to reduced chronic disease prevalence and enhanced life expectancy. This diet also incorporates principles of "One Health" and ecological sustainability, promoting a holistic approach to well-being that integrates environmental health with human health. Future research directions proposed include further refining the Apennine Diet's formulation based on ongoing biochemical analyses and expanding the study to include genetic factors influencing longevity. This research not only contributes to the academic field of nutritional epidemiology but also serves as a basis for public health interventions aimed at improving the quality and length of life in mountainous populations. The implications of this study extend beyond local health promotion, suggesting potential for broader applications of adapted dietary practices in similar ecological and demographic settings globally. This aligns sustainable health practices and supports the preservation of cultural dietary heritage while enhancing population health outcomes.

INTRODUCTION

The supplemental grant for the PhD course in Medical Biotechnology was financed under the National Operational Program 'Research and Innovation' 2014-2020, specifically Axis IV 'Education and Research for Recovery,' subsection IV.5 'Doctoral Programs on Green Themes.' This funding signifies the doctoral project's alignment with the strategic directions outlined in the SNSI and the NRP. It also confirms compliance with Legislative Decree 240/2010 and Ministerial Decree 45/2013 regarding doctoral studies, aiming to foster innovation and enhance collaboration between the academic research sector and industry, particularly in advancing research contributions within the domains of innovation and environmental sustainability.

This research project focused on the analysis of typical foods from the Abruzzese Mediterranean diet, particularly their micro and macronutrient composition, and the exosomes they release along with their transport of bioactive molecules. The investigation concentrated on the biological activity of these components in human cellular models. The aim was to promote the use of local raw materials, enhancing biodiversity and integrating gastronomic, touristic, health, and social aspects, within the broader framework of fostering 'ecological projects.'

Study Objective

The aim of this project was to develop an 'adapted Mediterranean diet' tailored to the unique nutritional and metabolic needs of a mountainous rural environment, characterized by lower temperatures and higher energy expenditure, to establish a suitable healthy diet. Originating from Abruzzo, we propose to name this diet the 'Abruzzese Mediterranean diet' or more broadly, the 'Apennine Diet/DIETapp'. The goal of this study was to recommend nutritional supplements to our 'Abruzzese' Mediterranean diet to better align it with the intrinsic characteristics of the 'healthy aging' and longevity diet. This will be connected to psychological aspects of aging within the frameworks of 'lifestyle' and 'One Health'.

The purpose of the research was to develop the 'Apennine Diet,' an adapted version of the Mediterranean diet, specifically designed for the population of rural mountainous environments. This dietary regimen aims to respect the principles of the Mediterranean diet, adapting them to the unique features of rural and mountainous environments, particularly those of the Apennines. The Mediterranean diet is a balanced nutritional model recognized for its beneficial properties in preventing chronic diseases. It is based on the consumption of typical foods from Mediterranean basin countries, such as fruits, vegetables, whole grains, legumes, nuts, olive oil, fish, lean dairy products, and red wine, consumed in moderation. The Abruzzo region, characterized by 65% mountains and

35% hills and bordered to the east by the Adriatic Sea, boasts 3 national parks and 38 protected areas, earning it the title of 'Green Region of Europe.'

Background

The formulation of a dietary regimen dedicated to the inhabitants of mountainous areas, inspired by the conference 'Nutrition in the Mountains from 800 to 8000 meters' (Supplement to *De rerum Natura*, 2018), highlighted the need to adjust caloric and nutritional intake to the specific demands of the mountain environment. The symposium, organized by the Italian Society of Mountain Medicine (SIMeM) and the University of Chieti-Pescara "G. d'Annunzio," emphasized how altitude significantly affects critical metabolic variables such as blood oxygenation and thermoregulation, thereby increasing daily energy expenditure. These factors, along with harsh climatic conditions and the greater physical effort required for movement in mountainous environments, necessitate a nutritional approach designed to support an increase in basal metabolism and a higher caloric demand. Consequently, the dialogue among experts led to the definition of dietary guidelines capable of supporting not only optimal physical performance but also the prevention of specific health risks associated with life in the mountains. The proposed approach integrates scientific updates to promote health and well-being, considering the challenges imposed by altitudinal conditions.

Convegno Nazionale della SIMeM-UdA
L'ALIMENTAZIONE IN MONTAGNA
da 800 a 8000 metri

sabato 21 aprile

8.00 Registrazione dei partecipanti "ingresso gratuito"

Presentazione **Mauro Berra** e **Vittorio Vercelli**

9.00 **Saluti**
Palmerino Fagnoli (Indirizzo di Fondazione)
Sergio Caporali (Indirizzo di Fondazione)
Antonio Pizzoli (Indirizzo di Fondazione)
Paolo Carretelli (Indirizzo di Fondazione)

Moderatore **Mauro Berra** - **Angela Cichelli** - **Carmine Di Rita**

9.00 **Daniela Casperini** (Tavola dei nutrizionisti della nutrizione umana)
9.30 **Chiara Pizzoli** (La dieta, la nutrizione e la salute: la dieta e la salute)
9.45 **Aurelio Manti** (La dieta e la salute: la dieta e la salute)
10.00 **Mauro Pizzoli** (La dieta e la salute: la dieta e la salute)
10.30 **Anna De Antoni** (La dieta e la salute: la dieta e la salute)
10.45 **Federica Pizzoli** (La dieta e la salute: la dieta e la salute)

11.00 PAUSA CAFFÈ (30 minuti)

Moderatore **Mauro Berra** - **Angela Cichelli** - **Carmine Di Rita**

11.30 **Nicola Pizzoli** (La dieta e la salute: la dieta e la salute)
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13.00 PAUSA PRANZO (30 minuti)

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domenica 22 aprile

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20.00 CENA SOCIALE

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Pizzoferrato
21 e 22 aprile 2018
Centro Avifauna
località Chiarenza
Provinciale 166, Pizzoferrato - Palena

Presidente del convegno
Prof. Paolo Carretelli

Con la partecipazione di
Jacopo Fo

Mostra permanente
del Maestro Luciano Emiliani

Arti visive di
"Transumanza Artistica"

SALUBER04
 Depurazione acque

VALORI
 vini

COCCOSTRE

www.societaitalianamedicinamontagna.it - **www.unich.it**

Figure 1: Poster of the conference held in Pizzoferrato (CH) in April 2018 'Nutrition in the Mountains from 800 to 8000 m' (Supplement to *De rerum Natura*, 2018).

Furthermore, within this context, the epidemiological study led by Professor Serafini's research group explored the impact of certain dietary patterns on the longevity of ultra-nonagenarians and centenarians residing in the Abruzzo region. Through a cross-sectional survey, the group highlighted how adopting an early dinner combined with a general reduction in daily caloric intake significantly contributes to longevity in this specific demographic cohort. These findings suggest that targeted modifications in dietary habits can have a significant impact on life extension and the reduction of aging-related diseases such as cardiovascular, cerebrovascular disorders, and some forms of cancer.

This study makes a significant contribution to the growing body of research linking specific dietary regimes to health and longevity (Angelino et al., 2022; Rimm et al., 2004).

In our demographic investigation, ISTAT data analysis for the years 2010-2020 and 2022 revealed that several municipalities in the Abruzzese Apennine region stand out nationally for their considerable presence of nonagenarians and centenarians. This phenomenon suggests that these mountain areas possess environmental, social, and lifestyle characteristics particularly conducive to longevity. The intrinsic living conditions of these communities, possibly characterized by greater daily physical activity, a locally sourced diet rich in nutritionally advantageous elements, and a strong sense of social cohesion, may significantly contribute to this trend. The significance of these results indicates a potential area of interest for further research into the correlation between environmental factors and lifestyles with the exceptional longevity observed in these Apennine communities (ISTAT <https://www.istat.it/it/popolazione-e-famiglie>).

In the context of our study, we explored the potential protective impact that mountain living might have on longevity. Our research focused on analyzing the peculiarities of mountainous lifestyle, examining how variables such as air quality, the specificity of the local diet, and social cohesion within mountain communities might favorably influence life quality and expectancy. We paid particular attention to comparing these data with those related to urban and lowland populations, hypothesizing that the distinctive elements of mountain areas could translate into a significant longevity advantage. This interdisciplinary approach allowed us to outline a broader picture, suggesting that the mountain environment might offer optimal conditions not only for a longer life but also for healthier aging, thanks to the integration of environmental and sociocultural factors well incorporated into the daily routines of these groups.

Research Hypothesis

Could living in the mountains be a contributory factor to longevity? Scientific literature emphasizes the importance of environmental factors, in addition to genetic and lifestyle factors, in profoundly influencing mortality and life expectancy (Passarino et al., 2016). Available evidence suggests that residence in medium-high altitude areas is associated with reduced mortality from cardiovascular diseases, stroke, and some types of cancer (Burtscher et al., 2021). Conversely, there is high mortality from COPD and, likely, from lower respiratory tract infections. It might be hypothesized that moderate altitudes offer greater benefits compared to extremely high elevations. Although life at medium-high altitudes may reduce the risk of developing certain diseases, it might have a negative impact on mortality in the advanced stages of these illnesses. Confirming and deepening these findings would be essential for optimizing medical care and disease management in elderly

populations residing in mountainous areas. However, epidemiological data on the effects of high-altitude living on mortality caused by severe diseases are still inconsistent, likely due to ethnic, behavioral differences, and complex interactions with the environment. It is estimated that about 500 million people live permanently at altitudes above 1500 meters (Tremblay et al., 2021).

Living at high altitude, in addition to genetic factors and lifestyles, can influence mortality and life expectancy, correlating with reduced mortality from cardiovascular diseases, stroke, and some types of cancer (Burtscher et al., 2013). The study presented here examined whether living in the mountains, with the consequent higher air quality, active lifestyle, local diet, and social cohesion, might represent a protective cofactor for longevity compared to populations residing in urban or non-mountainous environments.

Phenomenology of Longevity

Human longevity represents a phenomenon of extraordinary complexity, stemming from the interaction of multiple factors that span socioeconomic, biological, and behavioral dimensions. Recent multidisciplinary research has progressively unveiled how diverse variables such as educational level, income, social context, lifestyle habits, diet, physical activity, and genetic factors directly influence both life expectancy and the quality of aging. These studies emphasize the importance of a holistic approach in gerontological research. Analyzing these factors within innovative research models allows for the identification of levers through which aging might be modulated, thereby promoting longevity and the quality of a healthy life (Beck et al., 2020; Dziechciaż et al., 2014). The following are key aspects of quality aging.

Socio-Economic Factors

The relationship between economic prosperity and longevity has been explored and confirmed through multiple studies indicating that in higher-income countries, there is a notable increase in life expectancy from the age of 60 (Anna-Maria et al., 2023). This increase is primarily attributable to a reduction in smoking rates among males and a decrease in mortality from cardiovascular diseases in both sexes. These advances in longevity are often linked to improvements in health conditions and more equitable and extensive access to healthcare services. These combined factors reflect how economic well-being can positively influence public health, promoting a range of interventions and health policies capable of significantly improving the quality of life and life expectancy of the population (Spiteri et al., 2019).

Physiology of Aging

Scientific research has made significant strides in studying aging, focusing not only on increasing life expectancy but also on the quality of this extension. Preventing premature deaths was an important initial step, but now the focus has shifted to how to slow the degenerative processes associated with aging, thereby improving health and vitality in the later stages of life.

Cardiorespiratory Fitness

Cardiorespiratory capacity, quantifiable through the measurement of VO₂max (maximum oxygen consumption), has proven to be a robust independent predictor of all-cause mortality, including disease-specific outcomes. A high level of cardiorespiratory fitness reflects a healthy heart and lungs, essential factors for healthy aging (Strasser et al., 2018).

Genetic and Dietary Factors

The Japanese dietary culture, with its low intake of saturated fats and high consumption of antioxidants, is often cited as a reference model for a long and healthy life. Despite the presence of risk factors such as hypercholesterolemia and hypertension, Japan boasts an exceptional average life expectancy of 85 years (Kinae et al., 2007). This longevity is not solely attributable to diet but also to a favorable genetic makeup that, in synergy with lifestyle, contributes to longevity. Thus, longevity is the result of a delicate balance between genetics, environment, lifestyle, and health interventions. This holistic understanding allows for the identification of targeted strategies to promote healthy and active aging on both an individual and collective level, with the goal of enhancing not just years of life but the quality of life within those years.

Factors Influencing Longevity in Mountainous Contexts

Research on longevity in mountainous areas could uncover unique and valuable factors that contribute to a longer and healthier life expectancy. To enrich and deepen our hypotheses, we now turn to the distinctive aspects of these contexts:

Environmental Quality: The mountain environment, characterized by cleaner air, reduced levels of industrial pollutants, and lower population density, offers a habitat that could significantly reduce the risk of respiratory and cardiovascular diseases (Mallet et al., 2021).

Active Lifestyle: The geographic context of mountains naturally fosters greater daily energy expenditure through outdoor activities such as walking and trekking, which strengthen the cardiovascular and muscular systems, cornerstones of good health (Morris et al., 1997).

Local Diet: The sourcing of natural, often organic, and locally-produced foods contributes to a nutritious, balanced diet free of preservatives and additives, positively influencing health and longevity (Niso-Santano et al., 2019).

Stress and Mental Health: Life in the mountains is often associated with a more relaxed pace, lower levels of urban stress (Li et al., 2023), and more intense contact with nature, which, combined with physical activity, promotes the production and release of endorphins, beneficially reflecting on psychological well-being.

Social Cohesion: Mountain communities are often characterized by a cohesive social fabric, where bonds between individuals are strong and interpersonal interactions frequent. This environment facilitates the creation of a network of emotional and social support, essential for overall well-being. This support is crucial not only for mental health, helping to combat feelings of loneliness and depression but also for physical health, through encouraging an active lifestyle and sharing knowledge on healthy practices. Moreover, solidarity among community members can improve resilience in the face of daily challenges and mutual aid in times of need, enhancing a sense of belonging and individual self-esteem. Therefore, social cohesion is an important variable in supporting healthy aging and longevity (Chen et al., 2015).

The study of epigenetics in mountain populations provides valuable insights into how the environment influences gene expression and, consequently, longevity. Exposure to diverse climatic conditions, such as those characteristics of high altitudes, can modify epigenetic patterns regulating genes involved in aging and disease processes (Julian et al., 2017). Identifying these epigenetic variations enhances our understanding of the molecular foundations of longevity and allows for the assessment of how specific environmental factors contribute to health and lifespan in mountain communities.

Epigenetic Factors

The study of epigenetics in mountain populations provides valuable insights into how the environment influences genetic expression and, consequently, longevity. Exposure to different climatic conditions, such as those typical of high altitudes, can modify epigenetic patterns that regulate genes involved in aging and disease processes (Julian et al., 2017). Identifying these epigenetic variations aids in understanding the molecular basis of longevity and assessing how specific environmental factors contribute to health and life span in mountain communities.

Epidemiological Research

Only through comprehensive epidemiological studies can it be determined whether there is a causal correlation between mountainous habitats and increased life expectancy, considering all the demographic and socioeconomic variables that may influence the outcomes. The compilation of these factors provides a complex and detailed picture, suggesting how life in the mountains may be intrinsically linked to greater longevity. A systematic exploration of these dynamics could not only provide significant insights for public health but also encourage targeted policies to enhance and preserve mountain lifestyles.

Mountains as Natural Climatic Refuges for Healthy Aging

Climate change represents one of the greatest challenges of our time, profoundly affecting the planet and human societies. The rise in global temperatures is closely linked to anthropogenic activities, including the intensive use of fossil fuels, deforestation, and air pollution. These factors have contributed to an unprecedented acceleration of climate alterations, with significant impacts on the environment and public health. The increase in global temperatures has led to a rise in the frequency and intensity of extreme weather events, such as heatwaves, hurricanes, floods, and prolonged droughts. Such phenomena have devastating effects on ecosystems and infrastructures, increasing the risk of economic and human losses. Furthermore, air pollution is a critical factor in the onset of numerous pathologies, exacerbating respiratory and cardiovascular problems, as highlighted in studies by De Blois et al. (2015) and Moghadamnia et al. (2017). Recent research has demonstrated a link between exposure to pollutants and the deterioration of cognitive functions, increasing the risk of dementia and cognitive deficits (Rocque et al., 2021). Similarly, extreme climatic events not only cause material damage but also profoundly impact mental health, contributing to the onset of disorders such as PTSD (Post-Traumatic Stress Disorder), depression, and anxiety (Hrabok et al., 2020).

Considering these findings, it is essential to adopt concrete measures to mitigate the effects of climate change. Reducing greenhouse gas emissions, investing in renewable energies, and implementing sustainable policies are fundamental steps to counter this global crisis. The future of the planet depends on the actions we take today, making a collective commitment imperative to preserve the environment and ensure the well-being of future generations. Although climate change affects all of humanity, the population over 65 years old is particularly vulnerable to adverse health effects, primarily due to two factors:

- *Growing Population*, which will double in just 50 years. In 2020, for the first time, people over 60 outnumbered children under 5 years old (United Nations Department of Economic and Social Affairs PD, 2022).
- *Reduced Adaptability*, due to physiological changes associated with aging (Filiberto et al., 2009); the cumulative effect of repeated exposures (Stein et al., 2008); and social determinants such as low economic/educational level and insufficient family and social networks (Geller et al., 2005).

Extreme temperatures, particularly intense heat, represent a significant health risk for the elderly. It has been shown that heat can reduce renal function in elderly patients taking antihypertensive medications (Sagy et al., 2016). Additionally, there is an increased risk of hospitalization for renal, respiratory causes (Gronlund et al., 2016), and for hyperthermia (Noe et al., 2012), especially in people over 85 years old. This risk is particularly high in individuals with low socioeconomic status, who live in older homes or in cities with scarce air conditioning (Gronlund et al., 2016). Concurrently, there is a significant increase in the risk of hospitalization for cardiovascular diseases during heatwaves (Giang et al., 2014). These data highlight the importance of climate mitigation policies and targeted interventions to protect the most vulnerable populations from the consequences of global warming.

Increases in diurnal temperature variation are associated with a range of medical emergencies, particularly cardiovascular (Wang et al., 2013; Zheng et al., 2016), digestive, genitourinary, and respiratory conditions (Anderson et al., 2013; Wang et al., 2013). The acute effects of such thermal variations are particularly pronounced in certain subgroups of the population, such as individuals aged 75 years and older, and women (Wang et al., 2013; Zheng et al., 2016). Furthermore, the impact of diurnal temperature excursions appears to vary with the season, highlighting the need for heightened vigilance during the most critical seasonal periods (Zheng et al., 2016). High temperatures represent a significant risk factor for mortality among the elderly, especially for cardiovascular causes (Lim et al., 2016; Rocklöv et al., 2010; Vutcovici et al., 2014). This risk is significantly amplified in the presence of chronic diseases or comorbidities, including Chronic Obstructive Pulmonary Disease (COPD), diabetes, chronic heart failure, and a history of acute myocardial infarction (Tam et al., 2009; Zanobetti et al., 2012). These findings underscore the importance of targeted prevention strategies for the most vulnerable populations during periods of extreme heat.

The higher the proportion of the population over 65 in a region, the lower the summer temperature threshold at which excess mortality occurs (Zanobetti et al., 2012). This implies that with an increasing proportion of elderly, the temperature at which significant increases in mortality are recorded is lower. Rising summer temperatures, linked to climate change, will lead to a significant

increase in mortality during the summer months, accompanied by a concurrent decrease in temperature-related deaths during the winter (Bennett et al., 2014; Shi et al., 2016). The increased variability of summer temperatures affects the survival of the elderly with certain diseases. For every 1°C increase in summer temperature, the risk of death increases by 2.8% for individuals with heart failure and by 4% for those with diabetes (Zanobetti et al., 2012).

Heat stress is a significant risk factor for cognitive decline in individuals over 65. Recent studies have shown that exposure to high temperatures is associated with a significant reduction in cognitive abilities, particularly verbal and mathematical reasoning skills (Yi et al., 2021). Moreover, prolonged exposure to high temperatures, especially in the presence of large thermal variations, increases the risk of hospitalization for dementia (Wei et al., 2019). These findings highlight how climate change, through the increase in extreme temperatures, can have a direct impact on the cognitive well-being of the elderly population, underscoring the need for preventive strategies to mitigate the adverse effects of heat stress. The elderly are more susceptible to health risks caused by heatwaves (European & Kaźmierczak, 2019; Romanello et al., 2021). The most common reasons for hospitalization, ordered from highest to lowest risk, include:

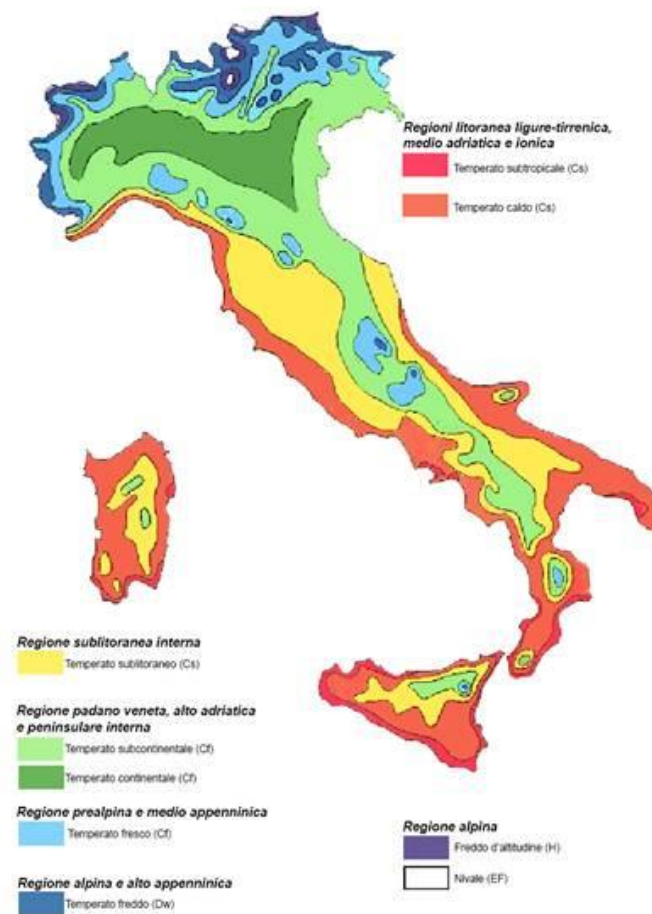
- *Heat stroke and sunstroke*
- *Anhidrotic heat exhaustion (without sweating)*
- *Heat exhaustion*
- *Acute and unspecified renal failure*
- *Dehydration*
- *Fluid and electrolyte disorders*
- *Renal insufficiency*
- *Urinary tract infections*

It is evident that climate change severely impacts the health of the elderly. In this regard, it is imperative to develop sustainable practices and educational strategies to mitigate these impacts and promote healthy aging. The increase in variability of summer temperatures, attributable to climate change, significantly affects the survival of the elderly, especially those suffering from specific chronic diseases. The effect is more pronounced in the older segments of the population, who are more vulnerable to thermal fluctuations. However, this impact is mitigated in cities with a greater presence of green spaces, which play a protective role by mitigating the effects of extreme temperatures. This phenomenon highlights how the rise in summer temperature variability can increase mortality risks in vulnerable elderly populations, underlining the urgency of targeted interventions such as the expansion of urban green areas and the implementation of preventive measures to counteract the negative effects of climate change on public health (Zanobetti et al., 2012).

The diverse climatic regions of Italy reflect the geographical and altitudinal variety of the territory, significantly influencing environmental conditions and their effects on the population. In the coastal regions of Liguria, Tyrrhenian, central Adriatic, and Ionian, a subtropical temperate climate (CS) prevails, characterizing the warmer coastal belts of southern and insular Italy, with average annual temperatures above 17°C, mild winters with temperatures above 10°C, and five months with average temperatures above 20°C, accompanied by an annual temperature range between 13°C and 17°C. In the Tyrrhenian coastal areas, from Liguria to Calabria, in the southern belt of the Adriatic coast, and in the Ionian zone, the warm temperate climate (Cs) dominates, with average annual temperatures between 14.5°C and 16.9°C, cooler winters (6°C-9.9°C), four months with temperatures above 20°C, and an annual range of 15°C-17°C.

The inland sublittoral regions, such as the hilly areas of the Tuscan-Umbrian-Marchean Apennines and the lower slopes of the Southern Apennines, exhibit a sublittoral temperate climate (Cs), with average annual temperatures between 10°C and 14.4°C, cooler winters (4°C-5.9°C), three months with temperatures above 20°C, and an annual range between 16°C and 19°C. In the Padano-Venetian region, the upper Adriatic, and the interior areas of the peninsula, the subcontinental temperate climate (Cf) affects the Venetian and Friulian plains, with annual averages between 10°C and 14°C, cold winters (-1°C to 3.9°C), two months with temperatures above 20°C, and an annual range of 16°C-19°C. The Po Valley and parts of the Venetian plain are dominated by the continental temperate climate (Cf), with annual averages between 9.5°C and 15°C, harsher winters (-1.5°C to 3°C), three months with temperatures above 20°C, and an annual range exceeding 19°C.

In the pre-Alpine and mid-Apennine areas, the cool temperate climate (Cf) prevails, characterized by average annual temperatures between 6°C and 9.9°C, harsh winters (0°C to -3°C), moderate summers with temperatures between 15°C and 19.9°C, and an annual range of 18°C-20°C. Finally, the Alpine and high Apennine regions are characterized by the cold temperate climate (Dw), affecting a belt of the Alps and the highest peaks of the Apennines, with annual averages between 3°C and 5.9°C, harsh winters with temperatures below -3°C, cool summers (10°C-14.9°C), and an annual range between 16°C and 19°C. This climatic diversity plays a crucial role in local ecology and climate impacts on human health, requiring region-specific approaches in managing risks associated with climate change.

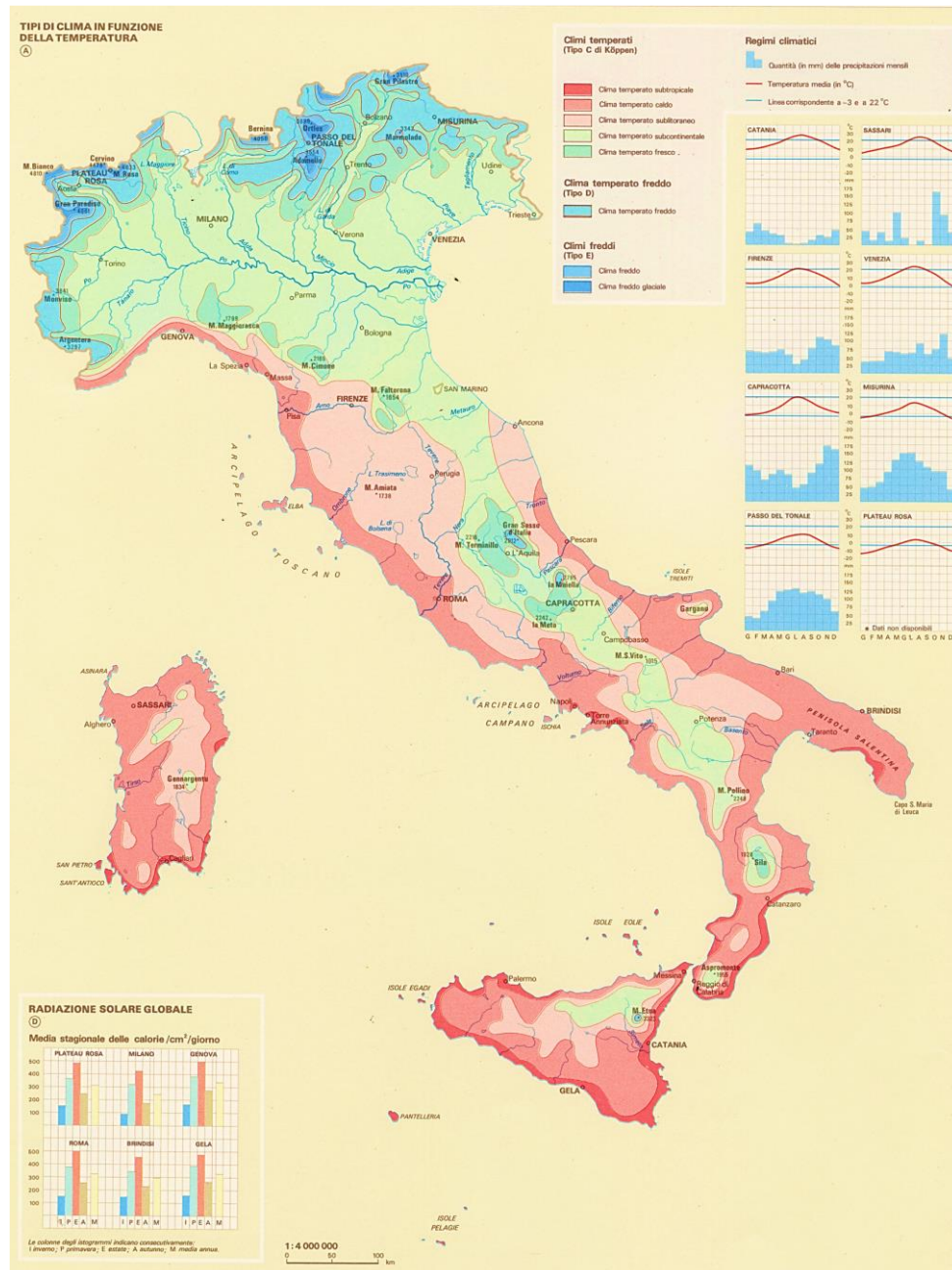


Mountains as Natural Climatic Refuges

The mountainous context offers a distinctive microclimate that, compared to flat and urban areas, experiences less intense effects of global warming. This climatic phenomenon, characterized by a progressive increase in global temperatures, manifests a mitigated impact in mountainous regions where temperatures tend to remain cooler. This climatic feature directly improves the quality of life for residents, playing a crucial role in modulating aging processes.

Extreme heat is known for its negative impact on human health, causing immediate discomfort and long-term complications, particularly for vulnerable populations such as the elderly. Moreover, mountain air, often cleaner and less subject to pollution, further contributes to a healthy habitat. Atmospheric pollution is a well-known catalyst for respiratory and cardiovascular diseases and can exacerbate existing health conditions. Reducing exposure to these pollutants is another factor that promotes longevity and healthier aging in mountain communities.

These observations can be attributed not only to the physical characteristics of the mountainous environment but also to a lifestyle that often includes increased physical activity, such as hiking and mountain farming, which contribute to maintaining physical and mental well-being. Mountains offer a living context that, through a combination of cooler temperatures, cleaner air, and active lifestyles, supports healthier aging and improved quality of life. These factors make mountain regions areas of great natural beauty as well as important enclaves for promoting public health in an era of growing global concern about climate change.



Atlante Tematico realizzato dall'Ufficio Cartografico del Touring Club Italiano con il CNR-Consiglio Nazionale delle Ricerche (1989-1992).

MATERIALS AND METHODS

Design of study

This study was conducted using an ecological observational approach, wherein the investigator does not alter the participant's normal behavior in carrying out their daily activities. The primary objective of the study is to assess the general health status of ultra-longevous elderly individuals in the Abruzzo region (90–99 years and 100+ years old), with a particular focus on aging-related factors such as nutrition, lifestyle, cognitive decline, sarcopenia, and the role of local and zero-kilometer food consumption in the prevention of these processes. Within the scope of this research, aimed at investigating the correlations between the traditional Abruzzese diet, the nutritional profiles of its foods, and their biological effects on the participants, the rigorous definition of inclusion and exclusion criteria was essential to ensure the homogeneity and reliability of the collected data. The study was conducted in the Abruzzo region during the period 2021–2025.

The study comprises two main components: the first part entails an analysis of data from ISTAT/TUTTITALIA surveys on the prevalence of the elderly population in the Abruzzo region; the second part, employing a cross-sectional design, examines a sample of 36 nonagenarians.

The study assessed the general health status of elderly people over 90 years (from 90 to 99 years) and centenarians (from 100 to 109 years) both men and women living in Abruzzo, in the four provinces: Chieti, Pescara, L'Aquila and Teramo, for the year 2021.

In the first step the research project has clarified how many are the nonagenarians and centenarians living in the four provinces of Abruzzo in 2021; the project has clarified in which centers live the nonagenarians and centenarians that is in countries with what density? in Small Villages <1000, Medium Villages 1000-3000, Big Villages >3000. Finally, the project investigated the nineties and centenarians at what altitude they live? High Altitude >600m, Medium Altitude 300-600m or Low Altitude <300m?

The second step was to take a historical photograph of the Province of Chieti from 2010 to 2020, evaluating the trend of the population of our interest, asking the same questions as before:

- What kind of population resides in the province of Chieti?
- Where do nonagenarians and centenarians live in the province of Chieti?

Small Villages <1000, Medium Villages 1000-3000, Big Villages >3000

- At what levels do nonagenarians and centenarians live in the province of Chieti?

High Altitude >600m, Medium Altitude 300-600m, Low Altitude <300m

Altitude, Diet, and Longevity: A Comparative Study

In the second part of the study, the variables which according to the research hypothesis may affect longevity were analyzed. Through an interview was analyzed the type of nutrition that ultra-longevous practice and have practiced in their lives, emphasizing the importance of consuming fresh, organic and at km 0. About 60 dishes were collected representative of the Apennine Diet. Of 53 subjects, as shown in Table 1: 12 subjects of which 6 men and 6 women at low altitude LA (<300m); 12 subjects of which 6 men and 6 women at medium altitude MA (between 300-600m) and 12 subjects of which 6 men and 6 women at high altitude HA (>600m); 17 subjects of which 9 men and 8 women, Control Group, CG from urban areas at sea level (0 m).

Biological samples were collected by salivette to analyze the biomarkers: appetite hormones: leptin and ghrelin, inflammatory status IL-6, and nitric oxide levels: NOx. The 53 subjects were also administered the Hand Grip, the Geriatric Depression Scale (GDS), Mini Nutritional Assessment (MNA), Pittsburgh Sleep Quality Index (PSQI), Food Frequency Questionnaire (FFQ), Euro Qol-5D.

Inclusion and exclusion criteria

Inclusion Criteria

Participants eligible for inclusion in the study must meet the following criteria:

1. *Residence and Familiarity with the Local Environment:* Participants must be permanent residents of the study areas, particularly the mountainous regions of Abruzzo, with a continuous presence in the territory for at least twenty years. This requirement ensures prolonged exposure to specific environmental and socio-cultural factors under investigation, as well as dietary habits deeply rooted in the traditional Mediterranean diet of Abruzzo.
2. *Age Range:* Participants must fall within a predefined age category, specifically ultra-longevous individuals, including nonagenarians (aged 90–99 years) and centenarians (aged 100–109 years), to allow an appropriate evaluation of variables related to longevity and aging quality.
3. *General Health Status:* Participants must be in a stable clinical condition, free from acute or active chronic diseases that could interfere with the biochemical and physiological parameters under analysis. This criterion ensures that any observed biomarker alterations can be primarily attributed to the effects of diet and lifestyle.
4. *Informed Consent:* Participants must be able to understand and autonomously sign the informed consent form after receiving adequate information regarding the study's benefits, risks, and procedures, in accordance with current ethical guidelines (e.g., the Declaration of Helsinki).

5. *Willingness to Collaborate*: Participants must demonstrate their ability and willingness to actively engage in all phases of the research, including completing the anamnesis form, providing biological samples (e.g., saliva), and undergoing additional tests or interviews as required.

Exclusion Criteria

Participants presenting one or more of the following conditions will be excluded from the study:

1. *Active Diseases or Confounding Clinical Conditions*: The presence of acute or chronic illnesses (e.g., inflammatory disorders, active malignancies, or critical cardiovascular conditions) that could alter the study parameters or interfere with data interpretation.
2. *Non-Conforming Environmental or Geographical Conditions*: Individuals residing in areas that do not meet the geographical and environmental characteristics defined in the study, as this could introduce extraneous variables compromising the homogeneity of the sample.
3. *Use of Confounding Pharmacological Therapies*: Participants undergoing pharmacological treatments (e.g., corticosteroids, immunosuppressants) known to significantly modify the biomarkers under investigation, to avoid confounding effects.
4. *Inability to Provide Informed Consent*: Individuals with cognitive or communication impairments that prevent a proper understanding of the provided information and the subsequent signing of the informed consent form.
5. *Non-Adherence to Sample Collection Procedures*: Participants who fail to accurately complete the anamnesis form or who do not comply with standardized procedures for biological sample collection (e.g., failure to adhere to fasting or other pre-collection requirements).
6. *Severe Diseases and Acute Clinical Conditions*: The presence of advanced-stage chronic diseases (e.g., severe heart failure, end-stage renal disease, advanced neurodegenerative disorders).
7. *Use of Specific or Restrictive Diets*: Adoption of dietary regimens incompatible with the traditional Abruzzese diet (e.g., strict hypocaloric diets, vegan/vegetarian diets, or medically prescribed restrictive diets).

The adoption of these criteria, duly validated and approved by the University Ethics Committee, aims to ensure the methodological quality of the study and the robustness of the results, it guaranteeing that observed differences can be primarily attributed to the effects of diet and lifestyle specific to the Abruzzese mountain areas.

The sensitive and personal data of the study participants were processed in compliance with Legislative Decree 196/2003 and the guidelines established by the Italian Data Protection Authority (Resolution No. 52 of 24-07-2008) concerning the protection of individuals.

Characteristics of Study Participants (a cross-sectional design)

The research involved ultra-longevous individuals residing in the mountainous regions of Abruzzo, specifically nonagenarians and centenarians, as shown in Table 1. This cohort was selected to delve into the influence of local lifestyle and dietary habits on longevity. Participants were aged between 90 and 99 years or were over one hundred years old, having lived in the mountainous areas for at least two decades, thus ensuring substantial exposure to the region's characteristic lifestyles.

	LA n = 12	MA n = 12	HA n = 12	CG n = 17
Females /Males (%)	50/50	50/50	50/50	47/53
Average Age females (years)	92 ± 3,7	95 ± 4,6	92 ± 2,2	96 ± 3,6
Average Age males (years)	92 ± 2,9	93 ± 2,0	92 ± 5,0	94 ± 4,1
Average Age M/F	92 ± 3,2	94 ± 3,7	92 ± 3,7	95 ± 3,9
Average Height females (cm)	148 ± 11,4	152 ± 6,5	152 ± 8,3	160 ± 10,4
Average Height males (cm)	163 ± 4,7	160 ± 7,8	163 ± 10,4	158 ± 8,4
Average height M/F	155 ± 11,4	156 ± 8,1	158 ± 10,5	159 ± 9,1
Average Weight females (kg)	58 ± 18,8	60 ± 8,4	57 ± 9,0	69 ± 8,9
Average Weight males (kg)	67 ± 7,4	65 ± 6,8	69 ± 9,4	84 ± 13,8
Average Weight M/F (kg)	63 ± 14,3	63 ± 7,4	64 ± 10,5	77 ± 13,9
Average females BMI (kg.m⁻²)	26 ± 4,9	28 ± 1,9	28 ± 4,5	27 ± 1,5
Average male BMI (kg.m⁻²)	26 ± 2,9	24 ± 1,8	26 ± 3,3	33 ± 3,0
Average BMI M/F (kg.m⁻²)	26 ± 3,9	26 ± 2,4	27 ± 3,9	30 ± 4,0

Note: Data are presented as mean ($\pm$ DEV-ST) for continuous data and as proportions for categorical data: Low Altitude-LA (<300m); Medium Altitude-MA (between 300-600m); High Altitude-HA (>600m); Control Group-CG (at sea level - 0 m), from urban areas.

Table 1. *Demographic and Physical Characteristics of Ultra-Centenarian Subjects Segregated by Altitude Groups and Control Group. LA: Low Altitude (<300m); MA: Medium Altitude (300-600m); HA: High Altitude (>600m); CG: Control Group, from urban sea-level areas. Data encompasses gender ratios, mean age, height, weight, and Body Mass Index (BMI) for each group.*

Their participation facilitated the collection of valuable data concerning the impact of an adapted Mediterranean diet to high-altitude conditions on health and longevity.

Demographic Survey of the Abruzzo Region in 2021

In 2021, a comprehensive demographic survey was conducted by the Italian National Institute of Statistics (ISTAT) across the Abruzzo region to gather essential population data. The survey encompassed all four provinces of the region, namely Chieti, L'Aquila, Pescara, and Teramo. The total number of municipalities surveyed amounted to 305, which collectively harbored a population of 1,281,012 inhabitants. Specifically, the Province of Chieti reported 375,215 inhabitants across 104 municipalities, making it the most populous province. It was followed by the Province of Pescara with 313,882 inhabitants in 46 municipalities, the Province of Teramo with 301,104 inhabitants across 47 municipalities, and the Province of L'Aquila, hosting 290,811 inhabitants in 108 municipalities. This demographic data forms the basis for assessing regional population distribution and density, which are pivotal for our study's focus on environmental and lifestyle factors influencing longevity in these areas.

Institution	Number of municipalities	Number of Inhabitants
Province of Chieti (Ch)	104	375.215
Province of L'Aquila (Aq)	108	290.811
Province of Pescara (Pe)	46	313.882
Province of Teramo (Te)	47	301.104
Total	305	1.281.012

Utilizing data from the Italian National Institute of Statistics (ISTAT, accessible via: <https://www.istat.it/it/popolazione-e-famiglie>), we extrapolated the distribution of nonagenarians and centenarians within the Abruzzo region for the year 2021, categorized by gender and province. This survey encompassed the provinces of Chieti, L'Aquila, Pescara, and Teramo. The details of the data include age segmentation, specifically individuals aged between 90 and 99 years, and those aged 100 years and older, with a further breakdown by gender.

Number of nonagenarians and centenarians in the Abruzzo provinces in 2021

Institution	People aged 90-99	People aged 100+	Nonagenarian men	Nonagenarian female	Centenarian men	Centenarian female
Province of Chieti	5.981	157	1.762	4.219	27	130
Province of L'Aquila	4.762	126	1.401	3.361	24	102
Province of Pescara	4.632	113	1.376	3.256	25	88
Province of Teramo	4.298	89	1.266	3.032	16	73
Total	19.673	485	5.805	13.868	92	393

Utilizing data from the Italian National Institute of Statistics (ISTAT, available at: <https://www.istat.it/it/popolazione-e-famiglie>), we have examined the distribution of nonagenarians and centenarians in the province of Chieti from 2010 to 2020. The data were categorized by gender and age groups, encompassing both male and female individuals aged 90 to 99 years, as well as those aged 100 years and above.

The collection of this data was pivotal for assessing the impact of socio-economic and environmental conditions on longevity across different areas within the region. Analyzing the number of ultra-long-lived individuals enhances our understanding of the factors that influence longevity within specific geographical and demographic contexts. This information was crucial for exploring the demographic dynamics related to aging and for directing future epidemiological research and targeted health interventions, thereby enhancing our understanding of gender-related longevity trends.

Distribution of nonagenarians and centenarians in the province of Chieti from 2010 to 2020

	Year 2010	Year 2011	Year 2012	Year 2013	Year 2014	Year 2015	Year 2016	Year 2017	Year 2018	Year 2019	Year 2020
Age 90-99 men	1.008	1.171	1.286	1.351	1.388	1.411	1.511	1.616	1.661	1610	1.687
Age 90-99 female	2.409	2.708	3.047	3.274	3.437	3.570	3.683	3.859	3.971	4006	4.090
Age 100+ men	18	23	28	25	21	29	29	29	26	20	20

Age 100+ female	75	99	93	114	102	122	133	139	102	102	101
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Demographic Data Collection

In the context of a scientific inquiry aimed at establishing the existence of a causal correlation between mountainous habitats and an increase in life expectancy, it was crucial to precisely define the methods and materials used. Two essential resources for collecting the data necessary for the study are the websites of ISTAT and Tuttitalia (ISTAT website: <https://www.istat.it/it/popolazione-e-famiglie>; Tuttitalia website: <https://www.tuttitalia.it/abruzzo/>), which provide extensive databases on Italian populations and the demographic and socio-economic characteristics of various geographic areas, including mountainous territories.

ISTAT (The Italian National Institute of Statistics)

The Italian National Institute of Statistics (also known as Istat) is a public research institution responsible for conducting general censuses on population, services, industry, and agriculture, as well as sample surveys on households and nationwide economic studies. The institute's activities are supervised by the Commission for the Guarantee of Statistical Information, under the Presidency of the Council of Ministers, which ensures the impartiality and completeness of the data collected and published. All content published by Istat is available under the Creative Commons Attribution 3.0 license. Additionally, Istat is a member of the European Statistical System (ESS).

TUTTITALIA

Tuttitalia.it is a web portal that provides detailed information on municipalities, provinces, and regions of Italy. The platform collects and publishes demographic, statistical, and geographical data from official sources, such as the Italian National Institute of Statistics (Istat), allowing users to access up-to-date information on population, economy, public services, and other territorial characteristics.

The portal is utilized for consulting data on:

- Population and Demographics: population trends, population density, and average age.
- Local Economy: employment, Gross Domestic Product (GDP), and average income.
- Public Services and Infrastructure: schools, hospitals, and transportation networks.
- Geography and Environment: land area, altitude, and protected areas.

It serves as a valuable resource for researchers, local administrators, and citizens seeking updated data on various territorial aspects of Italy.

Demographic and Socio-Economic Data Collection

We utilized the ISTAT website to obtain updated information on population, average age, income distribution, education level, employment, and other relevant socio-economic indicators. This will allow us to analyze demographic and socio-economic factors that may influence longevity in mountain areas.

Territorial and Environmental Data

The Tuttitalia site was consulted to acquire specific data related to Abruzzo, such as population density, the percentage of mountainous territory, the presence of protected areas, air quality, and accessibility to services. These data have provided us with a detailed view of the mountain habitat and its peculiarities.

Statistical Methodology

We applied advanced statistical methods to analyze the data collected. This included the use of multivariate regression models to control for confounding variables and isolate the effect of the mountain habitat on longevity. The models were calibrated to account for variables such as gender, age, lifestyle, and socio-economic status.

Comparison with Non-Mountainous Areas

To establish a meaningful comparison, it was necessary to include a control group in our study, consisting of populations residing in urban or non-mountainous environments. Data for these groups were collected in the same manner and analyzed to highlight differences or similarities.

Longitudinal Analysis

Data was analyzed in a time series to observe trends over time and assess the long-term impact of the mountain habitat on longevity.

Qualitative Evaluation

In addition to quantitative data, interviews and focus groups with residents of mountain areas were conducted to collect qualitative information that can provide further insights into perceptions of quality of life, access to healthcare services, and the impact of the environment on health.

Cooperation with Local Authorities and Institutions

Collaboration was established with local authorities and health institutions to access more specific health and mortality data, which might not be publicly available but are crucial for our analysis.

Literature Review

Finally, I conducted a systematic review of the existing literature on the topic to compare our findings with those of previous studies and to contextualize our discoveries within the current research landscape on longevity.

In summary, our methodological approach was characterized by rigorous and multidimensional analysis that integrates both quantitative and qualitative data, aimed at providing a comprehensive and reliable answer to our research hypothesis.

Identification of Traditional Abruzzese Recipes

Data Collection Methodology

We utilized a standardized telephone interview protocol to collect recipes from elderly individuals (nonagenarians and centenarians) in the rural mountainous areas of Abruzzo, distributed across the four provinces: Chieti, Pescara, Teramo, and L'Aquila. We ensured that the questions were open-ended to allow for the sharing of anecdotes and traditional details of the recipes.

Recipe Selection

A committee of experts in Abruzzese gastronomy and culture was formed to evaluate the collected recipes based on authenticity, rarity, and cultural significance, prioritizing those that best represent the culinary traditions of the areas under study.

Selection of Raw Materials

We collaborated with local farmers and botanists to identify and select the most authentic and high-quality raw materials, such as Navelli saffron, wild spinach (orapi), and Avezzano potatoes, thereby enhancing regional agrobiodiversity.

Replication of Recipes with a Chef

We worked with Chef Cristopher Pelusi (of the RED Academy) to faithfully replicate the collected recipes, documenting the process with videos or photographs to preserve the cooking techniques and traditional methods.

Nutritional Analysis and Environmental Impact

We collaborated with CREA (Council for Agricultural Research and Economics) to analyze the recipes in terms of nutritional values and environmental impact, aiming to promote food sustainability.

Exosome Extraction

Experiments were conducted on the extraction of exosomes both in our own laboratories and at Exolab (Aq) from selected raw materials. This could open new scenarios in understanding the beneficial properties of local products such as saffron and Avezzano potatoes.

These steps form the foundation of an in-depth research project that not only preserves the culinary cultural heritage of Abruzzo but also explores the potential of local food traditions in terms of health and sustainability.

Typical Recipes from the Province of L'Aquila and Chieti

First course

Fagioli in salsa
Je Frascareje
Minestra ceci e funghi porcini
Minestra fagioli e patate
Paccheri con quaglia
Pane al cotturo
Pasta con le rape
Polenta verza e fagioli
Quagliatelli
Ravioli con orapi
Sagne e recell
Sagne stracc e mitt

Main course

Baccalà con cipolla
Capretto in bianco
Cipolla e uovo
Crispino ad insalata/cicoria
Frittata con lupoli
Frittata con orapi
Frittata con vitavili
Pecora alla callara
Pizza rustica mais e salsiccia
Rattarella
Trippa con fagioli trippa con uovo

Dessert

Pan dell'orso
Pizzelle morbide
Fiadoni dolci

Typical Recipes from the Province of Teramo and Pescara

First course

Minestra fagioli e castagne
Pan cotto rape e salsiccia

Polenta di farro
Virtù teramane
Scrippelle ‘mbusse
Pasta con le voliche
Minestra ceci e castagne

Main course

Agnello acqua e sale
Mazzarelle
Tacchino alla canzanese

Side dish

Fuij strascinete

Dessert

Castagnole
Bocconotti di M.
Pastuccia
Sfogliatella
Mostaccioli
Fritti di latte

Execution of Dishes at ReD Academy

Following the collection of recipe data, dishes were reproduced by a team of professional chefs at the ReD Academy Culinary School in Treglio (Chieti, Italy) to measure the ingredients used before, during, and after cooking. Versions of each recipe were compared, and the most representative synthetic version of traditional dishes was defined, considering the type and quantity of ingredients, preparation, and cooking techniques (type, temperature, and utensils). Ingredients were sourced from local supermarkets, fruit vendors, butchers, and fishmongers.

Ingredient weighing was meticulously performed using two electronic kitchen scales, both subjected to rigorous precision and accuracy checks before use. Before beginning the preparation of each dish, we calibrated the scales using test weights ranging from 10 to 200 grams. The scale used, the Smart Weigh- Model PL11B, with a resolution of 1 gram, achieved impeccable results across four weight ranges: 10 g, 20 g, 100 g, and 200 g, conducting three independent measurements for each range to ensure the consistency of the results. The mini scale, used for weighing lighter ingredients such as salt and spices, with a resolution of 0.01 grams, demonstrated an accuracy of 99.86% and a precision

of 99.96%, with a mean absolute error (MAE) and a greatest possible error (GPE) of 0.01 grams for weighing 10 grams.

In the dish preparation process, ingredients were weighed in their raw state and freed from any non-edible waste. For specific preparations, such as those including bones, these were removed by post-cooking as indicated in the recipes. Weighing was meticulously documented for both solids and liquids, expressed in grams. During preparation, intermediate weighings were conducted to monitor changes in weight due to processes like boiling, culminating in the recording of the final weight of the completed dishes and individual reference portions.

Recipe Cards and Macronutrient Evaluation of the Abruzzese Apennines

Below are the recipe cards for Centenarians living in the mid-high altitude towns of the Abruzzese Apennines: Sheep in Callara Pot, Omelette with Orapi, Mazzarelle, Scrippelle 'mbusse (soaked/wet), Eggs and Onion, Quagliatelli, Bean and Potato Soup, Cute de Majo (May harvest), Cod and Onion, Horse, Mostaccioli, Pupa, Lamb with Water and Salt, Omelette with Avezzano Potatoes and Peppers, Fuje Strascinat, Bean and Chestnut Soup, Pasta with Voliche, Pastuccia, Sagn e Recell, Sagne fritte e b(basta), Tripe with Beans.

Pecora alla callara

Ingredients (4 serving portions)

Sheep meat: deboned sheep (leg) 400g, laurel, rosemary, tap water 2 liters.

Sheep in oil sauce: tomato sauce 500g, oregano, laurel, rosemary.

Salt, tap water.



Preparation:

Cut the sheep into pieces like a stew, put the meat in a pot with cold water, bay, and rosemary, boil it, remove the foam that forms, and let it boil for about two hours, when the meat is tender, it is ready. Aside in a pan put oil with oregano, bay, and rosemary, pour the sheep in the oil, and brown a little, add tomato sauce if it needs a bit of cooking broth, salt, and cook until the sauce is not withdrawn.

Nutritional properties:

Allergen content



Environmental impact

6,47 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Deboned sheep (leg) 240g, 2.53g laurel, 3.96g rosemary, 2061 liters tap water.

-Sheep in oil sauce 18.3g, tomato sauce 208.5g, oregano 2.82g, bay 1.25g, rosemary 2.6g, salt 2.5g, tap water 85g.

Typical values	Per 100g	Per serving portion (289)	%DRVs * per serving portion
Energy (kcal)	175	505	25.2
Protein (g)	18.39	53.15	83**
Fat (g)	8.99	25.98	11.7
of which saturated (g)	1.77	5.12	2.3
Carbohydrates (g)	3.63	10.49	2
of which sugars (g)	3.63	10.49	2
Fiber (g)	1.41	4.07	16.3
Sodium (mg)***	80.28	232	11.6 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§] % WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Frittata con orapi

Ingredients (4 serving portions)

- Orapi 20 g
- Omelet: eggs 2, a little extra virgin olive oil, salt: a hint, half of poached garlic, pecorino cheese grated 40g.



Preparation:

- Orapi: Wash the orapi with running water, blanch the orapi for a few minutes, squeeze them, and cut them into pieces.
- Omelet: Sauté the orapi in a pan with oil and poached garlic, after a few seconds remove the garlic, apart from beating eggs with pecorino, pour everything on the orapi, and cook the omelet on both sides for 7 minutes.

Nutritional properties:

Allergen content



Environmental impact

0,42 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

- Orapi 49 g,

-Omelet: eggs 119g, oil 19.4g, salt 1g, garlic with peel (only for perfuming) 4g, pecorino cheese grated 40g.

Typical values	Per 100g	Per serving portion (82.7)	%DRVs * per serving portion
Energy (kcal)	301	249	12.5
Protein (g)	16.17	13.37	20.9**
Fat (g)	25.90	21.42	9.6
of which saturated (g)	8.50	7.03	3.2
Carbohydrates (g)	0.91	0.75	0.1
of which sugars (g)	0.17	0.14	0.0
Fibre (g)	0.56	0.46	1.9
Sodium (mg)***	563	466	23.3 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Mazzarelle

Ingredients (1 serving portions)

-Lamb coratella: 250g; heart: a little, liver: a little, lung: a little, Bowel 2, vinegar 1 glass, salt, lemon half.

-Vegetable: wild beet 50 g, parsley, fresh garlic 1, fresh onion leaves 1, celery 1 coast, marjoram 3 leaves, a drop of water.

-To cook Mazzarelle: Extra virgin olive oil, water, white wine.

Preparation:

- Lamb Coratella: clean the guts, cut them vertically, rinse under running water, and put them. Clean the casings, cut them vertically, rinse under running water, put them in a container with glasses of vinegar, and salt, and rub well the casings until they are clear, repeat the operation, and leave them to soak with vinegar and lemon.

Cut the coratella into strips and dry it.

-In a bowl put marjoram, parsley, fresh garlic, and coratella cut into strips.

Spread the leaf of endive, put a little bit of wild beet to raw, filled with the smells of chopped and coratella, close forming a roll, and tighten everything with the guts.

- In a pan put oil and brown the mazzarelle, just fry, smoke with a little white wine, and let cook, add a little water, brown very well.



Nutritional properties:

Allergen content



Environmental impact

1,04 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Coratella: of lamb: 254g, heart 57g, liver 101g, lung 97g, clean casings 32g, salt 1g.

-Vegetable smooth scarola 7.9 g, wild beet 2.3g, parsley 2.2g, fresh garlic 2.3g, onion 10.6g, celery 11g, marjoram 0.8g.

-To cook mazzarelle: extra virgin olive oil 9g, water 10g, white wine 41g.

Typical values	Per 100g	Per serving portion (57)	%DRVs* per serving portion
Energy (kcal)	580	330	16.5
Protein (g)	38.19	21.77	34.0**
Fat (g)	46.05	26.25	11.8
of which saturated (g)	18.29	10.43	4.7
Carbohydrates (g)	3.24	1.85	0.3
of which sugars (g)	3.22	1.84	0.3
Fibre (g)	0.57	0.32	1.3
Sodium (mg)***	314	179	9.0 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged 18-59 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Scrippelle 'mbusse

Ingredients (2 serving portions)

-Scrippelle: 2 eggs, flour 00 38g, a little of tap water

-For the broth: one piece of veal about 250g, half celery, half carrot, half potato, 2 ripe cherry tomatoes, half onion, and a pinch of salt.

-For filling: 100g of grated parmesan cheese.

Preparation:

-Scrippelle: put together all the ingredients, knead. The dough must be liquid. Pour a ladle of dough into a small pan, cook for a few minutes on both sides. Let rest 20 minutes. Use a pan 20 - 25 cm in diameter.

-For the broth: put the chicken (or veal) into pieces, after having washed them well and put in a pot with celery, carrot, onion, tomatoes and potatoes, cover everything with cold water; cook on low heat for two hours, salt.

-To grease the pan: use extra virgin olive oil.

-To fill the skittles: sprinkle on each crepe a little grated parmesan cheese and roll them very tight.

Nutritional properties:

Allergen content



Environmental impact

14.95 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Scrippelle: whole hen eggs 227.6g, flour 00 58g, tap water 85g

-Broth: 600g tap water, 52.6g veal piece, 5g celery, 10.6g carrot, 23.2g potatoes, 12.8g ripe cherry tomatoes, 15g onion, salt up to 4g

-To grease the pan: extra virgin olive oil 4.2g

-For filling scrippelle: grated parmesan cheese 127.2g



Typical values	Per 100g	Per serving portion (403.6g)	%DRVs * per serving portion
Energy (kcal)	283	1142	57
Protein (g)	21.8	88	140-163**
Fat (g)	16.8	67.9	31
of which saturated (g)	8.7	35.1	16
Carbohydrates (g)	11.7	47.3	9
of which sugars (g)	0.6	2.4	0
Fiber (g)	1.2	5	20
Sodium (mg)***	963.6	3889	194 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Uova e cipolla

Ingredients (2 serving portions)

-Onions: 2 medium-sized white onions, 200 g of sheep's ricotta, 2 eggs, 60 g of grated parmesan cheese, half a sweet bell pepper, tap water, and a pinch of salt.



Preparation:

Cut the onion into slices. Add salt and water, cook the onion. When the water has evaporated add chopped sweet pepper. Add the ricotta cheese cut into large pieces and cook for a few minutes. Apart from beating eggs and parmesan cheese, incorporate to ricotta, and finish cooking until the egg is completely coagulated.

Nutritional properties:

Allergen content



Environmental impact

3.84 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation:

-white onions 230g, sheep ricotta 201.7g, eggs 98.5g, grated parmesan 73.1g, sweet bell pepper 0.6g, tap water 502.6g, salt 2.97g.

Typical values	Per 100g	Per serving portion (251.5g)	%DRVs * per serving portion
Energy (kcal)	156	393	20
Protein (g)	11.6	29.1	46-54**
Fat (g) of which saturated (g)	10.4 6.3	26.3 15.8	12 7
Carbohydrates (g) of which sugars (g)	4.3 4.3	10.8 10.8	2 2
Fiber (g)	0	1	5
Sodium (mg)***	153.21	385.3	19 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Quagliatelli

Ingredients (4 serving portions)

- Dough: "flour 00" 150g, tap water, flour (for spreading dough).
- Sauce: extra virgin olive oil, garlic half, celery half, carrot half, basil, skinned, salt, beans Borlotti cooked 200g, tap water for cooking pasta, salt.
- To garnish: grated pecorino cheese



- Dough: After making the dough, spread the dough and form long strips with a thickness of 3 mm and 5 cm wide. Put one on top of the other strips of pasta and cut out strips of 5 cm.
- Sauce: fry oil and garlic with peeled season, apart from mixing celery, carrot, and basil, and put in the sauce after a few minutes of cooking add beans. Boil the pan with water, and salt, and pour the quagliatelli. when the pasta comes to the surface, it is ready, drain and pour in the sauce with beans, and add pecorino grated cheese.

Nutritional properties:



Environmental impact

0,30 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

- Dough: flour 00 171g, tap water 93g, flour (for spreading dough) 12g.
- Sauce: oil 34,7g, garlic 2.43g, celery 25.8g, carrot 42.7g, basil 2.75g, peeled 297g, salt 2.8g. Borlotti cooked 201g.
- Tap water for cooking pasta 2315g, salt 5g, pecorino to garnish 40g.

Typical values	Per 100g	Per serving portion (247.4)	%DRVs * per serving portion
Energy (kcal)	151	373	18.7
Protein (g)	5.94	14.70	23**
Fat (g)	5.56	13.76	6.2
of which saturated (g)	1.44	3.56	1.6
Carbohydrates (g)	20.30	50.22	9.4
of which sugars (g)	2	4.95	0.9
Fibre (g)	1.97	4.87	19.5
Sodium (mg)***	89.68	221.87	11.1 ⁵

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

⁵% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Minestra fagioli e patate

Ingredients (2 serving portions)

Soup: extra virgin olive oil, celery half, carrot half, onion half, potato without skin half, pork fat 30g, garlic half, cooked beans borlotti 175g, Vialone Nano rice 150g, Parmigiano peel 25g, salt, tap water 1.5 l.



Preparation:

-In a pan, sauté oil, celery, carrot, and onion, beat the piece of bacon with garlic and fry, add chopped potatoes and water that must be brought to boiling, add beans, when the water boils pour the rice and cook, for the last 5 minutes, add Parmigiano zest.

Nutritional properties:

Allergen content



Environmental impact

0,43 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

Soup: oil soup Evo 32g, celery 23g, carrot 29g, onion 25g, potato without peel 69g, pig fat 30g, garlic 5g, cooked beans borlotti 175g, Vialone Nano 150 rice g, parmigiano skin 25g, salt 4g, tap water 755g.

Typical values	Per 100g	Per serving portion (455.5)	%DRVs * per serving portion
Energy (kcal)	156	710	35.5
Protein (g)	4.15	18.90	29.5**
Fat (g)	7.86	35.80	16.1
of which saturated (g)	1.65	7.52	3.4
Carbohydrates (g)	18.25	83.13	15.5
of which sugars (g)	0.78	3.55	0.7
Fibre (g)	1.38	6.29	25.1
Sodium (mg)***	25.14	114	5.7 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Cute de majo

Ingredients (2 serving portions)

-Legumes : Maize 100g; beans borlotti 100g; chickpeas 100g, and tap water and salt.



Preparation:

- Soak the grain, beans, and dried chickpeas for a day. Rinse them under running water, cook them in a pot for at least 2/3 hours, and season with salt.
- *It was not used to season with oil only salt

Nutritional properties:

Allergen content



Environmental impact

0,28 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Dry corn grain 100g, dried borlotti beans 100g, dried chickpeas 100g, tap water 3179g and salt 3g.

Typical values	Per 100g	Per serving portion (403)	%DRVs * per serving portion
Energy (kcal)	142	474	23.7
Protein (g)	7.56	25.22	39.4**
Fat (g)	1.82	6.08	2.7
of which saturated (g)	0.29	0.97	0.4
Carbohydrates (g)	25.47	85.07	15.9
of which sugars (g)	1.47	4.91	0.9
Fiber (g)	4.94	16.50	66
Sodium (mg)***	126.80	423.51	21.2 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Baccalà e cipolla

Ingredients (1 serving portion)

-Cod fillet: defrosted cod fillet, 1 blonde onion, extra virgin olive oil, and a pinch of salt.



Preparation:

-Put oil in a pan to fry the cod, remove the cod once browned, and put the finely chopped onion. When the onion is finished cooking, add cod and finish cooking, adding a little salt.

Nutritional properties:

Allergen content



Environmental impact

0,23 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Defrosted cod fillet 96g, blonde onion 122g,
extra virgin olive oil 19g, salt 1g.

Typical values	Per 100g	Per serving portion (171)	%DRVs * per serving portion
Energy (kcal)	69	118	14,7
Protein (g)	12.83	21.94	34.3**
Fat (g)	11.72	20.04	9.0
of which saturated (g)	1.91	3.27	1.5
Carbohydrates (g)	4.06	6.94	1.3
of which sugars (g)	4.06	6.94	1.3
Fiber (g)	0.71	1.21	4.9
Sodium (mg)***	1154	1973	98.7 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§] % WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Cavallo

Ingredients (4 serving portions)

- *Dough*: flour 0, eggs, sugar, butter, potato starch, lemon grater, sachets of yeast.
- *To garnish*: 2 eggs with shells, and colorful sugar.



Preparation:

Beat eggs and sugar, and grate lemon. Add all the other ingredients, mix, cover, and refrigerate for at least one hour. Put the egg in the middle of the pupa to decorate and add the colored sugar. Form the pupa and the horse. Bake in the oven at 180 ° for 15 minutes.

Nutritional properties:

Allergen content



Environmental impact

0,19 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-*Dough*: "flour 0" 500g, eggs 153g, sugar 200g, butter 102g, potato starch 25g, lemon grater 1.2g, yeast sachets 24g.

-*To garnish*: 2 raw eggs with shell 115g, colored sweets 5g.

Typical values	Per 100g	Per serving portion (89)	%DRVs* per serving portion
Energy (kcal)	358	319	15.2
Protein (g)	9.12	812	12.1**
Fat (g)	10.91	9.71	4.2
of which saturated (g)	5.56	5.03	2.2
Carbohydrates (g)	59.57	53.02	9.5
of which sugars (g)	21.83	19.43	3.5
Fibre (g)	1.39	1.24	4.7
Sodium (mg)***	38.32	34.10	1.6 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§] % WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Mostaccioli

Ingredients (6 serving portions)

- **Dough:** flour, honey 150g, roasted almonds 150g, powder bitter cocoa 15g, sugar 150g, seed oil, lemon peel, liqueur Strega, ammonia, ground cinnamon, ground cloves of the carnation, nutmeg, dark chocolate 125g.



Preparation:

In a bowl put flour, sugar, and cocoa. Toast the unpeeled almonds for 5/10 min in the oven. When they are cold, chop them coarsely. Add all the ingredients (except for the chocolate that we will use for the coating). When the mostaccioli are cooked, let them cool.

Melt the chocolate in a bain-marie and cover each mostaccioli. Arrange the frosted mostaccioli and let the chocolate dry.

Roll out the dough with a rolling pin (I keep a thickness of about 1 cm) and cut out the mostaccioli with a turnip-shaped mold. Place them on a baking sheet lined with baking paper and let them cook at 160° for about 20 min. Finally, cover them with melted dark chocolate, and let them dry.

Nutritional properties:

Allergen content



Environmental impact

0,06 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-**Dough:** flour 125g, honey 47g, toasted almonds 38g, bitter cocoa powder 10g, sugar 45g, oil seeds 56g, lemon peel 1.75g, liqueur Strega 8g, ammonia 2,5g, cinnamon 1g, cloves 1g, nutmeg 1g, dark chocolate 79g.

Typical values	Per 100g	Per serving portion (171)	%DRVs * per serving portion
Energy (kcal)	504	191	9.6
Protein (g)	7.96	3.02	4.7**
Fat (g)	28.29	10.72	4.8
of which saturated (g)	6.72	2.55	1.1
Carbohydrates (g)	57.60	21.83	4.1
of which sugars (g)	33.95	12.87	2.4
Fiber (g)	4.68	1.77	7.1
Sodium (mg)***	31.97	12.12	0.6 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§] % WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Pupa

Ingredients (4 serving portions)

- *Dough*: flour 0, eggs, sugar, butter, potato starch, lemon grater, sachets of yeast.
- *To garnish*: 2 eggs with shells, and colorful sugar.



Preparation:

Beat eggs and sugar, and grate lemon. Add all the other ingredients, mix, cover, and refrigerate for at least one hour. Put the egg in the middle of the pupa to decorate and add the colored sugar. Form the pupa and the horse. Bake in the oven at 180 ° for 15 minutes.

Nutritional properties:

Allergen content



Environmental impact

0,18 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-*Dough*: "flour 0" 500g, eggs 153g, sugar 200g, butter 102g, potato starch 25g, lemon grater 1.2g, yeast sachets 24g,

-*To garnish*: 2 raw eggs with shell 115g, colored sweets 5g.

Typical values	Per 100g	Per serving portion (85)	%DRVs * per serving portion
Energy (kcal)	358	304	15.2
Protein (g)	9.12	7.75	12.1**
Fat (g)	10.91	9.27	4.2
of which saturated (g)	5.56	4.80	2.2
Carbohydrates (g)	59.57	50.63	9.5
of which sugars (g)	21.83	18.56	3.5
Fibre (g)	1.39	1.18	4.7
Sodium (mg)***	38.32	32.57	1.6 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Agnello acqua e sale

Ingredients (1 serving portions)

-Meat: Lamb stew 500g, white onion 1, a little oil, a little salt a bay leaf, 3 marjoram leaves (for perfuming), laurel (to smell), tap water.



Preparation:

-Fry a lot of oil and onion in the pan. When the onion has wilted add water, boil, add the lamb stew, combine bay leaf, marjoram and salt; after cooking the lamb must remain moist and tender.

Nutritional properties:

Allergen content



Environmental impact

5,93 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Ragout of lamb 230g, white onion 34g, oil 33g, salt 3g, laurel (for perfuming) 1.59g, marjoram (for perfuming) 0.24g, tap water 896g.

Typical values	Per 100g	Per serving portion (230)	%DRVs * per serving portion
Energy (kcal)	292	673	33.6
Protein (g)	20.15	46.35	72.4**
Fat (g)	23.20	53.36	24
of which saturated (g)	6.61	15.20	6.8
Carbohydrates (g)	0.84	1.93	0.4
of which sugars (g)	0.84	1.93	0.4
Fibre (g)	0.15	0.35	1.4
Sodium (mg)***	91.62	210	10.5 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§] % WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Frittata con patate di Avezzano e peperoni

Ingredients (2 serving portions)

-Omelet: extra virgin olive oil, 1 red pepper, 2 medium-sized Avezzano potatoes, a pinch of salt, 3 eggs, and grated Parmigiano Reggiano.



Preparation:

- Peel the potatoes, cut into slices, and wash them; wash the peppers and clean them.
- Cut the peppers into slices. Fry the peppers and potatoes together in hot oil and season with salt. Apart from beating eggs with parmesan and pour everything in a pan on potatoes and peppers, mix and cook on both sides.

Nutritional properties:

Allergen content



Environmental impact

2,57 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Omelet: extra virgin olive oil 32.1g, pepper 125g, potatoes Avezzano 150g, salt 4.8g, eggs 153g, grated parmesan cheese 29.2g

Typical values	Per 100g	Per serving portion (151.5g)	%DRVs * per serving portion
Energy (kcal)	248	376	19
Protein (g)	10.9	16.5	26-31**
Fat (g) of which saturated (g)	18.3 5.2	27.7 7.9	12 4
Carbohydrates (g) of which sugars (g)	10.6 1.9	16 2.9	3 1
Fiber (g)	1.6	2.4	10
Sodium (mg)***	129.71	196.5	10 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Fruje strascinati

Ingredients (2 serving portions)

-*Verza*: half a cabbage, 1 clove of garlic, some white wine, some water, some oil, bay leaves (just to flavor), black pepper in grains, a pinch of salt.



Preparation:

- Cut the cabbage coarsely and wash it, in a pan of aluminum put oil, white wine, garlic, bay leaves, some pepper and water.
- You boil water and wine and oil, immerse the vegetable, cover with lid, salt chili at will, you must withdraw water, fry well, almost burn the vegetable.

Nutritional properties:

Allergen content



Environmental impact

0,85 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Cabbage 288.1g, clove of garlic 3.9g, white wine 55g, tap water 310.2g, extra virgin olive oil 20.8g, bay leaves (only for seasoning) 1.28g, black pepper in grains 0.3g, salt 1.9g

Typical values	Per 100g	Per serving portion (121g)	%DRVs * per serving portion
Energy (kcal)	120	145	7
Protein (g)	2.6	3.2	5-6**
Fat (g)	8.8	10.6	5
of which saturated (g)	1.4	1.7	1
Carbohydrates (g)	3.5	4.2	1
of which sugars (g)	3.2	3.8	1
Fiber (g)	3	4	16
Sodium (mg)***	29	35.1	2 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

§% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Minestra fagioli e castagne

Ingredients (2 serving portions)

- Beans: beans borlotti 500 g, chestnuts 350 g, celery a sprout, chestnuts, bay 2 leaves, extra virgin olive oil, a pinch of salt, tap water in which to cook soup
- For garnish: oil to season the final dish



Preparation:

Soak the beans in water the night before, drain them, rinse them, put them in water and boil for a few minutes, change the water and boil again with boiling water. Roast the chestnuts for about 35 minutes in a 180° oven, peel them, add to the beans, salt, bay and chestnuts, bring to the boil like a soup, it must be not too liquid, garnish with oil.

Nutritional properties:

Allergen content:



Net quantities (g) used for nutrient calculation

-beans borlotti dried beans 250
chestnuts with skin 185g, celery to flavor 15g, bay leaf to flavor 1g, salt 3g, oil for seasoning 12g,
-tap water to blanch beans for a few minutes 3091
-tap water in which to cook soup 2723

Typical values	Per 100g	Per serving portion (478)	%DRVs * per serving portion
Energy (kcal)	105	500	25
Protein (g)	5.46	26.10	40.8**
Fat (g)	2.04	9.75	4.4
of which saturated (g)	0.38	1.82	0.8
Carbohydrates (g)	17.14	81.93	15.3
of which sugars (g)	2.14	10.23	1.9
Fibre (g)	5	23.90	95.6
Sodium (mg)***	3.77	18.02	0.9 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Pasta con le voliche

Ingredients (Serving portions)

-Pasta: "flour 00" 250g, eggs 2, tap water.

-Seasoning: extra virgin olive oil, carrot half, celery, onion, boiled voliche (orapis) 75g, tomato puree 250g, a bit of pepper, a bit of nutmeg, a bit of salt, a bit of onion.
Tap water for pasta, and salt.



Preparation:

-Pasta: Pour the flour, add eggs, knead, and let rest for half an hour. The sheet can be pulled with the machine, 1.5 cm thick, cut the sheet with a knife giving the shape of rhombuses or coarse squares here are the miscuts.
- Boil the mountain vegetables. Mix carrot, celery, and onion and fry in a pan with oil, add to the base the chopped voliche, add simple tomato, pepper, nutmeg, and salt, and cook. Once the water boils, put the pasta then drain when it comes to the surface and sauté in a pan with the voliche.

Nutritional properties:

Allergen content



Environmental impact

0,56 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Pasta: "flour 00" 250g, eggs 58g, tap water 72g.
extra virgin olive oil 46g, carrot 32g, celery 14g, onion 19g,
Voliche (Orapi) blanched 76g, tomato paste 248,7g, pepper 0.4g, nutmeg 0.6g, salt 1.3g, onion 19g,
-Tap water for pasta 1391g, salt 6g

Typical values	Per 100g	Per serving portion (403)	%DRVs * per serving portion
Energy (kcal)	174	701	35.1
Protein (g)	5.06	20.39	31.9**
Fat (g)	6.69	26.96	12.1
of which saturated (g)	1.23	4.96	2.2
Carbohydrates (g)	24.15	97.32	18.2
of which sugars (g)	2.17	8.75	1.6
Fibre (g)	1.73	6.97	27.9
Sodium (mg)***	28.59	115	5.8 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Pastuccia

Ingredients (6 serving portions)

Dough: "flour 0" 150g, dried figs 10, sourdough 13g, a pinch of salt, a little lukewarm water, a little olive oil.



Preparation:

- Make a water fountain with flour, warm water, and yeast mother, knead until you get a soft loaf, let it rest and let rise until doubled.
- Soak the figs, once soaked dry them and cut into four pieces.
- Once you have doubled the dough, grease a round baking sheet and spread the mass on the bottom, then a layer of figs and still leavened mass above, grease very well the final layer and let rise for another 1 hour. Bake at 160° for 30 minutes.

Nutritional properties:

Allergen content:



Environmental impact

0,03 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Dough: "flour 0" 146.7 g, dried figs 88g, sourdough 12.7g, salt 1.96g, warm water 100.7g, extra virgin olive oil 1.96g.

Typical values	Per 100g	Per serving portion (59,3)	%DRVs * per serving portion
Energy (kcal)	225	134	6.7
Protein (g)	5.92	3.51	5.5**
Fat (g)	1.75	1.04	0.5
of which saturated (g)	0.28	0.17	0.1
Carbohydrates (g)	47.79	28.34	5.3
of which sugars (g)	15.34	9.10	1.7
Fiber (g)	4.51	2.67	10.7
Sodium (mg)***	33.94	20.13	1 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Sagne e recell (*cardoncello mushroom*)

Ingredients (4 serving portions)

-Dough for pasta: 200 gr flour (100 gr white flour and 100 gr wheat or whole wheat flour), 1 egg * (weigh egg, if 70 gr one egg, there will be the same of water), 75 ml water.

- Condiment: - 250 g of Cardoncello mushrooms called recell, a little oil, a little parsley, garlic 1, grated parmesan cheese 50 g.

- Tap water to cook pasta



Preparation:

- Knead flour, eggs and water. Roll out the dough with a rolling pin, the dough should be 1.5 or 2 mm thick, wrap the pastry around the rolling pin and cut it in half with the knife, the dough opens, cut the sagne 10 cm long further, cook, drain, Pour into a frying pan, sauté with mushrooms and season with parmesan.
- In a frying pan fry the mushrooms with oil and garlic, once ready pour sagne into the pan and sauté them with the mushrooms.

Nutritional properties:

Allergen content



Environmental impact

0,66 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Dough for pasta: white flour 00 104g, durum wheat flour 80g, eggs 41.6g, tap water 61g.

-Seasoning: Cardoncello mushroom (called recell) 241g, oil 25g, parsley 2.09g, garlic 3.52g, parmesan 52g, salt 2 g, tap water to boil pasta 1841 l.

Typical values	Per 100g	Per serving portion (305)	%DRVs * per serving portion
Energy (kcal)	173	528	26.4
Protein (g)	8.09	24.71	38.6**
Fat (g)	7.38	22.55	10.1
of which saturated (g)	2.51	7.67	3.5
Carbohydrates (g)	19.52	59.63	11.2
of which sugars (g)	0.82	2.51	0.5
Fiber (g)	1.61	4.92	19.7
Sodium (mg)***	63	192	9.6 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

§% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Sagne fritte e b (basta)

Ingredients (4 serving portions)

- Dough for pasta: water bottle 75 ml, flour semolina durum wheat 150g, a little bit of salt.
- Condiment: abundant extra virgin olive oil, wild asparagus dressing 75g, half garlic, a little saffron, and a little salt.



Preparation:

- On a pastry flour and water at a time and salt, knead. Pull the dough with a pasta machine, cut a sheet of pastry into strips 5 cm wide and give the shape of the sagna, cutting the strips with a knife.
- In a frying pan pour extra virgin olive oil and garlic, wild asparagus and saffron and fry, meanwhile when the water boils, pour the sagne in salted water, after draining pasta, pour it into the frying pan and fry for a few minutes (oil must be boiling and the pasta should not be brodosa but rather dry).

Nutritional properties:

Allergen content



Environmental impact

0,19 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Dough: water 80g, flour semolina durum wheat 150g, salt 3g.
-Seasoning: wild asparagus 42g, extra virgin olive oil 57g, garlic 2.84g, saffron 25g, salt 2g, tap water to boil pasta 1453g, salt 3g.

Typical values	Per 100g	Per serving portion (202)	%DRVs * per serving portion
Energy (kcal)	276	558	27.9
Protein (g)	5.35	10.81	16.9**
Fat (g)	14.67	29.63	13.3
of which saturated (g)	2.43	4.91	2.2
Carbohydrates (g)	32.66	65.97	12.3
of which sugars (g)	3.73	7.53	1.4
Fiber (g)	1.80	3.64	14.5
Sodium (mg)***	14.08	28.44	1.4 ^δ

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

^δ% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Trippla con fagioli

Ingredients (2 serving portions)

-Tripe: beef tripe 250 g,

-Dressing: a little oil, onion, celery, 500 g tomato puree, 2 eggs, grated parmesan cheese, a little salt, boiled beans, water for sauce.

To garnish: basil leaves



Preparation:

Soak tripe in boiling water, hunt after having boiled for 1 minute, and dry, cut into pieces. Put oil, onion, tomato, and celery sauce in a pan, dilute with a little water, add tripe, cook for 15 minutes, and add beans and let taste. Apart from beating the eggs and grated parmesan cheese in a container and pouring on tripe, cook the eggs and adjust the basil.

Nutritional properties:

Allergen content



Environmental impact

18.20 KgCO₂ eq/serving portion (g)

Net quantities (g) used for nutrient calculation

-Tripe: beef tripe 250g

-Condiment: oil 39g, onion 86g, celery 64g, tomato puree 500g, eggs 107g, grated parmesan cheese 50g, salt 4g, beans cooked 101g, water for sauce 92g

-For garnish: basil 0.72g

Typical values	Per 100g	Per serving portion (405g)	%DRVs * per serving portion
Energy (kcal)	153	618	31
Protein (g)	10.8	43.7	69-81**
Fat (g)	9.5	38.4	17
of which saturated (g)	3.2	12.9	6
Carbohydrates (g)	5.8	23.5	4
of which sugars (g)	3.1	12.5	2
Fiber (g)	2.1	8.4	34
Sodium (mg)***	104.48	423.1	21 [§]

*Dietary References Value on an average adult (2000 kcal)

** % Dietary References Value for males-females aged >60 years

*** Natural sodium content in food

[§]% WHO recommendation for cardiovascular prevention

Graphic design and photo editing: Antonello Vespoli and Elvira Desiata

Allergen Assessment in Traditional Recipes of the Abruzzese Apennines

The allergen content of traditional recipes was evaluated using the "Healthy Meals" web application, consistent with previous studies (Mandracchia et al., 2021), developed by a team of nutritionists at the Rovira i Virgili University (Tarragona, Spain) within the context of a Europe-funded project called PECT-Turistic en Família. This tool has recently been evaluated and validated, proving to be usable, of high quality, and effective in aiding food industry professionals in identifying the 14 most common food allergens (Mandracchia et al., 2022). The identified allergen content relates to the composition of the food ingredients used and does not consider cross-contamination that may occur during cooking.

CREA Analysis in Traditional Recipes of the Abruzzese Apennines

The nutritional content of the ingredients was analyzed by the Research Center for Food and Nutrition of the Council for Agricultural Research and Economics (CREA), using the FOODCONS database based on the cFCDB (Leclercq et al., 2009). The assessment of macro and micronutrients was conducted following the standardized EUROFIR procedures, without calculating the retention factor (Bell et al., 2006; Heli Reinivuo et al., 2007). Given the variety of dish types (appetizers, first courses, main courses, side dishes, and desserts), the nutritional content of portions for each dish was compared with the Dietary Reference Values (DRV) for the Italian population over 60 years, to better understand the quality of the nutritional contribution of traditional Abruzzese cuisine. The nutritional content information for 100 g of each recipe is described in detail in individually prepared recipe cards and includes data on the energy value of each dish. The nutritional variability of the analyzed dishes is significantly influenced by the nature of the ingredients used and the cooking techniques employed. For the final calculation of the post-processing nutritional content, our study excluded the nutrient retention factor, preferring to use the yield factor, which considers weight retention.

The cards illustrate the contribution of available proteins, fats, carbohydrates (CHO), fiber, sodium, and the total energy of the recipe.

Micro/Macronutrient Evaluation Cards of Apennine Abruzzese Recipes

Moisture, energy, macronutrients, and alcohol content in 100g of recipe.

	Moisture (g)*	En (Kcal/ 100g)	Pro (g/100g)	Fat (g/100g)	SFA (g/100g)	Oleic acid (g/100g)	MUFA (g/100g)	Linoleic acid (g/100g)	Linolenic acid (g/100g)	PUFA (g/100g)	Chol (mg/100g)	CHO (g/100g)	Starch (g/100g)	Sugars (g/100g)	Dietary fibre (g/100g)	Alcohol (g/100g) **
<i>Frittata con orapi</i>	52	301	16,17	25,90	8,50	11,86	12,55	1,64	0,30	2,21	291,92	0,91	0,68	0,17	0,56	0,00
<i>Baccalà e cipolla</i>	69	172	12,83	11,72	1,91	8,04	8,35	0,78	0,08	1,19	28,05	4,06	0,00	4,06	0,71	0,00
<i>Cavallo e pupa</i>	22	358	9,12	10,91	5,65	2,68	3,04	0,60	0,14	0,78	120,22	59,57	34,34	21,83	1,39	0,00
<i>Cute de majo</i>	60	142	7,55	1,82	0,29	0,42	0,42	0,80	0,15	0,95	0,00	25,47	21,83	1,47	4,94	0,00
<i>Mazzarelle</i>	9	580	38,19	46,05	18,29	17,49	17,31	3,20	0,30	4,18	525,47	3,24	0,02	3,22	0,57	0,00
<i>Minestra fagioli e patate</i>	66	156	4,15	7,86	1,65	5,14	5,34	0,56	0,10	0,75	2,46	18,25	15,88	0,78	1,38	0,00
<i>Mostaccioli</i>	3	504	7,96	28,29	6,72	11,88	12,35	7,30	1,05	8,42	0,00	57,60	21,50	33,95	4,68	0,00
<i>Quagliatelli</i>	66	151	5,94	5,56	1,44	3,02	3,15	0,42	0,07	0,57	4,47	20,30	16,63	2,00	1,97	0,00
<i>Sagne fritte e b</i>	47	276	5,35	14,67	2,43	10,17	10,55	1,09	0,18	1,46	0,00	32,66	26,41	3,73	1,80	0,00
<i>Agnello acqua e sale</i>	53	292	20,15	23,20	6,61	13,49	14,05	1,19	0,32	1,69	70,00	0,84	0,00	0,84	0,15	0,00

En: Energy; Pro: Protein; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; Chol: Cholesterol; CHO: Available Carbohydrates;

tr: trace amount of nutrient.

*water after cooking (EUROFIR, 2007)

**alcohol content after estimated evaporation process

Micronutrients content as minerals in 100g of recipe.

	Iron (mg/100g)	Calcium (mg/100g)	Potassium (mg/100g)	Phosphorus (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)
<i>Frittata con orapi</i>	2,11	204,49	274,29	312,12	2,10	36,79
<i>Baccalà e cipolla</i>	1,60	35,22	337,68	340,24	0,43	19,47
<i>Cavallo e pupa</i>	1,00	24,37	155,62	142,43	0,77	19,58
<i>Cute de majo</i>	2,67	42,34	397,03	170,25	1,26	62,63
<i>Mazzarelle</i>	20,16	45,97	822,69	609,30	5,82	55,24
<i>Minestra fagioli e patate</i>	0,84	54,92	205,15	74,47	0,59	45,18
<i>Mostaccioli</i>	2,26	49,96	241,46	141,19	1,06	70,37
<i>Quagliatelli</i>	0,95	48,84	261,09	90,64	0,57	51,57
<i>Sagne fritte e b</i>	1,33	17,71	198,77	85,00	0,32	35,65
<i>Agnello acqua e sale</i>	1,83	51,06	372,16	195,18	3,25	28,73

Micronutrients content as vitamins in 100g of recipe.

	Tiamin (mg/100g)	Riboflavin (mg/100g)	Niacin (mg/100g)	Ascorbic acid (mg/100g)	Pyridoxine (mg/100g)	Retinol Equiv. (mcg/100g)	Retinol (mcg/100g)	Beta Carotene (mcg/100g)	Vit E (mg/100g)	Vit D (mcg/100g)	Vit K (mcg/100g)	Vit B12 (mcg/100g)
<i>Frittata con orapi</i>	0,09	0,44	0,24	15,98	0,16	339,81	184,86	929,79	4,10	1,38	150,73	1,20
<i>Baccalà e cipolla</i>	0,04	0,18	1,71	3,57	0,20	0,33	0,00	2,11	2,90	0,00	7,19	0,56
<i>Cavallo e pupa</i>	0,16	0,13	0,60	0,13	0,10	137,06	126,08	65,80	0,64	0,53	1,00	0,35
<i>Cute de majo</i>	0,18	0,07	0,93	1,20	0,23	14,25	0,00	85,50	0,64	0,00	3,60	0,00
<i>Mazzarelle</i>	0,82	3,57	17,25	74,13	0,58	13352,16	13279,37	436,83	2,71	0,61	71,69	96,98
<i>Minestra fagioli e patate</i>	0,08	0,03	0,96	4,15	0,10	49,99	3,67	277,89	1,61	0,01	6,63	0,03
<i>Mostaccioli</i>	0,06	0,08	0,83	0,86	0,08	2,47	0,00	14,81	7,07	0,00	8,37	0,00
<i>Quagliatelli</i>	0,07	0,06	1,09	8,97	0,09	94,54	5,89	531,95	1,32	0,02	7,65	0,05
<i>Sagne fritte e b</i>	0,10	0,11	0,94	6,92	0,15	12,28	0,00	73,78	3,38	0,00	30,12	0,00
<i>Agnello acqua e sale</i>	0,15	0,28	6,07	0,74	0,32	0,43	0,00	2,74	3,35	0,40	8,73	2,77

Vit: Vitamin; tr: trace amount of nutrient.

Environmental Sustainability in Traditional Recipes of the Abruzzese Apennines

Environmental sustainability analysis was conducted by CREA. Carbon footprint data, representing greenhouse gas emissions (GHGE) responsible for climate change and expressed as kilograms of CO₂-equivalent (Kg CO₂-eq), were derived from the study "A multilevel carbon and water footprint dataset of food commodities" (Petersson et al., 2021). This took into account the Life Cycle Assessment (LCA) of food products up to the final product, excluding storage, transport, cooking, and other off-site production activities. The environmental sustainability analysis of 30 traditional recipes involved assessing the impact in terms of carbon dioxide equivalents (Kg CO₂ eq), starting from known values (Petersson et al., 2021; Ferrari et al., 2020) and calculating the impact per 100g and per portion of each recipe. Generally, traditional recipes showed moderate CO₂ eq values, being minimally processed and primarily composed of local products. Among the evaluated dishes, Tripe with Beans recorded the highest CO₂ eq value per portion, followed by Scrippelle 'mbusse, Sheep in Callara Pot, Lamb with Water and Salt, Eggs and Onion, Omelette with Avezzano Potatoes and Peppers, and Mazzarelle. Notably, ruminant livestock accounts for 73.5% of greenhouse gas emissions from livestock, increasing the environmental impact for meat and dairy products, whose production requires high milk usage. Higher impact values were also associated with particularly large portions; lower quantities were observed per 100g of recipe, suggesting that portion sizes and consumption frequency also influence the final environmental impact. On the other hand, food groups with moderate impact include olive oil, pasta, milk, and eggs, as observed for portions of Sagne e recell, Fuje strasciniti, and Pasta con le voliche. Moreover, lower greenhouse gas emissions stem from seasonal fruits and vegetables, legumes, potatoes, and cereals, as observed for vegetable-based first courses and sides, such as Sagne Fritte e (b) basta, Cod and Onion, Pastuccia, Pupa e Cavallo, and Mostaccioli. In this context, it has been demonstrated that foods frequently recommended in the Mediterranean diet, such as fruits and vegetables, also have lower environmental impacts, while foods recommended for less frequent consumption, such as red meat, also have higher environmental costs.

Isolation of Nanovesicles

Samples were centrifuged at 500×g for 10 minutes; the supernatants were filtered through 100 µm filters and serially centrifuged at 2000×g for 20 minutes to remove cellular debris, and then at 15,000×g for 30 minutes to eliminate the microvesicle-enriched fraction. The supernatants were then ultracentrifuged in a Sorvall WX ultracentrifuge (Thermo Fisher Scientific) at 110,000×g for 1 hour and 30 minutes to collect the nanovesicles. The pellet was resuspended in an appropriate buffer for subsequent analyses (Logozzi et al., 2021).

Western Blotting

Human satellite cells were cultivated in differentiation medium together to exosome derived from Ganoderma and Orapi for Western blotting analysis. After 7 days of differentiation, myotubes were lysed in RIPA buffer (89900 thermo scientific) supplemented with 1:100 protease inhibitor cocktail (Sigma), and 1:100 phosphatase inhibitor cocktail (Sigma). Protein extract was quantified by Micro BCA Protein Assay Kit (# 23235, Thermo scientific) following the manufacturer instructions. Total protein mixes (20µg) were separated on a 4-12% Bis-Tris Plus Gels (Cat.NW04120BOX, life technologies) and transferred to a nitrocellulose membrane (iBlot 2 Dry Blotting System (Cat. IB21001, life technologies). After blocking for 1 h at room temperature (RT) in 5% Skim-milk (Sigma) in TBS buffer supplemented with 0.1% Tween-20 (Sigma), the membrane was incubated overnight at 4 °C with primary antibodies, as follows: 1:1000 Myosin 4 Monoclonal Antibody (MF20), eBioscience (Catalog # 14-6503-82, invitrogen), 1:1000 MyoD (D8G3) XP Rabbit mAb (#13812, Cell Signalling Technology), 1:500 myogenin ((F5D): sc-12732 Santa Cruz Biotechnology, INC), 1:300 VDAC1 ((B-6): sc-390996 Santa Cruz Biotechnology, INC), 1:600 GAPDH ((6C5): sc-32233; Santa Cruz Biotechnology, INC). After incubation with anti-rabbit IgG, HRP-linked Antibody (diluted 1:2000) (#7074, Cell Signalling Technology) or anti-mouse IgG, HRP-linked Antibody (diluted 1:2000) (#7076, Cell Signalling Technology) for 1 h at RT. Immunodetection was performed with the SuperSignal West Pico PLUS Chemiluminescent Substrate (Cat # 34579, Thermo scientific). Pictures were taken at Uvitec (Cambridge, UK). Densitometry analyses were performed with Uvitec software (Di Filippo, 2016).

Subject Anamnestic Record and Informed Consent

All participants involved in the research protocol freely signed an informed consent and completed an anamnestic record. The informed consent and the anamnestic record are fundamental documents in research protocols concerning the information and acceptance of treatments by the patient and the collection of data on their medical history, respectively.

The anamnestic record is a document that gathers detailed information about a patient's medical history. This includes data on past illnesses, treatments and interventions undergone, allergies, reactions to medications, lifestyle habits, and information about health conditions present in the family. We required the anamnestic record to be filled out accurately as it was crucial to help us understand the patients' overall conditions and identify risk factors.

The informed consent is a document through which we informed the patient about the details of our research. This includes information about benefits, risks, available alternatives, and possible consequences of abstaining from treatment. This process respects the ethical principle of patient

autonomy, ensuring that the decision is made freely and with an adequate understanding of the information. Consent must be obtained without pressure or coercion, and the patient has the right to withdraw consent at any time.

This study was conducted following the Declaration of Helsinki and approved by the Bioethics Committee of the University of Chieti-Pescara. Informed consent was obtained from all subjects involved in the study.

Number of subjects who participated in the psychological tests and salivary tests

	experimental group	control group
Low Altitude	12	
Medium	12	
High	12	
Urban Area		12

The chart delineates the distribution of subjects who participated in psychological and salivary assessments, categorized by experimental and control groups across different environmental conditions. In the experimental group, 12 subjects each were allocated to low, medium, and high-altitude categories. In the control group, 12 subjects from an urban area were tested. This uniform distribution facilitates a controlled comparison between varying altitude environments within the experimental group and a comparative analysis between high-altitude and urban settings across the experimental and control groups.

Saliva Sample Collection

To assess the biological response to the Apennine diet and thus evaluate purely biological aspects in ultra-long-lived subjects from different geographical areas and altitudes, we adopted a non-invasive approach. Instead of resorting to blood sample collection or muscle biopsy, we opted for saliva sample collection, thus ensuring a zero-invasiveness method.

Subjects were instructed on the correct use of the “Salivette” device (Sarstedt, Nümbrecht, Germany) for collecting saliva: in the 30 minutes before saliva collection, they were to refrain from drinking, eating, smoking, brushing their teeth, and using mouthwash. Each Salivette was centrifuged (10 minutes at 3000 g) and yielded approximately 1 mL of saliva. Subsequently, the saliva was aliquoted and stored at -80°C until analyses and thawed only once before analysis. (Mrakic-Sposta et al. 2020; Giacon et al. 2022; Bosco et al. 2023).

Biochemical Assays

Appetite Hormones

Leptin levels were measured using an enzymatic immunoassay kit (ELISA) (cat. No. EH0216; FineTest, Wuhan, China), while ghrelin was determined through an enzyme-linked immunosorbent assay kit (cat. No. EH0355; FineTest, Wuhan, China). The analysis was performed following the manufacturer's instructions, and concentrations were measured spectrophotometrically at a wavelength of 450 nm by comparing the optical density of the samples against standard curves (Micarelli et al. 2022). Adipokines are believed to be involved in the pathogenesis of diabetes and depression. Some studies suggest that irisin and leptin may play a role in the pathogenesis of concurrent depression in the elderly population (J Clin Med. 2023 Jun 26;12(13):4283. doi: 10.3390/jcm12134283.). Ghrelin is an orexigenic digestive hormone that plays roles in sleep and memory. It is hypothesized that ghrelin may have a role in depression, particularly for certain specific endophenotypes (Encephale. 2018 Jun;44(3):256-263. doi: 10.1016/j.encep.2017.10.012.). It has been suggested that numerous nutrients, foods, and dietary compounds may be involved in the onset and maintenance of depressive disorders and the severity of depressive symptoms. Nutritional compounds might modulate biomarkers associated with depression and concurrently influence the development of depression, obesity, and diabetes. For instance, IGF-1, NPY, BDNF, ghrelin, leptin, CCK, GLP-1, AGEs, glucose metabolism, and the microbiota act in the gut-brain circuits (Cell Physiol Biochem. 2015;37(3):1029-43. doi: 10.1159/000430229).

Nitric Oxide Metabolites

The determination of nitric oxide metabolites ($\text{NO}_x = (\text{NO}_2^-) + (\text{NO}_3^-)$) was performed using a spectrophotometric method with the Griess reagent, in saliva samples using a commercial colorimetric analysis kit (Cayman Chemical, Ann Arbor, MI, USA, cat. No. 780001) as previously described [Mrakic Sposta et al 2024]. Samples and standards were read in duplicate at a wavelength of 540 nm. The coefficient of variation (CV) indicated by the manufacturer: inter-assay CV 3.4%; intra-assay CV 2.7%. A linear calibration curve was calculated starting from pure standards of nitrite and nitrate.

Inflammatory State

The systemic level of inflammation was assessed by analyzing interleukin 6 (IL-6) in saliva using an ELISA kit (IL-6: No. 501030, Cayman Chemical, Ann Arbor, MI, USA), following the manufacturer's instructions. These methods have been previously described (Giacon et al, 2022). Briefly, a monoclonal antibody specifically for IL-6 was pre-coated onto a microplate. Standards and samples (100 μL) were pipetted into the wells and the immobilized antibody bound any IL-6 present. After the washing procedure, an enzyme-linked polyclonal antibody specific for IL-6 was added to

the wells. Following another wash, a substrate solution was added to the wells and color developed in proportion to the amount of IL-6 bound initially. Samples were measured at 450 nm. All samples and standards were read with a microplate spectrophotometer (Infinite M200, Tecan Group Ltd., Männedorf, Switzerland). Determinations were evaluated in duplicate, and the inter-assay coefficient of variation was within the range indicated by the manufacturer.

ELISA technique

Enzyme-linked immunosorbent assays (ELISAs) are widely utilized for the quantification of specific biomolecules in diverse sample matrices, including serum, plasma, and cell lysates. These assays rely on the immobilization of a target-specific capture antibody within 96-well microplates, facilitating the selective binding of the analyte of interest. Following incubation, a biotinylated or enzyme-conjugated detection antibody recognizes a distinct epitope on the target, forming a highly specific antibody-analyte-antibody complex, commonly referred to as a “sandwich” in sandwich ELISA formats.

The addition of an enzymatic substrate, such as tetramethylbenzidine (TMB) for horseradish peroxidase (HRP)-conjugated assays, results in a colorimetric or chemiluminescent reaction proportional to analyte concentration. Optical density or luminescence measurements are subsequently performed using a microplate reader, allowing precise quantification based on a standard curve.

The superior sensitivity, specificity, and reproducibility of ELISAs render them invaluable for biomedical research, clinical diagnostics, and pharmaceutical development. However, the reliability of results depends on optimized assay conditions, including proper antibody pairing, rigorous validation, and strict adherence to quality control measures. Minimizing background noise, ensuring batch-to-batch consistency, and implementing appropriate controls are crucial for maximizing assay performance. Through careful design and execution, ELISAs provide a powerful tool for detecting biomarkers, assessing immune responses, and monitoring disease progression with high precision and accuracy.

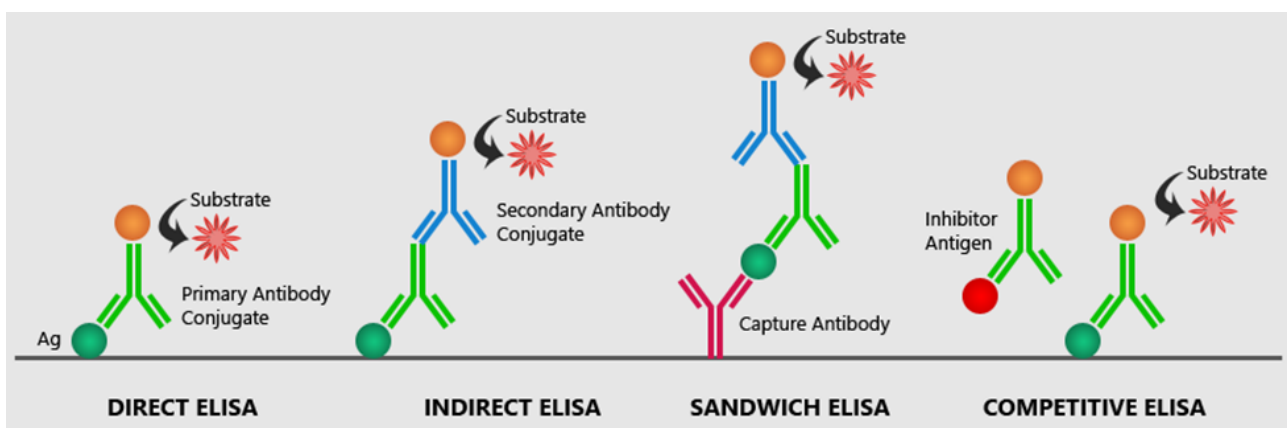
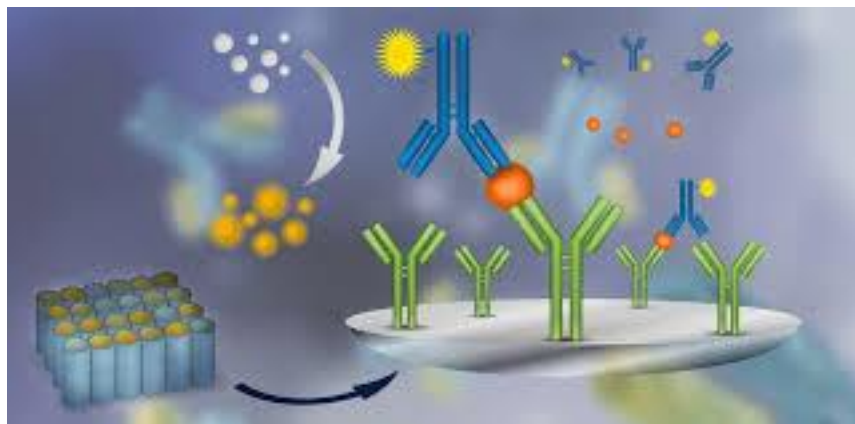
The four main types of ELISAs:

1. *Direct*: an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.
2. *Indirect*: is like a direct ELISA in that an antigen is immobilized on a plate, but it includes an additional amplification detection step. First, an unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the

host species of the primary antibody is then added. Substrate then produces a signal proportional to the amount of antigen bound in the well.

3. *Sandwich*: Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.
4. *Competitive*: used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. Similar to a sandwich ELISA, a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to compete for binding with the antigen present in the sample. The more antigen presents in the sample, the less conjugated antigen will bind to the capture antibody. Substrate is added and the signal produced is inversely proportional to the amount of protein present in the sample.

Each type of ELISA has its own advantages and disadvantages



Finally, ELISA is a highly sensitive technique, capable of detecting analytes at picogram to nanogram levels. Its sensitivity is primarily attributed to the high affinity and specificity of antibody-antigen interactions, as well as enzymatic amplification of the detection signal. The use of optimized antibody pairs and advanced detection methods, such as chemiluminescence or fluorescence, further enhances sensitivity. Proper assay optimization, including minimization of background noise and rigorous validation, ensures accurate and reproducible results. Additionally, signal amplification strategies, such as biotin-streptavidin systems, can improve detection limits. However, sensitivity may vary depending on the assay format, reagents, and sample preparation. By carefully selecting antibodies and optimizing reaction conditions, ELISA remains one of the most sensitive and reliable immunoassays available for biomarker detection.

Scales

During our study, we have considered additional cofactors that might influence the longevity of nonagenarians and centenarians in the Abruzzo region. To deepen our understanding of the variables affecting the longevity of the Abruzzese population, we administered assessments to investigate:

- **Quality of Life:** Examining how overall well-being and life satisfaction impact longevity.
- **Education:** Investigating the association between education level and lifespan, considering how education may influence other lifestyles and cognitive variables.
- **Anxiety/Depression:** Assessing the impact of mental health, specifically levels of anxiety and depression, on elderly subjects.
- **Active/Sedentary Life:** Determining the role of physical activity and sedentary behaviors in health promotion and prevention of age-related diseases.
- **Economic Availability:** Analyzing how economic conditions influence access to quality healthcare, adequate nutrition, and other essential resources for healthy aging.

These areas of study are crucial to outline a comprehensive framework of conditions that promote a long and healthy life.

Geriatric Depression Scale (GDS)

The geriatric depression scale (GDS-15) was administered by investigators to 12 subjects (6 men and 6 women) living at sea level (0-300m) 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects. The questionnaire was administered within minutes.

The Geriatric Depression Scale (GDS-15) is composed of these questions:

1. Are you satisfied with your life? Yes [0] ☐ No [1] ☐

2. Did you interrupt many of the things you were doing before? Yes [1] ☐ No [0] ☐
3. Do you feel empty in your life? Yes [1] ☐ No [0] ☐
4. Do you get bored often? Yes [1] ☐ No [0] ☐
5. Is he in a good mood most of the time? Yes [0] ☐ No [1] ☐
6. Are you afraid that something bad might happen to you? Yes [1] ☐ No [0] ☐
7. Do you generally feel happy? Yes [0] ☐ No [1] ☐
8. Do you often feel abandoned? Yes [1] ☐ No [0] ☐
9. Would you rather stay at home than go out and do new things? Yes [1] ☐ No [0] ☐
10. Do you feel that you have more memory problems than others? Yes [1] ☐ No [0] ☐
11. Do you think it's nice to be alive today? Yes [0] ☐ No [1] ☐
12. Do you feel a little useless as it is today? Yes [1] ☐ No [0] ☐
13. Do you feel full of energy? Yes [0] ☐ No [1] ☐
14. Have you lost all hope that things will improve? Yes [1] ☐ No [0] ☐
15. Do you think others are better off than you? Yes [1] ☐ No [0] ☐

Total score:

The scoring of the Geriatric Depression Scale (GDS-15) is as follows:

- 0-5: Normal (no significant depression)
- 6-10: Mild depression
- 11-15: Moderate to severe depression

The Geriatric Depression Scale (GDS) is an assessment tool used to identify symptoms of depression in older adults. It was originally developed by researchers Yesavage JA. in 1982 to facilitate the assessment of depression, specifically in the geriatric population. The GDS is designed to be easy to administer, score, and interpret, making it a practical tool for use in various settings. It is available in several versions, including a long form of 30 questions and a short form of 15 questions, both consisting of binary (yes/no) response questions. The most common versions of the GDS are:

- GDS-30: This is the original, more comprehensive version that assesses various aspects of depressive symptoms commonly experienced by the elderly.
- GDS-15: An abbreviated version that includes 15 of the original questions. This version is widely used for research and care, providing an effective balance between comprehensiveness and practicality.

The GDS is particularly valued for its high reliability and validity and is used in a wide range of clinical and research settings to identify depression in the elderly. It is also a freely available and easily accessible tool for health professionals. Below are the main areas that the GDS investigates:

- *Mood*: The GDS assesses feelings of sadness, despair, and discouragement, as well as loss of self-esteem.
- *Vitality*: It probes the reduction of energy, fatigue, and feeling of tiredness, common symptoms of depression.
- *Interest in Activities*: It evaluates the loss of interest or pleasure in daily activities and hobbies, a typical symptom of depression called anhedonia.
- *Socialization*: It examines social withdrawal and decreased interaction with friends and family.
- *Concentration*: It investigates possible difficulties in concentration and decision-making that can occur in depressed individuals.
- *Sleep*: It assesses sleep disturbances, such as insomnia and hypersomnia, which can be related to depression.
- *Appetite*: It monitors changes in appetite, which can include significant loss or increase in appetite.
- *Optimism about the future*: It detects feelings of pessimism or lack of hope about the future, which are commonly associated with depression.

The GDS is structured to avoid including questions directly related to physical symptoms, as these can be common in the elderly population regardless of the presence of depression. This feature makes the GDS a particularly useful and accurate tool for identifying depression in elderly individuals, as it reduces the risk of false positives due to physical conditions.

EuroQol-5D (EQ-5D)

The Euro Qol (EQ-5D) was administered by investigators to 12 subjects (6 men and 6 women) living at sea level (0-300m) 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects. The questionnaire was administered within minutes. Applicable to a wide range of health conditions and treatments, it provides a simple descriptive profile and a single index value for health status that can be used in the clinical and economic evaluation of health care as well as in population health surveys. EQ-5D is designed for self-completion by respondents and is ideally suited for use in postal surveys, in clinics, and in face-to-face interviews. It is cognitively demanding, taking only a few minutes to complete. Assessment The EuroQol-5D (EQ-5D) (Balestroni et al., 2012) is a standardized instrument for measuring health-related quality of life, employed as a simple and generic questionnaire in clinical

evaluations, economic assessments, and population health surveys. This instrument assesses health status across five dimensions:

- *Mobility*: This dimension evaluates an individual's ability to move without assistance. Respondents must answer based on their ability to walk unaided, highlighting any limitations such as difficulty in walking long distances or the need for aid like canes or wheelchairs.
- *Self-care*: Concerns the individual's ability to take care of oneself in basic daily activities, such as washing, dressing, and feeding. Responses in this category reflect a person's independence in their daily routines.
- *Usual Activities*: This dimension examines how health conditions affect the ability to perform usual activities, including work, study, household tasks, and leisure activities. It assesses how health problems may restrict these activities.
- *Pain/Discomfort*: Assesses the presence and intensity of pain or physical discomfort. This dimension helps to understand the impact of pain on daily life quality, considering both mild and debilitating pain.
- *Anxiety/Depression*: Measures levels of anxiety and depression, considering the emotional impact of health conditions. Responses can range from no symptoms of anxiety or depression to an extremely severe condition that significantly impacts daily life.

These five dimensions are designed to cover the essential aspects of health that can be affected by a wide range of diseases and conditions. Each dimension has various levels of severity, generally measured on a scale from 1 to 5, where 1 indicates "no problems" and 5 indicates "extreme problems." The choice of these dimensions reflects the questionnaire's aim to provide a comprehensive and sensitive assessment of the major areas that can influence quality of life, thus allowing its application in diverse healthcare contexts for a variety of populations.

Mini Nutritional Assessment (MNA)

The Mini Nutritional Assessment (MNA) was administered by investigators to 12 subjects (6 men and 6 women) living at sea level (0-300m) 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects. The questionnaire was administered within minutes.

Mini Nutritional Assessment (MNA) is a widely utilized tool for evaluating the nutritional status of the elderly. It was originally developed in 1999 by Vellas B. and is specifically designed to identify elderly individuals at risk of malnutrition or those already malnourished.

Assessment of nutritional status:

from 24 to 30 points: normal nutritional status

from 17 to 23.5 points: risk of malnutrition

less than 17 points: poor nutritional status

- *Structure and Scoring:* The MNA comprises 18 questions that encompass various aspects related to nutrition, including anthropometric assessments (weight, height, wrist circumference), overall assessment (health status, mobility), dietary assessment (number of meals, consumption of proteins and fruits/vegetables), and subjective evaluation, namely self-perception of health and nutritional status. The total score ranges from 0 to 30, with lower scores indicating malnutrition.
- *Versions:* There are two versions of the MNA: the complete version (MNA® Full Form) and a short form (MNA®-SF) (Vellas et al., 1999). The short form is useful for quick screening and includes six questions from the complete version. If the score suggests a risk of malnutrition, using the full version is recommended for a more detailed analysis.
- *Application:* MNA is used in various settings, including hospitals, long-term care facilities, and community contexts. It is particularly valued for its ease of use and reliability in predicting nutritional risk in the elderly.
- *Validity and Reliability:* Numerous studies have confirmed the validity and reliability of the MNA as a nutritional assessment tool. It has shown good correlation with other nutritional assessment methods and has been adapted in many countries and is available in several languages.
- *Clinical Importance:* Utilizing the MNA can help to identify risks of malnutrition in the elderly early on, allowing for timely interventions that can significantly improve quality of life and reduce complications associated with malnutrition.

Thus, the MNA is an essential tool in geriatrics and dietetics to ensure that the elderly receive appropriate nutritional care, based on an accurate and scientifically validated assessment of their nutritional status.

Food Frequency Questionnaire (FFQ)

The Food Frequency Questionnaire (FFQ) was administered by investigators to 12 subjects (6 men and 6 women) living at sea level (0-300m) 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects.

The Food Frequency Questionnaire (FFQ) is a standardized questionnaire that includes a list of commonly consumed foods and beverages, accompanied by questions about consumption frequency and, in some cases, portion sizes. A typical FFQ consists of:

- *Food list:* A list of 80-150 common foods, grouped by category (e.g., cereals, dairy products, fruit, meat, beverages, etc.).
- *Frequency questions:* For each food item, the respondent indicates how often they consume it (e.g., "Never," "1-3 times per month," "1-2 times per week," "Every day," etc.).
- *Quantity consumed (optional):* Some FFQs include questions about portion sizes to improve the estimation of nutrient intake.

FFQ is an essential tool in nutritional research for analyzing dietary habits in large populations in a practical and cost-effective way. Although it has some limitations, it is widely used in nutritional epidemiology to understand the impact of diet on health.

The Food Frequency Questionnaire (FFQ) is a dietary assessment tool used to gather information on how frequently a person consumes specific foods over a given period, typically over the last year. It is one of the most common tools used in nutritional epidemiological studies to estimate nutrient intake. The FFQ (Cui Q et al., 2023) consists of a list of foods and beverages with associated frequency options that the respondent must select to indicate how often each item is consumed. Frequency options can range from "never or less than once a month" to "six or more times a day."

- *Reference Period:* The FFQ asks respondents to reflect on their usual intake over the past months or year, providing a long-term dietary intake estimate.
- *Nutritional Components:* Once collected, the data can be used to calculate estimated nutrient intakes based on reported frequencies and amounts, often with the help of food composition tables. The FFQ is widely used in epidemiological studies to examine correlations between diet and the risk of various chronic diseases such as cancer, diabetes, and cardiovascular diseases. This questionnaire is used to assess the effectiveness of dietary interventions by modifying food intake over time and also serves to identify prevailing dietary patterns in different populations or demographic groups. The questionnaire can cover a wide range of foods and beverages, providing a comprehensive overview of dietary patterns.

Participants may have difficulty accurately recalling their usual intake or may interpret questions or portion sizes differently, and respondents may alter their responses to conform to what they believe are desirable eating behaviors. Additionally, seasonally consumed foods may not be accurately represented. The food frequency questionnaire is a valuable tool in large-scale nutritional data collection, and adequate training of participants can help minimize errors and enhance the reliability of the data collected.

Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) was administered by investigators to 12 subjects (6 men and 6 women) living at sea level (0-300m) 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects. The questionnaire was administered within minutes.

Evaluation of scores:

>5= bad sleep

<5= good sleep

The Pittsburgh Sleep Quality Index (PSQI), devised by Buysse et al. in 1989, is a standardized tool used to assess sleep quality and patterns. PSQI is a questionnaire that provides a comprehensive measure of sleep quality by considering various aspects over a one-month period. It consists of 19 individual questions grouped into seven components, each rated on a scale from 0 to 3, where 0 indicates no difficulty and 3 indicates severe difficulties:

- *Subjective Sleep Quality:* Assesses general perception of sleep quality.
- *Sleep Latency:* Measures the time it takes to fall asleep.
- *Sleep Duration:* Accounts for the total number of hours of sleep per night.
- *Sleep Efficiency:* Calculates the percentage of time spent in bed actually sleeping.
- *Sleep Disturbances:* Records the frequency of sleep disruptions, such as waking up in the middle of the night or too early in the morning.
- *Use of Sleeping Medication:* Records the frequency of using medication to aid sleep.
- *Daytime Dysfunction:* Assesses the degree of difficulty experienced during the day due to nighttime sleep, including fatigue and daytime sleepiness.

Total PSQI Score: The scores of the seven components are summed to obtain a total score ranging from 0 to 21. A total score of 5 or more suggests significant sleep pattern dysfunction indicative of poor sleep quality. PSQI is extensively used in clinical and research studies to evaluate insomnia and other sleep disorders. It is beneficial in various clinical settings, including sleep medicine, psychiatry, and geriatric research. Additionally, it can be used to monitor changes in sleep quality before and after therapeutic interventions. In summary, PSQI is a valuable and reliable tool for health professionals to systematically and quantitatively assess sleep quality and identify potential sleep disorders in their patients.

Hand Grip Strength

The values of Hand Grip Strength were collected by investigators from 12 subjects (6 men and 6 women) living at sea level (0-300m), 12 subjects (6 men and 6 women) at medium altitude (300-600m) and 12 subjects (6 men and 6 women) at high altitude (>600m), all residents in the province of Chieti aged between 90 and 109 years. The test was administered to a total of 36 subjects. The administration lasted just enough time to detect the values of the subject.

The measurement of hand grip strength is a widely used clinical indicator to assess health status and physical functionality during aging. This measurement tool provides valuable insights for several reasons.

Procedure for performing handgrip strength. Preparation (Vaishya R. et al., 2024): ensure the participant is in a comfortable seated position with their feet flat on the floor and their arm resting on a flat surface. Some investigators have used other positions including standing and found strength measurement to be similar to sitting position, while others have found higher HGS values in standing position. **A review of the measurement of grip strength in clinical and epidemiological studies: toward a standardized approach** **Shoulder position:** The shoulder should be in a neutral position, and the elbow should be bent at 90 degrees **Wrist position:** Wrist should be between 0 and 30° of dorsiflexion **Calibration:** Before starting, make sure the dynamometer is calibrated according to the manufacturer's instructions to ensure accurate measurements **Grip Position:** Instruct the participant to hold the dynamometer in their hand so that the handle rests comfortably against their palm. The handle should be adjusted for a snug fit, neither too tight nor too loose **Grip Technique:** The participant should be encouraged to grip the handle as firmly as possible without straining excessively. The four fingers should wrap around the handle, while the thumb rests on the other side. Ensure that the wrist is maintained in a neutral position **Measurement:** Ask the participant to squeeze the dynamometer with maximal effort for about 3 to 5 s. During this time, encourage them to exert their maximum strength. The participant should avoid any sudden jerks or movements during the squeeze **Encouragement:** "I want you to squeeze as hard as you can for as long as you can until I say stop". (When the needle stops rising) **Rest Between Trials:** Allow a brief rest period (about 1 min) between each trial to prevent muscle fatigue that could affect subsequent measurements **Repeat Measurements:** Typically, three trials are conducted for each hand alternately. This provides a more accurate representation of the participant's handgrip strength. However, some investigators see one trial as equally reliable.

Health Status Assessment:

- **Muscle Strength Indicators:** Hand grip strength is considered a reliable indicator of overall muscle strength. As people age, muscle strength diminishes, and regular measurements can help monitor this decline.

- *Disease Predictor:* Several studies have linked low hand grip strength with an increased risk of cardiovascular incidents, diabetes, and other chronic conditions. Reduced hand grip strength has also been associated with an increased risk of mortality.
- *Functionality Assessment:* Hand grip strength is often used as a marker of physical functionality in the elderly. It is related to the ability to perform independent daily activities, and a reduction in strength can be an early sign of functional decline.
- *Nutritional and Overall, Health Indicators:* Hand grip strength can also reflect nutritional status and general health. For instance, malnutrition can lead to a decrease in muscle mass and consequently reduced hand grip strength.



Figure 2: *Measurement of Grip Strength in an Ultracentenarian from Gessopalena (CH)* "Grip strength is an indicator of muscular health and a potential predictor of longevity. Studies indicate that weaker grip strength is associated with an increased risk of frailty, cardiovascular diseases, and early mortality. Measuring it aids in assessing physical functionality and preventing muscular decline.

Grip strength has been studied as a possible predictor of longevity. Research has demonstrated that older individuals with stronger grip strength tend to have a longer life expectancy. This may be due to its association with better overall health quality and lower rates of chronic diseases.

Measuring grip strength can help healthcare professionals identify elderly individuals at risk of physical deterioration and implement timely interventions to improve strength and overall health, potentially prolonging their quality of life.

The measurement of grip strength is a non-invasive, quick, and cost-effective test that can be easily implemented in various clinical and research settings. In conclusion, the use of hand grip as an assessment tool provides a valuable insight into the physical and functional health of the elderly,

contributing to the understanding and improvement of strategies for healthy aging and potentially to the prediction of longevity.

RESULTS

Demographic ISTAT analysis. *Bar charts represent data in percentage:*

- In 2021 Nonagenarians (90+ years): highest prevalence in provinces: Chieti, Pescara, L'Aquila, and Teramo (See Figure 4.).
- In 2021 Centenarians (100+ years): order of prevalence in provinces: Pescara, L'Aquila, Chieti e Teramo (See Figure 4.).
- Small Villages: higher percentage of elderly, both nonagenarians and centenarians (See Figure 5).
- Areas above 600 meters altitude (inland and mountain zones): higher percentage of 90+y and 100+y compared to hill and coastal areas. (See Figure 6).
- Province of Chieti (2010-2020): increase in both absolute number and percentage of the elderly population, especially among nonagenarians and centenarians (See Figure 7).
- The trend of nonagenarians and centenarians has been increasing from 2010 to 2020. Nonagenarians and centenarians live in small villages (<1000 inhabitants), medium villages (1000-3000 inhabitants), and large villages (>3000 inhabitants). (See Figure 8)
- Prominent increase observed in less populated areas and areas above 600 meters altitude (See Figure 9)
- Gender Distribution in Chieti (2010-2020): majority of the elderly population, both nonagenarians and especially centenarians, are female (See Figure 10).

For the year 2021, we analyzed both the population numbers and the number of municipalities in the four Abruzzo provinces. The results are displayed in two separate charts (below) that illustrate the population and the number of municipalities of the 4 provinces of Abruzzo for the year 2021.

Demographic Analysis The point of the analysis was an in-depth examination of the demographics across the four provinces of Abruzzo: Chieti, L'Aquila, Teramo, and Pescara, to assess their degree of longevity. This historical study required the collection of demographic data from istat.it, spanning from 2010 to 2021.

The collection of saliva was carried out on ultra-long subjects, aged 90 years or over, permanently resident in Abruzzo municipalities located in three different elevations: below 300 meters (low altitude (LA)), between 300 and 600 meters (medium altitude (MA)) and higher than 600 metres (high altitude (HA)), compared with a group of healthy Romans of the same age.

Due to the heterogeneity of the subjects, and therefore a very high standard deviation, no significant difference was found for the biomarkers analyzed: appetite hormones: leptin and ghrelin; inflammatory status: IL-6, and nitric oxide levels: NOx. (see figures 13-15-16)

We report the differences as a percentage. Ghrelin is higher in the control groups. It may be the quality of life, high-calorie diet, or stress factors, life in cities, there is an increase in feelings of «hunger». Leptin levels are high especially in the control group, these levels are related to overeating (see Figure 16).

For IL-6, the population living at high altitude would seem to be more protected than the inhabitants at low altitude, and therefore suggests a lower risk of pathological events, (perhaps less refined foods, less processed meats, less red meats, less carbonated soft drinks and sweet reduction of systemic inflammation), while in the Ctr group the inflammatory state is high. (see Figure13)

In order to evaluate other factors that may influence the aging process, we administered the Pittsburgh Sleep Quality Index (PSQI), a good quality of sleep is closely related to better physical and mental health.

Geriatric Depression Scale (GDS): The GDS scores are distributed in a similar way among the different high-altitude bands, without significant discrepancies. (See Figure 17)

Mini Nutritional Assessment (MNA) MNA scores are also evenly distributed across altitudes, with a slight concentration of higher high altitude (HA) scores, which may indicate slightly better nutritional status. (See Figure17)

The results show the distribution of hand grip force (hand grip) measured in kg between ultralights living at different altitudes: low (LA), medium (MA) and high (HA). Although there are no statistically significant differences between the groups, we can observe some visual trends in the data.

Low Altitude (LA): The grip force distribution in this group shows a considerable variation, with a shape extending mainly between 0 and 30 kg, and the median visually close to 20 kg. (See Figure 21)

Through pie charts show that the 69.44% of ultralights do not have depressive symptoms (by Geriatric Depression Scale (GDS)), suggesting that despite their advanced age, most of this population maintains a good state of mental health. The lower incidence of depression, 5.56% recorded at high altitude (HA) raises interesting considerations on how this factor can positively influence aging. (See Figure 18)

For the quality of sleep is shown by the pie chart as the 58.33% of the subjects have a normal quality of sleep and 72.22% of the subjects do not use sleeping drugs. (see Figures 19 and 20).

Number of inhabitants and number of municipalities in the four Abruzzese provinces for the year 2021

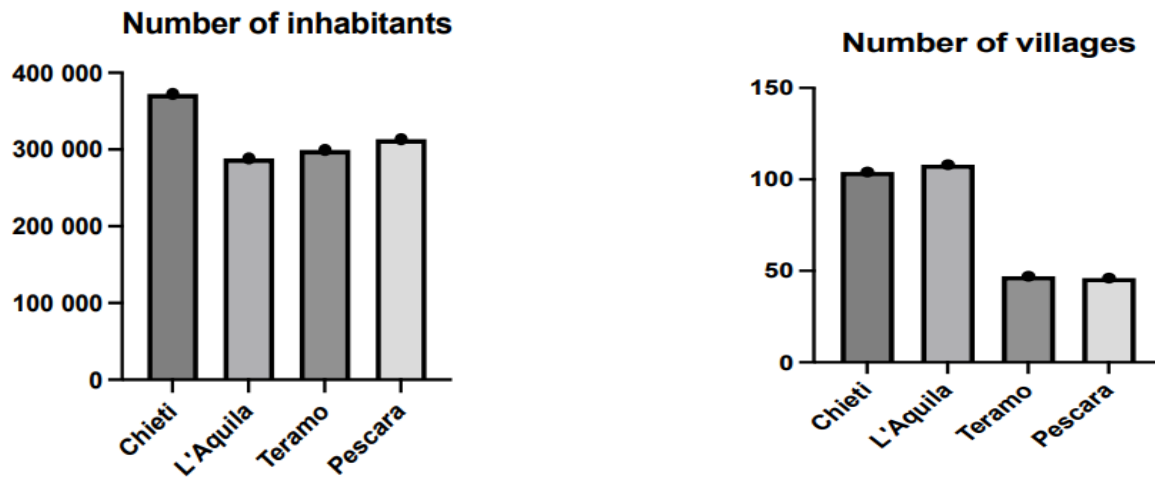


Figure 3. Population and Number of Municipalities in the Four Provinces of Abruzzo for the Year 2021. Source: ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

- The left chart displays the population numbers per province, with Chieti having the largest population, followed by Pescara, Teramo, and finally L'Aquila.
- The chart on the right, on the other hand, shows the number of municipalities for each province, highlighting that L'Aquila has the most municipalities, followed by Chieti, Teramo, and with the fewest, Pescara.

These data provide an overview of the geographic and demographic distribution of the population in the Abruzzo provinces and serve as a basis for further analyses on longevity in these areas.

Percentage of Nonagenarians and Centenarians in 2021 in the Abruzzo Provinces

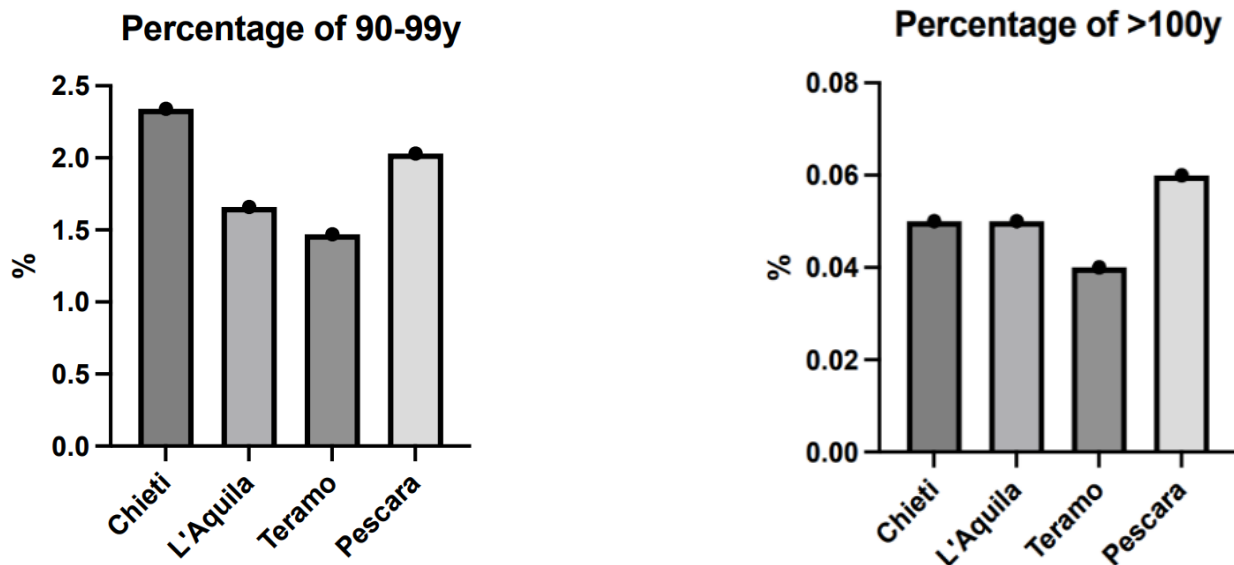


Figure 4. Bar Graphs Displaying the Percentages of Nonagenarians (ages 90-99) on the Left and Centenarians on the Right Across the Four Abruzzo Provinces: Chieti, L'Aquila, Teramo, and Pescara. The bars represent the proportion of individuals between the ages of 90 and 99 in each province, highlighting variations in the regional demographic distribution of this age group. Source: ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

The left graph shows that the province of Chieti has the highest percentage of nonagenarians, followed respectively by the provinces of Pescara, L'Aquila, and Teramo.

The right graph reveals that the province of Pescara holds the highest percentage of centenarians, followed in order by L'Aquila, Chieti, and finally Teramo.

It is important to note that the higher percentage of centenarians in the province of Pescara may be attributed to its larger population size compared to the provinces of Teramo and L'Aquila. This demographic factor can influence the representativeness of centenarians in the total population, resulting in a relatively higher percentage.

Distribution of Population and Ultra-Elderly by Size of Municipalities in the Abruzzo Provinces

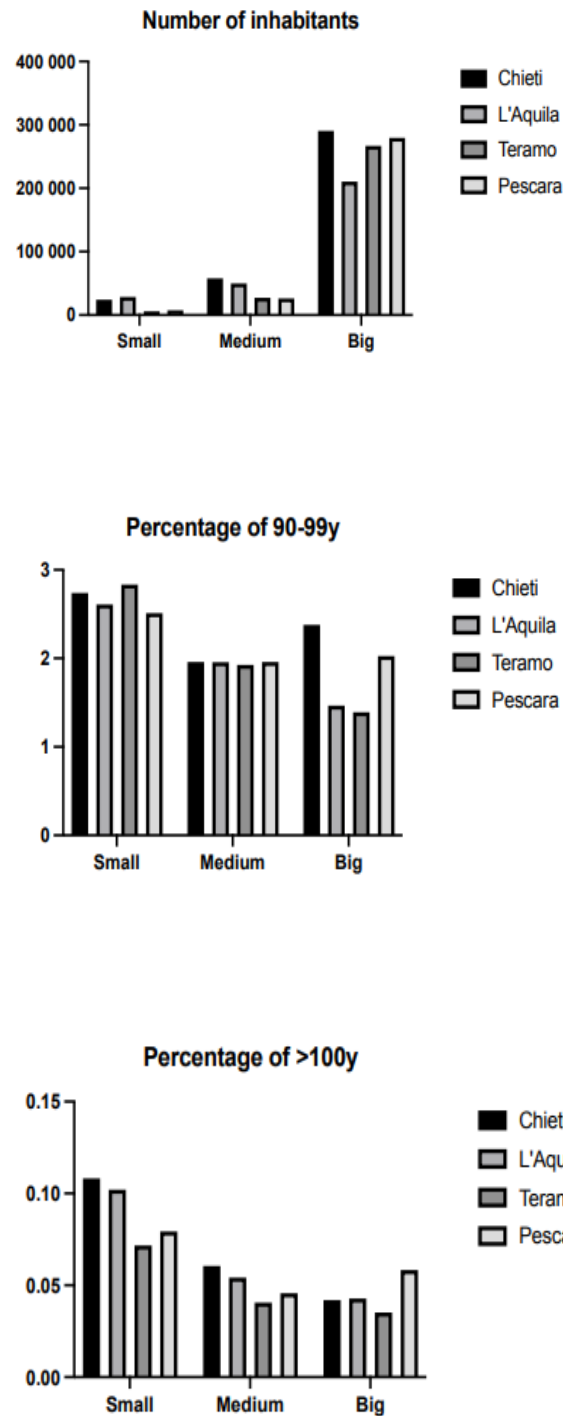


Figure 5. *Graphs depicting three distinct aspects of demographics across the four Abruzzo provinces (Chieti, L'Aquila, Teramo, Pescara) relative to the size of municipalities (small, medium, large). Population Number: The first graph displays the total number of inhabitants in each province, subdivided by municipal size. It provides a clear demographic breakdown, highlighting variations in population distribution across different municipal sizes. Percentage of Ages 90-99: The second graph shows the percentage of nonagenarians within the various provinces, again categorized by municipal*

size. This visualization helps in understanding the age structure and the distribution of older populations in relation to municipal dimensions. Percentage Over 100 Years Old: The third graph details the percentage of centenarians. Notable variations are evident, particularly in the small and medium municipalities of Chieti and the medium municipalities of Teramo, indicating a relatively higher presence of centenarians in these areas compared to others within the same provinces.

Source: ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

These graphs offer a detailed view of both the general population distribution and the very elderly across the Abruzzo provinces, showing how the size of municipalities can influence both the total number of residents and the proportion of the very old. Such data are crucial for urban planning and the targeting of health and social services towards these age groups.

Year 2021: Small Villages <1000; Medium Villages 1000-3000; Large Villages >3000

Top Graph - Population Number:

- Chieti and Pescara exhibit larger populations in medium and large municipalities.
- L'Aquila, despite its mountainous terrain, shows a significant population spread in large municipalities.
- Teramo displays a more evenly distributed population across various municipal types.

Middle Graph - Percentage of 90-99 Years:

- The percentage of nonagenarians is highest in the small municipalities for Chieti, followed by slightly lower percentages for L'Aquila and Teramo.
- In medium-sized municipalities, Chieti and L'Aquila have the highest percentages, whereas Pescara shows a lower percentage.
- In large municipalities, L'Aquila and Teramo exhibit the highest percentages, suggesting a significant portion of their elderly population resides in more urban areas.

Bottom Graph - Percentage Over 100 Years:

- Percentages of centenarians are generally lower compared to nonagenarians.
- Chieti has the highest percentage of centenarians in small municipalities.
- L'Aquila shows the highest percentage of centenarians in medium and large municipalities, notable as it is a mountainous region, which could provide better quality of life or access to care for the elderly.

Theoretical Interpretation

In small municipalities, which may offer a more tranquil and less stressful lifestyle, Chieti shows a notably high percentage of nonagenarians, suggesting favorable living conditions for longevity.

The significant presence of nonagenarians and centenarians in L'Aquila, even in large municipalities, might indicate that despite challenges associated with mountainous living, there are protective factors related to these environments, such as air quality and lifestyle, contributing to longevity.

Teramo and Pescara, with a more homogeneous distribution of the elderly population, might offer a good balance of services and quality of life in both urban and rural areas.

These insights could suggest significant differences in how various sizes of municipalities impact the lives of the elderly, with potential implications for urban planning and the provision of health and social services.

Demographic Distribution by Altitude in the Abruzzo Provinces

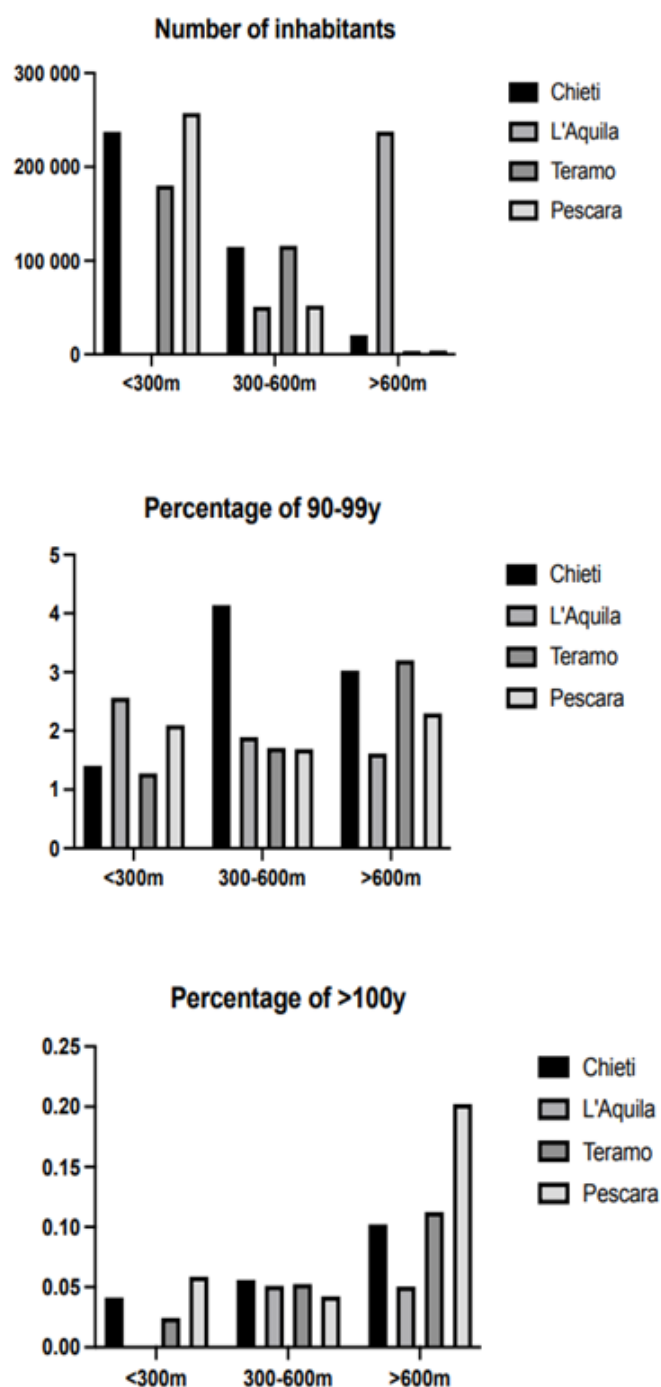


Figure 6. Demographic Distribution by Altitude in the Abruzzo Provinces. This figure shows the population distribution and the percentages of nonagenarians (90-99 years old) and centenarians (over 100 years old) in the Abruzzo provinces of Chieti, L'Aquila, Teramo, and Pescara, categorized by altitude bands (<300m, 300-600m, >600m). The charts illustrate how altitude affects demographic distribution and longevity across different provinces. Year 2021: High Altitude >600m; Mid Altitude 300-600m; Low Altitude <300m. ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

First Chart - Number of Inhabitants

- Chieti and Pescara have the majority of their population residing below 300m.
- L'Aquila has a significant distribution of population even above 600m, which is notable given its predominantly mountainous territory.
- Teramo shows a more balanced distribution across the lower and mid altitude ranges.

Second Chart - Percentage of 90-99y

- The percentage of nonagenarians is generally higher in the lower altitude bands (<300m) across all provinces, with Chieti and Pescara showing the highest percentages.
- Interestingly, L'Aquila also has a significant percentage of nonagenarians in the highest altitude band (>600m).

Third Chart - Percentage of >100y

- The percentage of centenarians is lower compared to nonagenarians, as expected given the rarity of reaching such age.
- L'Aquila again stands out with the highest percentage of centenarians over 600m, although the number is small in absolute terms.
- The other provinces show lower percentages of centenarians, with numbers more concentrated below 300m altitude.

Speculative Interpretation

- L'Aquila may offer a living environment that supports longevity, despite or perhaps because of its mountainous setting, which might promote a more active and less stressful lifestyle.
- Chieti might benefit from a combination of positive socioeconomic factors and access to medical care, especially in the more populated and low-altitude areas.
- The lower percentage of nonagenarians and centenarians in the higher altitudes of Teramo and Pescara might indicate that these environments pose specific challenges impacting longevity, such as limited access to essential services or harsher climatic conditions.

These data provide a detailed map correlating altitude with longevity across the different Abruzzo provinces, suggesting that altitude may influence the distribution of nonagenarians and centenarians in the population.

Demographic Trends and Distribution of the Very Old in the Province of Chieti (2010-2020)

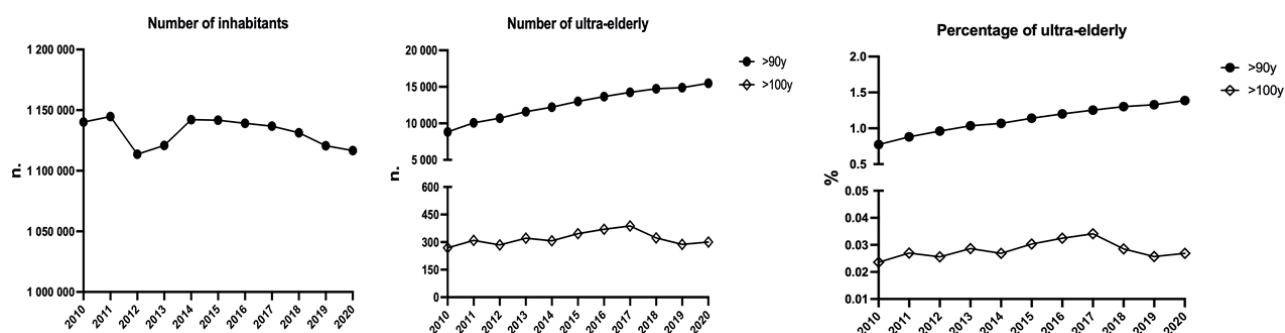


Figure 7. Data on the Total Population and the Very Old Population, Nonagenarians (>90 years) and Centenarians (>100 years). Demographic Trends in the Province of Chieti from 2010 to 2020. ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

First Chart on the Left - Number of Inhabitants:

- A slight decrease in the total population over the decade is observed, with a more marked decline between 2010 and 2012, followed by stabilization and a slight downward trend thereafter.

Second Chart in the Center - Number of the Very Old:

- The number of nonagenarians is increasing, indicating that the population within this age group has grown over time.
- Conversely, the number of centenarians has remained relatively stable, with minor fluctuations but no clear trend of increase or decrease.

Third Chart on the Right - Percentage of the Very Old:

- The percentage of nonagenarians relative to the total population is steadily increasing, suggesting that the proportion of people reaching 90 years is on the rise.
- For centenarians, the percentage shows an initial increase until around 2012, then stabilizes and slightly decreases towards the end of the observed period.

Interpretation

The increase in both the number and percentage of nonagenarians could be attributed to improvements in public health, access to medical care, and quality of life that allow people to live longer.

The stability of the number of centenarians might indicate that there are limiting factors preventing a significant increase in people surpassing 100 years, which could include biological limits or the presence of health conditions that are not easily treatable or preventable even with modern medical technology.

The decrease in the percentage of centenarians over time could suggest that while more people are reaching 90 years, there isn't a corresponding percentage increase of those reaching 100 years, possibly due to a combination of socioeconomic, genetic, environmental, or lifestyle-related factors.

Distribution of the Population and the Very Old Age Group (Nonagenarians and Centenarians) in Small, Medium, and Large Villages of the Province of Chieti from 2010 to 2020

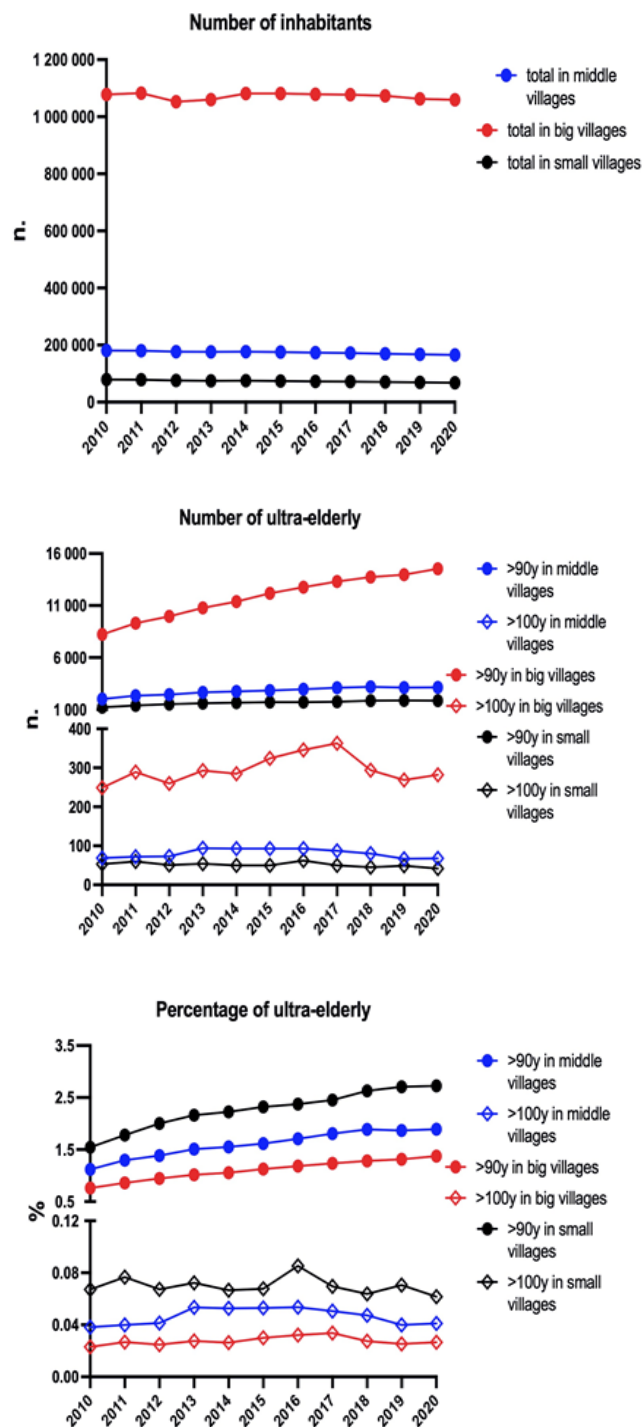


Figure 8. *Demographic Trends and Proportion of the Very Old (>90 years and >100 years) in Villages of Varying Sizes (small, medium, large) from 2010 to 2020. The first chart displays the total number of inhabitants, while the second and third charts respectively show the absolute numbers and percentages of nonagenarians and centenarians. Small Villages <1000; Medium Villages 1000-3000; Large Villages >3000. ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].*

First Chart at the Top - Number of Inhabitants

- The red line (totals in large villages) indicates that the majority of the population resides in larger urban centers, a trend that has remained constant throughout the decade.
- The blue line (totals in medium-sized villages) and the black line (totals in small villages) indicate a smaller population in smaller-sized villages, which has also remained relatively stable over time.

Second Chart in the Middle - Number of the Very Old

- The number of nonagenarians (over 90 years old) is higher in large villages (solid red line), followed by medium-sized villages (solid blue line) and then small villages (solid black line).
- For centenarians (over 100 years old), the numbers are lower than for nonagenarians in all categories of villages. Some fluctuations are noted over the years, but without a clear trend of increase or decrease.

Third Chart at the Bottom - Percentage of the Very Old

- Analyzing the percentage of the very old in relation to the total population of each village can provide a more precise indication than the absolute number of the impact of village size on longevity.

The percentage of nonagenarians (>90y) in medium-sized villages is the highest, followed by those in large and then small villages. This may suggest that medium-sized villages offer an optimal balance between access to services and quality of life, favorable to longevity.

For centenarians (>100y), the percentage is generally lower in villages of each size compared to nonagenarians. However, in small villages, the percentage of centenarians is slightly higher compared to medium and large villages, which could indicate that specific factors in small centers, such as tighter communities and possibly healthier lifestyles, might contribute to extreme longevity.

Generally, the percentage of nonagenarians is increasing in villages of all sizes, suggesting that longevity up to 90 years is growing regardless of village size. This could reflect general improvements in public health and quality of life affecting the entire population.

For centenarians, the percentage seems to be more stable or slightly declining, which might indicate that reaching 100 years is influenced by a combination of factors, including genetics and individual lifestyles, in addition to environmental and community characteristics.

These data suggest that in large and medium-sized villages, which are likely to offer more services, healthcare, and social opportunities, there is a higher percentage of nonagenarians. This might indicate that access to these services contributes to longevity. In small villages, despite the smaller total population, the percentage of centenarians is slightly higher compared to larger-sized villages. This could be due to a quieter, less stressful lifestyle or greater social cohesion that promotes the well-being of the elderly. The trend of declining percentages of centenarians in all three types of villages might reflect difficulties in obtaining advanced medical care or other support services for the extremely old population. Overall, the data could suggest that a more urbanized and resource-rich environment, such as that of large and medium-sized villages, fosters longevity up to 90 years, but not necessarily beyond 100 years, whose maintenance might be influenced by other factors beyond available services. In summary, medium-sized villages appear to be the most favorable environment for living longer beyond 90 years, while small villages might have specific conditions that support life up to 100 years and beyond. This data could be useful in guiding public policies and investments in healthcare and social infrastructure that support longevity in different urban and rural contexts.

Population and the Very Elderly in the Province of Chieti Categorized by Altitude

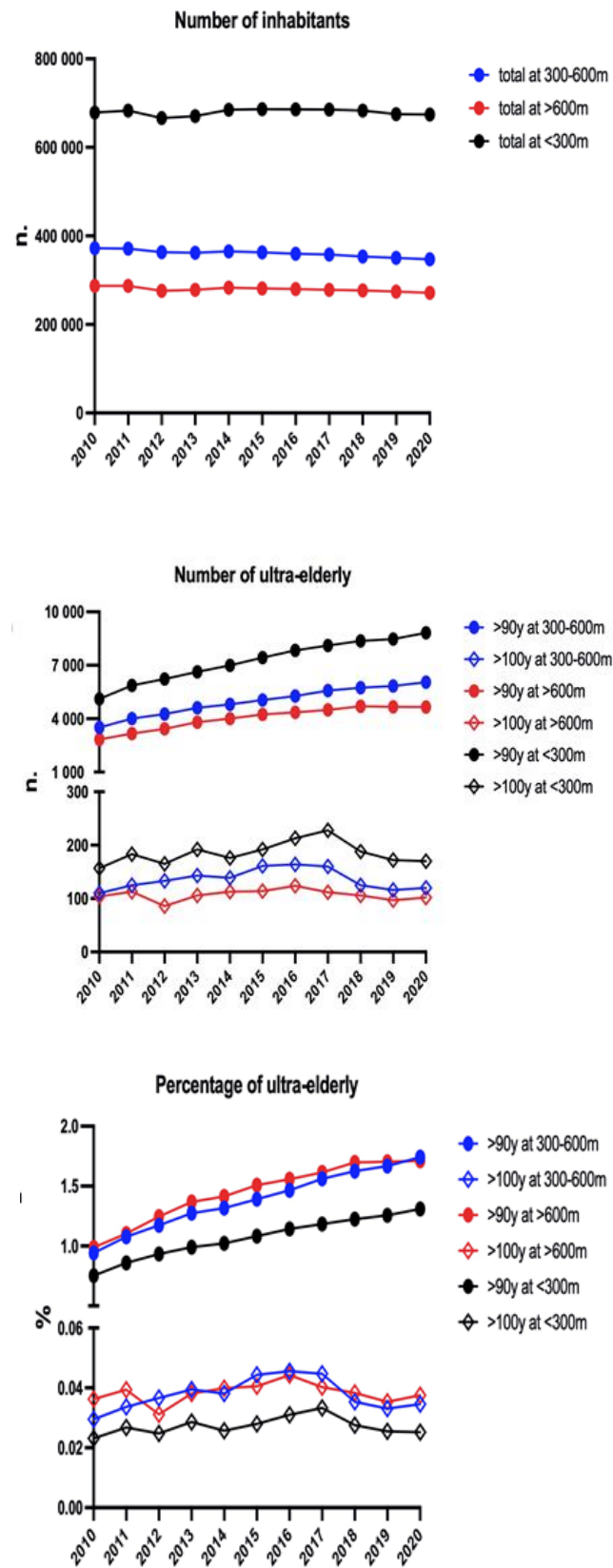


Figure 9: Demographic Distribution by Altitude in the Province of Chieti. The data presented illustrates the general population and the segment of the very elderly (nonagenarians aged 90-99 and

centenarians over 100 years old) in the Province of Chieti, divided according to their residential altitude. High Altitude >600m; Medium Altitude 300-600m; Low Altitude <300m. The demographic trend of the province of Chieti from 2010 to 2020 is analyzed. ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>].

The three charts display the distribution of the general population and the very elderly (both nonagenarians and centenarians) according to the altitude of their residence, from 2010 to 2020. First *Chart at the top - Number of Inhabitants*

- This chart shows the distribution of the general population from 2010 to 2020 across three altitude bands: below 300m, between 300 and 600m, and above 600m.
- The majority of the population resides below 300m (depicted by the black line).
- Populations between 300 and 600m (blue line) and above 600m (red line) are smaller compared to those below 300m, with the population above 600m being the smallest of the three.
- The number of inhabitants across these three altitude bands remains relatively stable over the period considered.

Second Middle Chart - Very Elderly

- This chart illustrates the number of nonagenarians (over 90 years) and centenarians (over 100 years) from 2010 to 2020 in the same altitude bands as the first chart. Both the number of nonagenarians (>90y) and centenarians (>100y) show growth in the higher altitude bands (300-600m and >600m), while in the villages below 300m, the number of nonagenarians is stable, and that of centenarians shows a slight decline.
- Nonagenarians are mostly found below 300m, followed by those between 300 and 600m, and finally, those above 600m.
- The trend of growth is observable in all three altitude bands for nonagenarians.
- For centenarians, the number is lower and shows a more variable trend compared to nonagenarians across the three altitude bands.

Third Bottom Chart - Percentage of Very Elderly

- This chart provides the percentages of nonagenarians and centenarians relative to the total population in the three altitude bands from 2010 to 2020.
- The percentage of nonagenarians consistently increases in all three altitude bands, with a more marked trend for those living between 300 and 600m and above 600m. The percentage of centenarians is higher in villages situated at higher altitudes, although the growth is not as marked as for nonagenarians.

- For centenarians, the percentage is lower and shows an increase from 2012 to 2017, followed by a more unstable trend in subsequent years. Overall, the data suggests that a significant proportion of the very elderly choose to live at lower altitudes, but it is noteworthy that the rate of percentage growth of nonagenarians and centenarians is higher at higher altitudes. This could indicate a relationship between altitude and longevity, warranting further investigation.

The data might suggest that living in villages located at higher altitudes, characterized by a more "human-sized" environment, may contribute positively to longevity. These villages tend to have a smaller population, which could mean less stress and a tighter community life, factors often associated with better quality of life. The increasing percentage of nonagenarians and centenarians in higher areas might also be related to a healthier lifestyle, including a better diet that could come from greater availability of local, less processed food products, and non-industrial agriculture or livestock. Cleaner air, less pollution, the intrinsic physical activity of a mountainous environment (such as walking on sloped terrain), and perhaps isolation from some modern diseases might be additional factors contributing to greater longevity. Although these findings are intriguing, it is important to note that there may be many confounding variables, and correlation does not necessarily imply causality. However, these data can be a starting point for further research on the relationship between environment, lifestyle, and longevity.

Demographic Trends of the Province of Chieti from 2010 to 2020 Divided by Sex

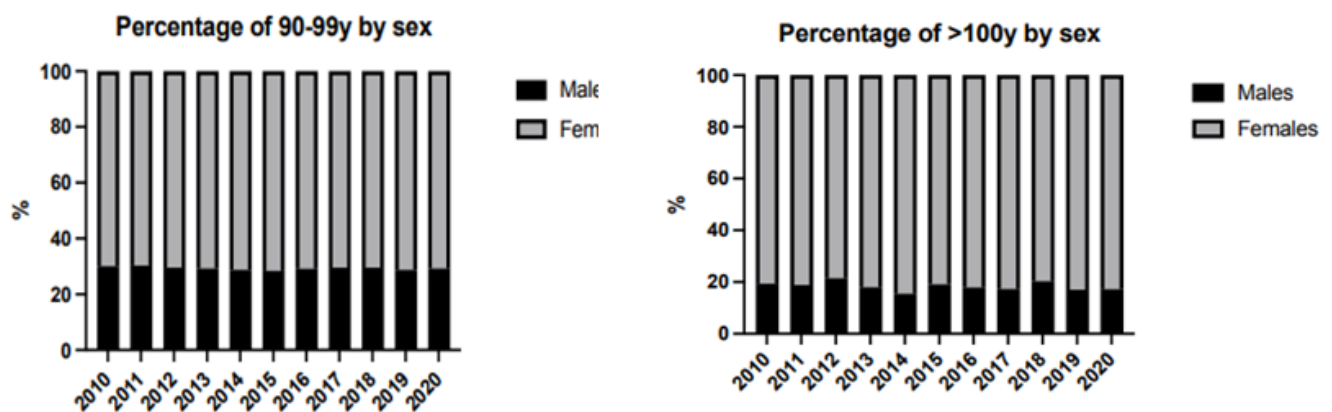


Figure 10: *Percentage of Nonagenarians and Centenarians Divided by Sex, from 2010 to 2020. ISTAT [<https://www.istat.it/it/popolazione-e-famiglie>]. The left graph displays the percentage of nonagenarian individuals, divided by sex, from 2010 to 2020. Black bars represent males, while white bars represent females.*

From the analysis of the chart, the following observations can be made:

1. The percentage of female nonagenarians is consistently higher than that of males in every year depicted. This aligns with demographic data that typically indicate greater longevity in females.
2. For both sexes, there appears to be no significant year-on-year variation in percentages, suggesting that the ratio between the number of male and female nonagenarians has remained relatively stable over the decade.
3. There is no clear trend of increase or decrease over time for either sex. The chart does not provide absolute numbers or the total number of nonagenarians, so it is not possible to deduce whether the total number of nonagenarians has increased or decreased over the years. What is clear is that, in percentage terms, there are more females than males in the 90-99 age group.

The right chart illustrates the percentage of centenarian individuals, divided by sex, from 2010 to 2020. Black bars indicate the percentage of male centenarians, while white bars represent female centenarians.

From the analysis of this chart, the following can be deduced:

1. In each represented year, the percentage of female centenarians is significantly higher than that of males. This suggests that, among those who have surpassed the age of 100, there is a female predominance.
2. Despite annual fluctuations, female predominance among centenarians is a constant throughout the period considered.
3. There are no significant changes in the trend for male centenarians, whose percentage remains relatively low and stable over the decade.
4. The percentages shown do not indicate the absolute number of centenarians but express the gender ratio within this very advanced age group. These observations are consistent with global demographic data, which generally show greater longevity for women compared to men, a phenomenon that is also reflected in the age group of centenarians.

Representative image of a Western Blot analysis

The research involved analyzing the activity of biomolecules and exosomes extracted from traditional products on muscle cell cultures subjected to a low-grade oxidation protocol (Ferguson C., et al., 2021). This analysis was aimed at investigating the antioxidative and trophic effects on specific tissue, namely muscle. The antioxidant effect was assessed through enzymatic activity assays for catalase, superoxide dismutase, and glutathione peroxidase. The trophic effect was evaluated via experiments in skeletal muscle differentiation and quantitative expression assays of the myosin heavy chain.

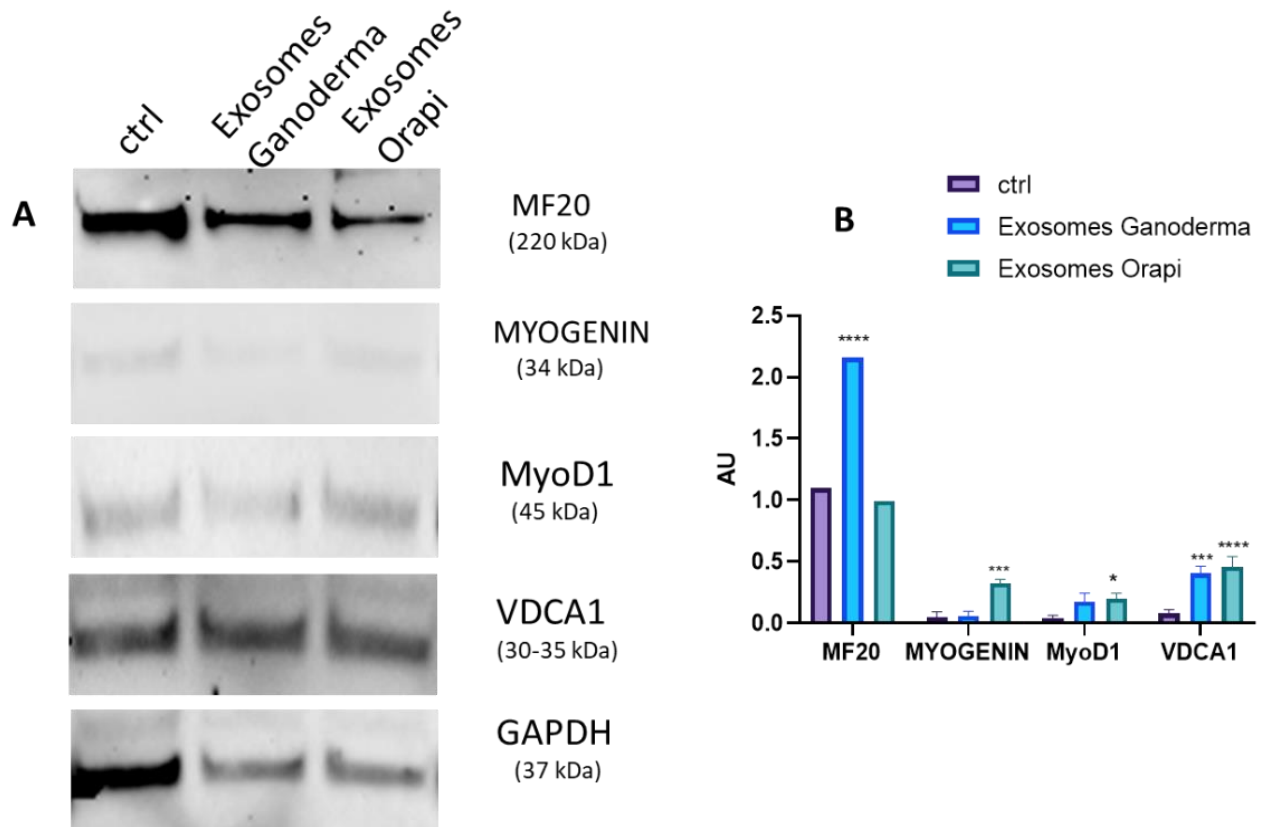


Fig. A) Representative image of Western blotting analysis of MF20, MYOGENIN, MyoD, VDCA1 and GAPDH (as loading control) expression in myotubes after incubation for 7 days with exosome of Ganoderma and Orapi.

Fig B) Densitometric analysis expressed as AU (arbitrary units). Values are shown as mean $\pm$ SD; n = 3; Turkey's multiple comparisons test, ctrl vs exosomes treatment, *p < 0.05, ***p < 0.001, ****p < 0.0001. ctrl: control without exosomes treatment.

Muscle Stem Cell Incubation with Ganoderma and Orapi Exosomes: Muscle stem cells were incubated in differentiation medium enriched with exosomes extracted from Ganoderma and Orapi. After seven days of differentiation, we assessed the expression of the proteins MF20, MYOGENIN, MyoD, and VDCA1. These proteins were found to be more expressed in cells treated with exosomes from Ganoderma and Orapi compared to control cells, which were not treated with exosomes. Notably, there was an increased expression of myogenic proteins (MF20, MYOGENIN, MyoD) in cells incubated with the exosomes (Kim S.Q, 2022), suggesting an enhanced push towards muscle regeneration due to the treatment. Additionally, we observed an increase in mitochondrial activity, indicating a possible enhancement in cell functionality due to exosome treatment.

Pilot Evidence from EVs Isolated from Ganoderma and Orapi: The results on extracellular vesicles (EVs) isolated from Ganoderma and Orapi serve as pilot evidence for potential studies and applications aimed at confirming the biostimulatory effect on myogenesis by EVs extracted from edible sources, with hopeful implications for the muscular health of the elderly.

The biostimulatory effect of the EVs on myogenic processes and myotube maturation could be due to the vesicular content affecting the protein expression pathways studied; further studies are needed to evaluate the role of specific bioactive compounds within the EVs and their timing of action on different stages of the myogenic process, as well as their potential bioactive role on mature muscle cells.

The feasibility of obtaining EVs from edible sources to deliver bioactive compounds or therapeutic molecules aligns with the goals of enhancing local heritage within a one-health context, as local edible sources not derived from intensive agriculture or other procedures limit the likelihood of encountering toxic compounds or undesirable materials in EVs, which also represent a biological route for the elimination of toxic molecules (Logozzi et al., 2022).

Biomarkers

Analysis of Biomarkers: Appetite Hormones Leptin and Ghrelin

Due to the heterogeneity of subjects, and thus a very high standard deviation, no significant difference was detected for the analyzed biomarkers, namely the appetite hormones: leptin and ghrelin; inflammatory state: IL-6, and nitric oxide levels: NOx. Therefore, we report the differences in percentages. Saliva collection was carried out on ultra-elderly subjects, aged 90 years and older, residing permanently in municipalities located at three different altitudes: below 300 meters (low altitude (LA)), between 300 and 600 meters (mid altitude (MA)), and above 600 meters (high altitude (HA)) in the Abruzzo region.

Ghrelin and leptin are two hormones that play crucial roles in regulating appetite, metabolism, and energy balance in the human body. Ghrelin, often referred to as the "hunger hormone," is a peptide primarily produced by the epithelial cells of the stomach, and to a lesser extent by the intestine, pancreas, and brain. Its main function is to stimulate appetite and promote food intake. Ghrelin acts on the hunger center in the brain, particularly the hypothalamus, signaling the body to increase food intake. Metabolic Regulation: In addition to stimulating appetite, ghrelin affects the release of growth hormone, fat metabolism, and energy homeostasis. Ghrelin levels rise before meals and decrease afterward, in relation to stomach fullness.

Leptin is known as the "satiety hormone" and is primarily produced by adipose cells, the fat cells of the body. This hormone informs the brain about the adequacy of energy reserves, i.e., body fats.

Leptin plays a crucial role in inhibiting appetite as it communicates with the hypothalamus, signaling that the body has accumulated enough energy and does not need additional food. Leptin regulates body weight because it plays a critical role in maintaining long-term weight stability by regulating both food intake and energy expenditure. In obese individuals, leptin resistance often develops as the brain does not adequately respond to the hormone's satiety signals, leading to increased appetite despite ample energy reserves. Ghrelin and leptin work in a delicate balance to regulate appetite and energy balance. When this balance is disrupted, such as in leptin resistance or anomalies in ghrelin levels, it can contribute to eating disorders, obesity, or metabolic conditions like type 2 diabetes. Proper regulation of these hormones is essential for maintaining healthy body weight and good metabolic health. The percentage differences in salivary levels of leptin (ng/mL) and ghrelin (pg/mL) between different altitudes are indicated below.

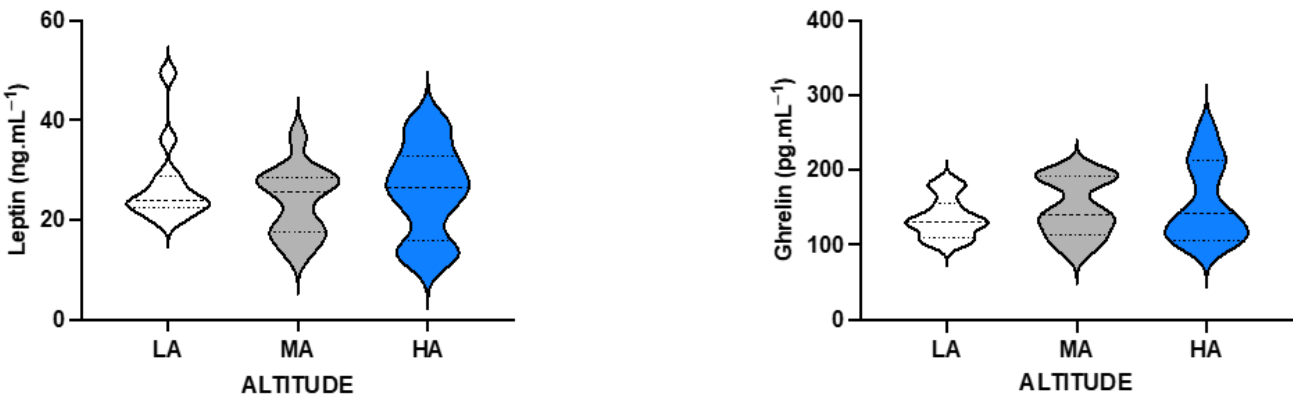


Figure 12. *Leptin (ng mL⁻¹) and Ghrelin (pg mL⁻¹) Charts: Violin plots depicting the distribution of leptin (left) and ghrelin (right) levels in subjects exposed to different altitudinal zones LA-MA-HA. Dashed lines in the charts represent the median and interquartile ranges, highlighting the variations in hormonal levels in relation to altitude. In violin plots, the broader regions of the density plot signify values that occur more frequently, whilst the narrower regions indicate values that are less common. The three lines denote the positions of the quartiles (third and first quartiles), with the median situated between them.*

	LEPTINA	GRELINA
LA vs MA	12%	14%
LA vs HA	6%	19%
HA vs MA	9%	6%

Leptin Chart (ng mL-1)

- *Distribution:* Leptin exhibits variable distributions depending on altitude, with the distribution at HA (high altitude) appearing wider and longer, suggesting greater variability in leptin levels compared to LA and MA.
- *Levels:* The violin shape for LA and MA is similar, but the peak at HA is slightly higher, indicating that average leptin levels tend to increase with altitude.

Ghrelin Chart (pg mL-1)

- *Distribution:* Ghrelin shows similar variability, with a wider distribution and a more pronounced peak at HA, indicating a greater variation in ghrelin levels at this altitude.
- *Levels:* Unlike leptin, the peak of the ghrelin distribution at HA is much higher compared to LA and MA, suggesting that ghrelin levels significantly increase with altitude.

These percentage differences illustrate how leptin and ghrelin levels vary across different altitude bands. Notably, Ghrelin shows a significant increase in HA vs LA with a 19% difference, while leptin shows less marked variations. These findings could suggest a differentiated hormonal adaptation in response to living conditions at different altitudes, with potential implications for metabolic and psychological health in the elderly.

The observation of a significant increase in ghrelin levels in ultra-elderly subjects as altitudinal bands rise from less than 300 meters to more than 600 meters suggests the existence of a physiological adaptation even in contexts of modest altitude differences.

- *Metabolic Adaptation:* Even in modest altitude bands, the increase in ghrelin could reflect a metabolic adaptation to slight environmental changes, such as microclimatic variations or biodiversity, which might indirectly influence the availability of food resources, and the energy required for daily activities.
- *Disease Protection:* Although the altitudinal variations considered do not induce hypoxic conditions, the increase in ghrelin could still play a role in protecting against metabolic and cardiovascular disorders through its anti-inflammatory and metabolically beneficial properties.
- *Influence on Sleep and Mood:* By positively influencing sleep and mood, Ghrelin could contribute to a better quality of life, a relevant factor for longevity. Even at modest altitudes, improvements in sleep and mood can have a significant impact on overall well-being.
- *Interaction with the Microenvironment:* The modification of ghrelin levels could be a response to individuals' interaction with their specific microenvironment, which varies slightly between altitudinal bands, thus subtly influencing homeostasis and overall well-being.

This observed increase in ghrelin levels at different altitudinal bands, although not associated with significant hypoxic stress, could indicate a physiological adaptation mechanism to the variation of

more subtle environmental factors. Such adaptation could be beneficial for maintaining health and longevity, indicating that even modest environmental changes can elicit significant biological responses. These results underscore the importance of further studies to better understand how relatively minor environmental variations can influence health in elderly populations.

Higher levels of ghrelin may promote longevity through various mechanisms primarily associated with caloric restriction, which is known to delay aging and extend life. Ghrelin, secreted during fasting, can play a significant role in calorie restriction-mediated longevity. It is involved in anti-aging mechanisms by regulating the growth hormone secretagogue receptor, which can have positive effects on health and longevity. Indeed, ghrelin is known to be a potent secretagogue of growth hormone (GH), which has several beneficial effects, including those related to longevity and aging delay (Amitani et al., 2017).

Biomarker Analysis of Inflammatory State: Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is a cytokine, a protein of the immune system, playing a pivotal role in the body's inflammatory processes. It is not the state of inflammation itself, but one of the key biochemical mediators involved in inducing and regulating inflammation.

The primary roles of IL-6 include:

- *Inflammation Mediation:* IL-6 is produced by various cell types, including macrophages, T cells, endothelial cells, and fibroblasts, in response to infections and tissue injuries. This cytokine contributes to the acute phase of the inflammatory response by stimulating the production of acute phase proteins from the liver, such as C-reactive protein (CRP), which serves as an inflammation marker in the body.
- *Immune System Regulation:* IL-6 assists in regulating immune cell activities, stimulates the differentiation of B and T cells, playing an essential role in both innate and adaptive immunity.
- *Impact on Other Conditions:* Elevated levels of IL-6 have been associated with various chronic pathological states, such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and some forms of cancer and cardiovascular diseases. In chronic disease contexts, IL-6 can contribute to the perpetuation of the inflammatory state, leading to long-term tissue damage and other systemic symptoms.
- *IL-6 as a Therapeutic Target:* Given its centrality in inflammatory processes, IL-6 has become a significant therapeutic target. Monoclonal antibodies that inhibit the action of IL-6 or its receptor are currently used to treat diseases such as rheumatoid arthritis and other autoimmune conditions.

IL-6 is a critical mediator of inflammation and immune response, and its regulation is crucial for the management of many chronic inflammatory diseases."

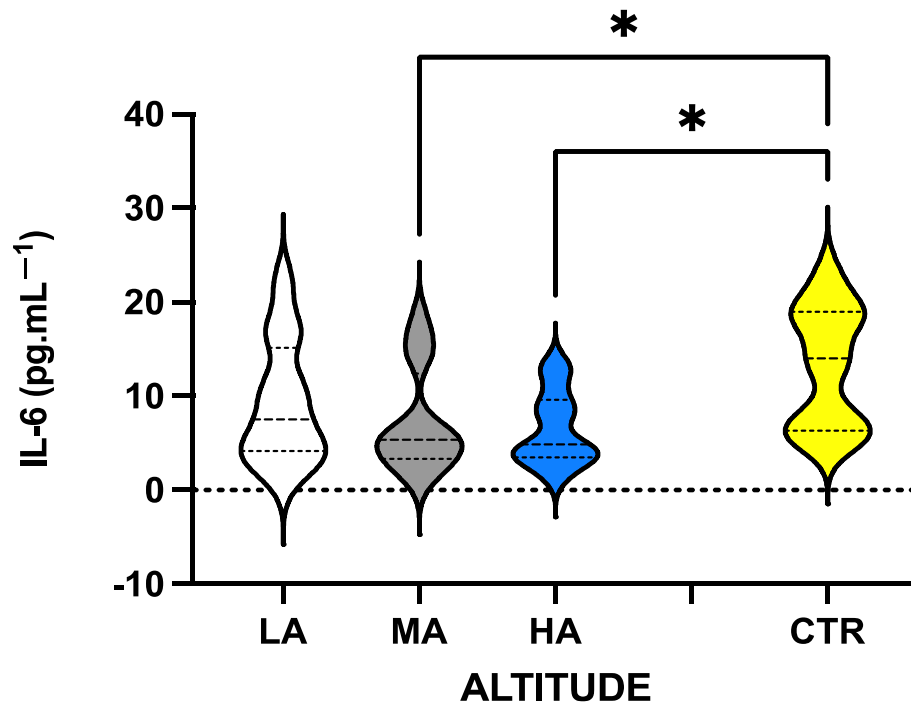


Figure 13. Distribution of Interleukin 6 (IL-6) Levels Across Experimental Groups, Including Control and Groups Exposed to Different Altitudes. The data are represented using violin plots that illustrate the variability within each group. Dashed lines indicate the median and interquartile ranges. The analysis highlights significant variations in IL-6 levels in relation to exposure to hypoxia at altitude.

Despite the sample's heterogeneity, a significant difference in baseline inflammatory state is observed among the three groups analyzed at low altitude (LA), medium altitude (MA), and high altitude (HA). The population residing at high altitude appears to enjoy greater protection compared to inhabitants of lower altitude areas, suggesting a lower risk of pathological events. This may be related to nutritional status, as described in subsequent sections.

Furthermore, as evident from the graph, the general "Abruzzese" sample exhibits a better systemic inflammatory state compared to a matched sample by age and sex but residing outside Abruzzo.

Dietary differences may play a key role in this context. It is possible that high-altitude residents consume less refined foods, less processed meats in smaller quantities, and lower amounts of red meats, carbonated drinks, and sweets. These dietary factors contribute to the reduction of systemic inflammation, offering a possible explanation for the lower risk of pathological conditions observed in these individuals. This hypothesis also seemed to be a good explanation for the differences obtained

in the sample between the two Italian regions. Dietary habits and lifestyle may have significantly influenced the outcome.

Nitric Oxide Metabolites (NOX) and Nitrites (NO₂)

Nitric oxide (NO) metabolites, nitrites (NO₂), and nitrates (NO₃), are substances partly composed of nitrogen (N) and oxygen (O) that are naturally occurring.

NO is a potent vasodilator, meaning it helps relax the walls of blood vessels, increasing blood flow and lowering blood pressure. Additionally, NO acts as a neurotransmitter in the central and peripheral nervous systems, influencing various biological processes, including the functioning of the immune system and pain response.

In the food industry, NO₂ and NO₃ are essential for the growth of plants, which use nitrogen to synthesize their protein structures with the help of sunlight.

Metabolically, nitrites have the unique property of binding with hemoglobin (Hb), transforming it into methemoglobin, and thus reducing the transport of oxygen to tissues. This aspect is critical and particularly harmful; indeed, they can combine with amines (e.g., organic compounds found in meat, cured meats, and cheeses) to produce substances called nitrosamines, which have carcinogenic potential.

In foods, nitrites are also used as additives in canned meats, cured fish, and dairy products. On the other hand, the concentration of NO₂ in plant-based foods is high in certain vegetables such as cabbage, lettuce, turnip, chard, rhubarb, spinach, and moderately high in celery, kale, fennel, and cabbage; and finally, a low content of nitrates is found in eggplant, chicory, potatoes, pepper, mushrooms, leek, carrots, asparagus, tomato, and onion.

Distribution of NO₂ and NO_x Levels at Various Altitudes

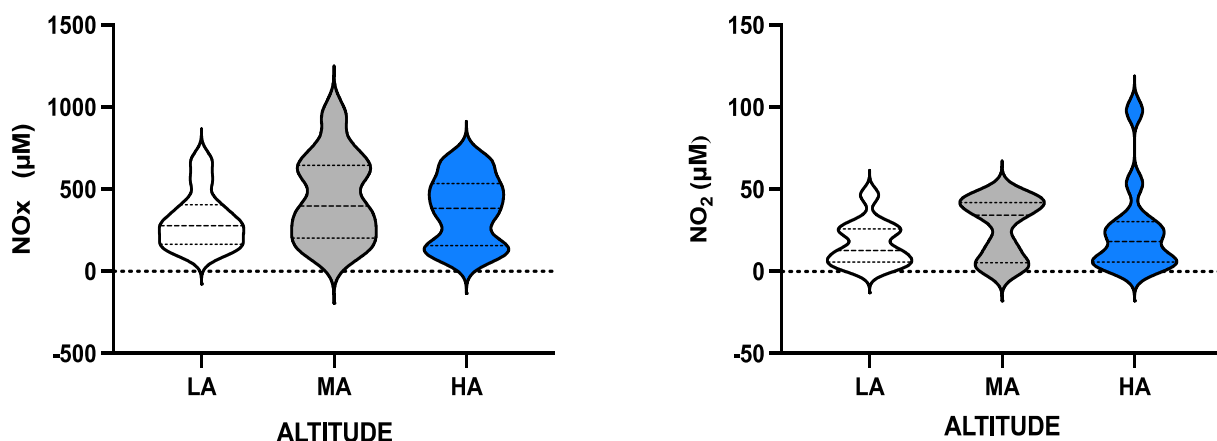


Figure 14. This is illustrated using violin plots. Dashed lines indicate the median and interquartile ranges. The analysis reveals variations in the levels of NO_2 and NO_x in relation to altitude exposure, suggesting a potential effect of hypoxia on the modulation of nitric oxide metabolism.

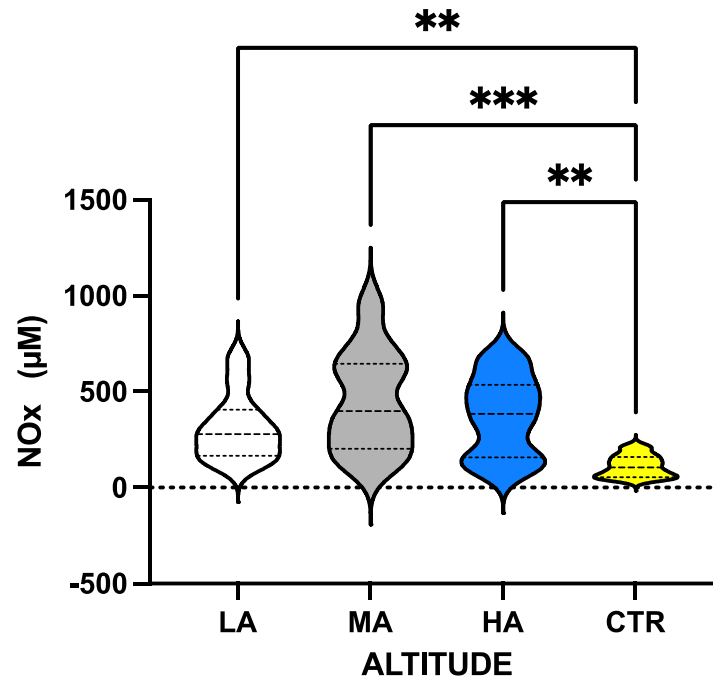


Figure 15. Distribution of Nitric Oxide Metabolites (NO_x , including Nitrites (NO_2^-) and Nitrates (NO_3^-)) Levels. This figure illustrates the concentration of nitric oxide metabolites measured in individuals residing at different altitudes (Low Altitude LA, Medium Altitude MA, High Altitude HA) and a control group (CTR) from an urban environment. The analysis highlights variations in NO_x levels in response to altitude, suggesting a possible effect of hypoxia on the regulation of nitric oxide metabolism.

Nitric Oxide Metabolites (NO_x)

Levels: NO_x levels demonstrate a significant increase with altitude. Individuals living at medium altitude (MA) exhibit the highest levels, followed by those at high (HA) and low altitude (LA).

Control Group: The control group (CTR) shows markedly lower levels compared to the altitude groups, suggesting that environmental exposure and/or living conditions at different altitudes may influence the production or metabolism of NO_x .

Nitrites (NO_2)

Similarly to NO_x levels, NO_2 levels are higher in residents at medium altitude compared to those at low and high altitudes, with the control group displaying the lowest levels.

Possible Role of NO_x and NO₂ in Longevity

Cardiovascular Health Effects: NO is known for its beneficial effect on the cardiovascular system. It contributes to vasodilation, improving blood flow and decreasing blood pressure, crucial factors in preventing cardiovascular diseases and promoting longevity. Studies indicate that NO plays a vital role in regulating cellular functions and mitigating oxidative stress, key elements in healthy aging.

Antioxidant Properties: By reducing oxidative stress, NO helps protect cells from damage, which can accelerate the aging process.

Immune Regulation: NO plays a role in regulating immune responses, essential for keeping the body resilient against infections and diseases.

These data suggest that increased NO_x levels at medium and high altitudes might contribute to an environment favorable for healthier aging and potentially greater longevity. Analyzing NO_x levels at different altitudes provides intriguing insights into possible connections between environment, physiological responses to living at high altitudes, and longevity. These findings serve as a basis for further research into the benefits of nitric oxide and its metabolites in the context of aging and longevity.

Effects of Altitude on NO_x Levels

The increase in NO_x levels with altitude can be attributed to various physiological and environmental factors. Reduced oxygen exposure at higher altitudes may stimulate the body to produce more nitric oxide as a compensatory mechanism to facilitate tissue oxygenation and vasodilation.

Cardiovascular Health: Nitric oxide is renowned for its beneficial effects on cardiovascular health, including blood pressure regulation and atherosclerosis prevention (Fu et al., 2024). Elevated NO_x levels in high-altitude communities might therefore suggest a potential cardiovascular benefit associated with these environments.

Implications for Research and Public Health: These findings could have significant implications for understanding physiological responses to altitude and for developing targeted health strategies for mountain populations. They might also influence cardiovascular disease research, suggesting that high-altitude environments could offer favorable conditions for heart and vascular health. In summary, the graph depicts a clear gradient of increased nitric oxide metabolites with rising altitude, contrasting significantly with the low levels observed in the urban control group. These results emphasize the importance of the environment and altitude in biological responses and human health.

Leptin, Ghrelin, and BMI in Subjects at HA, MA, LA, and Control Group

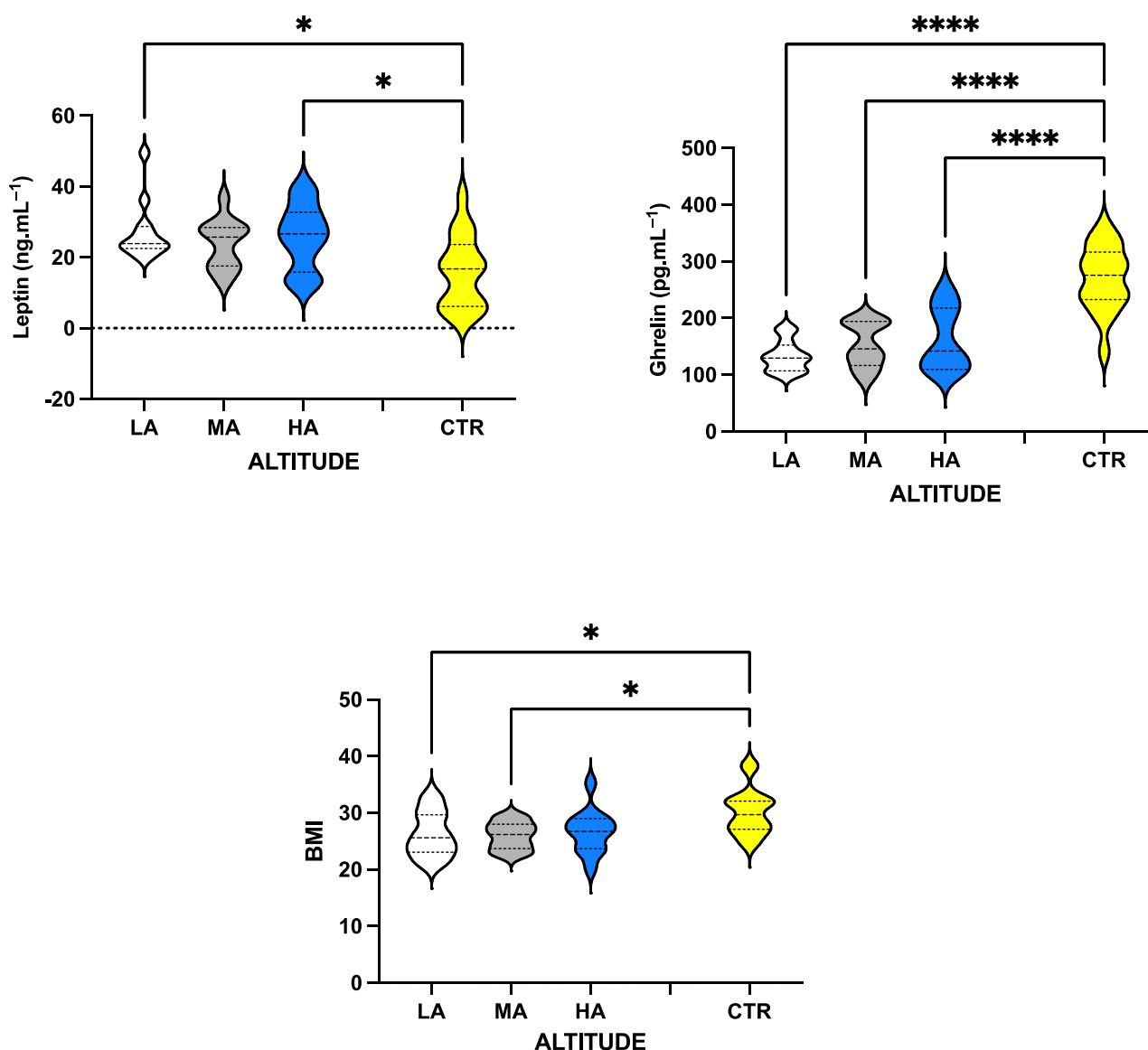


Figure 16: Distribution and Statistically Significant Differences in Leptin and Ghrelin Levels, and Body Mass Index (BMI) among Ultra-Longevity Individuals Living at Different Altitudes (Low, Medium, High) and a Control Group from Rome. This figure presents the distribution and statistically significant differences in leptin and ghrelin levels, alongside Body Mass Index (BMI), among ultra-longevity individuals residing at various altitudes, compared to a control group (CTR) from Rome, which is matched by age and has an equal male and female composition.

Leptin Levels

- *Distribution:* Leptin levels are highest in the control group (CTR) compared to other groups, showing a progressive increase from low to high altitude.

- *Significance:* The high leptin concentration in the Rome control group may reflect a greater prevalence of adipose tissue than in other groups. This aligns with the known association between leptin and adipose tissue.
- *Implications:* The correlation between leptin and adipose tissue in the urban control group suggests that factors such as a richer urban diet and lower physical activity may contribute to higher BMI, thereby positively influencing leptin levels.

Ghrelin Levels

- *Distribution:* Subjects in the Rome control group exhibit significantly higher levels of ghrelin compared to those living at different altitudes.
- *Significance:* This pronounced difference may indicate variations in dietary regimes or appetite regulation influenced by an urban lifestyle.
- *Implications:* Elevated ghrelin levels in the urban group suggest a potential increase in appetite stimulation, which could be linked to easier access to food and nutritional resources in an urban setting.

Body Mass Index (BMI)

- *Distribution:* The BMI is significantly higher in the control group from Rome compared to other groups, with a marginal increase in BMI from low to high altitude.
- *Significance:* The notable difference in BMI between individuals from Rome and those living at various altitudes underscores the impact of urban lifestyle on body weight.
- *Implications:* The higher BMI in Rome may reflect the influence of an urban environment that promotes a sedentary lifestyle and consumption of higher-calorie diets, thereby increasing the risk of obesity and related diseases. The analysis highlights how the urban environment of Rome can significantly impact metabolism and physical health, with clear differences in regulatory hormone levels and BMI compared to individuals living at different altitudes. These results underscore the importance of considering the urban context in public health strategies and gerontological research, aiming to address specific health challenges associated with urban lifestyles.

The analysis of leptin, ghrelin, and BMI levels in relation to living environments (Rome and various altitudes) provides key insights into the understanding of aging processes and factors that may contribute to favorable aging. The results highlight how the environmental context and lifestyle significantly influence biological markers directly linked to the physical and metabolic health of the elderly.

Influence of the Urban Environment on Metabolic Markers

Individuals in Rome exhibit elevated levels of leptin and ghrelin, associated with a higher BMI. These findings suggest that the urban environment, likely characterized by high availability of calorie-dense foods and a sedentary lifestyle, may contribute to obesity or greater fat mass. Obesity is well-known for its association with multiple chronic diseases such as type 2 diabetes, cardiovascular diseases, and certain types of cancer, which can compromise both the quality and expectancy of life.

These findings are crucial for understanding the environmental and biological influences of altitude and lifestyle on metabolism and physical well-being. The differences in hormone levels and BMI between the control group and those at varied altitudes highlight how urban environments may impact physiological and metabolic health significantly.

Effects of Altitude on Aging

Individuals living at various altitudes exhibit lower levels of leptin and ghrelin and a reduced Body Mass Index (BMI) compared to an urban control group. This may suggest a more active lifestyle or a less calorific diet, both of which are known to promote healthy aging. Moreover, exposure to an environment with less pollution and greater daily physical activity, typical of mountainous areas, may enhance cardiovascular health and reduce the incidence of metabolic diseases.

Correlations Between Hormones, Diet, and Sleep Quality

Ghrelin, known for its role in stimulating appetite, shows an intriguing correlation with sleep quality at high altitudes. This suggests that proper regulation of appetite, influenced by the living environment, is crucial for maintaining a stable circadian rhythm, essential for healthy aging.

A better nutritional assessment (MNA) at different altitudes compared to Rome may reflect a higher consumption of balanced diets, with adequate intake of essential micronutrients for the prevention of cellular aging and the promotion of longevity.

These findings emphasize the need for public health policies that promote active lifestyles and healthy diets, especially in urban settings, to mitigate the negative effects of a sedentary lifestyle and a high-calorie diet. Awareness programs and specific interventions can help the elderly make healthier choices that support favorable aging. The data highlight that encouraging residence in environments with low pollution levels and ample opportunities for physical activity, such as mountainous areas, can be an effective strategy to enhance the overall health of the elderly.

Reactive Analysis

Analysis of PSQI, GDS, and MNA reagents for subjects living at LA, MA, and HA

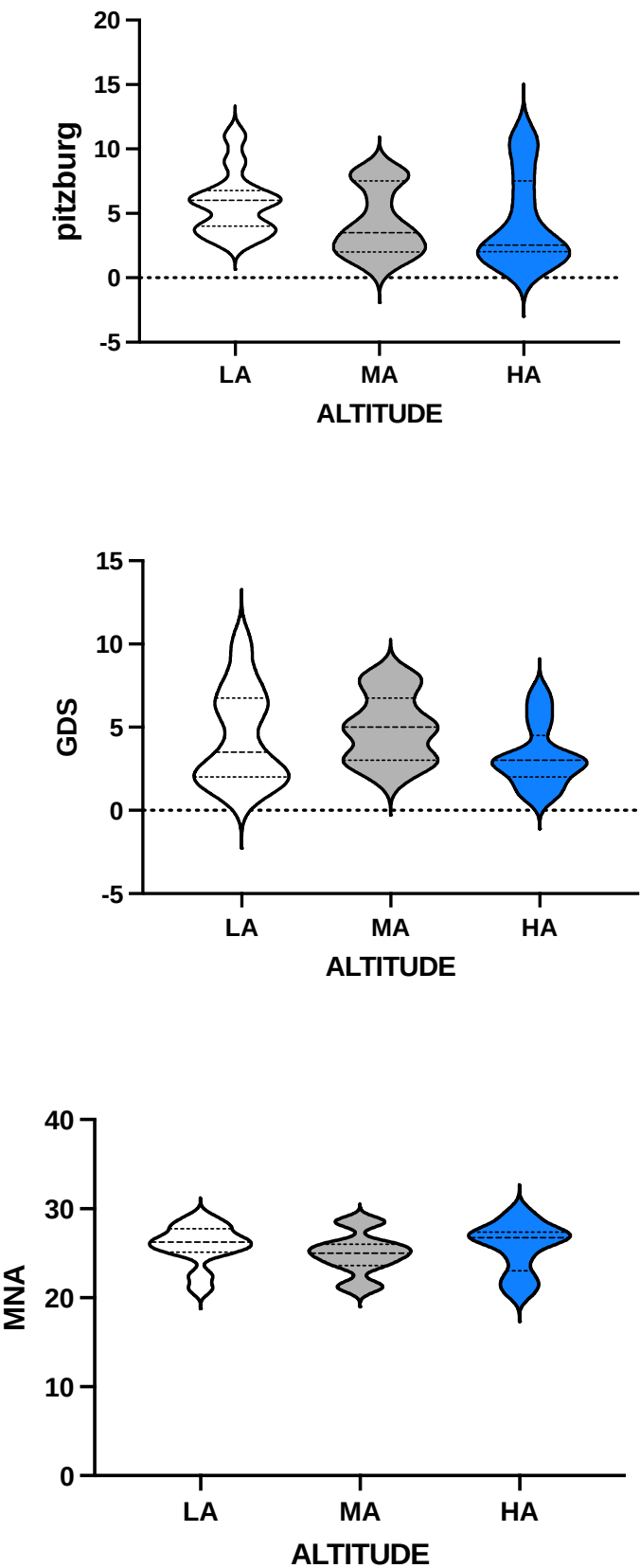


Figure 17: *Distribution of Pittsburgh Sleep Quality Index (PSQI), Geriatric Depression Scale (GDS), and Mini Nutritional Assessment (MNA) Scores Among Supercentenarians at Different Altitudes. The violin plots illustrate the variability of data for each parameter, with dashed lines indicating the median and interquartile ranges. The analysis highlights differences in sleep quality, geriatric depression, and nutritional status relative to residential altitude.*

Data Analysis Overview: No significant differences are observed in the scores of the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), Geriatric Depression Scale (GDS) (Yesavage et al., 1982-1983), and Mini Nutritional Assessment (MNA) (Bauer et al., 2008) among supercentenarians living at low (LA), medium (MA), and high altitudes (HA).

Pittsburgh Sleep Quality Index (PSQI):

- Distribution at LA is wider with a lower peak; at MA, sample distribution resembles LA but is slightly more concentrated. At HA, the peak rises, and distribution narrows, suggesting better sleep quality (range +26-32%).

Geriatric Depression Scale (GDS):

- Similar distributions across all altitudinal bands, though not statistically significant, show important percentage variations, indicating higher levels of depressive states in populations at low (+27%) and medium altitude (+35%) compared to those at high altitude.

Mini Nutritional Assessment (MNA):

- At LA and MA, distributions are similar with a slight trend towards lower scores at MA. At HA, the highest peak and tighter distribution suggest better nutritional status.

Percentage Differences Considered:

- PSQI indicates improved sleep quality with increasing altitude.
- GDS does not show variations suggesting an impact of altitude on depression levels.
- MNA suggests improved nutritional status at higher altitudes.

Implications for Supercentenarian Longevity: These observations might suggest that better sleep and nutritional status at higher altitudes could contribute to greater longevity and quality of life, while depression does not appear to be influenced by altitude in this population. However, further research is necessary to confirm these trends and explore their underlying causes. Despite the visual differences in the plots, statistically, there are no significant differences in test results across the altitude bands. This implies that other factors, beyond altitude, might play more significant roles in determining sleep quality, depressive state, and nutritional status in a supercentenarian population. Nonetheless, the indication of better sleep quality and slightly improved nutritional status at high

altitudes may warrant further investigation to better understand aging dynamics under these conditions.

Distribution of Depression by Altitude

The chart offers a detailed overview of the percentage distribution of supercentenarians with depression based on their altitude of residence. It compares those exhibiting depressive symptoms to those deemed without depression (normal).

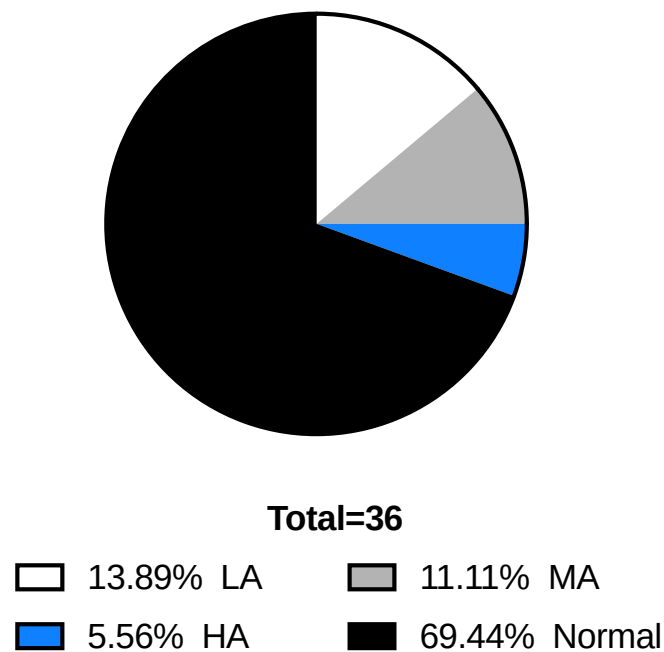


Figure 18: *Percentage Distribution of Supercentenarians with Depression Based on Their Altitude of Residence, Comparing Those with Depressive Symptoms and Those Considered Depression-Free (Normal)*

Depression Distribution by Altitude

- Low Altitude (LA): 13.89% of supercentenarians exhibit symptoms of depression.
- Medium Altitude (MA): 11.11% of supercentenarians exhibit symptoms of depression.
- High Altitude (HA): 5.56% of supercentenarians exhibit symptoms of depression.
- Normals (Without Depression): 69.44% of supercentenarians show no depressive symptoms.

This distribution provides insights into the prevalence of depression among supercentenarians residing at different altitudes, indicating a lower incidence of depressive symptoms as altitude increases.

Increased Incidence of Depression at Lower Altitudes

The highest incidence of depression is observed among supercentenarians residing at low altitudes (LA). This may suggest suboptimal environmental, social, or healthcare access conditions at lower

elevations adversely affecting the psychological well-being of the elderly. Conversely, a lower incidence of depression is noted among individuals at high altitudes (HA), where only 5.56% exhibit depressive symptoms. This could indicate that, despite potential challenges related to climate and service access, there might be protective factors associated with high-altitude living, such as greater social cohesion, a more active lifestyle, or reduced exposure to urban stressors.

Majority without Depression

Interestingly, 69.44% of supercentenarians do not show depressive symptoms, implying that despite their advanced age, most maintain good mental health. This serves as a positive indicator of general well-being among the elderly.

Research Implications

These findings provide a basis for further investigation into the causes of variations in depression prevalence across different altitudinal zones. Exploring differences in social support networks, daily activities, exposure to environmental pollutants, and access to medical care among these areas could enhance our understanding of the dynamics behind these numbers.

Considerations for High Altitude

The notably lower incidence of depression at high altitudes raises interesting considerations on how this factor might positively influence aging. High-altitude environments may offer several features contributing to elderly mental well-being:

- *Serene Environment and Lower Pollution:* High-altitude areas typically have lower population density and reduced levels of both air and noise pollution. This cleaner and quieter environment can lessen daily stress factors, contributing to overall well-being.
- *More Active Lifestyles:* Communities at higher altitudes often engage in lifestyles involving more physical activity, such as farming or hiking. Physical exercise is well known for its positive effects on mental health, including reducing depressive symptoms through the release of endorphins and improving overall physical health.
- *Social Cohesion:* Many mountain communities are characterized by strong social cohesion and an intense community support network, providing better emotional and social support for the elderly, a key factor in preventing depression.
- *Contact with Nature:* High altitudes often offer spectacular natural landscapes and constant contact with nature, which has calming effects and stress reduction. Simply observing nature can decrease cortisol levels, stress hormones, and improve mood.
- *Reduced Environmental Noise:* Less exposure to urban noise and chaos can contribute to a more tranquil and less stressful living environment, positively influencing mental health.

This reduced prevalence of depression in high-altitude areas suggests significant advantages for healthy aging. Health authorities and policymakers might explore how the positive environmental and social factors of these regions could be integrated or emulated in other areas to better support the elderly, especially concerning mental health. This could include promoting more cohesive communities, increasing green spaces and areas for physical activity, and implementing strategies to reduce pollution and noise in denser urban areas.

Distribution of sleep quality by altitude

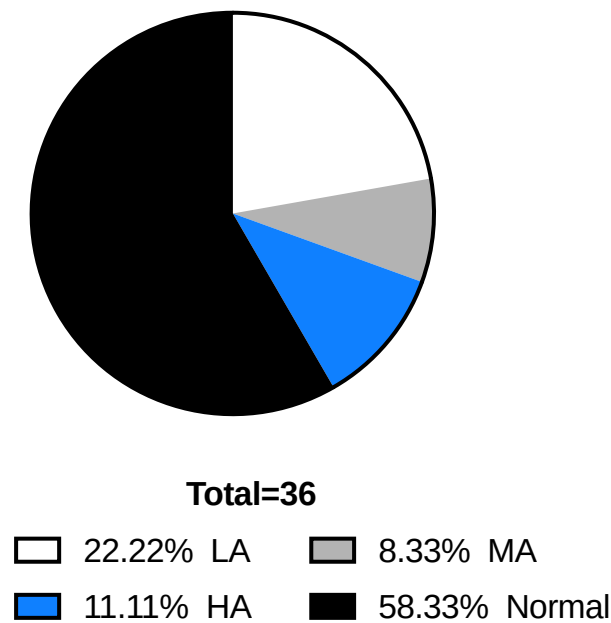


Figure 19: *Distribution of Sleep Quality Among Supercentenarians Across Altitudinal Zones. This graph illustrates the distribution of sleep quality among supercentenarians across different altitudinal zones, specifically highlighting the percentage of individuals experiencing poor sleep quality versus those enjoying good sleep quality.*

- Low Altitude (LA): 22.22% of individuals report poor sleep quality.
- Medium Altitude (MA): 8.33% of individuals report poor sleep quality.
- High Altitude (HA): 11.11% of individuals report poor sleep quality.
- Normal (Good Sleep Quality): 58.33% of all individuals report good sleep quality.

The highest incidence of poor sleep quality occurs at low altitude (LA). This may suggest that environmental or social factors at low altitudes could be less favorable, adversely affecting sleep. Factors such as noise, pollution, and population density could play a role. Both MA and HA exhibit lower percentages of poor sleep quality compared to LA, indicating that environmental conditions or reduced urban stress at these altitudes may contribute to better sleep quality. Most individuals,

irrespective of altitude, appear to enjoy good sleep quality (58.33%). This is a positive indicator for overall well-being, as quality sleep is crucial for physical and mental health, especially for the elderly. *Implications for Health and Aging:* Sleep quality is a critical factor in healthy aging. Studies have shown that good sleep quality is associated with a lower incidence of chronic diseases, improved mental health, and increased longevity. The observed differences across altitudinal bands may highlight the importance of considering environmental interventions and health policy initiatives aimed at enhancing sleep quality, especially in low-altitude areas where poor sleep quality is more prevalent. Further research may be necessary to identify and mitigate the specific causes of poor sleep quality at lower altitudes.

Sleep Aid Use Among the Ultra-Long-Lived Across Different Altitudes

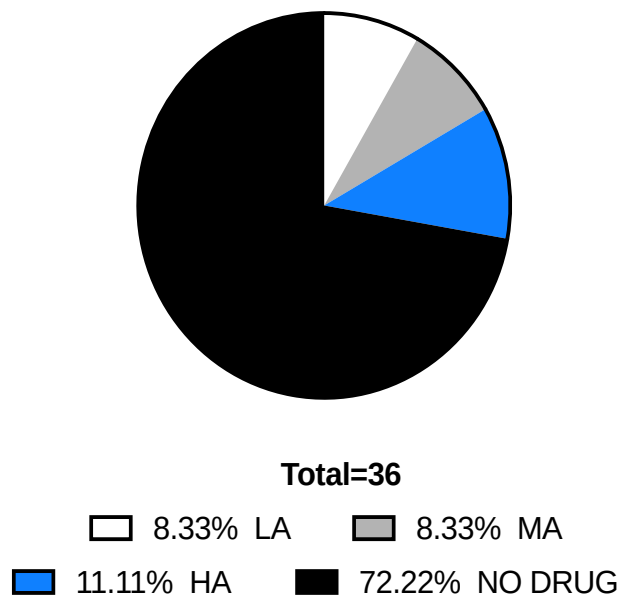


Figure 20: *Percentage Distribution of Ultra-Centenarians Using and Not Using Sleep Aids Across Altitudinal Bands.*

This graph illustrates the percentage distribution of ultra-centenarians who do and do not use sleep aids, categorized by altitudinal bands:

- LA (Low Altitude): 8.33% of ultra-centenarians using sleep aids reside at this altitude.
- MA (Medium Altitude): 8.33% of ultra-centenarians using sleep aids reside at this altitude.
- HA (High Altitude): 11.11% of ultra-centenarians using sleep aids reside at this altitude.
- No Drug (No Medication): 72.22% of ultra-centenarians do not use sleep aids.

The graph highlights that the majority of ultra-centenarians (72.22%) do not use sleep aids, indicating a preference or limited possibility for using such medications in this population. Among those who do use sleep aids, a slight majority reside at high altitude (HA). This may suggest that ultra-centenarians at high altitudes may experience more sleep difficulties or have greater access to sleep aid compared to those living at lower and medium altitudes. Similar percentages of sleep aid usage between the low and medium altitude bands (both at 8.33%) suggest a uniform distribution of sleep aid usage in these areas.

This pattern of sleep aid usage may reflect differences in living conditions, sleep quality, or access to healthcare services across different altitudinal bands. It could be beneficial to further investigate the reasons behind the variable use of sleep aids, such as differences in the prevalence of sleep disorders or local health policies affecting the prescription and purchase of medications.

Hand Grip

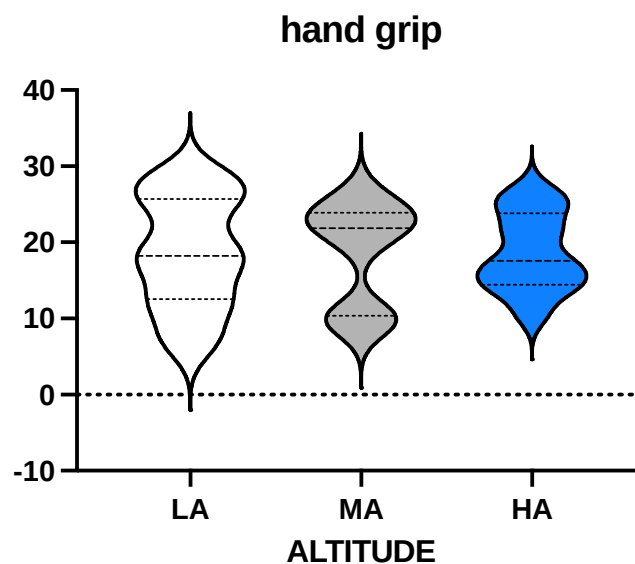


Figure 21: *Distribution of Hand Grip Strength Measured in Kilograms Among Ultra-Centenarians Residing at Different Altitudes in the Abruzzo Region. The graph displays the distribution of hand grip strength (measured in kilograms) among ultra-centenarians residing at different altitudes: low (LA), medium (MA), and high (HA). Although there are no statistically significant differences between the groups, some visual trends can be observed in the data.*

Analysis of Trends in the Graph:

- Low Altitude (LA): The distribution of hand grip strength in this group shows considerable variation, with a range primarily between 0 and 30 kg, and the median visually close to 20 kg.

The graph significantly widens in the middle, indicating substantial variation in hand grip strength among individuals.

- Medium Altitude (MA): The distribution here is like that of LA but appears slightly more constrained. The median is also around 20 kg, but the distribution is slightly more symmetrical, and uniform compared to LA, indicating less variability.
- High Altitude (HA): This distribution visually differs from LA and MA, with a shape suggesting a higher concentration of data around the median, also located around 20 kg. The distribution appears narrower and taller, suggesting that most individuals at this altitude have similar hand grip strength.

Variation Between Altitudes: Although statistically not significant, visually, the distributions show that individuals at high altitude tend to have less variability in their hand grip strength compared to those at low and medium altitudes. This might indicate greater homogeneity in physical conditions or levels of physical activity among ultra-centenarians living at higher altitudes.

Implications for Ultra-Centenarians: Hand grip strength is a well-known indicator of general health and functional capacity in the elderly. Although the differences are not statistically significant, the observed trends could have implications for targeted interventions to improve physical strength and general health, especially in low and medium altitude environments where variability is greater. While the statistical analysis shows no significant differences, the visual examination of the distributions suggests that the high-altitude environment might foster greater uniformity in physical strength among the elderly. Further research could explore the causes of this uniformity, such as differences in diet, exposure to environmental factors, or levels of daily physical activity. This could help develop specific strategies to enhance the physical health of ultra-centenarians at all altitudes.

Correlation Analysis

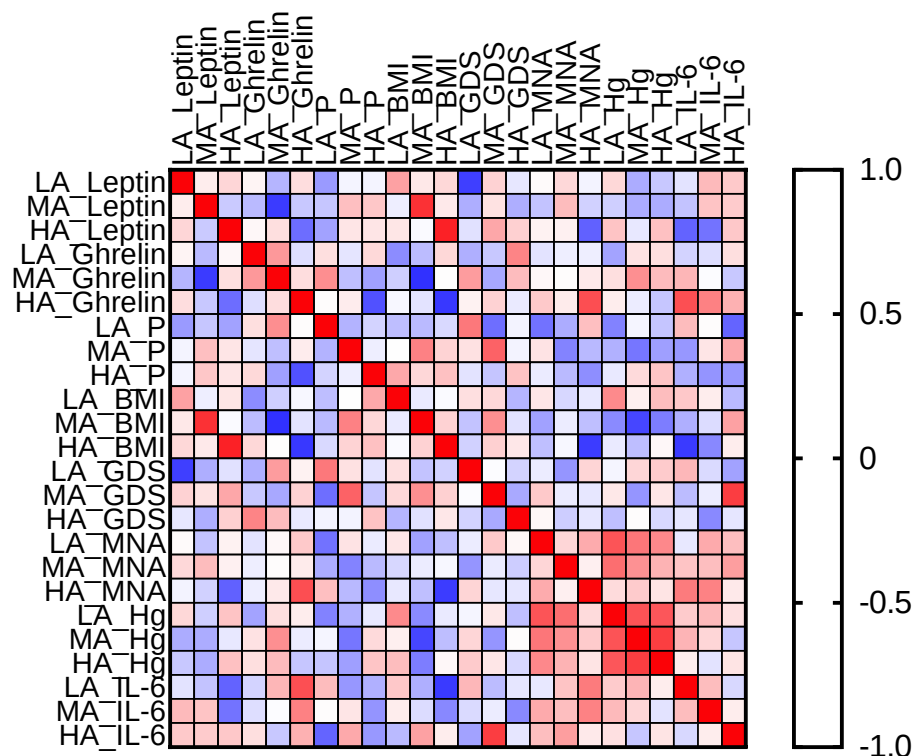


Figure 22. Correlation Matrix of Variables Examined at Different Altitudes. *This matrix displays the correlation values between biophysiological indicators in ultra-elderly individuals across varying altitudes, with color scale values ranging from -1 to 1. It is important to note that a value of -1 indicates a perfect inverse linear relationship, meaning as one variable increases, the other decreases; a value of 1 signifies a perfect direct linear relationship, where both variables increase in unison; and a value of 0 means that no linear trend can be established between the two variables.*

Correlation Matrix Analysis for an Ultra-Elderly Population Across Various Altitudinal Zones

This subsequent correlation matrix explores specific interactions between variables related to health, environment, and metabolic functions in an ultra-elderly population distributed across different altitudinal ranges. Focusing on key points, the analysis centers on the direct correlations between variables, observing particular trends that emerge from the data:

Hormonal Correlations (Leptin and Ghrelin)

Leptin and BMI: Consistent positive correlations across all altitudinal bands indicate that increases in BMI are associated with increases in leptin levels, supporting the role of this hormone in energy balance and satiety regulation.

Ghrelin and MNA: Ghrelin shows predominantly negative correlations with the MNA, suggesting that higher levels of ghrelin, associated with appetite stimulation, might coexist with a lower nutritional assessment.

Physical Health and Capability (BMI, MNA, Hand Grip)

BMI and MNA: The correlation between BMI and MNA varies, with some bands displaying slight positive correlations, implying that a higher BMI could be slightly related to better nutritional status.

Hand Grip and BMI: Mixed relationships are evident, with some bands showing slight positive correlations, indicating that greater hand grip strength might be associated with a higher BMI, possibly reflecting greater muscle mass.

Mental Health (GDS) and Sleep (Pittsburgh Sleep Quality Index)

GDS and PSQI: Moderate and positive correlations in various altitudinal bands emphasize a relationship between higher depression scores and poorer sleep quality, reflecting the interconnection between mental health and sleep.

GDS and MNA: Variable interactions, with some altitudes showing negative correlations, could suggest that better nutritional states are associated with lower levels of depression.

Influence of Altitude

Psychological and Environmental Variables (GDS, PSQI) at Different Altitudes: Display a pattern of correlations that varies with altitude, potentially reflecting how environmental conditions influence mental health and sleep quality.

This correlation matrix reveals how complex interactions between physical state, mental health, nutrition, and hormones modulate the well-being of the ultra-elderly. The variability of correlations across different altitudes underscores the importance of the environment in modulating these effects. These findings not only emphasize the need for personalized approaches in elderly care but also the potential impact of targeted interventions that consider the physical and altitudinal environment. These correlations provide a foundation for further investigations into how environmental and behavioral modifications can optimize longevity and quality of life in this age group.

DISCUSSION

Discussions on the First Phase of Work Concerning the Distribution of the Ultra-Elderly in the Abruzzo Region.

The demographic analysis of Abruzzo in 2021 provides an overview of the distribution of the ultra-elderly within the region. The year in question marked an exceptional presence of aged individuals,

particularly evident across the four provinces of Abruzzo. The sequence of prevalence for nonagenarians (individuals aged over 90 years) is as follows: Chieti at the forefront, followed by Pescara, L'Aquila, and Teramo. As for centenarians, we observe a slight variation in order, with Pescara emerging as the leader, again followed by Chieti, L'Aquila, and Teramo.

A fascinating aspect of this demographic dynamic is manifested in the smaller centers of Abruzzo, where the percentage of ultra-elderly, both nonagenarians and centenarians, is surprisingly higher compared to that recorded in medium and large urban centers. Another noteworthy observation is the higher concentration of ultra-elderly centers located above 600 meters in altitude, characteristic of mountainous areas and hinterlands, as opposed to those in hill and coastal areas.

Focusing specifically on the province of Chieti, the decade 2010-2020 has shown growth both in absolute numerical terms and in the percentage of the ultra-elderly population. This increase was particularly marked among ultra-nonagenarians and centenarians, especially in less populated centers and those situated at higher altitudes. It is also significant that, in the province of Chieti, during the same period, the majority of the ultra-elderly, both nonagenarians and centenarians, turned out to be female, outlining an interesting demographic profile that warrants further studies and insights.

The present doctoral work offers a sharp and detailed analysis of factors influencing longevity, especially in mountain contexts, emphasizing the essentiality of detailed epidemiological research for a deeper understanding of these dynamics. It explores the interaction of various elements, from the quality of the environment, active lifestyle, and local diet to stress and mental health, to social cohesion, physiological adaptations, and epigenetic factors, highlighting how each contributes to promoting a healthy and active aging process.

Comprehensively, it emerges that medium-sized villages represent an ideal habitat that balances access to services with a quality of life conducive to longevity, especially up to 90 years. In contrast, small villages, despite having a smaller overall population, show a slightly higher percentage of centenarians. This suggests that specific characteristics of these environments, such as a more united community and a healthier lifestyle, may be crucial for exceptional longevity. It is interesting to note how the size of the villages influences longevity. It is hypothesized that a more urbanized and better-served environment, typical of large and medium-sized villages, favors longevity up to 90 years. However, surpassing a hundred years seems to be influenced by additional factors, such as genetics and personal lifestyles. From the data collected on different age groups in the general population and among the ultra-elderly of the Abruzzo provinces, there is a tendency for the ultra-elderly to live at lower altitudes. However, it is remarkable that the percentage growth rate of nonagenarians and centenarians is higher at higher altitudes. This might indicate a correlation between altitude and longevity that merits further studies, suggesting that living in villages located at higher altitudes could

positively contribute to longevity, perhaps due to factors like less stress, a more cohesive community, and a better diet. This work emphasizes the importance of considering a wide range of interconnected factors in analyzing the impact of environment and lifestyle on longevity. These factors can vary significantly from one context to another and are influenced by specific geographical, socioeconomic, and cultural conditions. Moreover, it highlights the need for thorough statistical and qualitative analysis to support hypotheses and guide targeted interventions. This research proposes a holistic view of longevity, highlighting the importance of a multidisciplinary approach that considers the complex interactions between genetics, environment, lifestyle, and healthcare. This perspective offers not only a better understanding of longevity but also valuable insights for the development of public policies and investment in infrastructure to support the quality of life of older generations.

Exploring the Link between the Abruzzese Mountain Diet and Longevity

The study of nutritional aspects of the Apennine diet in the mountainous areas of Abruzzo represents a promising ground to investigate its potential as a cofactor in the longevity of local populations. This analysis goes beyond nutrition and dietetics, to also cover the socio-cultural and environmental dimensions that define the hinterland of Abruzzo.

The diet in question, steeped in historical heritage, reflects the balance between ancient culinary traditions and current studies on longevity. Composed of whole grains, legumes, fresh fruits and vegetables, and dairy products from sustainable pastoralism, this diet favors foods that are not processed and low in additives, maintaining a high nutritional value. Moderate consumption of meat, mainly from grazing, together with the use of herbs and spices in the preparations, enhances the anti-inflammatory and antioxidant properties of dishes.

This diet rich in antioxidants and fiber, and low in saturated fats and refined sugars, could explain the protection against chronic-degenerative diseases and support good intestinal health. Active lifestyles and the lower stress characteristic of mountain communities, together with food practices that emphasize seasonality and biodiversity, are other synergistic factors contributing to well-being and longevity (Gantenbein et al., 2021). It is crucial to consider the impact of the cultural context, including the conviviality of meals and attention to food preparation, in promoting psychosocial well-being. Therefore, the analysis of the Apennine diet can reveal the keys to a healthy diet and inspire nutritional policies that enhance local traditions, contributing to healthy aging at global level.

The exploration of this diet offers an opportunity to broaden the scientific debate on optimal nutrition in ageing, providing a replicable model for public policies targeting food education and infrastructural support for high quality of life in advanced age.

Traditional Abruzzese Diet and Longevity of Populations Living in Mountain Areas

This study aims to interpret and evaluate the results of research on the correlation between the traditional diet in Abruzzo and the longevity of populations living in mountain areas. The Mediterranean diet, with its local variants, has shown a close association with increased life expectancy in several international studies, and the Abruzzo context provides an interesting geographical and cultural perspective to explore this phenomenon.

The Mediterranean diet is rich in whole grains, fruits, vegetables, legumes, olive oil and moderate consumption of meat and dairy products (Schwingshackl et al., 2020). Typical foods from Abruzzo, as documented in our study, include fresh and local ingredients that are grown and consumed in the same areas, reducing the presence of preservatives and improving the nutritional profile. Analysis of the composition of traditional dishes revealed high concentrations of fibre, antioxidants and mono-unsaturated fatty acids, all factors that contribute to reducing the risk of chronic diseases such as type 2 diabetes, cardiovascular diseases and certain types of cancer (Jiang et al., 2023).

The lifestyles in the mountain areas of Abruzzo are characterized by increased daily physical activity, mainly due to the logistical needs of a predominantly hilly and mountainous territory. This aspect of daily life supports the theory that regular exercise, naturally integrated into everyday activities, contributes significantly to longevity (Gremeaux et al., 2012). In addition, living in small communities promotes strong social cohesion, which has been shown to have positive effects on mental health and general well-being (Williams et al., 2020).

The quality of the environment in these mountain areas is better than in densely populated urban areas. Cleaner air, less noise and visual pollution, and direct access to natural spaces contribute to better physical and mental well-being, reducing stress and improving the quality of sleep. Factors both associated with a lower risk of chronic disease and increased life expectancy (Kumar et al., 2019).

In this sense, the integration of these factors clearly demonstrates that longevity is not only influenced by diet but is also the result of a complex web of interactions between nutrition, physical environment, lifestyles and social cohesion (Global Roadmap for Healthy Longevity. 2022).

This holistic approach enriches our understanding of healthy living practices, suggesting that public policies and health recommendations should take these interactions into account to promote a longer and healthier life.

Correlation Between the Abruzzese Mediterranean Diet and Longevity

The Mediterranean diet, characterized by a high intake of vegetables, whole grains, fruits, and olive oil, is universally recognized for its beneficial effects on health and longevity (Tosti et al., 2018). In the specific context of the mountainous regions of Abruzzo, our research has explored how this diet directly influences the longevity of its inhabitants, yielding notably positive results. The analysis

revealed that individuals who adhere strictly to this diet tend to live longer, with lower rates of chronic diseases compared to those who follow more Westernized dietary patterns.

The staple foods of the Abruzzese Mediterranean diet, enriched by the biodiversity of a mountainous environment, are naturally rich in essential nutrients that promote health. In particular, olive oil, a cornerstone of this diet, is well known for its high content of monounsaturated fatty acids, particularly oleic acid, which provides significant cardiovascular protection (Chrysant et al., 2024). Research has demonstrated that these monounsaturated fats help reduce LDL cholesterol levels (commonly referred to as "bad cholesterol") while increasing HDL cholesterol ("good cholesterol"), thereby improving the lipid profile.

Moreover, the antioxidants abundantly present in fruits, vegetables, and whole grains—such as flavonoids and vitamins A, C, and E—play a crucial role in neutralizing free radicals. These bioactive compounds help mitigate oxidative stress and inflammation at the cellular level, conditions often associated with aging and the pathogenesis of many chronic diseases, including cancer and type 2 diabetes (Rahaman et al., 2023).

This phenomenon is further enhanced by the mountainous environment of Abruzzo, where local production and the freshness of food ensure maximum nutrient retention. For example, the daily harvesting of vegetables and fruits guarantees that antioxidants are consumed at their peak efficacy. Moreover, mountain life often entails greater daily physical activity, which, when combined with the Mediterranean diet, synergizes to promote optimal metabolism and weight reduction—both well-known contributors to longevity.

The adoption of this diet influences disease prevention, quality of life, and ultimately longevity. Our research has found that elderly individuals in the mountainous areas of Abruzzo exhibit higher longevity rates and better overall health compared to those in other regions who do not adhere to the Mediterranean diet with the same rigor. These findings align with other longitudinal studies conducted across various parts of the Mediterranean world, where a direct correlation has been observed between adherence to this diet and a reduced risk of all-cause mortality.

Thus, the Abruzzese Mediterranean diet fosters a longer and healthier life for the inhabitants of the Abruzzo mountains, offering a sustainable and health-promoting dietary model that could be more widely adopted to counteract the modern epidemic of non-communicable diseases.

Impact of Environmental Quality and Lifestyle

The intrinsic characteristics of the mountainous regions of Abruzzo, with their less polluted environment compared to congested urban areas, play a crucial role in shaping a lifestyle that promotes longevity. The superior air quality, lower exposure to industrial pollutants, and the presence of lush flora significantly contribute to reducing the risk of respiratory and cardiovascular diseases

conditions often exacerbated by prolonged exposure to adverse environmental factors typical of urban settings. The very nature of the mountainous terrain necessitates greater daily physical activity. Residents of these areas tend to cover longer distances on foot, often traversing rugged landscapes, which increases their daily energy expenditure and enhances cardiovascular and muscular capacity (Miko et al., 2020; Pinckard K et al.; Pes et al., 2018). This unintentional form of exercise aids in maintaining body weight and stimulates the production of endorphins, commonly known as "happiness hormones," which improve mood and reduce stress (Amir et al., 1980).

Future Implications and Research Directions

The findings of this study open intriguing avenues for future research on the relationship between diet and longevity, particularly within the framework of the Abruzzese Mediterranean diet. These results not only enrich the existing body of knowledge but also outline new trajectories for more in-depth investigations and targeted interventions. One of the most promising directions for future research is the detailed analysis of individual components of the Abruzzese diet and their direct impact on health biomarkers. By examining how specific foods or nutrients—such as olive oil, legumes, whole grains, and fresh produce—influence key biological indicators, including markers of inflammation, oxidative stress, lipid profiles, and glycaemic regulation, it would be possible to isolate the mechanisms through which diet contributes to longevity. Furthermore, assessing how the integration of elements of the Abruzzese Mediterranean diet could benefit individuals in urban settings represents another critical area of interest. The transition from a typically urban lifestyle, often characterized by an energy-dense diet and low physical activity, to one incorporating principles of the Mediterranean diet could present new opportunities to combat the epidemic of non-communicable diseases such as obesity, type 2 diabetes, and cardiovascular conditions in metropolitan areas. Future research could explore the behavioral changes required to facilitate this transition and their long-term effects on public health.

A crucial aspect emerging from this research concerns the adaptation of public policies to promote healthier lifestyles across different geographical contexts. Based on the findings of this study, policymakers could be encouraged to develop programs that support local and sustainable agriculture, enhance access to healthy foods, and promote physical activity through urban planning and the reinforcement of socially cohesive communities. The design of such policies should take into account the cultural and socio-economic specificities of different populations to ensure the effectiveness of interventions.

Vision: From the Abruzzese Mediterranean Diet to the "Apennine Diet"

The transition towards the Apennine Diet represents a critical adaptation of the principles of the Abruzzese Mediterranean Diet, modified to meet the specific needs of the mountain communities of

the Apennines. This new dietary model directly addresses the unique environmental factors of these regions, such as the variable climate and mountainous terrain, which influence the seasonal availability of food products and increase caloric requirements due to colder temperatures and the greater energy expenditure required for movement across challenging landscapes. Consequently, the Apennine Diet emphasizes the importance of foods that not only provide a higher energy intake but are also well-suited to growing in these harsh climatic conditions.

The valorization of local species plays a fundamental role in the Apennine Diet. By utilising and promoting the use of native plants and animals or those well-adapted to the Apennine environment, this diet helps to preserve local biodiversity. This approach supports environmental sustainability while encouraging a diverse and nutrient-rich diet that makes the most of what the mountainous ecosystem naturally provides. Moreover, the Apennine Diet places strong emphasis on sustainability and local economic support, promoting a short supply chain and the "Km Zero" philosophy, which prioritizes local producers. This sustainable approach not only supports the regional economy but also contributes to a food system that enhances both health and economic resilience.

By adapting the principles of the Mediterranean Diet to incorporate greater use of legumes, whole grains, nuts and seeds, almonds, chestnuts, persimmons, and wild herbs—foods that are particularly nutritious and well-suited to the Apennine climate—the Apennine Diet effectively addresses common health challenges in mountain areas, such as vitamin and mineral deficiencies or cardiovascular issues.

The development of the "Apennine Diet" represents an innovative and strategically adapted integration of the Mediterranean Diet, specifically designed to meet the distinct nutritional needs of rural mountain populations in the Apennine regions. As highlighted, this adaptation is necessary to accommodate the unique environmental conditions, which include harsh temperatures, increased physical energy demands due to intensive manual labor, and the metabolically demanding nature of living at high altitudes. The Apennine Mountain environment, characterized by a colder climate and topographical conditions that require greater physical exertion, significantly alters individuals' energy and nutritional requirements. The low temperatures increase basal caloric expenditure, as the body must work harder to maintain optimal body temperature. Similarly, physical labor, often intensified by agricultural activities and other forms of manual work typical of mountain communities, necessitates a higher caloric and nutritional intake to adequately sustain energy levels and muscle repair.

In this context, the Apennine Diet aims to recalibrate the balance between macro- and micronutrients to meet these specific needs. It emphasizes an increased intake of complex carbohydrates, derived from whole grains, which provide a sustained release of energy essential for supporting prolonged

periods of physical activity. Proteins also play a central role, with a higher intake from legumes, dairy products, and lean meats, to support muscle repair and growth while ensuring optimal immune function in a climate that may predispose individuals to respiratory illnesses.

Moderate meat consumption, combined with a high intake of fruits and vegetables, remains a cornerstone of the diet, ensuring the supply of essential vitamins and minerals that might be lacking in less varied diets typical of certain mountainous areas. Additionally, the predominant use of healthy fats, such as olive oil, not only aligns with Mediterranean tradition but also enhances the absorption of fat-soluble vitamins, which are crucial for overall health in cold living conditions.

The Apennine Diet preserves the health benefits of the Mediterranean Diet while adapting them to effectively meet the challenging conditions of mountain environments. It emphasizes an increased caloric intake, an appropriate balance of macronutrients to support intense physical activity, and the inclusion of specific micronutrients to compensate for extreme environmental conditions.

The diet also includes a substantial number of high-quality proteins, essential for muscle repair and development, particularly important for individuals engaged in physically demanding labor. These proteins primarily come from legumes such as lentils and beans, which are a traditional component of the rural Abruzzese diet, as well as from small quantities of locally reared meat, ensuring freshness and reducing the environmental impact of food transportation.

At the same time, the use of healthy fats derived from local sources such as nuts, seeds, and, most notably, olive oil, supports an adequate caloric intake while contributing to cardiovascular health. These fats, rich in mono- and polyunsaturated fatty acids, are well known for their beneficial effects on cholesterol levels and overall inflammation in the body.

The Apennine Diet places a strong emphasis on sustainability, integrating principles that not only promote individual well-being but also respect and enhance the natural environment. This holistic vision embraces the use of local and seasonal products, supporting agricultural practices that preserve the land and minimize environmental impact.

Research Experience Abroad at Charles University

The activities carried out at Charles University, in collaboration with the Department of Biomedical Foundations in Kinanthropology, involved a comparative study of the diet practiced by the population of Prague and its surrounding areas.

To compare the traditional diet of native Czech families in Prague and its vicinity with that offered by modern food services, a comprehensive analysis of dish composition was conducted. Traditional Czechoslovak recipes were collected from families with roots spanning at least two generations. Additionally, a survey and analysis of the gastronomic offerings of modern restaurants in Prague and

nearby areas were carried out, with particular attention to contemporary reinterpretations of traditional dishes. A comparative evaluation was then carried out, focusing on ingredients, portion sizes, and the caloric/nutritional content of traditional home-cooked meals versus modern restaurant cuisine.

Furthermore, in collaboration with Prof. Jandova Tereza, advanced body composition analysis techniques were applied. Techniques such as DEXA (Dual-Energy X-ray Absorptiometry) and bioelectrical impedance analysis (BIA) were used to assess body composition in elderly individuals and athletes, focusing on fat mass, lean mass, and bone density. These body composition data were then correlated with dietary habits to determine the impact of a traditional versus modern diet.

Regarding the evaluation of metabolic and nutritional needs, nutritional interviews and dietary assessments were conducted to determine the specific requirements of the Czech elderly population, taking into account physical activity levels, clinical history, and socioeconomic conditions. The collected data were analyzed to identify potential nutritional deficiencies or excesses that could be addressed by incorporating traditional dishes or adopting modern dietary practices.

In terms of nutritional status and its correlation with overall health, regular nutritional screenings were conducted on the elderly population to monitor nutritional status and prevent both malnutrition and overnutrition. The association between adherence to the traditional Czech diet and key health indicators—such as the incidence of chronic diseases, quality of life, and longevity—was also evaluated.

Finally, health education and promotion played a crucial role. Awareness campaigns were organized to highlight the benefits of a balanced diet that integrates elements of traditional Czech cuisine with modern nutritional principles. A collaboration was established with chefs and nutritionists to develop menus that combine traditional flavors with contemporary dietary needs.

These key points provide a comprehensive framework for assessing the impact of diet on the health of Prague's population and its surrounding areas, with a particular focus on the elderly, while simultaneously preserving the rich culinary heritage of the Czech Republic.

Additional Activities

As part of my doctoral studies, in order to compensate for the six-month suspension, and following approval from the MUR for the implementation of supplementary activities, I undertook additional academic and research activities starting in December 2022.

During this period, in parallel with my PhD research, I carried out additional tasks that enriched my academic and professional development. Specifically, I contributed to health and nutritional assessments of individuals involved in the 2022 high-altitude scientific expedition at the Pyramid

Laboratory in Nepal, aiming to gather data on physiological adaptation to high altitude. My role included data analysis and interpretation at 5050 m altitude, as well as comparative studies involving different mountain populations, including communities acclimatized to high-altitude environments for millennia. This contribution added significant value to the ongoing PON project, which focuses on the sustainability and biodiversity of human nutrition and promotes the de-industrialized use of biologically diverse foods within a green framework.

Additionally, I provided support for research thesis in Nutritional Physiology, assisted in the drafting and development of research projects, both domestically and during my time abroad, with the aim of enhancing my training and enriching my doctoral research. Furthermore, I collaborated with colleagues in the writing of scientific articles, engaging in discussions on research findings and interpretations.

Lastly, I actively participated in additional seminars and webinars beyond those already included in my doctoral program, further broadening my academic and professional knowledge.

CONCLUSIONS

This doctoral thesis has rigorously explored the intersections between the Abruzzese Mediterranean diet and the biotechnological aspects of bioactive substances derived from foods, with particular focus on their composition in micro and macronutrients and the release of exosomes. This study has purposefully employed a holistic approach, integrating advanced analytical methodologies with demographic and socio-economic analysis of the mountain populations of Abruzzo, offering innovative insights into how traditional foods can influence longevity and health.

In this work, we have sought to demonstrate that the "Apennine Diet," a local adaptation of the Mediterranean diet, has the theoretical basis to significantly contribute to health promotion, reducing the risks of age-related chronic diseases such as cardiovascular pathologies and dementias. The foods studied, rich in antioxidants, fiber, and essential fatty acids, have proven fundamental in modulating favorable physiological responses, an aspect highlighted by the analysis of the ultra-nonagenarian and centenarian populations of Abruzzo, which have exhibited exceptional longevity rates.

In the development of this work, it emerged how diet influences not only the biological aspect of health but also the socio-cultural fabric of the mountain communities. The preservation of traditional recipes, the use of local products, and the transmission of culinary knowledge have proven to be effective tools for strengthening social cohesion and cultural identity. Furthermore, the adopted approach has promoted environmental sustainability, highlighting how local agricultural practices contribute to reducing ecological impact and maintaining biodiversity.

Looking to the future, the research suggests adopting the Apennine Diet as a nutritional model and strategy for sustainable rural development. It is imperative to consider expanding this dietary model to other mountain regions, not only in Italy but also in the European context, to promote health through diet and at the same time enhance and protect natural and cultural resources.

Finally, we hope that future research may include broader longitudinal studies to validate the benefits of the Apennine Diet on a larger scale, as well as to explore the interaction between diet and specific genetic factors of mountain populations. These studies could provide further confirmation of how targeted dietary adaptations can improve the quality of life and increase longevity.

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