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UNIVERSITÀ DI ROMA

ESCUELA INTERNACIONAL DE DOCTORADO
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Department of Physiology and Pharmacology V. Erspamer
PhD Program in Pharmacology and Toxicology
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Author:
Paola Aiello

Supervisors:
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Murcia, mes de Junio 2022

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AUTORIZACIÓN DE LO/S DIRECTOR/ES DE LA TESIS
PARA SU PRESENTACIÓN

La Dra. Dña. Débora Villaño Valencia y la Dra. Dña. Patrizia Campolongo como Directores de la Tesis Doctoral titulada "Dietary habits, lifestyle and self-medication in an Italian and Spanish university population." realizada por Dña. Paola Aiello en el Programa de Doctorado de Ciencias de la Salud a la UCAM y en el Programa de Doctorado de Farmacología y Toxicología de la Sapienza, **autorizan su presentación a trámite** dado que reúne las condiciones necesarias para su defensa.

Lo que firmo, para dar cumplimiento al Real Decreto 99/2011 de 28 de enero, en Murcia a 29 de Junio de 2022.

Fdo: Dra. Dña. Débora Villaño Valencia Fdo: Dra. Dña. Patrizia Campolongo

ABSTRACT

BACKGROUND: Over the past 20 years there has been an increase in obesity rates among university students, therefore they should be seen as a group that requires special attention regarding health promotion. The interest in a healthy diet can lead to a psychological obsession known as orthorexia nervosa (ON), frequent among students in the biomedical field and in the sports context. The high levels of stress recorded in university students have been related to the use of drugs to enhance their cognitive abilities. However, a high adherence to the Mediterranean diet (MD) can bring cognitive benefits, with an improvement in depressive symptoms and anxiety, enabling a better academic outcome.

AIM: The aim of this study was to evaluate self-medication, adherence to MD, and the relationship between lifestyle and biomarkers of metabolic status in a university population.

METHODS: Students, doctoral students, post-docs and specialists have been recruited in Italy (N = 108) and Spain (N = 86). Data were collected through questionnaires in order to evaluate lifestyle, self-medication, alcohol consumption (AUDIT), eating habits, in particular adherence to MD and food neophobia (FN), level of physical activity (IPAQ), orthorexia (ORTO-15 and subscores), body concerns (MBSRQ and BUT), psychological distress (K10), eating attitude (EAT-26) and malnutrition (SSI). Participants have been evaluated with clinical parameters of metabolic status (glycaemia, cholesterol, triglycerides, and ketones).

RESULTS: Italian females had higher MED-55 and FNS (Food Neophobia Scale) scores, and a lower AUDIT score than Spaniards ($p < 0.01$). Students who stayed with their family (resident) were more adherent to MD than those who moved away from home. Resident Italians consumed less beer, hard liquors, and cocktails than Spaniards on Saturday nights ($p < 0.01$). There were negative correlations between AUDIT and QueMD (R^2: 0.137, $p < 0.05$), and AUDIT and non-typical MD foods score (ntMED) (R^2: 0.201, $p < 0.01$) in Spaniards; however, there was no relationship between AUDIT and other MD scores. Most of the sample (72.8% of IT and 62.3% of SP) used medicines without medical prescription, with a higher tendency to

self-medication among Italian females (IT-F) and Spanish males (SP-M). Moreover, 47.6% of IT and 31.1% of SP read the leaflet before taking a drug ($p < 0.05$). The ORTO-15 positive subjects, assessed with the originally proposed cut-off, were above 70% in both IT and SP students, with a higher prevalence in the Spanish sample (96-97%). According to ORTO-7, about 30% of Italian and 48% of Spanish students were positive to ON, with not significant gender differences. When excluding students underweight, overweight or obese, as well as those potentially at risk of eating disorders or presenting mild, moderate, and severe distress ($K10^{neg}$-$EAT\text{-}26^{neg}$ subgroup), we did not find many correlations between ORTO scores and BUT, SSI, total MBSRQ and some of its components. ORTO-7 resulted the only ON score unrelated with body mass index, MBSRQ components and IPAQ-assessed intense activity, in the NW - $K10^{neg}$ -$EAT\text{-}26^{neg}$ subgroup. After this sort of "exclusion diagnosis", the prevalence of ON of these students on the overall sample resulted of 16.9%, 12.2%, 15.2% and 25.9% for IT-F, IT-M, SP-F and SP-M, respectively. As regards the metabolic status, Spaniards had higher blood glucose levels than Italians (IT-F vs SP-F, $p < 0.01$; IT-M vs SP-M, $p < 0.001$), whereas a state of ketosis has been observed in SP-M.

CONCLUSION: Results of this study suggest that non-typical MD foods and Saturday night consumptions, related to being far from home, have a great impact on alcohol consumption. In some university students ON could be a symptom of other conditions related to body image concerns and distress, as well as to high PA and appearance, fitness, health, or illness orientation. However, ORTO-7 became independent from these confounding, after the exclusion of underweight, overweight, obese and students positive to EAT-26 and K10, suggesting the possibility of identifying orthorexic subjects with this specific questionnaire.

RESUMEN

ANTECEDENTES: En los últimos 20 años ha habido un aumento de las tasas de obesidad entre los estudiantes universitarios, por lo que deben ser vistos como un grupo que requiere una atención especial en materia de promoción de la salud. El interés por una alimentación saludable puede conducir a una obsesión psicológica conocida como ortorexia nerviosa (ON), frecuente entre los estudiantes del ámbito biomédico y del ámbito deportivo. Los altos niveles de estrés registrados en estudiantes universitarios se han relacionado con el uso de drogas para potenciar sus capacidades cognitivas. Sin embargo, una alta adherencia a la dieta mediterránea (DM) puede traer beneficios cognitivos, con una mejoría en los síntomas depresivos y de ansiedad, posibilitando un mejor rendimiento académico.

OBJETIVOS: El objetivo de este estudio fue evaluar la automedicación, la adherencia a la DM y la relación entre el estilo de vida y los biomarcadores del estado metabólico en una población universitaria.

MÉTODOS: Estudiantes, doctorandos, posdoctorados y especialistas han sido reclutados en Italia (N = 108) y España (N = 86). Los datos fueron recolectados a través de cuestionarios para evaluar estilo de vida, automedicación, consumo de alcohol (AUDIT), hábitos alimentarios, en particular adherencia a la DM y neofobia alimentaria (NA), nivel de actividad física (IPAQ), ortorexia (ORTO-15 y subpuntuaciones), preocupaciones corporales (MBSRQ y BUT), malestar psicológico (K10), actitud alimentaria (EAT-26) y desnutrición (SSI). Los participantes han sido evaluados con parámetros clínicos del estado metabólico (glucemia, colesterol, triglicéridos y cetonas).

RESULTADOS: Las mujeres italianas tenían puntuaciones más altas en MED-55 y ENA (Escala de Neofobia Alimentaria), y una puntuación en AUDIT más baja que las españolas ($p < 0,01$). Los estudiantes que se quedaron con su familia (residente) se adhirieron más a la DM que aquellos que se mudaron fuera de casa. Los italianos residentes consumieron menos cerveza, licores fuertes y cócteles que los españoles los sábados por la noche ($p < 0,01$). Hubo correlaciones negativas entre AUDIT y QueMD (R^2: 0,137,

$p < 0,05$), y AUDIT y alimentos no típicos de la DM (ntMED) (R^2: 0,201, $p < 0,01$) en españoles; sin embargo, no hubo relación entre AUDIT y otras puntuaciones de adherencia a la DM. La mayor parte de la muestra (72,8% de IT y 62,3% de ES) usaba medicamentos sin prescripción médica, con mayor tendencia a la automedicación entre las mujeres italianas (IT-M) y los hombres españoles (ES-H). Además, el 47,6% de los IT y el 31,1% de los ES leyeron el prospecto antes de tomar un fármaco ($p < 0,05$). Los sujetos ORTO-15 positivos, evaluados con el punto de corte originalmente propuesto, estaban por encima del 70% tanto en estudiantes de IT como de ES, con mayor prevalencia en la muestra española (96-97%). Según ORTO-7, alrededor del 30% de los estudiantes italianos y el 48% de los españoles fueron positivos para ON, sin diferencias significativas por género. Al excluir a los estudiantes con bajo peso, sobrepeso y obesos, así como aquellos estudiantes potencialmente en riesgo de trastornos alimentarios o que presentan estrés leve, moderado y severo, en el resultante subgrupo ($K10^{neg}$-EAT-26^{neg}), no encontramos muchas correlaciones entre ORTO y BUT, SSI, MBSRQ total y algunos de sus componentes. ORTO-7 resultó el único índice de ON no relacionado con el índice de masa corporal, los componentes MBSRQ y la actividad intensa evaluada por IPAQ, en el subgrupo NW - $K10^{neg}$ -EAT-26^{neg}. Después de este tipo de "diagnóstico de exclusión", la prevalencia de ON de estos estudiantes en la muestra total resultó de 16,9%, 12,2%, 15,2% y 25,9% para IT-M, IT-H, ES-M y ES-H, respectivamente. En cuanto al estado metabólico, los españoles tenían niveles de glucosa en sangre más altos que los italianos (IT-M vs ES-M, $p < 0,01$; IT-H vs ES-H, $p < 0,001$), y se observaron estado de cetosis en el subgrupo de hombre españoles.

CONCLUSIÓN: Los resultados de este estudio sugieren que los alimentos no típicos de la DM y el patrón alimentario de los sábados por la noche, relacionados además con estar lejos de casa, tienen un gran impacto en el consumo de alcohol. En algunos estudiantes universitarios, la ON podría ser un síntoma de otras condiciones relacionadas con la preocupación por la imagen corporal y la angustia, así como con una alta actividad física y orientación sobre la apariencia, el estado físico, la salud o la enfermedad. Sin embargo, ORTO-7 es un índice independiente de estas

variables confundentes, lo que sugiere que puede ser útil en la identificación de individuos ortoréxicos.

Key words: lifestyle, dietary habits, Mediterranean diet, orthorexia nervosa, body image, metabolic status, nutritional status, physical condition

Palabras clave: estilo de vida, hábitos dietéticos, dieta mediterránea, ortorexia nerviosa, imagen corporal, estado metabólico, estado nutricional, condición física

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"Los humanos somos lo que comemos". Ludwig Feuerbach (1804-1872).

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ABBREVIATIONS

A: Avoidance
ADS: alcohol dependence syndrome
AE: Appearance Evaluation
aMED: alternate Mediterranean diet score
AN: anorexia nervosa
AO: Appearance Orientation
ARFID: avoidance/restrictive food intake disorders
AQUA: Allergy Questionnaire for Athletes
AUDIT: Alcohol Use Disorders Identification Test
BASS: Body Area Satisfaction Scale
BDD: body dysmorphic disorder
BIA: bioelectrical impedance analysis
BIC: Body Image Concerns
BMI: body mass index
BOT: Bratman Orthorexia Test
BUT: Body Uneasiness Test
CIISCAM: International Center for Studies on Mediterranean Food Cultures
CSM: Compulsive Self-Monitoring
CVD: cardiovascular diseases
D: Depersonalization
DPOC: obsessive-compulsive personality disorder
DSM-V: Diagnostic and Statistical Manual of Mental Disorders
EAT-26: Eating Attitudes Test
ED: eating disorder
EHQ: Eating Habits Questionnaire
EFSA: European Food Safety Authority
F: females
FAO: Food and Agriculture Organization of the United Nations
FBDG: food-based dietary guidelines

FE: Fitness Evaluation
FM: fat mass
FNS: Food Neophobia Scale
FO: Fitness Orientation
FSs: Factor Subscales
Glu: glucose
GSI: Global Severity Index
HE: Health Evaluation
HO: Health Orientation
IBD: irritable bowel syndrome
ICD-10: International Statistical Classification of Diseases and Related Health Problems
IO: Illness Orientation
IPAQ: International Physical Activity Questionnaire
IT-F: Italian females
IT-M: Italian males
K10: Kessler Psychological Distress Scale
Ket: ketone
LARNs: Reference Intake Levels of Nutrients and Energy
LDL: low-density lipoprotein
MBSRQ: Multidimensional Body-Self Relations Questionnaire
MBSRQ-AS: Multidimensional Body-Self Relations Questionnaire-Appearance Scale
MED-55: Mediterranean Score
MD: Mediterranean Diet
MDS-14: Mediterranean Diet Score
MET: Metabolic Equivalent Task
MM: muscle mass
MetS: metabolic syndrome
MUFA: monounsaturated fatty acids
NCGS: non-celiac gluten sensitivity
NIT: nitrite
ntMED: non-typical Mediterranean diet foods
NW: normal weight

OB: obese
OCD: obsessive-compulsive disorders
OECD: Organization for Economic Co-operation and Development
ON: orthorexia nervosa
ON-TF: Task Force of Orthorexia Nervosa
OP: Overweight Preoccupation
OW: overweight
PA: physical activity
PSDI: Positive Symptom Distress Index
PST: Positive Symptom Total
QueMD: Questionnaire to measure Mediterranean diet
RDA: Recommended Dietary Allowances
SCW: Self-Classified Weight
SEE: Spanish Society of Epidemiology
SENC: Spanish Society of Community Nutrition
SINU: Italian Society of Human Nutrition
SM: self-medication
SMA: self-medication with antibiotics
SP-F: Spanish females
SP-M: Spanish males
SSI: Starvation Symptom Inventory
T2DM: type 2 diabetes mellitus
TC: total cholesterol
TG: triglycerides
USDA: United States Department of Agriculture
UW: underweight
VF: visceral fat
WC: waist circumference
WHO: World Health Organization
WHR: waist-to-hip ratio
WHtR: waist-to-height ratio
WP: Weight Phobia

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I - INTRODUCTION

I - INTRODUCTION

1.1 DIETARY HABITS AND LIFESTYLE

Alimentation is the choice and conscious consumption of foods and beverages by the individual in their various combinations, and it is influenced by biological and non-biological factors (FESIN, 2010). On the other hand, the concept of nutrition concerns the use of nutrients for all those biological processes that allow or condition the growth, development and integrity of the living organism. A nutrient is any substance that is absorbed in the gastrointestinal tract and has a defined role in the physiological processes and metabolism of the human body. Nutrients are divided in macronutrients and micronutrients: the first are present in the diet in high quantities that have distinct nutritional effects, a certain metabolic role, and are a source of energy for the human body, such as proteins, fats, and carbohydrates; the second are minerals or vitamins present in the diet in small quantities, with defined nutritional effects and a certain role in the metabolism of the human body, but without being a source of energy (FESIN, 2010). Energy needs vary from individual to individual based on age, sex, physical activity (PA), environment, and physiological conditions, and they are generally in the range of 1200 – 4000 kcal (SINU, 2014). According to the lifestyle, the total energy requirement is represented in a variable percentage by the basal metabolism, defined as the energy expenditure necessary in the awake subject for the functioning of the organism, physical and mental rest, and thermal balance with the environment, and it is calculated in standardized fasting conditions from the previous evening (FESIN, 2010). The proportion of food types we consume and their quality are the basis of a complete human development, both physical and mental. On the other hand, poor quality, improperly stored or contaminated foods can constitute important risk factors and cause disease and death for millions of people every year (ISS, 2019). Therefore, a varied and balanced diet is the basis of a healthy life.

1.1.1 Dietary guidelines

The aim of food-based dietary guidelines (FBDG) is to provide preventive evidence-based nutrition recommendations regarding the minimum nutritional needs that should be guaranteed as part of a balanced, varied and moderate diet, which play an important role in setting nutrition policies and public health strategies and educating population about healthy food choices (EFSA, 2010; FAO/WHO, 1996). In order to be significant for the target audience, FBDG have to be appropriate for the region or country, culturally acceptable, easy to adopt, and include practical advice. Furthermore, food production, distribution, cost, and access in the concerned country or region are also crucial in the development process, as well as salient food beliefs, preferences, culinary practices, gastronomic culture, and other relevant factors among the people for which they are intended (Bechthold *et al*., 2018; Aranceta Bartrina & Serra Majem, 2006). In fact, for better adherence in the case of Spain and Italy, it is critical to consider the food production structure as a Mediterranean country with genuine food traditions, but also with food diversity and a rich variety of gastronomic cultures within the country (Varela Moreiras *et al*., 2019; Bechthold *et al*., 2018; Altomare *et al*., 2013).

The idea of providing reference nutritional standards was born in 1941, when on the initiative of the United States Department of Agriculture (USDA) the first Recommended Dietary Allowances (RDA) were codified, being the earliest indications regarding the intake of energy, proteins, iron, calcium, and some vitamins (Davis & Saltos, 1999). Since then, USDA has worked in synergy with the Department of Health and Human Services to publish the Guidelines for proper nutrition for the United States population every 5 years.

In Italy, a consideration of these qualitative and quantitative standards appeared in the seventies with LARNs (Reference Intake Levels of Nutrients and Energy), recommendations developed periodically by the Italian Society of Human Nutrition (SINU), with the aim of providing dietary information relating to the minimum intake of energy, micro and macronutrients for a good functioning of the organism, on the basis of scientific and epidemiological studies related to the basic necessities, consumption and relationship between nutrition and health. Since then, LARNs constitute a fundamental tool for the nutritional planning of Italian population (CREA, 2018).

Contrary to Italy and other countries, neither the Spanish Ministry of Health nor the Spanish Ministry of Agriculture developed national dietary guidelines. In 1995 the Spanish Society of Community Nutrition (SENC) designed the first comprehensive dietary guidelines for the Spanish population based on the best scientific evidence available (Majem *et al*., 1995), that were updated periodically (Aranceta & Serra-Majem, 2001; Dapcich *et al*., 2004) and, more recently, in 2016 (Aranceta Bartrina *et al*., 2016). Furthermore, in 2018, a more practical version of the guidelines was developed, with an emphasis on food sustainability (Aranceta-Bartrina *et al*., 2019) and the importance of PA, emotional status, energy balance, healthy cooking procedures and adequate hydration (Aranceta Bartrina *et al*., 2016).

FBDG refer to the healthy population, discerning by age group and sex; therefore, the identified standards do not concern individuals in particular pathological conditions, whose needs must be reviewed and assessed case-by-case.

1.1.2 Food pyramids and Mediterranean diet

One of the main references for identifying the elements of a healthy and balanced diet is the food pyramid, a model born as a nutritional information and education activity promoted by USDA since the earlies nineties, which led to the publication of the first edition of the food pyramid in 1992, re-proposed by FAO (Food and Agriculture Organization of the United Nations) in 1997, to synthetically translate the scientific indications contained in the Dietary Guidelines for Americans. Over the years, various institutions and research centers, including World Health Organization (WHO), International Center for Studies on Mediterranean Food Cultures (CIISCAM), and the Harvard School of Public Health, have developed communication campaigns based on the image of food pyramid, in which food is located within the perspective of proportionality and health impact by means of a background colour code, position in the different strata, and comments on recommended frequency of consumption. The organization of the different layers was framed considering the Mediterranean diet (MD) as the reference dietary pattern (Bach-Faig *et al*., 2011), consistent with the prevailing culture in Spain and Italy, and its food habits and culinary practices. It involves a significant consumption of cereal products (bread, pasta), fruits and vegetables, together with a moderate consumption of legumes, fish, and red wine,

and olive oil as the main dressing (Debellis & Poli, 2019). Since MD is based on the balanced consumption of foods rich in fiber, antioxidants, unsaturated fats, and on the reduction of dietary fats and cholesterol, on November 2010 it was included in the List of Intangible Cultural Heritage of Humanity recognizing this heritage belonging to Italy, Morocco, Greece and Spain, and in November 2013 this recognition was extended to Cyprus, Croatia and Portugal, contributing to the promotion of the lifestyle and typical Mediterranean products in the world, and encouraging traditional local productions according to high quality standards (Debellis & Poli, 2019). The link between MD and health has an important role on the quality of life of individuals both on physical and psychological status (Godos *et al*., 2019; Galilea-Zabalza *et al*., 2018; Zaragoza-Martí *et al*., 2018 Pérez-Tasigchana *et al*., 2016; Henriquez *et al*., 2012; Bonaccio *et al*., 2013), determining the state of health in every stage of life (Konstantinidou *et al*., 2014). MD has been shown to be effective in weight reduction and appears to prevent the development of cardiovascular diseases (CVD), breast cancer, depression, colorectal cancer, diabetes, obesity, asthma, erectile dysfunction, and cognitive decline (CREA, 2018; Davis *et al*., 2015; Psaltopoulou *et al*., 2004). MD carries out its beneficial activity through a synergistic effect of all its nutrients and non-nutrients. The features that make it unique are:

- High intake of both soluble and insoluble fibers from fresh and of local origin products, such as legumes, whole grains, and fresh fruits as a daily dessert.
- Excellent balance in the intake between simple and complex carbohydrates, with little use of foods rich in simple sugars, and complex carbohydrates at the base of the diet as the main source of energy.
- Extra virgin olive oil (monounsaturated fatty acids, MUFA) as a source of fats, and moderate or low use of saturated fats of animal origin.
- Dairy products consumed daily in modest quantities.
- Consumption of drinks with low alcohol content near meals.
- A protein intake of animal origin consisting of red meats in minimum quantity, fish and white meat 2-3 times a week, often replaced by vegetable proteins.

The frugality of MD guarantees a daily balanced total calorie intake with about 2500 kcal for men and 2000 kcal for women (CREA, 2018).

Furthermore, in recent years, the food pyramid scheme has often been supplemented by recommendations for a correct lifestyle, such as the amount of water to drink, the time to devote to physical activity, etc (BCFN, 2016).

The most recent Italian food pyramid was published in 2016 (fig. 1.1) with the specific objective of shifting the perception of the benefits of this model from the particular attention to humans to a focus on the benefits for the planet and its populations, in order to provide a unified representation of the MD as a sustainable and representative food model of the entire Mediterranean area, to be adapted for each country to their contexts and traditional cuisine (Vitiello *et al*., 2016).

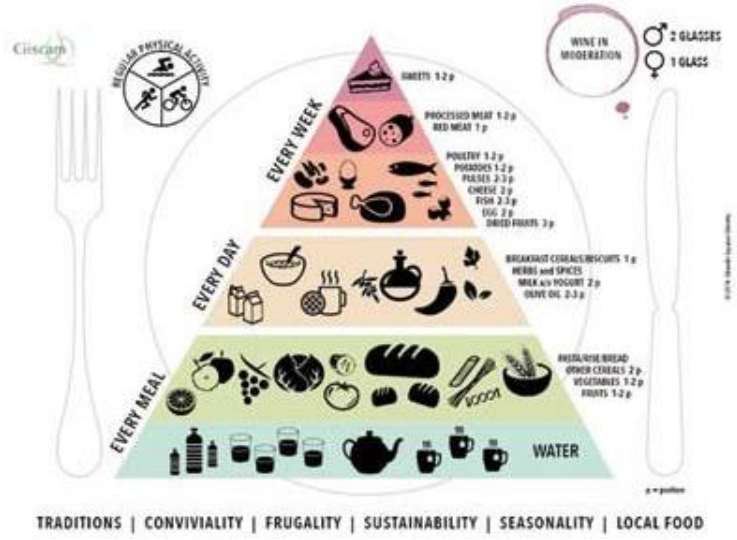

Figure 1.1. The new modern Mediterranean diet Italian pyramid (Vitiello *et al*., 2016).

Sustainable diets are defined by FAO as diets with low environmental impact, which contribute to food and nutrition security and to healthy life for present and future generations (FAO, 2012). They are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair, and affordable. In addition, such diets are nutritionally adequate, safe, and healthy**,** while optimizing natural and human resources. Therefore, the sustainability of diets is not only focused on nutrition and the environment, but it also relates to economic and sociocultural dimensions.

In recent years, several countries have developed guidelines to promote and protect traditional food cultures, and consider the impact of dietary patterns and food systems on the environment (Herforth *et al*., 2019; Bechthold *et al*., 2018; Erve *et al*., 2017).

SENC published the new healthy eating pyramid (fig. 1.2) in 2017, based on the most recent dietary guidelines for the Spanish population (Aranceta Bartrina *et al*., 2016). An orange, dotted line delimits the two bottom layers including the cereal group, starchy vegetables, fruit, vegetables, and olive oil as the food groups to include in each main eating occasion. The proportion among the layers represents the different contribution to the total energy intake by the different food groups.

Figure 1.2. Healthy eating pyramid (Aranceta-Bartrina *et al*., 2019).

This new version of the Spanish pyramid incorporates clear messages regarding different determinants of food intake, criteria for sustainable diets, suggestions for consulting an appropriate healthcare professional (doctor, pharmacist, nurse practitioners, and dietitians/nutritionists) for advice on the use of special foods, herbs, pharmacological or nutritional supplements, nutraceuticals, among others (Garcia-Alvarez *et al*., 2014), dose, duration, potential interactions, and changes in food intake that would make their consumption unnecessary (Serra-Majem *et al*., 2017; Biesalski & Tinz, 2017; Shenkin *et al*., 2013), and limitations on the consumption of low-grade fermented alcoholic beverages (Serra-Majem *et al*., 2017).

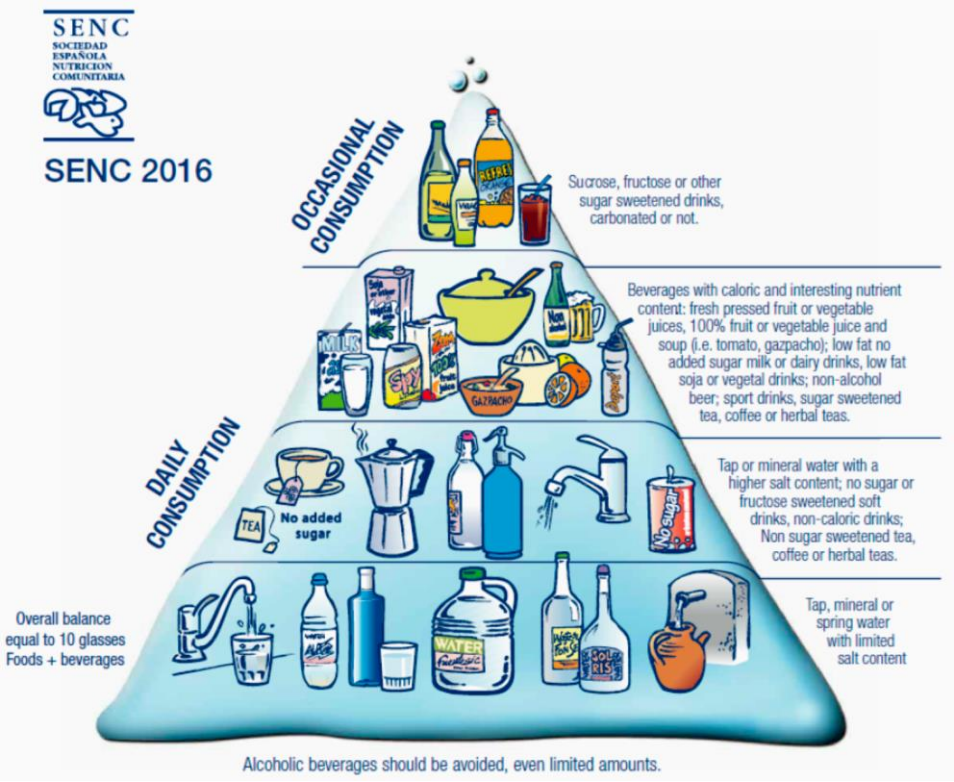

Figure 1.3. Healthy Hydration Pyramid (Aranceta-Bartrina *et al*., 2019)

Both Spanish and Italian pyramid base are completed with a jug and glasses of water that advise on ensuring adequate fluid intake for an optimal hydration status as fundamental to achieving and maintaining health and well-being (EFSA, 2010; Tetens, 2010). Figure 1.3 shows the latest proposal for the Healthy Hydration

Pyramid (SENC, 2016), which includes advice for healthy hydration in the context of a healthy diet. It was designed considering water intake levels in different population groups, sources, and contribution of beverage consumption to energy and nutrient intake in addition to their contribution to water intake.

The general basic recommendation encourages the consumption of water, tap water wherever available, that is safe to drink and palatable. Recommendations regarding other beverages consider their contribution to energy and nutrient intake as well as to water intake in light of the current consumption practices in the population. The hydration pyramid base mainly suggests water of varying mineralization grades and then, at the second level, different beverages without caloric content as a priority for proper hydration. Sport drinks, located in the third level of this pyramid, are increasingly consumed to replace conventional soft drinks for the lower sugar content, particularly among adolescents and young people in Spain (Samaniego-Vaesken *et al*., 2018; Nissensohn *et al*., 2016).

Consumption of alcoholic beverages is a controversial issue. Since alcoholic beverages do not contribute to hydration, they should not be used with this function (Maughan *et al*., 2016). The guidelines do not encourage or recommend alcohol consumption for health, but acknowledge the fact that a high proportion of citizens use alcoholic beverages on a daily basis (Van Horn *et al*., 2016; Costanzo *et al*., 2011). The only evidence supporting alcoholic beverages is bounded in the context of the MD to limited amounts (15 g/day) of low-grade fermented alcoholic beverages (wine, beer) with meals (Bazal *et al*., 2019; Gea *et al*., 2014). Such tolerance refers exclusively to adults with a consumption limited to no more than 2 glasses of wine or equivalent (200 ml; <40 g alcohol/day) for men, and no more than 1 glass for women (100 ml; <20 g alcohol/day) (Bazal *et al*., 2019; Gea *et al*., 2014). Spirits and liquors should be avoided in the context of a healthy diet and lifestyle. In Spain, there are good-tasting, socially acceptable alternatives for private or social consumption of alcoholic beverages, such as non-alcoholic beer, de-alcoholised wines, and similar products available in virtually all establishments in the country (Aranceta-Bartrina *et al*., 2019).

Some studies have previously examined the cross-sectional association between adherence to the SENC recommendations and body mass index (BMI), suggesting that the Spanish dietary guidelines may be an effective tool for obesity prevention (Rodríguez-Rodríguez *et al*., 2017; Molina-Montes *et al*., 2014).

The guide offered by both food pyramids on the proportions and frequencies of food consumption can be completed by a healthy lifestyle and cultural elements, in order to acquire all the benefits of MD. The reference elements are (Aranceta-Bartrina *et al*., 2019; Vitiello *et al*., 2016):

- Moderation: portions should be adequate, adapting them to energy needs for urban and modern sedentary lifestyles. Energy balance has to be considered in order to maintain body weight within the healthy range: higher food intake with increased physical activity, and paying more attention to a healthier diet while limiting portion sizes if we move less (Manore *et al*., 2017). Important remarks related to healthier culinary techniques are included as well.
- Socialization: the aspect of conviviality is important for the social and cultural value of the meal, beyond the nutritional aspects. Cooking, sitting around the table and sharing food in the company of family and friends is a social support and gives a sense of community. Conviviality at the table has been rediscovered as an element for the prevention of eating disorders (Monsivais *et al*., 2014; Bach-Faig *et al*., 2011; Burgesse-Champoux *et al*., 2009).
- Seasonality, biodiversity, eco-compatibility, and local and traditional food products are presented at the bottom of the pyramids to highlight how the modern MD is comparable with the development of a sustainable food model for present and future generations. The preference for seasonal, fresh, and minimally processed foods maximizes the content of nutrients and protective substances in the diet (Serra-Majem *et al*., 2019).
- Mindful eating and emotional balance are determinant of an adequate diet, but also a conditioning factor when buying, cooking, and eating food (Warren *et al*., 2017). Mindful eating consists of making conscious food choices, developing an awareness of physical versus psychological hunger and satiety cues (Dalen *et al*., 2010), and eating healthfully in response to those signals (Miller *et al*., 2014).
- Rest: sleeping the right number of hours every day contributes to the health of the individual.
- Physical activity: practicing regular PA (at least 30 minutes per day) in association with a varied and balanced diet has many health benefits,

including a correct energy balance and maintenance of body weight (WHO, 2020; CSEP, 2011; HHS, 2008). Engaging in recreational activities outdoors, and preferably with others, makes them more enjoyable and strengthens a sense of community.

However, currently available evidence suggests that greater dietary diversity is not necessarily beneficial in terms of promoting an optimal body weight and healthy eating patterns (Otto *et al*., 2015; de Oliveira *et al*., 2018).

Although Italian and Spanish dietary guidelines include the possibility of a moderate and responsible consumption of fermented alcoholic beverages (CREA, 2018; Aranceta Bartrina *et al*., 2016), it is inconsistent with the positioning of the Spanish Society of Epidemiology (SEE) (SEE, 2016) and other scientific evidence in the Spanish population (Fresán *et al*., 2016; Barrio-Lopez *et al*., 2013; Sayon-Orea *et al*., 2011). Additionally, there is a global concern regarding the potential presence of commercial bias in nutrition research and an undue influence in the elaboration of dietary guidelines (Blake, 2018; Nestle, 2018; Rey-López & Gonzalaez, 2018; Bero, 2017; Fabbri *et al*., 2017; Chartres *et al*., 2016; Mandrioli *et al*., 2016).

1.1.3 Dietary habits and lifestyle in university students

University is a crucial stage in life that often causes a change in students' routine and consequent eating habits (Peltzer *et al*., 2014), since they have to face a variety of challenges, such as a new environment, study stress, lack of proper time management, and busy class schedules, which can lead to a lack of interest in their own health (Ganasegeran *et al*., 2012; King *et al*., 2007). During the university period, students tend to practice some unhealthy eating and lifestyle patterns, such as meal skipping, snacking, fast-food intake, smoking, and sedentary behaviour (Ganasegeran *et* al., 2012; Gan *et al*., 2011), with resulting health problems and nutritional deficiencies (El Ansari *et al*., 2015; Papadaki *et al*., 2007).

A distinction needs to be made between students who live away from home and the resident ones. The former seem to abuse ready-to-eat or packaged foods, and make a moderate use of milk, chips, beer and spirits. The latter seem to be less sensitive to this change, play more sports and have a healthier and more balanced diet, with greater consumption of raw and cooked vegetables, fresh fruit, meat and

poultry, fish, eggs, and bread/cereals (Muñoz de Mier *et al*., 2017; Lupi et al., 2015; Teleman *et al*., 2015). Most of the students who live alone have more difficulty adopting a healthy diet and have reported a change in their eating habits. The reasons that influence food choices made by students include a change in lifestyle, the convenience of fast-food, and the taste (Lupi *et al*., 2015). It has been observed that lifestyle changes directed towards unhealthy habits, especially in the young age groups, are due to an increasingly evident deviation towards a Western diet richer in saturated fats, refined cereals, simple carbohydrates and processed foods (Tsakiraki & Grammatikopoulou, 2011), with a consequent high prevalence of overweight and obesity in countries that should adopt a traditional MD (Grosso & Marventano, 2017; Belahsen, 2014).

It has been shown that around 11.3% of college students consume excessive amounts of caffeine, less than 50% of students eat at least one serving of fruit a day, and less than 1 in 4 eat at least two servings of vegetables per day. Therefore, most of university students do not follow national recommendations (Norman *et al*., 2018). Additionally, in recent years, the consumption of alcohol among university students has become a problem of health concern at an international level (Arria *et al*., 2016; White & Hingson, 2013). As reported in the Global Status Report on Alcohol and Health prepared by the WHO, on average about 2.3 billion people in the world consume alcoholic beverages (WHO, 2018). A report by the Italian Ministry of Health from 2015 highlighted that 64.5% of Italians over the age of 11 years consumed an alcoholic beverage at least once in their life, with a clear majority among males compared to females (Ministero della Salute, 2016). Furthermore, it was reported that between 11 and 24 years the consumption of alcoholic beverages occurred often outside meals (Ministero della Salute, 2016). According to the OECD (Organization for Economic Co-operation and Development), Spanish women with high education are more likely to be hazardous drinkers than less educated women, while men with low education are more likely to drink at risk (OECD, 2021). College students report a greater increase in drinking than their peers who do not attend colleges and universities (Yockey *et al*., 2020). In addition to alcohol abuse, binge drinking, especially on weekends, is an increasing phenomenon among university students (ISTAT, 2017). At the European level, it has emerged that two-thirds of university students are high-risk drinkers (Davoren *et al*., 2016). There are marked differences in the type of drink

consumed. Worldwide, 44.8% of alcohol is consumed in the form of spirits, the most consumed beverage in the regions of Southeast Asia and the Western Pacific, followed by beer (34.3%) and wine (11.7%) (Ministero della Salute, 2016). In the last 20 years, the portion of liters of wine consumed has decreased, while beer has increased, in Italy (MS, 2016). In 2016, 54% of alcohol per capita consumption among the Spanish population aged 15 years and older was of beer, followed by 28% of spirits, and 18% of wine (WHO, 2016).

Although the daily consumption of moderate quantities of red wine is a well-established custom of the eating habits of the Mediterranean area (WHO, 2018), the adverse effects remain predominant (Forouzanfar *et al*., 2015). Moreover, it has been recently pointed out that messages on the benefits of moderate wine drinking should be avoided, also considering the other sources of polyphenols in the MD (Santos-Buelga *et al*., 2021). Besides, the perceived utility of warnings on alcoholic beverages in Italy was high among university students with moderate intake, but low among those with an at-risk consumption (Annunziata *et al*., 2017). When the compliance with the recommendations of the MD pyramid was evaluated among Italians, the regular consumption of fruits and vegetables was significantly more common in females, and it increased with age and education (84.7% of those with a university degree, Bracale *et al*., 2020). Controversial results came from studies that evaluated the relationship between alcohol consumption and adherence to the MD in Spain (López-Moreno *et al*., 2021; Rodríguez-Muñoz *et al*., 2021; Carlos *et al*., 2020); however, in addition to high alcohol intake, factors that predicted a worse diet in Spanish university students included male gender and living alone (Ramón-Arbués *et al*., 2021).

Alcohol abuse over a prolonged period of time is associated with long-term negative health outcomes, and can cause liver cirrhosis, heart disease and cancer. The study carried out in the United Kingdom involved about 5,832 students in order to evaluate their alcohol consumption at the beginning and during the university course (after 1 week and after six months, respectively). The results showed that, regardless of demographic conditions (age, sex, ethnicity), alcohol consumption tended to increase during university studies (Ahmed *et al*., 2019), as well as the consumption of fast food and sugary soft drinks. The main reasons justifying the consumption of fast foods are the lack of time to cook healthy foods

(51%), the pleasant taste (26%) and the influence of friends (15%) (Ahmed *et al*., 2019).

The university period can be an exciting moment, but it is often characterized by anxiety and stress since they must cope with changes in academic workloads, and this is an additional risk factor for eating disorders and other pathological conditions. Stress, defined as "a complex physiological state that embodies a range of integrative physiological and behavioural processes that occur when there is a real or perceived threat to homeostasis", can be related to the pressures of work, family and other daily responsibilities, which could affect many aspects of life (e.g. physical, behavioural and psychiatric manifestations) explained by excessive and prolonged secretion of major stress mediators, and their effects. A systematic review found higher levels of psychological distress among medical students compared to the general population and peers. Furthermore, a number of factors, including academic pressure, workload, sleep deprivation, as well as changes in eating habits, were considered to be the main causes of increased stress levels in this population. Recent studies have reported that stress increases cortisol levels and the intake of high-fat foods. Additionally, a study conducted in animal models found rewarding properties of palatable sweet foods used to relieve stress. A study reported that women under severe stress preferred sweet and high-fat foods. Furthermore, males were more likely to consume large amounts of fat than females (Vidal *et al*., 2018), reporting a higher prevalence of overweight or obesity, and a more moderate physical activity (Ahmed *et al*., 2019).

1.1.4 Eating disorders

A US study was carried out to analyse and verify how stress, anxiety, depression and insomnia affect proper nutrition. The results showed that 82.3% of students associated negative emotional states with an unhealthy diet, with a higher prevalence in women (84.8%) than men (76.4%) (Barnhart *et al*., 2020). Further studies confirm how emotions positively and negatively influence proper nutrition. One of these was conducted in the United States on a sample of 77 university students of mixed race/ethnicity and education. They were subjected to various questionnaires to evaluate negative and positive emotional eating, the

difficulty of regulating emotions, and the presence of eating disorders (EDs). The scores obtained suggested that negative emotional states are positively associated with EDs, such as anorexia nervosa (AN) (Schnepper *et al*., 2020).

Other studies have evaluated food intake not only as a response to physiological hunger signals that ensure survival, but as a reward, a response to boredom, stress, and emotions. A study conducted at the University of Grande-Fortaleza (Brazil) found that most of the negative stimuli appear to be due to family conflicts, followed by those with friends and partner. Only 20% are not motivated by social and personal issues (Uchôa *et al*., 2019).

EDs are also due to an overestimation of physical fitness and body weight, in particular dissatisfaction with one's body shape and the psychological problems associated with it, such as the fear of losing control over food consumption. It is interesting to highlight the role of the media in inducing adolescents to internalize models of physical beauty. In the constant search for an "ideal body", adolescents can begin to develop risky behaviours. From a study conducted on about 1,100 boys, it emerged that 45.3% of adolescents were moderately or heavily influenced by the media, with a higher percentage of girls (25.7%) than boys (19.6%) (Uchôa *et al*., 2019).

As regards eating behaviour, hypothalamus is the organ that integrates the homeostatic signals, related to nutritional needs, and the endocrine signals, related to feelings, emotions, memories, forecasts concerning the pleasure experience. The relationship between man and food is regulated by three components:

1. Affective: experience, pleasure and displeasure.
2. Cognitive: recognition, memorization, predictions of pleasure and displeasure.
3. Motivational: urge to repeat behaviours that cause pleasure and avoid those causing pain.

The relationship with food can take on compulsive characteristics similar to drug addict subjection to alcohol and drugs. Irresistible craving underlies all pathological addictions. Patients with anorexia, bulimia or obesity have dysregulations of the reward system; therefore, they present alterations in the release of dopamine, endogenous opioids, serotonin and acetylcholine (O'Hara *et al*., 2015).

Modalities of voluntary dietary restriction are many and include the exclusion of one or more meals of the day up to periods of total fasting, the selection of low energy density foods with the exclusion of foods richest in calories and fat or considered as such, including condiments above all, adherence to a vegetarian or vegan diet, rich in fiber and with a lower energy content. With voluntary dietary restriction, behaviours are often associated with the aim of reducing and better tolerating the feelings of hunger and discomfort associated with limiting calorie intake. Some of the most frequently adopted strategies are:

- Choice of unappetizing foods, restriction of variety and limitation of food availability.
- Intake of foods or drinks whose volume activates the gastric mechanoreceptors favouring a feeling of satiety, such as drinking plenty of water or sweetened drinks, consuming unsweetened vegetables or bran flakes, or introducing hot non-calorie drinks such as coffee, tea, herbal teas and vegetable broth. Tea and coffee are also stimulating drinks as they contain theine and caffeine, therefore their energizing effect is exploited as well.
- Creating the illusion of having a meal of a certain size by distributing the food over the entire plate, cutting it into small pieces, and eating extremely slowly or chewing sugar-free gum for a long time.
- Avoid family or social situations in which food is present, making excuses of commitments or having already eaten, or having digestive or health problems; eat alone, in order to choose freely and without controls or criticisms the foods to be consumed.
- Vicarious nutrition, or "eating through others" by observing them, passing them their own food, cooking energetic foods, for example cakes and sweets for family or friends.
- Practicing excessive and compulsive physical activity in order to burn more calories and lose weight.

Dietary restriction is associated not only with energy deficiencies but also with essential nutrients, such as amino acids, essential fatty acids, vitamins and minerals, and this leads to specific consequences of altering the body's homeostasis (Ministero della Salute, 2017).

As regards pathological conditions due to excess dietary imbalances, they can cause metabolic syndrome (MetS), defined by the WHO as a pathological condition characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidaemia. The prevalence of MetS is often higher in the urban population of some developing countries than in its Western counterparts. The identified causes of the spread of this condition are the increase in the consumption of low-fiber fast food and the decrease in physical activity due to mechanized transport and increasingly sedentary lifestyles (Sakalayen, 2018).

1.1.5 Physical activity

The relationship between nutrition, health and fitness constitutes a value of fundamental importance, also widely known by general population, to achieve a better quality of life, health promotion and disease prevention. The concept of physical activity is very broad and it includes all the forms of movement that are realized in the various spheres of life. Specifically, it means "any movement determined by the musculoskeletal system that results in an energy expenditure higher than that of resting conditions". This definition includes not only sporting activities, but also simple movements such as walking, cycling, dancing, playing, gardening and housework, that are part of spontaneous motor activity. The expression "motor activity" is essentially synonymous with PA. On the other hand, the term "physical exercise" means PA in a structured, planned and regularly performed form (WHO, 2020).

According to WHO, it is necessary to provide elements to encourage PA, aiming for equity, overcoming inequalities and the inclusion of vulnerable people. In support of this, WHO published the "Global recommendation on Physical activity for Health" in 2010, updated in 2017, with the aim of providing indications on the level of PA recommended for health, with reference to three age groups: children and teenagers aged 5 to 17, adults aged 18 to 64, and adults/seniors aged 65 and over. In addition, for all the various stages of life, the frequency, duration, intensity, type and amount of PA necessary to maintain health is taken into account. In all ages, the recommended levels should be understood as a minimum limit: those who manage to exceed them obtain additional benefits for their health (WHO, 2020).

Italy has contributed to the definition of the WHO documents by supporting a concept of PA understood as an expression of the relationship between the human being and the environment in which he lives his daily life, with the aim of increasing physical and psychological well-being in all age groups, in subjects in physiological conditions or suffering from pathologies. In adulthood, the health benefits deriving from PA include a lower risk of suffering from chronic diseases such as CVD, diabetes, arterial hypertension, some forms of cancer, such as breast, prostate and colon, and the improvement of bone mineralization at a young age, which contributes to the prevention of osteoporosis. PA improves digestive function and regulation of intestinal rhythm, and it is a determining factor for energy expenditure, essential for the purpose of controlling body weight. It has positive effects on mental health, contributing to the maintenance of cognitive functions and to the reduction of the risk of depression and dementia. It reduces stress and anxiety, improves sleep quality and self-esteem. Positive effects are also found in the school environment in terms of productivity (WHO, 2020).

The current WHO recommendations on PA for the health of the adult population recommend carrying out during the week a minimum of 150 minutes of moderate intensity aerobic PA or a minimum of 75 minutes of vigorous activity, plus strengthening exercises of the major muscle groups 2 or more times a week. This can be achieved, for example, through 5 exercise sessions per week lasting at least 30 minutes or by doing at least 25 minutes of vigorous intensity exercises 3 times a week. The recommendation can also be met by combining moderate and vigorous intensity activities. Moderate intensity activity is normally characterized by an energy expenditure of 3-6 Metabolic Equivalent Tasks (METs, 3-6 times the energy expenditure at rest) and induces a modest increase in heart rate and ventilation, usually allowing you to speak easily but not sing. The classic example is that of brisk walking. Vigorous intensity activity, on the other hand, induces a higher energy expenditure (>6 METs) and, consequently, a greater and substantial increase in heart rate and pulmonary ventilation. At this intensity it usually becomes more difficult to converse, so much so that you are unable to pronounce more than a few words before catching your breath. An example of this form of PA is jogging. The intensity can also be modulated on the basis of the perception of effort, therefore on a scale from 0 to 10, where 0 is the sitting position and 10 the maximum effort, the moderate intensity is equivalent to 5-6, while the vigorous one

is 7-8. The recommended amount of PA can be divided throughout the day to better include exercise in the routine of various daily activities. In addition to aerobic activity, adult individuals should perform exercises aimed at muscle conditioning (strength exercises, such as bending, push-ups, and suitable exercises with weights or gym machines) for a minimum of two sessions per week on non-consecutive days, structured to involve most of the muscle groups. Exceeding the minimum recommended amount of PA brings additional health benefits and more effective risk reduction for various chronic diseases (cardiovascular and metabolic), and it is also recommended for individuals who have difficulty maintaining their body weight constant, and for whom it is suggested to achieve at least 60-90 minutes of daily exercise (WHO, 2020).

Since it is difficult to arrive at a general recommendation on the minimum amount of exercise to guarantee health benefits, it is important to make it clear that "little is better than nothing", and some health benefits in sedentary adults can also be achieved with minimal amounts of PA (e.g., 60 minutes per week) (WHO, 2020). Therefore, regular PA is one of the most important element for maintaining good health. To support this, a pyramid of PA (fig. 1.4) has been drawn up (Ali, 2018).

It is necessary to distinguish the concept of sedentary lifestyle from that of inactivity. It is possible, in fact, that physically active individuals, i.e. who reach the minimum recommended amount of PA, can be sedentary at the same time, as they spend most of the day seated. The deleterious effects of hours spent in a sedentary lifestyle are independent of the level of PA in free time, and are also found in individuals who achieve the aforementioned amount of recommended PA. The riskiest condition is that of inactive and sedentary individuals. Sedentary activities are those characterized by an energy expenditure of less than 1.5 times that of rest (<1.5 METs), exemplified by sitting or in a reclined position (watching television, driving the car, reading, sitting at the desk, etc.). Therefore, it is advisable to recommend all adult individuals to frequently interrupt the sedentary activity, ideally at least every 30 minutes, with even short periods (2-3 minutes) of activity (active breaks). Equally effective can be short walks, low/moderate intensity aerobic exercises, bodyweight exercises performed on the spot (for example bending on the legs, carried out simply by getting up from a chair or sofa repeatedly), periodically alternating the sitting position with the standing one (for example every 30 minutes) (WHO, 2020).

Figure 1.4. Physical activity pyramid (Ali, 2018).

It has been demonstrated that interventions based on "active breaks" benefited glycemia control in adults with or at risk for metabolic diseases (Duvivier *et al*., 2017). People who are not physically active have an increased risk of all-cause mortality compared to those who engage in at least 30 minutes of moderate-intensity PA most days of the week. PA is able to prevent and/or treat numerous pathological states and is associated with multiple benefits for physical and mental health in both men and women. Furthermore, a significant reduction in risk occurs already for modest increases in the level of PA, below the recommended threshold for the adult population. In addition, it was highlighted that the risk of death from all causes can be effectively reduced in inactive individuals through the adoption of PA already practiced at low or moderate intensity (CONI, 2014).

1.1.5.1 Supplements

Supplements are food products intended to integrate the normal diet but, in athletes, they may have the purpose of ensuring a proper supply of nutrients and energy in order to minimize the stress induced by physical exercise. Food supplements are defined by sector legislation (FAO, 2004) as: "food products

intended to integrate the common diet and which constitute a concentrated source of nutrients, such as vitamins and minerals, or other substances having a nutritional or physiological effect, in particular, but not exclusively, amino acids, essential fatty acids, fibers and extracts of vegetable origin, both single and multi-compound, in pre-dosed forms". The latter are usually presented in small consumption units, such as capsules, tablets, sachets, vials and similar, and can contribute to the well-being by optimizing the condition or promoting normal body functions with the supply of nutrients or other substances with a nutritional or physiological effect (Ministero della Salute, 2021).

The acquisition of the best physical condition and the achievement of optimal sporting fitness derive from the interaction of many elements, and the diet plays a decisive role. Eating habits strongly affect, for better or for worse, physical and sporting performance (amateur, healthy or competitive) regardless of the level of training. Only an optimal state of health guarantees the maximum expression of an individual physical and athletic potential. At the base of this favourable psychophysical condition there is a healthy diet, accompanied by correct lifestyles, able to promote good physical efficiency (Ministero della Salute, 2021).

Food supplements include a wide and differentiated range of products:

- Vitamins and mineral salts: micronutrients with a nutritional effect for which is important to establish the maximum doses of daily use as they can cause undesirable effects. Vitamin supplements are only useful in deficient states (e.g., pregnancy, breastfeeding, the elderly) or in pathological conditions that cause impaired absorption, and also in individuals who adopt particular dietary regimes (vegans). Mineral supplements can be useful following high sweating, as they restore the electrolytes balance.
- Plant extracts: it is currently permitted the use of herbal substances and preparations authorized by the Italian Ministry of Health and the Belfrit List, which has been developed by Belgium, France, and Italy (Ministero della Salute, 2018).
- Prebiotics and probiotics: the former are non-digestible substances of plant origin that when consumed promote the growth of one or more bacteria already present in the intestine; the latter are live microorganisms that, taken in adequate quantities, are capable of

reaching the gut and multiplying, exerting a beneficial effect on the body.

- Supplements for sports practice: many of them have nutrients that are found normally in food (carbohydrates, proteins, creatine, L-carnitine, vitamin C, caffeine, etc.), marketed as extracts or synthesized industrially. Isotonic sports drinks are often simply made up of glucose and sodium; other drinks recommended for athletes are those based on maltodextrin, to provide carbohydrates rapidly assimilated and, at the same time, available for a longer time. Supplements for athletes based on creatine (6 g/day) (Tarnopolsky, 2010), taurine (1 g/day) (Waldron *et al*., 2018; Schaffer *et al*., 2010), and branched amino acids (5 g/day) (Phillips, 2016), such as valine, leucine, and isoleucine, are the most used nowadays.

These products are generally marketed in order to make up for any deficiencies in one or more nutrients, caused by their insufficiency intake with normal diet. The main difference with respect to food intake lies above all in the dosages commonly used for these substances, for which there are conflicting opinions regarding their ethics and long-term harmlessness (Ministero della Salute, 2021).

Energy drinks contain stimulant substances, such as glucose, taurine, caffeine and B vitamins, and in some cases plant extracts, such as guaranà, ginseng and Gingko Biloba, are also used. These are referred to as ergogenic substances, capable of improving physical performance (Katsung & Trevor, 2021; Tarnopolsky, 2010; Burke, 2008).

In general, in the sports field, reference is made to all those products that are taken to replenish what has been lost during intense physical exertion (e.g., mineral salts) or to obtain energy readily available during exertion (e.g., sugar). In addition to these, it is also important to remember all those products used to provide the body with an excess of energy substances in order to increase muscle mass, strength and/or endurance. Sometimes, these types of supplements contain prohibited substances, such as pharmacologically active ingredients (e.g., sildenafil), plant extracts (e.g., yohimbine) prohibited by current legislation, but also anabolic (e.g., testosterone), hormonal and metabolic modulators (e.g., tamoxifen, clomiphene) or stimulants (hydroxyiamphetamine, methylhexaneamine), included in the list of

prohibited substances for doping. The presence of pharmacologically active ingredients, not declared on the label, constitutes an important risk factor for the health of the consumer in general and, specifically, for the health of the sports practitioner. In fact, the intake, sometimes unconscious, of these substances, especially when in excess or in absence of a medical prescription, is often associated with the appearance of acute and/or chronic side effects, some of which are relevant and, in some cases, fatal. Indeed, the purchase of supplements through unofficial channels can lead to the obtaining of counterfeit supplements containing long or short-term risky substances not listed on the label. In addition to being a violation of current legislation, it can lead to side effects for the health of the individual (Bellante *et al*., 2017).

1.2 ORTHOREXIA NERVOSA: THE OBSESSION FOR A HEALTHY DIET

1.2.1 Orthorexia nervosa: definition

The spectrum of eating disorders (EDs) is very broad. These are closely related to each other by the presence of an abnormal relationship with food, by an excess of concern for physical fitness, by an altered perception of the body image and by a close correlation between all these factors and the levels of self-esteem, but with different clinical and psychopathological characteristics. This has led many researchers to focus more on subthreshold manifestations, atypical characteristics and behavioural traits rather than the actual disorder.

In recent years, especially within the sports environment, an ED called orthorexia has spread. The term orthorexia was coined by the American doctor Steven Bratman in 1997, and it is referred to the psychological obsession with a healthy, organic and pure diet, associated with the aversion to foods deemed dangerous, such as fast food, industrial products, preservatives, pesticides or foods perceived as contaminated. Such obsession is often based on stereotyped or erroneous nutritional beliefs, which implies concern and fixation in healthy eating. Whether this eating style can be considered a psychopathological syndrome is still under study. However, although ON shares several aspects in common with obsessive-compulsive disorders (OCD) and AN, it is not officially recognized as a specific ED by neither the DSM-V (Diagnostic and Statistical Manual of Mental

Disorders) nor the ICD-10 (International Statistical Classification of Diseases and Related Health Problems) (Garano *et al*., 2016). ON could be included among Avoidant/Restrictive Food Intake Disorders (ARFID), introduced in 2013 in the fifth edition of the DSM (APA, 2013). However, ARFID refer to a persistent avoidance of food for various reasons (depressed temperament, phobias, sensory repulsion of the appearance, smell, texture of food), not necessarily associated with the search for a healthy diet that characterizes the subject with ON. Therefore, it is assumed that the estimation of subjects affected by ON is very complex, given the lack of explicit diagnostic criteria. Furthermore, numerous researches are limited to certain samples, which may not be representative of the entire population.

The person who suffers from ON manifests cognitive distortions with a real obsession with a healthy and correct diet, showing a meticulous and perfectionist attitude, sometimes disabling, which leads to dietary restrictions with resulting nutritional deficiencies, severe malnutrition, pathological complications, impairment of social life, deterioration in the quality of life, and reduction of psychological well-being (Dunn & Bratman, 2016; Barthels *et al*., 2015; Moroze *et al*., 2015). Orthorexics become highly selective in choosing what to eat with a resulting loss of pleasure in eating, anxiety, and the need to control food intake. Therefore, ON usually begins as an interest in one's health followed by efforts to achieve optimal health through diet, but can lead to significant damage. Consequently, the state of health defined by WHO as "a condition of complete physical, mental and social well-being, and not just the mere absence of a disease" is altered. The orthorexic person carries out a very careful search for foods, analyzes them and always prepares them with the same ritual, with the aim of achieving self-esteem and self-realization by controlling food intake. Although the ON is still under study, it has been found that it could lead to complications similar to those observed in severe anorexia, namely osteopenia, anaemia, hyponatremia, metabolic acidosis, pancytopenia, testosterone deficiency and bradycardia. These pathological conditions are due to nutritional deficiencies, failure to take entire food groups and ultimately can lead to weight loss without having the intent (Garano *et al*., 2016).

1.2.2 Orthorexia Nervosa and Healthy Orthorexia

It has been recently pointed out that the interest in healthy eating could not be ranged from not interest at all to a pathological level without passing through a middle point of adaptive interest in healthy eating. Therefore, Barrada and Roncero (Barrada & Roncero, 2018) have proposed to distinguish between ON and healthy orthorexia. The latter, literally understood as "orthos orexis" (correct appetite), is the rational, non-pathological tendency to follow a healthy-balanced diet (Depa *et al*., 2019). As emerged from a study on 14,000 inhabitants of European countries, the idea of healthy nutrition refers mainly to a diet based on fruit, vegetables, fiber, and lower amounts of lipids, red meat, and sugars (Lappalainen *et al*., 1998). The orthorexic exasperates and takes the concept of a healthy diet to the extreme, obeying his personal convictions of purity. ON, unlike healthy orthorexia, is the condition of someone who spends much of his time reflecting on perfect nutrition, to the point that adherence to one's strict diet reinforces a sense of satisfaction and self-confidence (Brytek-Matera *et al*., 2015), which could lead to an elitist perception of the superiority of one's diet over that of others (Donini *et al*., 2004; Donini *et al*., 2005), perceived as impure and dangerous. The subject who develops ON initially pays particular attention to the quality of the food taken in order to treat or prevent pathologies (e.g., diabetes, asthma, arthritis, cardiovascular disorders), to lose weight, or to definitively exclude the products of fast food (Oberle *et al*., 2017). The diet gradually turns into a rigid ritual, the disobedience of which would determine a feeling of guilt in the subject, who feels a loss of control over food choices, revealing an anxiety-inducing condition at the base of the disorder (Donini *et al*., 2004). The state of anxiety is therefore compensated thanks to the reassuring attachment to a healthy diet, so to rigid rituals, such as researching the origin of the food product, planning future meals, focusing on the quality and not on the quantity of food (Koven & Abry, 2015), doubting the possible carcinogenicity of the food. Therefore, numerous psychological factors are involved in the development of orthorexia, but also anthropological, sociological, and pedagogical aspects.

1.2.3 Comorbidity and differential diagnosis of orthorexia

Orthorexia presents an overlap of symptoms with other psychopathological pictures, such as AN and OCD (fig. 1.5). Numerous similarities emerge between orthorexic and anorexic subjects: both exert excessive control over their diet and show significant weight loss (Fidan *et al*., 2010), but in the former there is anxiety for the quality of food, whereas in the latter for the quantity of food ingested. These two categories are geared towards a single goal and consider adherence to the diet as a marker of self-discipline. Food transgression is experienced as a failure of their self-control (Garano *et al*., 2016).

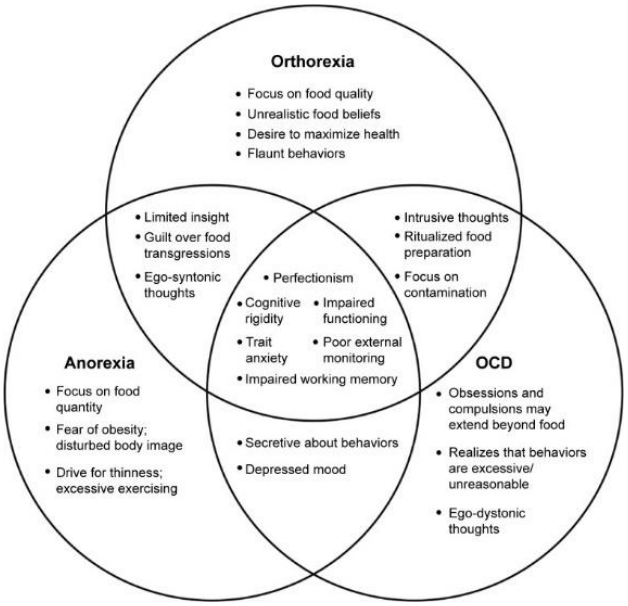

Figure 1.5. Venn diagram showing unique and overlapping features of orthorexia nervosa, anorexia nervosa, and obsessive–compulsive disorder (OCD) (Koven & Abri, 2015)**.**

Orthorexics also have symptoms similar to obsessive-compulsive ones. Among these, the focus on food and health at inappropriate times stands out. Foods are not chosen on the basis of the feeling of hunger, but on the basis of their caloric content and their presumed beneficial and harmful effects on health. All this interferes with the normal routine, since a control over the diet and food preparation prevails (Fidan *et al*., 2010). Outside of meals, a considerable amount of time is spent planning and making daily meals in order to be able to pay attention to thoughts

about what will be eaten, gathering information on ingredients, preparation and finally consumption of foods.

In addition to these two disorders, there are also correspondences with other diagnostic categories, including obsessive-compulsive personality disorder (DPOC), that shares perfectionism, rigidity of thought, excessive devotion, hyper-morality, and concern for details and rules with orthorexia.

Finally, the possibility that orthorexia may be characterized by symptoms attributable to the psychotic spectrum should be considered. To date, there is only one case described in the literature, in which orthorexic eating habits have manifested themselves as prodromal symptoms of schizophrenia (Saddichha et al., 2012).

1.2.4 Classification criteria

Currently, there is no universally agreed definition of ON and the diagnostic criteria are still under discussion. In this regard, in 2016, the Task Force of Orthorexia Nervosa (ON-TF) was established, made up of researchers from many countries who are actively involved in ON. The task of the ON-TF is to outline the concept of ON and verify which elements must be considered in its definition (Cena *et al*., 2019). The latter are: eating behaviour, compulsiveness, body image disorder, problems with body weight, intuition, medical complications and psychosocial functioning. However, there is still no official set of diagnostic criteria. Some studies have used the DSM classification (IV or V) trying to adapt the criteria used for AN, ARFID or body dysmorphic disorder (BDD) to ON. It is still a matter of debate whether ON should be considered as a distinct disorder, a variant of an existing eating disorder, an OCD or a disturbed eating habit (Garano *et al*., 2016).

All these studies have set as primary diagnostic criteria the obsessive or pathological preoccupation with healthy eating, the emotional consequences (e.g., distress, anxieties) for non-adherence to self-imposed nutritional rules, and psychosocial impairments in relevant areas of life, as well as malnutrition and weight loss.

Furthermore, over the years, additional criteria have been added, such as the elimination of foods considered unhealthy, the presence of positive effects due to

compliance with an eating behaviour considered by the subject as healthy, and compulsive behaviour (Dunn & Bratman, 2016).

The first diagnostic criteria for the ON date back to 2013 and have been modified over the years, up to those of Dunn and Bratman in 2016 (Setnick *et al*., 2013; Moroze *et al*., 2014; Barthels *et al*., 2015; Dunn & Bratman, 2016). To date, the diagnostic criteria on the ON should be considered as "working criteria"; in fact, there are still no characteristic symptoms and sufficient information to clearly distinguish it from other EDs. However, the current diagnostic criteria provide a useful starting point for trying to improve the description of the symptoms and behaviours of people who suffer from ON throughout their lives (Garano *et al*., 2016).

1.2.5 Characteristics of orthorexic subjects

Obsessiveness, insecurity, persistent uncertainty, perfectionism and anxiety are among the most common characteristics shared by subjects with orthorexia (Oberle *et al*., 2017), but also by other disorders such as anorexia and bulimia (Barnes & Caltabiano, 2017).

It has been hypothesized that orthorexia is associated with OCD due to recurrent and intrusive thoughts about the anxiety of contamination, connected to cognitive rigidity (Brytek-Matera *et al*., 2017). These negative emotions, typical of OCD patients, concern the perception of the well-being of their physique and body image. The latter has been defined, in psychological terms, as the mental photograph of our body perceived by our ego (Brytek-Matera *et al*., 2017). The body image, however, does not coincide with the body schema, which is an unconscious representation of one's body, "a system of sensory-motor skills that act without awareness". Cognitive biases relating to body image can lead to an altered judgment and obsessions regarding the size of one's physical appearance, to the point that the individual associates their value with the size of the body. A persistent dissatisfaction with the body could derive from an underestimation of the self, which corresponds to a sort of pathological inferiority complex, term coined by the psychoanalyst Alfred Adler in 1920. Adler argued that all men live trying to compensate for a feeling of inferiority, or a psychological perception of intense inadequacy. While the feeling of inferiority for some individuals becomes

a positive stimulus to motivate self-improvement in various fields, others are overwhelmed by it, transforming it into an inferiority complex, sometimes disabling. Adler also assumed that the superiority complex was a psychological defence mechanism, also originating from an inevitable feeling of inferiority offset by the tendency to perfectionism, a characteristic common to patients with OCD and ON. The orthorexic subject strives to strictly pursue a nutrition that he/she considers healthy and rejects the idea that his cognitive perception is wrong and unhealthy (Brytek-Matera *et al*., 2015). This attitude of "superiority" of one's diet and refusal towards that of others has sometimes been defined by some researchers as superior attitude, a concept that could correspond to the Adlerian superiority complex (Oberle *et al*., 2017). This psychological malaise affects the social life of the subject, who tend to avoid eating meals outside his home, as he is not sure of the quality or method of preparing food by others. Social isolation eventually follows, therefore a state of greater anxiety and depression. Literature suggests that self-esteem associated with strict compliance with food rituals is high in orthorexics (Barnes & Caltabiano, 2017). It has been observed that ON is related to neuroticism, a personality trait that refers to the pessimistic state of tending to negative emotions of depression, anxiety and anger (Oberle *et al*., 2017). In psychology, neuroticism is one of the so-called "Big Five" (Oberle *et al*., 2017), an expression that refers to one of the best known models that defines five personality traits: neuroticism (emotional vulnerability), extroversion (sociability), openness (availability to creativity), empathy (agreeableness, kindness, altruism, cooperativeness) and conscientiousness (respect for duty). It has been found that the symptomatology of ON unites the most depressed, pessimistic and suicidal subjects (Strahler *et al*., 2018).

So far most of the studies on orthorexia have focused on the emotional and physical consequences that the condition entails, rather than on the underlying neurological profile that would seem to induce this behaviour. From these studies it is clear that the desire to improve one's physical condition and well-being leads orthorexic subjects to continuously and carefully check the quality of food: for example, organic vegetables and fruit can be considered healthy and low-calorie foods, unlike sweeteners and artificial flavours or preservatives; equally, nutritional properties that could be lost during cooking must be well controlled. A considerable time is spent examining the origin of food: for example, if the plants

have been exposed to pesticides or if the dairy products come from cows subjected to the use of hormones. Orthorexics themselves analyse labels in order to find sufficient information to judge the quality of specific ingredients. Finally, processing and packaging are essential to assess whether the food contains carcinogenic components derived from plastic or other potentially harmful materials (Garano *et al*., 2016).

Orthorexic subjects often find themselves conducting complex food rites, with very long times that involve the preparation of meals. They are governed both by rules, regarding which foods can be eaten with each meal or at specific times of the day, and by beliefs, according to which the optimal digestion of a certain food must take a specific amount of time after the intake of another type of food. Over time, an orthorexic develops a highly specific, restrictive and rigid diet that causes negative consequences both on the physical, psychological and relational and working levels (Mathieu, 2005). Another characteristic of orthorexics is that they feel intense frustration when their food-related practices are interrupted or hindered. Deviations from the dietary regimen can trigger a desire for self-punishment, usually manifested by an even stricter diet (Mathieu, 2005).

However, studies on the neurological profile of orthorexic subjects were also carried out, and it emerged that orthorexics have disorders mainly in three cognitive domains:

1. Cognitive flexibility: it consists in being able to solve problems in an elastic way and in knowing how to move freely, according to need, from one situation or group of thoughts to another. Its impoverishment or cognitive rigidity could explain the inflexible approach and closely linked to the rules that individuals with orthorexic symptoms take towards the selection, preparation and consumption of food (Koven & Senbonmatsu, 2013).
2. External attention: it refers to the ability to focus on the external environment while being aware of one's social impact on other people. Since attention to inner aspects is associated with less processing of external stimuli, it is not surprising that individuals with orthorexic symptoms may have deficits in this area, since many of their attitudes signal a focus on themselves, especially in the concern for purity and health (Koven & Senbonmatsu, 2013).

3. Working memory: it consists in the ability to assimilate information for short periods of time in the service of other tasks. It can be easily disturbed by attention for irrelevant internal and external stimuli (Anticevic *et al*, 2010). Therefore, it is possible that concerns about food or continuously present health-related images and thoughts impair this ability in people with orthorexia.

1.2.6 Orthorexia in relation to gender and BMI

Several studies have reported conflicting results regarding the relationship between orthorexia and gender: in some cases there is a prevalence among men (Donini *et al*., 2004; Fidan *et al*., 2010), in others, instead, a greater prevalence among women (Arusoğlu *et al*., 2008; Keller & Konradsen, 2013), and in further studies it is found that there is no relationship, to the point that it can be assumed that ON and sex are not factors connected, also because the lifestyles proposed by the mass media affect both men and women (Parra-Fernandez *et al*, 2019).

Conflicting results also emerged for the association between BMI and ON. While in some studies it is statistically insignificant (Parra-Fernandez *et al*, 2019), in others it has been observed that a BMI lower than or equal to 18.5 kg/m^2 refers to those at greatest risk of ON (Gezer & Karban, 2013). Studies also suggest a higher prevalence of ON among subjects that tend to promote the body and environment well-being, such as vegetarians, environmentalists (Plichta & Jezewska-Zychowicz, 2020), sportsmen (Oberle C. *et al*., 2016). In a study on a sample of 2,826 subjects, it was pointed out that there is a relationship between ON and specific diets; in fact, vegetarians and vegans show a higher percentage of the symptoms of ON (Dell'Osso *et al*., 2016).

1.2.7 Orthorexia nervosa among university students

Several studies have analyzed the prevalence of ON in university students. A multicultural comparison study among 664 university students enrolled in Italy (N=216), Spain (N=242) and Poland (N=206), have tried to analyse the psychological

characteristics of subjects with EDs or altered eating habits, as in the case of the ON (Gramaglia *et al*., 2019). The presence of typical aspects of the ON has emerged in more than one third of the sample, especially in the female population. In Poland, there was a greater spread of ON among enrolled university students, a more frequent attention to the dietary regime, that includes supplements, such as vitamins, and less sporting activities (over 50% in the Polish sample). In recent years, healthy nutrition has become a very popular ideal in Poland. The differences in prevalence of the ON may be linked to the role of culinary culture, adherence to the MD, and the convivial and social role of nutrition (Gramaglia *et al*., 2019). In particular, in contexts in which the MD is highly appreciated (Spain and Italy), a lower frequency of orthorexic trends has been observed (Gramaglia et al., 2019).

Furthermore, some studies reported that students of dietetic institutes showed a higher prevalence of symptoms of ON and EDs, compared to subjects enrolled in degree courses not related to nutrition and health (Bo *et al*., 2014). Additionally, according to some researchers, ON is frequently present among nutritionist women, and orthorexic symptoms influence their career choice (Donini *et al*., 2004). While in an Italian study it was suggested that the population of athletes was at increased risk of developing ON (Segura-Garcìa *et al*., 2012), no differences between athletes and non-athlete students have emerged from another study. However, students who tended to engage in intense PA are more susceptible to developing ON, although there would be no direct correlation between the prevalence of ON and athletics (Clifford & Blyth, 2018).

From a study carried out at the University of Pisa enrolling 2,826 students from various degree courses, it emerged that symptoms of ON occurred with a frequency of 32.7% (Dell'Osso et al., 2016).

Studies available in the literature most of the time conclude with the clarification that further research is necessary to analyse the ON, since it is complex to deduce if an individual is affected by ON, or if he has a particularly strong interest in health and nutrition (Plichta & Jezewska-Zychiwicz, 2019). There is a lack of universal diagnostic criteria and it is complex to establish the percentage of the population affected by ON (Koven & Abry, 2015). However, it is possible to evaluate the trends in ON in specific samples of enrolled volunteers using psychometric questionnaires, such as the Bratman Orthorexia Test (BOT), the ORTO-15, adapted in different versions depending on the country, the Eating Habits Questionnaire

(EHQ), the Eating Attitudes Test (EAT-26), the Food Neophobia Scale (FNS), the Starvation Symptom Inventory (SSI), and deepening the psychological conditions of the volunteers through other tests, such as the Kessler Psychological Distress Scale (K10), the Body Uneasiness Test (BUT), the Alcohol Use Disorder Identification Scale (AUDIT), and the Multidimensional Body Self Relations Questionnaire (MBRSQ).

II - RATIONALE

II - RATIONALE

Young people are considered a population at risk for nutritional deficiencies due to poor dietary habits, especially among university students (Lupi *et al*., 2015). Since, over the last 20 years, there has been an increase in obesity rates among college students (WHO, 2014; Shah *et al*., 2014a), they should be seen as a group that requires special attention in terms of health promotion, especially in the dietary domain.

The high levels of stress experienced by university students have been related not only to the changes in food choices, but also to the use of so-called "smart drugs" and "smart nutrients" (Dietz *et al*., 2018), which university students, especially medical students (74.7%) (Pighi *et al*., 2018), use to improve their cognitive abilities. The phenomenon of self-medication represents one of the main problems for public health, since only 48% of the population of the European Union uses prescription drugs (Eurostat, 2015; Grappasonni et al., 2018; Petrelli et al., 2018). Another particularly worrying trend is the increased use of antidepressants among young people (Gualano *et al*., 2014), which many of them take as a result of disappointments in academic performance, and which have negative side effects, such as weight gain, altered lipid profile, and risk of diabetes (OECD, 2017), as well as the use of substances, such as amphetamines and other stimulants, alcohol abuse, or other drugs (Vo et al., 2015; DeSantis et al., 2010). A previous study revealed a high percentage of drugs use in Spanish students, 38.4% of whom take them in combination with alcohol. On the other hand, it was observed that Italian students rarely go to the pharmacist and rely more on the advice of family or friends (Scuri *et al*., 2019).

The role of lifestyle in the prevention of chronic low-grade inflammation and related diseases is well known. Unhealthy behaviours (eating habits, physical activity and lifestyle) are common in the European population and, in particular, university students abandon healthy eating patterns even in the Mediterranean area. Improvement of dietary habits of these population includes the increase of

the consumption of foods rich in bioactive compounds with known biological properties. Although labelling provides information leading to the reduction of the caloric and fat content of meals chosen by university students, without compromising the micronutrient composition, it should be taken into account that vegetable seasonings play an important role in the absorption of these micronutrients, as is the case of fat-soluble vitamins.

For the first time, an in-depth analysis of the lifestyle of Italian and Spanish university students will be carried out, with an assessment of the frequency of self-medication and the use of drugs and supplements/nutraceuticals, both in students who follow studies related to Health Sciences and those in the Humanistic area. In this way it would be possible to assess whether the perception of health problems, the appropriate use of medications and lifestyle habits in general may be influenced by their area of study.

This study should also confirm the results of previous studies on the high rate of orthorexia in students of health science faculties, especially among nutritionists and athletes. The results will be useful in the design of promotional programs for the appropriate use of medications/supplements and for healthy diets and lifestyles in university students, and will also provide a potentially useful basis for the recruitment of subjects in future intervention studies.

III - OBJECTIVES

III - OBJECTIVES

The main objective of this study is to evaluate the self-medication, the adherence to Mediterranean diet, and the relationship between lifestyle and biomarkers of metabolic status in a university population.

Specific objectives:

- To compare the adherence to the Mediterranean diet of Italian and Spanish university students
- To compare the levels of self-medication and the consumption of alcohol, energy drinks, supplements, dietary products and/or other types of nutraceuticals in Italian and Spanish university students
- To compare cardiovascular risk factors (obesity, hypertension, hyperglycemia, dyslipidemia, and smoking) in Italian and Spanish university students
- To evaluate the level of physical activity and the percentage of subjects with orthorexia nervosa in Italian and Spanish university students
- To compare the frequency and type of meals consumed outside home and the adherence to certain dietary regimes (vegetarian diet, ketogenic diet, etc.) in Italian and Spanish university students
- To record other characteristics of interest (diseases, anxiety/depressive symptoms, food phobias, eating disorders, vaccinations and opinion about vaccinations)

IV - MATERIAL AND METHODS

IV - MATERIAL AND METHODS

4.1 MULTI-CENTRE CROSS-SECTIONAL OBSERVATIONAL STUDY OF DIETARY HABITS AND LIFESTYLE: RECRUITMENT AND STUDY DESIGN

The present PhD project has been conducted in the framework of the project "Lifestyle, Self-medication and Use of Nutraceuticals in a Population of Italian and Spanish Students", acronym STANIS, which is a multi-centre cross-sectional observational study. It is registered on ClinicalTrials.gov (Identifier NCT04099420). The study protocol was approved by the Ethics Committee for Human Experimentation of the Department of Physiology and Pharmacology V. Erspamer of La Sapienza University of Rome, and by the Ethics Committee of the Catholic University of Murcia (UCAM), with the collaboration of the Research Centre for Food and Nutrition of the Council for Agricultural Research and Economics (CREA-AN). This study was carried out from July 2019 to September 2021. Participants (N=194, 108 Italian and 86 Spanish) were recruited through verbal disclosure, e-mails and notice boards at La Sapienza University of Rome and UCAM, applying the following criteria:

- EXCLUSION: age <18 years or inability to sign informed consent
- INCLUSION: university students of different academic degrees as doctoral, post-doc, and specialisation students, aged between 18 and 35 years, particularly from biomedical and pharmaceutical sciences.

All the volunteers included in the study signed the informed consent, accompanied by an informative note, and recruiters assigned them an alphanumeric code in order to guarantee privacy during the data processing and analysis phases. On-line questionnaires were administered to collect information about use of drugs and self-medication, eating habits, level of PA, presence of anxiety/depressive symptoms, and lifestyle of the subjects. Questionnaires are shown in Annex. Besides, students were evaluated with clinical parameters of

metabolic status (glycaemia, cholesterol, triglycerides and ketones), and underwent anthropometric and impedance measurements. Optionally, participants could fill in a 7-day food diary to assess energy and macronutrients consumption.

4.2 QUESTIONNAIRES FOR THE ASSESSMENT OF UNIVERSITY STUDENTS

4.2.1 Adherence to Mediterranean diet

To evaluate the grade of adherence to MD three different questionnaires were administered to the volunteers.

The Mediterranean Diet Score (MDS-14), validated in the PREDIMED study (Martínez-González *et al*., 2012), is a 14-item questionnaire (Appendix 1), of which 12 questions inquire on frequency of consumption of typical components of MD (items 2-12, 14), while the other 2 (items 1, 13) concern dietary habits, in particular the use of olive oil as the principal source of fat for cooking, and the preference in consuming white meats than red ones. A score of 0 (no adherence to MD) or 1 (adherence) can be assigned to each item, with a total score ranging from 0 to 14. In particular, three groups can be identified according to the final score:

1. low adherence with a score ≤ 5
2. medium adherence with a score between 6 and 9
3. high adherence with a score ≥ 10

A greater score is associated with a high consumption of olive oil, fruits and vegetables, wine, fish, legumes and dried fruit, and a scarce use of red meats, animal fats, sugary drinks and sweets.

The Mediterranean Score (MED-55) is another index proposed by Panagiotakos and colleagues (Panagiotakos *et al*., 2006) to estimate the adherence level to MD. It is a 11-item questionnaire (Appendix 2) that examines the frequency of consumption of the main components of MD: non-refined cereals, fruits, vegetables, potatoes, legumes, olive oil, fish, red meat, poultry, full fat dairy products and alcohol. For the intake of items supposed to be close to this dietary pattern scores 0, 1, 2 ,3, 4, and 5 were assigned when volunteers reported no consumption, rare, frequent, very frequent, weekly and daily, respectively;

whereas, for the consumption of food assumed to be away from this pattern the same scores were assigned on a reverse scale. Particularly for alcohol, score 5 was assigned for consumption of less than 300 ml per day, score 0 for consumption of more than 700 ml per day or for no consumption, and scores from 4 to 1 for consumption of 300-400, 400-500, 500-600, and 600-700 ml per day (100 ml = 12 g ethanol). The final score, ranging from 0 to 55, was calculated with a software program developed in Microsoft Visual Basic 6.0 (fig. 4.1). Higher values indicate a greater adherence to MD. In the main form the user is asked to state the frequency of intake of every listed food, by clicking at the respective option button. Every time that an option button is selected, the respective red circle turns to green colour indicating that selection has been made. When frequency of consumption for all listed foods has been selected, the program automatically calculates the diet score. The rationale of this score is based on the diet score proposed by Trichopoulou and colleagues in previous works (Trichopoulou *et al*., 2003).

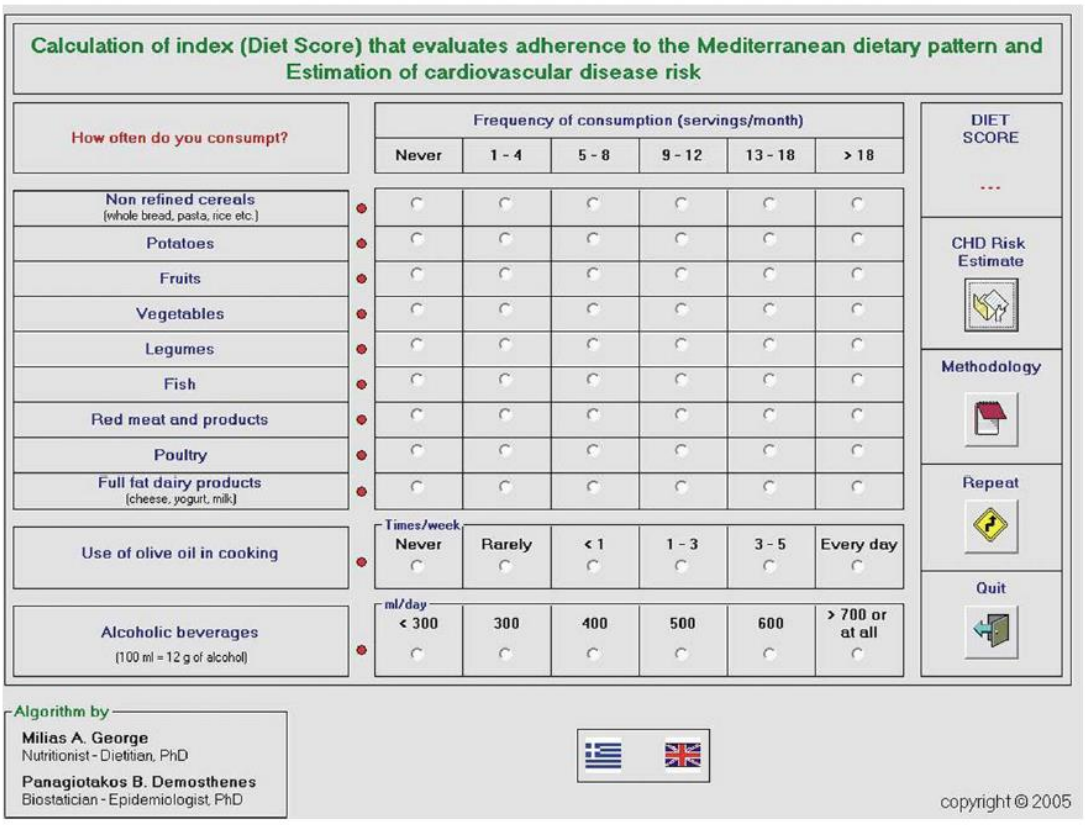

Figure 4.1. Main form of the software program to calculate MED-55 score (Panagiotakos et al., 2006).

Assuming that a score of 55 represents 100% adherence to the Mediterranean dietary pattern, a score equal to *k* represents (*k*/55) x 100% agreement to this nutritional model. Five classes of distribution of the MED-55 score have been developed (0-11, 12-22, 23-34, 35-44, and 45-55). Additionally, this program also calculates an estimation of the cardiovascular disease risk based on the values of the score (fig. 4.2) (Panagiotakos *et al*., 2002).

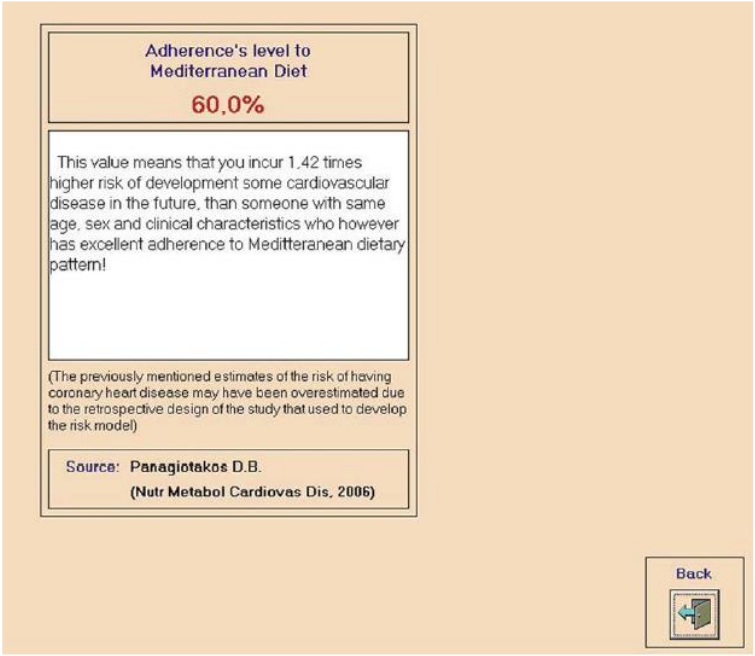

Figure 4.2. Sample of advice given after diet score has been calculated (Panagiotakos *et al*., 2006).

The Questionnaire to measure Mediterranean diet (QueMD) is a self-administered 15-item questionnaire (Appendix 3), with a score ranging from 0 to 30, that includes questions for the nine food items considered as key components of MD (wholegrain cereals, raw or cooked vegetables, legumes, fresh fruits, dried fruits, red or processed meat, fish, wine and olive oil), using two different questions to evaluate the intake of wholegrain cereals (wholegrain pasta or rice and wholegrain bread and substitutes) (Gnagnarella *et al*., 2018).

The alternate Mediterranean diet (aMED) score, ranging from 0 (minimal adherence to MD) to 9 (maximal adherence), was calculated from QueMD assigning 1 point to volunteers reporting consumptions above the average levels

for each of the nine foods that are characteristic of MD: vegetables and fresh fruits (≥2/day), dried fruits (≥2/week), wholegrain cereals (≥1/day), pulses and fish (≥2/week), and olive oil (≥3/day) intakes. Moreover, 1 point was also assigned to those consuming red and processed meat ≤1–3/week, and for men drinking 1–2 glasses of wine per day (corresponding to 125–250 mL) or women drinking a limited amount of wine (1/2–1 glass/day, corresponding to 62.5–125 mL) (Bach-Faig *et al*., 2011). Additional items are the consumption of white meat (chicken, turkey, and rabbit) and dairy products, focusing only on milk and yogurt (excluding cheese). Furthermore, questions for three additional food groups that are not typical of MD are included: carbonated and/or sugar sweetened beverages (soft drinks), butter, margarine or cooking cream, manufactured sweets, pastries, and cakes. A sub-score for these non-typical MD foods was evaluated (ntMED). For each of the food items, participants could choose between five consumption levels, ranging from "never or seldom" to a high frequency. For each question a standard portion was indicated to help reporting consumption as correctly as possible (Gnagnarella *et al*., 2018).

4.2.2 Prevalence of orthorexia nervosa

The ORTO-15 test (Appendix 4) was introduced to estimate the prevalence of ON (Donini *et al*., 2005). It is a questionnaire made up of 15 multiple-choice items based on a Likert scale (always, often, sometimes, never), and developed starting from a previous American model used by Bratman (Bratman & Knight, 2000). The items concern in particular three areas:

1. the cognitive-rational area (items 1, 5, 6, 11, 12, 14)
2. the clinical area (items 3, 7-9, 15), mainly related to anxiety-inducing and obsessive psychiatric disorders
3. the emotional area (items 2, 4, 10, 13)

A score of 1 was given to responses that were more indicative of orthorexia, whereas a score of 4 was attributed to those that indicated a normal eating behaviour. Therefore, lower scores correspond to a more pathological behaviour, assuming that in the Italian version total score ranges from 15 to 60 and the cut-off point is equal to 40 (Donini *et al*., 2005). This value has been questioned as it was considered too high; consequently, lower cut-offs, such as 35, were also chosen

(Plichta & Jezewska-Zychowicz, 2020). ORTO-15 is used to estimate obsessiveness in relation to the preparation and selection of nourishments, and in general to the relationship with food. However, it presents some limitations since it does not allow to always discern the limit between the healthy lifestyle and the pathological one. Furthermore, studies on ON are restrained to recruited volunteers (for example athletes and students), who are not representative of the entire population. In fact, Donini and colleagues admit these psychometric limits of the ORTO-15 (Donini *et al*., 2005). It has been pointed out that some questions may apply more to AN than to ON (*e.g*., "When eating, do you pay attention to the calories of the food?"), or more to thoughts than psychopathological behaviours (*e.g*., "Do you think that consuming healthy food may improve your appearance?"); some items may not be relevant to all geographic regions (*e.g*., "Do you think that in the market there is also unhealthy food?"), and some questions may simply be confusing in meaning (*e.g*., "When you go in a food shop do you feel confused?") (Oberle *et al*., 2017).

Additional versions of the ORTO test have been developed based on the selection of ORTO-15 items that more specifically could be indicative of the presence of symptoms of ON. ORTO-11 is the Spanish version that excludes items 5, 8, 14 and 15 of the ORTO-15 that cannot be associated with EDs. Final score ranges from 11 to 44 and a cut-off point <25 (efficiency 84%, sensitivity 75% and specificity 84%) is considered to be the most appropriate for suggesting the tendency to ON (Parra-Fernandez *et al*., 2018).

ORTO-12 is a French shorter version of 12 items, obtained by excluding items 5, 6 and 8 of the original ORTO-15 since they contribute less to the definition of the orthorexic context (Babeau *et al*., 2020). In fact, for item 5 ("Is the taste of food more important than the quality when you evaluate food?") the literature suggests that taste is not a decision criterion to choose food among binge eaters (Goldfield *et al*., 2008); for item 6 ("Are you willing to spend more money to have healthier food?") the answers seem to be strongly linked to financial well-being, while item 8 ("Do you allow yourself any eating transgressions?") was discarded by statistical analysis. For this questionnaire a specific cut-off point was not determined since it was elaborated as a structure validation study (Babeau *et al*., 2020).

ORTO-9 is the German version of ORTO test which excludes items 1,2 and 8, with a cut-off <26.7. This test was found to be ineffective in predicting orthorexic tendencies (Missbach *et al*., 2015).

ORTO-7 is based on items (1, 3, 4, 7, 9, 11 and 13) that mostly highlight the presence of ON with a cut-off ≤19 (Moller *et al*., 2019).

4.2.3 Food neophobia scale

Actually, the interest towards novel food, including insects, transgenic and uncommon products, has increased with market globalization, migration flows, and the spread of new dietary habits, particularly in Western society (Guidetti *et al*., 2018). Food neophobia scale (FNS) is a measure of food neophobia (FN), the reluctance to eat and/or avoidance of unfamiliar foods (Pliner & Hobden, 1992; Guidetti *et al*., 2018). In this study the revised FNS (Appendix 5) has been used. It is a 10-item questionnaire with answers given on a 5-point agreement scale (not at all, little, sufficiently, very, strongly), and the score can range from 10 to 50. Items 3 ("If I don't know what a food is, I won't try it"), 8 ("I am very particular about the foods I eat"), and 9 ("I will eat almost anything") could not discriminate between neophobic subjects and picky eaters (who reject a large amount of both familiar and novel foods), vegans/vegetarians, and intolerant/allergic people. Furthermore, item 4 ("I like foods from different cultures") refers to liking, therefore implies that those foods have already been tasted and cannot be considered novel food. As a consequence, the removal of these four items could represent an improvement of the measure. Scores ≥28 are associated with FN, between 12 and 27 were neutral, and less than 12 were associated with food neophilia (Guidetti *et al*., 2018).

4.2.4 Starvation Symptom Inventory

Starvation Symptom Inventory (SSI) is a 16-item questionnaire (Table 4.1) to evaluate the emotional state that can reveal the presence of malnutrition. Participants were asked to provide an estimate of the number of days out of the preceding 28 in which they had experienced symptoms of starvation (hunger, poor concentration, heightened satiety, dizziness, reduction in rate of weight loss) on a 7-point Likert scale: never (0), 1-5 days (1), 6-12 days (2), 13-15 (3), 16-22 (4), 23-27

(5), and always (6) (Calugi *et al*., 2017). Items for the SSI were generated by taking into consideration starvation symptoms recorded in the Minnesota Starvation Study, in which 36 young male volunteers underwent dietary restriction reporting starvation symptoms, such as mood lability, ritualistic eating, cookbook and recipe collection (Keys *et al*., 1950). The highest score indicates increased frequency of starvation symptom over the last 28 days. In subjects with AN the average score obtained is 55 while in healthy ones is 10 (Calugi *et al*., 2017).

Table 4.1. **SSI Questionnaire**

How many times in the last 28 days
1. Worried about food?
2. Collected recipes, menus or cookbooks?
3. Increased your consumption of tea, coffee or spices?
4. Felt depressed?
5. Felt anxious?
6. Felt irritable?
7. Had moon swings (between excited and depressed)?
8. Stayed away from other people?
9. Experienced a loss of concentration?
10. Felt apathetic?
11. Had disturbed sleep?
12. Felt weak?
13. Experienced a lack of interest in sex?
14. Felt cold?
15. Felt an increase in hunger?
16. Felt full early?

(Calugi *et al*., 2017)

4.2.5 Alcohol Use Disorders Identification Test

AUDIT is a 10-item screening tool (Appendix 6) developed by WHO to assess alcohol consumption, drinking behaviours, and alcohol-related problems. Items are selected to measure the three conceptually distinct dimensions of intake (items 1-3), dependence (items 4-6), and adverse consequences of drinking (items 7-10), which are highly related (Allen *et al*., 1997). However, some studies support an AUDIT structure aimed to evaluate the two different dimensions of consumption

(items 1-3) and adverse consequences (items 4-10) (Shields *et al*., 2004). With the exception of the last two items, AUDIT questions allude to the previous year, and the score assigned to each item that ranges from 0 to 4 is generally based on frequency of occurrence. A score of 8 or more is considered to indicate hazardous or harmful alcohol use (Allen *et al*., 1997; Babor *et al*., 2001). Nevertheless, a cut-off point of 6 has been suggested for women, since the original score yielded lower sensitivities and higher specificities for women than for men. The differentiated cut-off scores would correct the gender discrepancies in the sensitivity of the measure (Reinert & Allen, 2007). Scores ≥16 are associated with harmful drinking for men and women, while scores ≥20 with alcohol dependence (Babor *et al*., 2001).

4.2.6 International Physical Activity Questionnaire

The International Physical Activity Questionnaire (IPAQ) assesses PA undertaken across a comprehensive set of domains including:

a. leisure time PA
b. domestic and gardening (yard) activities
c. work-related PA
d. transport-related PA

The IPAQ short form made up of 9 items has been used in this study (Table 4.2), and it asks about three specific types of activity: walking, moderate-intensity activities and vigorous-intensity activities. The items are structured to provide separate scores for the three specific activities. Computation of the total score requires summation of the duration (in minutes) and frequency (days) of walking, moderate- and vigorous-intensity activities. Measure of the activity volume was computed by weighting each type of activity by its energy requirements defined in Metabolic Equivalent of Task (METs) to yield a score in MET-minutes. One MET is equal to energy expenditure during rest and is approximately equal to 3.5 ml O_2 kg^{-1} min^{-1} in adults (*Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire*, 2005; Hagströmer *et al*., 2006).

Table 4.2. IPAQ. The International Physical Activity Questionnaire, 2005

Vigorous-intensity activities
1a) In the past 7 days, how many days did you engage in INTENSE physical activity, such as weight lifting, heavy garden work, aerobic activity, or cycling at high speeds?
1b) How much time in total did you normally spend doing INTENSE physical activity on any of these days? (Minute)
Moderate-intensity activities
2a) In the past 7 days, how many days have you been in MODERATE physical activity, such as carrying light weights, cycling at regular speed, gym activities, garden work, prolonged physical work around the house? Don't consider walking.
2b) How much time in total did you normally spend doing MODERATE physical activity on any of these days? (Minutes)
Walking
3a) In the last 7 days, how many days did you walk for at least 10 MINUTES? Consider all the walks you have ever taken.
3b) How long in total have you normally walked one of these days? (minutes)
3c) At what pace did you mostly walk? o INTENSE step, which made you breathe at a faster rate than normal o MODERATE pace, which caused you to breathe at a rate only moderately faster than normal o SLOW step, with no change in the rhythm of your breathing
Sitting activity
4a) In the past 7 days, how much time (minutes) in total did you spend SITTING on a weekday? (includes activities at work, at home and in your free time)
4b) Over the past 7 days, how much total time did you spend SITTING on a weekend day? (minutes)

Available at http://www.ipaq.ki.se

The following values are used for the analysis of IPAQ data: Walking = 3.3 METs for an intense step, 3 METs for a moderate pace, and 2.5 METs for a slow step, Moderate PA = 4.0 METs, and Vigorous PA = 8.0 METs. Using these values, four continuous scores are defined:

- Walking MET-minutes/week = 3.3 * walking minutes * walking days (intense) or 3 * walking minutes * walking days (moderate) or 2.5 * walking minutes * walking days (slow)
- Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate-intensity days
- Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days
- Total PA MET-minutes/week = sum of Walking + Moderate + Vigorous MET-minutes/week scores

There are three levels of PA proposed to classify populations:

1. Low: it is the lowest level of PA and includes those individuals who not meet criteria for categories 2 or 3.
2. Moderate: 3 or more days of vigorous-intensity activity of at least 20 minutes per day, or 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day, or 5 or more days of any combination of walking, moderate- or vigorous-intensity activities achieving a minimum Total PA of at least 600 MET-minutes/week.
3. High: vigorous-intensity activity of at least 3 days achieving a minimum Total PA of at least 1500 MET- minutes/week, or 7 or more days of any combination of walking, moderate- or vigorous-intensity activities achieving a minimum Total PA of at least 3000 MET-minutes/week.

The IPAQ sitting question is an additional indicator variable of time spent in sedentary activity and is not included as part of any summary score of PA. All cases in which the total sum of all Walking, Moderate and Vigorous time variables is greater than 960 minutes (16 hours) have been excluded from the analysis since on average an individual spends 8 hours per day sleeping. Responses of less than 10 minutes and their associated days have been re-coded to “zero”. All Walking, Moderate and Vigorous time variables exceeding 180 minutes have been re-coded to be equal to 180 minutes. This rule permits a maximum of 21 hours of activity in a week to be reported for each category (3 hours * 7 days). In calculating

"moderately active", those volunteers who undertook activity on at least 5 days/week were identified and coded in a new variable called "at least five days" which includes all participants who meet criterions of the second category aforementioned. The same approach has been used to compute the "high category". In fact, all those participants who undertook a combination of walking, moderate- and/or vigorous-intensity activities on at least 7 days/week were coded in a new variable to reflect "at least 7 days" (*Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire*, 2005).

4.2.7 Multidimensional Body-Self Relations Questionnaire

MBSRQ is a 69-item self-report inventory (Appendix 7) for the assessment of self-attitudinal aspects of the body-image construct. The MBSRQ Factor Subscales (FSs) reflect two dispositional dimensions: "Evaluation" and cognitive-behavioural "Orientation" with respect to each of the three somatic domains of "Appearance", "Fitness", and "Health/Illness". The seven FSs are the following:

1. APPEARANCE EVALUATION (AE): feelings of physical attractiveness or unattractiveness; satisfaction or dissatisfaction with one's looks. High scorers feel mostly positive and satisfied with their appearance; low scorers have a general unhappiness with their physical appearance.
2. APPEARANCE ORIENTATION (AO): extent of investment in one's appearance. High scorers place more importance on how they look, pay attention to their appearance, and engage in extensive grooming behaviours. Low scorers are apathetic about their appearance; their looks are not especially important and they do not expend much effort to "look good".
3. FITNESS EVALUATION (FE): feelings of being physically fit or unfit. High scorers regard themselves as physically fit, "in shape", or athletically active and competent. Low scorers feel physically unfit, "out of shape", or athletically unskilled.
4. FITNESS ORIENTATION (FO): extent of investment in being physically fit or athletically competent. High scorers value fitness and

are actively involved in activities to enhance or maintain their fitness. Low scorers do not value physical fitness and do not regularly incorporate exercise activities into their lifestyle.

5. HEALTH EVALUATION (HE): feelings of physical health and/or the freedom from physical illness. High scorers feel their bodies are in good health. Low scorers feel unhealthy and experience bodily symptoms of illness or vulnerability to illness.
6. HEALTH ORIENTATION (HOr): extent of investment in a physically healthy lifestyle. High scorers are "health conscious" and try to lead a healthy lifestyle. Low scorers are more apathetic about their health.
7. ILLNESS ORIENTATION (IO): extent of reactivity to being or becoming ill. High scorers are alert to personal symptoms of physical illness and are apt to seek medical attention. Low scorers are not especially alert or reactive the physical symptoms of illness.

In addition to these FSs, the MBSRQ has three multi-item subscales:

1. BODY AREAS SATISFACTION SCALE (BASS): similar to the AE subscale, except that the BASS taps satisfaction with discrete aspects of one's appearance. High composite scorers are generally content with most areas of their body. Low scorers are unhappy with the size or appearance of several areas.
2. OVERWEIGHT PREOCCUPATION (OP): this scale assesses a construct reflecting fat anxiety, weight vigilance, dieting, and eating restraint.
3. SELF-CLASSIFIED WEIGHT (SCW): this scale reflects how one perceives and labels one's weight, from “very underweight” to “very overweight”.

Each of the MBSRQ scales has its corresponding items (Appendix 7) that can be answered by a primary number from 1 (strongly disagree) to 5 (strongly agree). The score of contraindicative items (6,15-17, 23, 25, 28, 32-34, 36-38, 40, 42, 43, 45, 47-49) is reversed (*i.e.*, 1=5, 2=4, 4=2, 5=1). MBSRQ subscale scores are the means of the constituent items (Brown *et al*., 1990; Cash, 2000). The MBSRQ-Appearance Scales (MBSRQ-AS) is the shorter form of MBSRQ that assesses only the

appearance-related components of the body image construct. It is a 34-item version that consists of two of the main FSs of the original version (AE and AO), and the three additional multi-item subscales (Cash *et al*., 2004).

4.2.8 Eating Attitudes Test

Eating Attitudes Test (EAT-26) is a standardized measure of symptoms and concerns characteristic of EDs. It is made up of 26 items (Appendix 8) and represent a screening tool to assess "eating disorder risk" in high school, college and other special risk, such as athletes (Garner & Garfinkel, 1979; Garner *et al*., 1982; Garner *et al*., 1998). However, it does not provide a diagnosis of an ED. The EAT-26 items form three subscales:

1. Dieting scale: items 1, 6, 7, 10, 11, 12, 14, 16, 17, 22, 23, 24, 26
2. Bulimia & Food Preoccupation scale: items 3, 4, 9, 18, 21, 25
3. Oral Control subscale: items 2, 5, 8, 13, 15, 19, 20

Four behavioural questions are included to determine the presence of extreme weight-control behaviours as well as provide an estimate of their frequency. These items assess self-reported binge eating, self-induced vomiting, use of laxatives, and treatment for an ED over the preceding 6 months. Participants were required to judge whether the item applied "always", "very often", "often", "sometimes", "rarely" or "never". Each extreme response in the "anorexic" direction is scored as worth 3 points, while the adjacent alternatives are weighted as 2 points and 1 point, respectively. If a score ≤9 is obtained, it can be excluded being affected by EDs, whereas for a score between 10 and 19 a low risk can be assumed. A score ≥20 on the EAT-26 do not necessarily mean that respondent has an ED. However, it indicates a high level of concern about dieting, body weight or problematic eating behaviours and it would be advisable to consult a specialist (Garner & Garfinkel, 1979).

4.2.9 Body Uneasiness Test

Body Uneasiness Test (BUT) is a 71-item psychometric questionnaire (Appendix 9) aimed to assess several areas: body shape and/or weight dissatisfaction,

avoidance, compulsive control behaviours, detachment and estrangement feeling towards one's own body, specific worries about particular body parts, shapes or functions. It is made up of two parts, BUT A (34 statements) and BUT B (37 body parts). Items are rated on a 6 point Likert-type scale (range 0-5, from "never" to "always") and high rates indicate greater body uneasiness (Cuzzolaro *et al*., 2006). BUT A include five FSs:

1. Weight Phobia (WP): fear of being or becoming fat
2. Body Image Concerns (BIC): worries related to physical appearance
3. Avoidance (A): body image related avoidance behaviour
4. Compulsive Self-Monitoring (CSM): compulsive checking of physical appearance
5. Depersonalization (D): detachment and estrangement feelings toward the body

As far as BUT B is concerned, it has a structural model with eight factors represented by Roman numerals:

I. eyebrow, eyes, nose, mouth, lips, teeth
II. shape of the head, shape of the face, forehead, ears, chin, neck
III. stomach, abdomen, hips, thighs, knees
IV. stature, legs, ankles, feet, hands
V. arms, shoulders, chest, breasts, genitals
VI. moustache, beard, hairs,
VII. hair, skin
VIII. sweating, blushing, noises, odours, buttocks

4.2.10 Kessler Psychological Distress Scale

K10 is a simple measure of psychological distress. It is a 10-item questionnaire (Appendix 10) about emotional states, each with a five-level response scale. The measure can be used as a brief screen to identify levels of distress. Each item is scored from 1 (none of the time) to 5 (all of the time). Scores of the 10 items are then summed, yielding a minimum score of 10 and a maximum score of 50. According to the total score, the likelihood of having a mental disorder (psychological distress) is established (Kessler *et al*., 2003). In particular:

- 10-19 likely to be well
- 20-24 likely to have a mild disorder
- 25-29 likely to have a moderate disorder
- 30-50 likely to have a severe disorder

4.2.11 Allergy Questionnaire for Athletes

The Allergy Questionnaire for Athletes (AQUA) is a 25-item tool (Appendix 11) developed for screening allergy in athletes. For all questions with a potential value in predicting allergic diseases (items 4-16), responses are related to an objective documentation of allergy (positive skin tests and/or specific IgE antibody to at least one allergen). A score ≥5 (range 0-30) indicates the presence of allergy with a specificity of 97,1% and a sensitivity of 58,3% (Bonini *et al*., 2009).

4.3 ANTHROPOMETRIC MEASUREMENTS AND IMPEDANCE ANALYSIS

To evaluate body composition, in particular body fat and muscle mass (MM), bioelectrical impedance analysis (BIA) has been used. Body weight, BMI, MM and fat mass (FM) percentages, and visceral fat (VF) level were measured by the impedance balance OMRON BF511 (figure 4.3) with the subject in fasting conditions or 2 hours or more after breakfast or lunch, and after evacuation and urination (Duren *et al*., 2008).

Figure 4.3. Impedance balance OMRON BF511

BIA (fig. 4.4) is a non-invasive method where an extremely weak electrical current of 50 kHz and less than 500 μA flows through the body and the voltage is measured in order to calculate impedance (resistance) of the body (Duren *et al*., 2008). The latter is greater in the adipose tissue, which has little electric conductivity, and lesser in the lean mass, since muscles, blood vessels and bones are body tissues with a high water content that conducts electricity easily (Fosbøl & Zerahn, 2015). In order for the scale to determine body composition, it uses the electrical impedance, along with height, weight, age and gender information of the volunteer to generate results based on OMRON's data of body composition. During the course of a day, the amount of water in the body tends to gradually shift to the lower limbs. The ratio of water in the upper body and lower body is different in the morning and evening, and this means that the electrical impedance of the body also varies. Since the BF511 uses electrodes for both hands and feet to take measurements, it can reduce the influence of these fluctuations on measurement results.

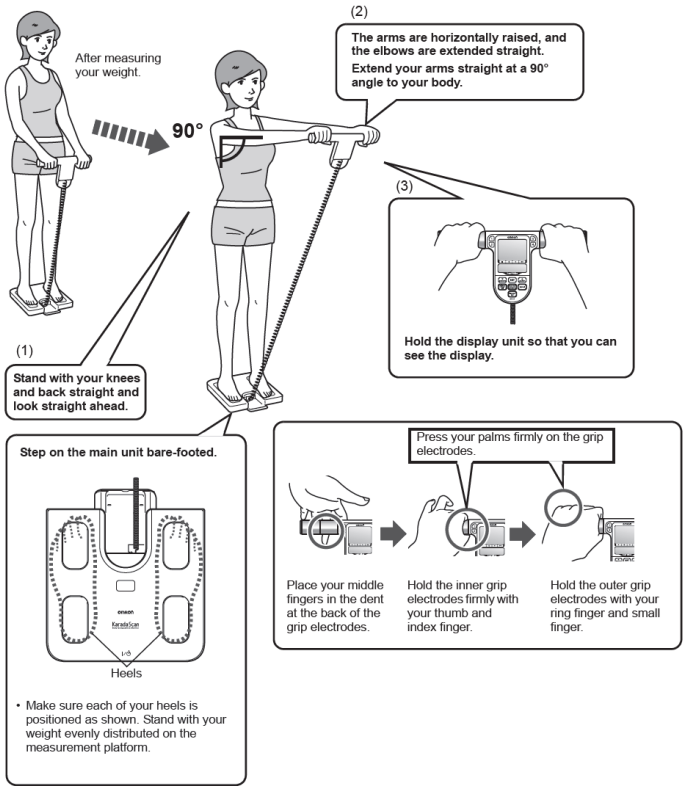

Figure 4.4. Representative scheme of BIA

BMI uses the following simple formula to indicate the ratio between weight and height of a person:

BMI = weight (kg) / height squared (m^2)

The OMRON BF511 uses the height information to calculate subjects BMI classification (underweight, normal, overweight or obese) according to the values for obesity judgement proposed by WHO (fig. 4.5).

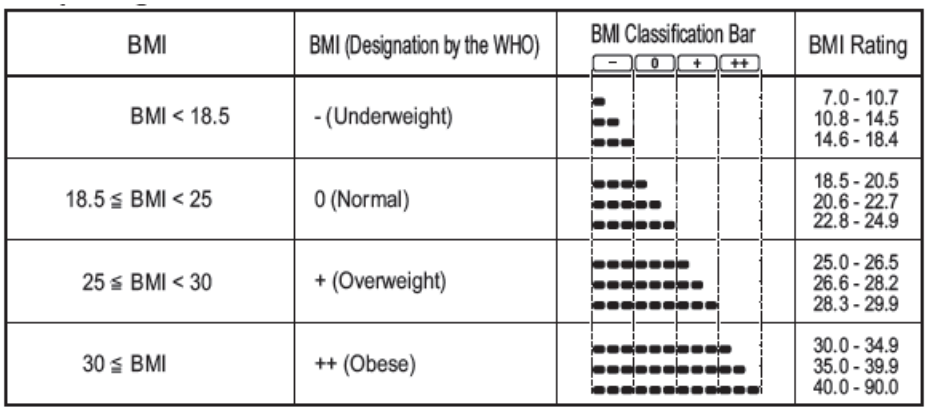

Figure 4.5. Interpreting the BMI result

Body fat percentage refers to the amount of body FM in regards to the total body weight expressed as a percentage.

Body fat percentage (%) = {Body FM (kg)/Body weight (kg)} x 100

The device uses bioelectrical impedance method to estimate volunteers body fat percentage that can be classified in four levels according to Omron Healthcare (Table 4.3). The value measured by the scale may significantly differ from the actual body fat percentage in body builders or highly trained athletes, and these differences may be related to changing ratios of body fluid and/or body composition.

Table 4.3. Interpreting body fat percentage result (Gallagher *et al*., 2000)

Gender	*Age*	*- (Low)*	*0 (Normal)*	*+ (High)*	*++ (Very High)*
Female	18-39	< 21.0%	21.0 – 32.9%	33.0 – 38.9%	≥ 39.0%
Male	18-39	< 8.0%	8.0 – 19.9%	20.0 – 24.9%	≥ 25.0%

Depending on where fat is distributed in the body, it is classified as visceral fat (VF, surrounding internal organs) or subcutaneous fat (below the skin). Based on the value measured by the scale, according to Omron Healthcare, VF level can be classified as normal, high or very high (fig. 4.6).

Visceral Fat Level	Level Classification
1 - 9	0 (Normal)
10 - 14	+ (High)
15 - 30	++ (Very High)

Figure 4.6. Interpreting the visceral fat level result

Lastly, according to Omron Healthcare, MM percentage measured by BIA can be classified in four levels represented in Table 4.4.

Table 4.4. Interpreting the skeletal muscle percentage result

Gender	*Age*	*- (Low)*	*0 (Normal)*	*+ (High)*	*++ (Very High)*
Female	18-39	< 24.3%	24.3 – 30.3%	30.4 – 35.3%	≥ 35.4%
Male	18-39	< 33.3%	33.3 – 39.3%	39.4 – 44.0%	≥ 44.1%

Height was measured with the SECA 217 portable stadiometer (fig. 4.7), with the measure approximated to the nearest 0.1cm (Lohman *et al*., 1988).

Figure 4.7. Portable stadiometer Seca 217

Waist and hips circumferences were measured with the anthropometric tape measure Anthroflex (fig. 4.8).

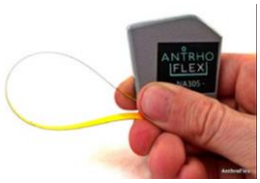

Figure 4.8. Anthropometric tape measure Anthroflex NA035. 6mm x 2m

The volunteer wore only underwear in order to facilitate the positioning of the tape measure. The subject was in an upright position, the abdomen was relaxed, the arms hanged at the side of the body and the feet were united. The inelastic meter was placed at the level of the waist, the narrowest part of the abdomen (fig. 4.9a), making sure the tape measure was in the horizontal plane. The measurement, approximated to the nearest 0.1 cm, was carried out at the end of a normal exhalation, without compress the skin with the measuring tape. The same procedure has been implemented to measure the maximum circumference of buttocks (or hips circumference) (fig. 4.9b) (Lohman *et al*., 1988).

(a) **(b)**

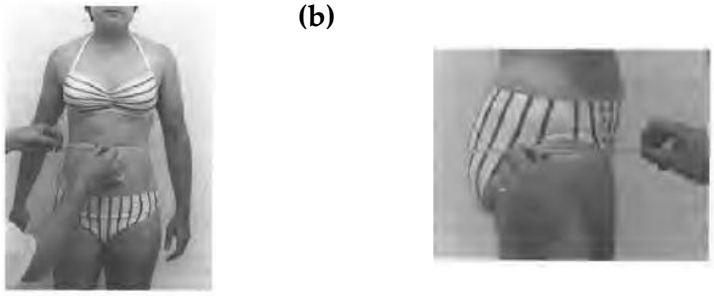

Figure 4.9. Measurement of waist (a) and hips (b) circumferences (Lohman *et al*., 1988).

The waist-to-hips ratio (WHR) has been evaluated since it is very useful to describe the adipose tissue distribution and its related risks. It should be less than 0.90 for men and 0.85 for women (CREA, 2018).

In addition to BIA, participants underwent plicometry, a simple and non-invasive body fat assessment technique that allows to define the topography of the subcutaneous fat. Body folds' figure as important variables of numerous anthropometric equations for the prediction of body composition (Durnin & Womerseley, 1974). Skinfold thickness was measured to the nearest 0.1 cm by the Harpenden caliper (fig. 4.10) at biceps, triceps, subscapular and supra-iliac areas

on the right side of the body with the subject standing in a relaxed condition (Lohman *et al*., 1988).

Figure 4.10. Harpenden caliper for body fat assessment

On a vertical line drawn between the anterior edge of the acromion and the centre of the antecubital fossa the midpoint was marked (fig. 4.11a). The bicipital fold was raised on the anterior surface of the arm at the protuberance of the biceps muscle (fig. 4.11b), 1 cm above the line marked. The subject was in an upright position, his arms relaxed at the hips and the palms of the hands faced forward (Lohman *et al*., 1988).

(a)  **(b)** 

Figure 4.11. Biceps skinfold. Localization of the measurement site of the skinfold **(a)** and its measurement **(b)** (Lohman *et al*., 1988).

The triceps skinfold was measured on the posterior surface of the arm, above the triceps muscle, in correspondence to the previous marked midpoint of the drawn line between the acromial process of the scapula and the lower margin of the olecranon of the ulna (fig. 4.12) (Lohman *et al*., 1988).

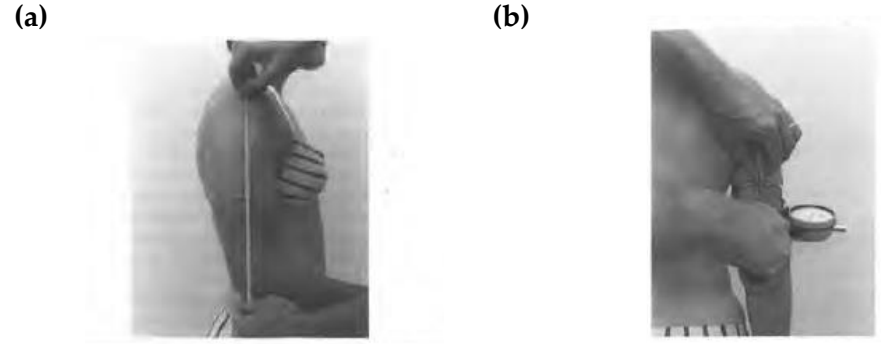

Figure 4.12. Triceps skinfold. Localization of the measurement site of the skinfold **(a)** and its measurement **(b)** (Lohman *et al*., 1988).

The subscapular skinfold was raised on the Langer lines according to a diagonal, with an infero-lateral inclination and forming an angle of about 45° with the horizontal plane. The measurement site is barely located below the lower corner of the shoulder blade (fig. 4.13a). The ends of the gauge were applied 1 cm inferior-laterally to the thumb and to the finger that lifted the skinfold (fig. 4.13b) (Lohman *et al*., 1988).

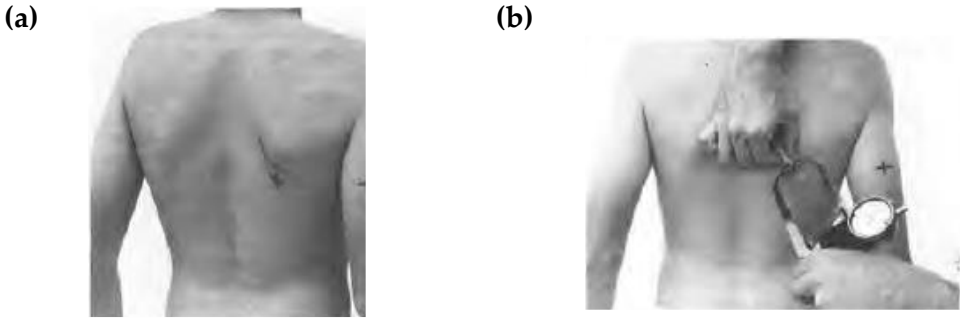

Figure 4.13. Subscapular skinfold. Localization of the measurement site of the skinfold **(a)** and its measurement **(b)** (Lohman *et al*., 1988).

The supra-iliac skinfold was measured on the mid-axillary line just above the iliac crest, as an oblique skinfold raised just posterior to the mid-axillary line, taking care to follow Langer lines. It is inclined infero-medially by 45° with respect to the horizontal plane (fig. 4.14a). The measurement was carried out 1 cm below the lifting point (fig. 4.14b) (Lohman *et al*., 1988).

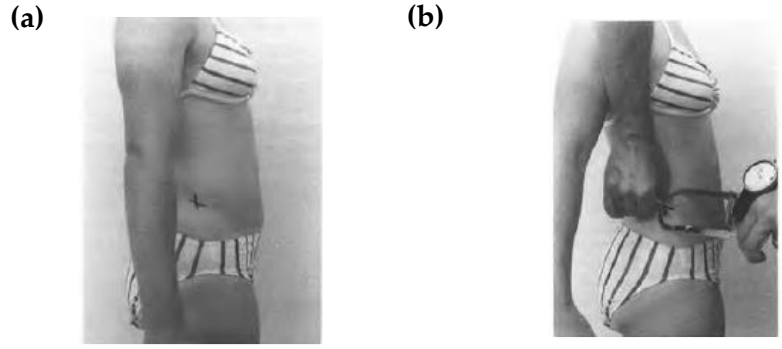

Figure 4.14. Supra-iliac skinfold. Localization of the measurement site of the skinfold **(a)** and its measurement **(b)** (Lohman *et al*., 1988).

Then body fat percentage was calculated from body density using the following linear regression equations:

$$\textbf{\% fat} = [(4.95/\text{density})-4.50)] \times 100$$

$$\textbf{density} = c - m \times \log \text{skinfold}$$

The regression coefficients *c* and *m* vary according to gender, age group and skinfold thickness taken into consideration (Table 4.5) (Durnin & Womersley, 1974). In this study values of *c* and *m* corresponding to all four skinfolds have been taken into consideration.

Table 4.5. Regression coefficients values for the estimation of body density

		Male			*Female*		
Age		**17-19**	**20-29**	**30-39**	**16-19**	**20-29**	**30-39**
All four skinfolds	**c**	1.1620	1.1631	1.1422	1.1549	1.1599	1.1423
	m	0.0630	0.0632	0.0544	0.0678	0.0717	0.0632

(Durnin & Womersley, 1974)

4.4 BIOCHEMICAL ANALYSIS

4.4.1 Capillary blood sampling

Clinical parameters of metabolic status were assessed from capillary blood samples. In particular, total cholesterol (TC), triglycerides (TG) and glucose (Glu)

blood levels have been evaluated with the MultiCare In System (fig. 4.15a). The MultiCare In meter uses a data-chip that is inside the strips box, which is necessary to change before using a new strips box or when another analyte has to be detected. To perform a measurement with MultiCare In System, it was necessary to withdraw a drop of capillary blood using a lancing device (fig. 4.15b) that had six different penetration depths, allowing to adjust the penetration depth to the texture of the volunteer skin.

Figure 4.15. Kit for the measurement of glucose, cholesterol and triglycerides levels. (a) MultiCare In kit**; (b)** Lancing device and sterile lancets.

MultiCare In has two different technologies: amperometric, with Glu electrodes strips, and reflectometric, with TC and TG strips. To determine Glu concentrations, a volume of at least 0.5 µl of only capillary blood is needed to use. Regarding TC and TG analysis, participants had to fast for at least 9 hours and 12 hours, respectively, and a volume of at least 10 µl of blood is required. In table 4.6 are reported the possible results displayed for Glu, TC and TG concentrations.

Table 4.6. Results displayed for Glu, TC and TG concentrations

Results	*Glucose*	*Cholesterol*	*Triglycerides*
*LO**	< 10 mg/dL (0.6 mmol/L)	< 130 mg/dL (3.3 mmol/L)	< 50 mg/dL (0.5 mmol/L)
Numerical result	10 – 600 mg/dL (33.3 mmol/L)	130 – 400 mg/dL (10.2 mmol/L)	50 – 500 mg/dL (5.6 mmol/L)
*HI***	> 600 mg/dL	> 400 mg/dL	> 500 mg/dL

Abbreviations: LO, low; HI, high. *Repeat the test; **Such results mean that the analyte concentration in the sample is very elevated and it indicates a serious risk.

To evaluate blood ketones (Ket) concentration the Glucomen® areo β-Keton Sensor kit (fig. 4.16) has been used. "HI" appears on the system meter if the test result is more than 8.0 mmol/L, whereas "0.0" appears if the result is less than 0.1 mmol/L. A volume of at least 0.8 µl of blood is required to detect Ket value.

Figure 4.16. Kit for the measurement of ketones concentration

4.4.2 Food consumption analysis

In order to correlate lifestyle, anthropometric and biochemical parameters with dietary habits, food consumption was evaluated using a 7-day food diary. Participants were asked to write day by day quality and quantity of food consumed and to report how they felt before and after eating. A short training was provided giving instructions in order to fill in the diary correctly. Furthermore, a photographic food atlas (fig. 4.17) was provided to subjects, which contained images of common dishes, so as to compare visually their dish at the time of evaluating the portion consumed. In this way, if the subject was unable to provide the exact weight, it would have been possible to estimate the quantity of food by means of the standardized portions.

Figure 4.17. Photographic atlas of food portions Scotti Bassani

Data collected by food diary made it possible to calculate food grams per day, total energy (kcal/day), and macronutrients (total grams of proteins, lipids, carbohydrates and alcohol, and respective energy contribution percentage) through Food Composition tables of CREA-AN.

4.5 STATISTICS

A descriptive statistical analysis (averages, standard deviations, percentages) has been first performed for all variables measured. Normal distribution of variables has been checked by Shapiro-Wilk test. Categorical variables have been expressed as percentages, while continuous variables as means with standard deviation (SD) or medians with interquartile interval. Pearson and Spearman correlations were used to evaluate relationships between variables. Bivariate analysis was conducted using unpaired t-test and Mann-Whitney test, for continuous variables, and chi-square test and Fisher's exact test, for categorical variables. Univariate analysis has been performed by Wilcoxon signed-rank test. Furthermore, results were analysed by analysis of variance (ANOVA, Shapiro–Wilk test passed), or by Kruskal-Wallis one-way analysis of variance on ranks (Shapiro-Wilk test failed). The significance of the differences between females and males within the same country, and those between the different countries within the same gender, were evaluated using the Student-Newman-Keuls method (Shapiro–Wilk test passed) or the Dunn's method (Shapiro-Wilk Test failed).

Statistical analysis has been performed using the Graph Pad software (GraphPad Prism 8 XML ProjectT, La Jolla, CA, USA), and variables with a p value <0.05 were considered statistically significant.

V - RESULTS

V - RESULTS

5.1 MULTI-CENTRE CROSS-SECTIONAL OBSERVATIONAL STUDY OF DIETARY HABITS AND LIFESTYLE: PARTICIPANTS

5.1.1 Distribution of participants

The sample, 65.8% females (F) and 34.2% males (M), comprised 194 participants (108 Italian and 86 Spanish students) with mean age 25.0 (± 3.4) years. A distribution of the sample for gender and age classes within the two groups is reported in figure 5.1. Most of the sample (53.1%) was aged between 24 and 29 years, with 62.8% of M (26.9% of Italians and 28.2% of Spaniards) and 45.5% of F (29.6% of Italians and 19.7% of Spaniards). It emerged no significant differences between the two groups according to gender and age.

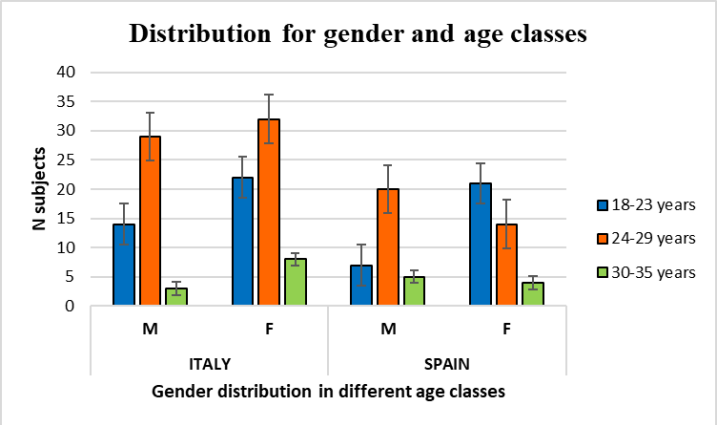

Figure 5.1. Distribution of the Italian and Spanish sample for gender and age classes. M: males; F: females; N: number. Chi-square test: not significant.

Participants were university, doctoral and postdoctoral, specialization and master students from both scientific (81.2% of Italians and 72.5% of Spaniards) and humanistic (18.8% of Italians and 27.5% of Spaniards) areas. In figure 5.2 is represented the distribution of the subjects in the two groups on the basis of their study area. Pharmacy, physiotherapy, and medicine were the most represented faculties within the sample with 26.6%, 12.4%, and 10.6%, respectively.

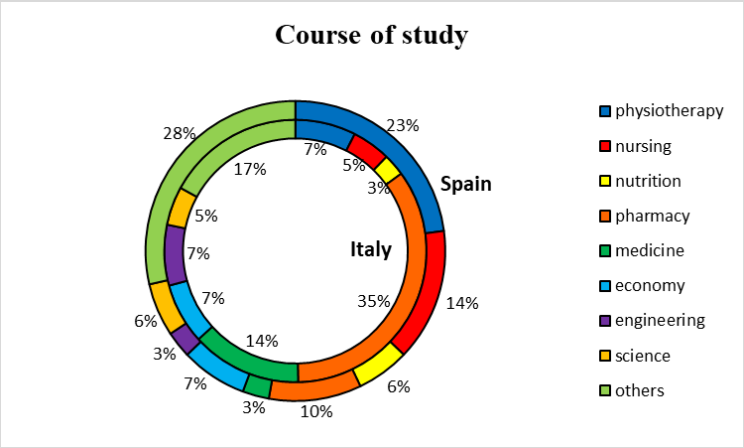

Figure 5.2. **Distribution of the sample on the basis of the course of study**. The category "others" includes students of Odontology, Criminology, Communication Sciences, Architecture, Culinary Arts, Veterinary, Law, Podiatry, Sports Science, Computer Science, Tourism, Physics, and Languages.

5.1.2 Physical condition assessment

The principal characteristics of the sample are reported in Table 5.1.

Table 5.1. Sample characteristics

	IT-M	IT-F	SP-M	SP-F
Sex (n,%)	46, 43.0	62, 57.0	35, 40.7	51, 59.3
Age	24.5 (23.0-7.3)	24.0 (23.0-27.3)	24.5 (24.0-26.0)	23.0 (21.0-28.0)
Height (m)	1.78 (± 0.08)††††	1.65 (± 0.06)	1.78 (± 0.07)††††	1.63 (± 0.06)
Weight (kg)	78.3 (± 12.2)††††	58.5 (± 10.1)	79.5 (± 13.8)††††	61.9 (± 10.3)
WC (cm)	84.3 (74.9–89.8)††††	67.0 (62.8–72.0)	81.4 (76.8-89.6)††	72.0 (65.6–83.6)
HC* (cm)	96.5 (90.0–101.4)††	92.0 (85.5– 96.5)	99.9 (92.8-103.5)	97.0 (89.3–100.4)

Categorical variable (gender) is expressed as percentage, and continuous variables as mean with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; WC: waist circumference; HC: hips circumference. *P <0.05 (Italians vs Spaniards); ††P <0.01; ††††P <0.0001 (Male vs Female within country).

Table 5.1. *(continued)*

	IT-M	IT-F	SP-M	SP-F
WHR	0.90 (0.80–0.90)††††	0.70 (0.70–0.80)	0.80 (0.80–0.90)††	0.80 (0.70–0.80)
WHtR	0.46 (0.42–0.50)	0.41 (0.37–0.44)	0.46 (0.42–0.48)	0.44 (0.40–0.51)
Pressure (mmHg)				
Systolic	120.0 (115.8–120.0)††	111.5 (103.0–120.0)	120.0 (116.0–125.0)†††	110.0 (101.3–120.0)
Diastolic	80.0 (70.0–80.0)†	70.0 (65.8–80.0)	75.0 (70.0–80.0)†	62.0 (60.0-70.0)
BMI (kg/m^2)				
Underweight	-	17.7 (± 0.4)	16.3 (± 0.0)	17.6 (± 1.2)
Normal weight*	22.5 (± 1.5)†††	20.9 (± 1.6)	23.2 (± 1.4)	22.2 (± 2.0)$^{\#\#}$
Overweight	27.2 (± 1.5)	27.1 (± 1.6)	27.9 (± 2.4)	27.1 (± 1.1)
Obese	32.5 (± 2.2)	33.9 (± 4.6)	-	32.7 (± 0.0)
%FM				
Plicometry	22.2 (± 20.9)††	24.8 (± 3.7)	14.2 (± 3.6)††††	25.6 (± 3.7)
Impedentiometry	21.0 (± 7.9)††††	29.1 (± 4.0)	20.7 (± 8.4)†††	30.9 (± 9.1)
%MM***	39.8 (37.0-42.7)††††	29.3 (28.5–30.2)	32.9 (21.3–41.5)††	27.3 (23.6–29.8)
Visceral fat (kg)	7.3 (± 3.6)††††	3.5 (± 0.9)	6.7 (± 3.9)††	3.7 (± 1.8)

Continuous variables are expressed as mean with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; WHR: waist-to-hips ratio; WHtR: waist-to-height ratio; BMI: body mass index; FM: fat mass; MM: muscle mass. *P <0.05; ***P=0.001 (Italians vs Spaniards); †P <0.05; ††P <0.01; †††P <0.001; ††††P <0.0001 (Male vs Female within country); $^{\#\#}$P <0.01 (Italian vs Spanish within gender).

Significant differences between countries were observed in hips circumference, with higher values in Spaniards.

As regards waist-to-hips ratio (WHR), it is very useful to describe the adipose tissue distribution and its related risks, and it should be less than 0.90 for men and 0.85 for women. It has emerged that 24.5% of Italians (IT, 16.1% of F and 36.4% of M, P=0.0223) and 21.2% of Spaniards (SP, 22.2% of F and 20.0% of M) showed a

WHR value higher than the 0.9 cut-off, linked to high cardiovascular risk. Regarding BMI, it was significantly higher in Spanish students within the normal range (P=0.0189). However, not significant differences have emerged in the frequency of population underweight (UW, 7.4% of IT and 8.2% of SP) and obese (OB, 7 IT and only one SP) subjects, whereas a similar prevalence of overweight (OW, 15.7% of IT and 16.3% of SP) was observed. Differences between fat mass (FM) values measured by plicometry and impedentiometry were statistically significant (P <0.0001), as well as their correlation (Spearman correlation: P <0.0001, r: 0.83; 95%CI: 0.73–0.89).

In figure 5.3 is reported the distribution of subjects for the two groups according to their percentage of FM. In particular, among Italian subjects, 4.1% presented a low percentage, 63.3% showed a normal level, and 16.3% had a high and very high percentage. As regards Spanish population, 12.8% presented a low percentage, 51.3% showed a normal level, 18.0% had a high percentage, and 30.8% reported a very high level of FM.

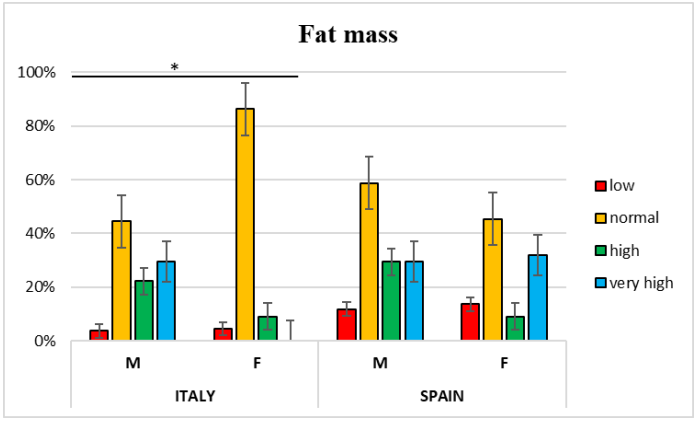

Figure 5.3. Fat mass (FM) level, according to impedentiometry, among Italian and Spanish university students. M: males; F: females. Low: %FM<8.0% in males and <21.0% in females; Normal: %FM between 8.0% and 19.9% in males, and between 21.0% and 32.9% in females; High: %FM from 20.0% to 24.9% in males, and from 33.0% to 38.9% in females; Very high: %FM≥25.0% in males and ≥39.0% in females. Chi-square test: *P value=0.0108 (Italian Males vs Females).

Among Italian students, those who studied at health sciences faculties (Medicine, Nutrition, Pharmacy, etc.) showed a higher percentage of FM than the other ones of humanistic faculties above all (25.9 ± 7.3 vs 19.7 ± 6.8). On the contrary,

Spanish students of humanistic areas reported a greater amount of FM than those of health sciences faculties (35.5 ± 9.6 vs 24.5 ± 9.6).

In both populations, tissue distribution was in accordance with sex differences, with male subjects showing lower content of total fat mass and higher content of visceral fat and muscle mass.

In figure 5.4 is reported the distribution of the subjects based on their amount of muscle mass (MM). Significant differences were observed between countries, with higher values reached for Italian students (Table 5.1). Regarding the Italian population, 8.2% of subjects (only men) showed a low percentage of MM, 53.1% had a normal percentage, 28.6% presented high values, and 10.2% reported a very high amount. Among Spanish subjects, 44.2% presented a low percentage of MM, 34.9% had a normal level, 20.9% showed a high percentage of MM, and 2.3%, represented by only men, reported a very high amount of MM. In accordance with what have emerged from the analysis of fat mass, within the Italian sample those who attended humanistic faculties reported a higher amount of MM (41.2, 37.4–42.8 vs 30.6, 29.1–38.1). Among Spanish students, those of humanistic area who have shown a higher percentage of FM, presented a greater amount of MM as well (30.7, 23.7–35.1 vs 29.4, 21.4–32.6).

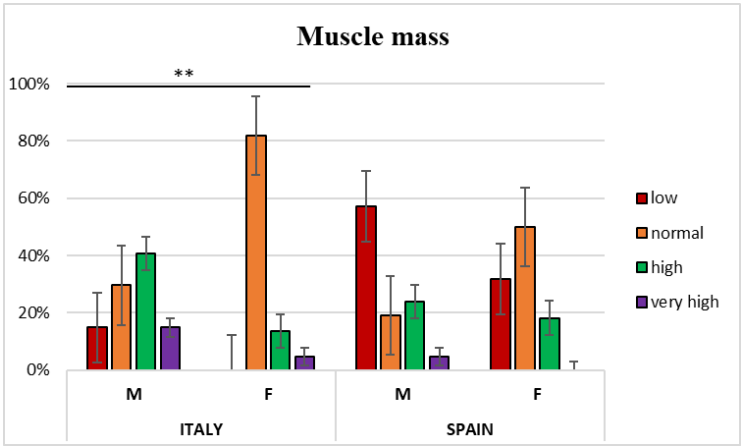

Figure 5.4. Distribution of the sample according to percentage of muscle mass (MM). M: males; F: females. Low: %MM<33.3% in males and <24.3% in females; Normal: %MM between 33.3% and 39.3% in males, and between 24.3% and 30.3% in females; High: %MM from 39.4% to 44.0% in males, and from 30.4% to 35.3% in females; Very high: %MM≥44.1% in males and ≥ 35.4% in females. Chi-square test: **P value = 0.0031 (Italian Males vs Females).

Both Italian and Spanish male subjects presented high amounts of visceral fat (VF) (fig. 5.5). As regards Italian subjects, 85.7% showed a normal amount of VF, and 10.2% and 4.1%, represented by only men, reported a high and very high level, respectively. Concerning the Spanish sample, 91.5% presented a normal amount of VF and 8.5%, all of them men, reported a high level, whereas, contrary to what has emerged on the Italian sample, no Spanish subject has showed a very high VF level.

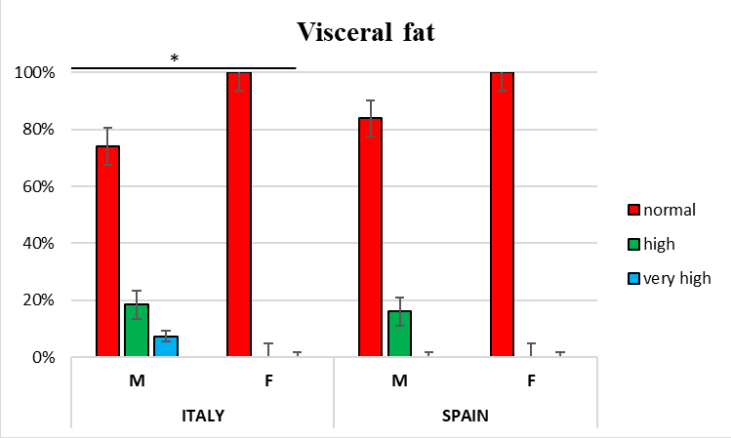

Figure 5.5. Visceral fat (VF) level among the Italian and Spanish sample. M: males; F: females. Normal: amount of VF between 1 kg and 9 kg; High: amount of VF from 10 kg to 14 kg; Very high: amount of VF between 15 kg and 30 kg. Ranges of values are the same for both males and females. Chi-square test: *P value = 0.0359 (Italian Males vs Females).

Within the Italian subjects those who attended humanistic faculties presented a slightly higher amount of VF than those of health sciences faculties (5.6 ± 2.6 vs 5.5 ± 3.5), whereas Spanish students of humanistic areas reported much greater differences (10.0, 6.5–11.5 vs 4.0, 2.0–6.0).

Concerning health condition, most of the sample (78.3% of IT and 88.1% of SP) declared to not suffer from pathologies, 4.7% of IT and 1.7% of SP reported to suffer from asthma, 4.7% of IT stated to be hypothyroid, 2.8% to have hereditary thrombophilia, and 1.9% to suffer from gastric reflux; one Spanish volunteer declared to suffer from Gilbert's syndrome. A volunteer from the group of Italy/Spain suffered from favism.

According to AQUA score, 48.4% of Italians (34.3% of M and 56.7% of F, P=0.0014) and 62.1% of Spaniards (46.2% of M and 75.0% of F, P=0.0490) showed an allergy. Among them, only 59.6% of Italians (66.7% of M and 57.1% of F) and 38.9% of Spaniards (58.3% of M and 29.2% of F) had allergic diseases diagnosed by a doctor, whereas the others, although had reported a significant score, did not perform a specific medical examination.

5.2 LIFESTYLE

5.2.1 Voluptuary habits

Among the *voluptuary habits*, the smoking one has been evaluated. More than half of the sample (66.0% of IT and 71.9% of SP) was non-smoker, of whom 11.2% (8.5% of IT and 6.3% of SP) were former smokers (fig. 5.6). In particular, 17.0% of Italian males (IT-M) and females (IT-F) were smokers, of whom 50.0% (22.2% M and 27.8% F) occasional smokers; 10.9% of Spanish males (SP-M) and 17.2% of Spanish females (SP-F) were smokers, of whom 55.6% (22.2% M and 33.3% F) occasional smokers. Furthermore, 8.5% of IT and 7.8% of SP reported to have used vape pens.

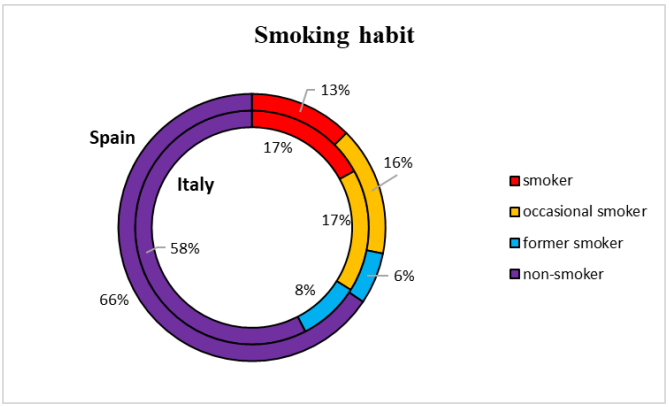

Figure 5.6. Percentage of smokers and non-smokers in the Italian and Spanish sample.

Among smoking subjects, half of the Italians stated to smoke between one and 5 cigarettes per day, 10.5% less than one cigarette and between 11 and 15 cigarettes per day, 23.7% between 6 and 10 cigarettes per day, and 5.3% 20 cigarettes per day. One female subject claimed to smoke only in company. On the contrary, 35.3% of Spanish smokers reported to smoke less than one cigarette per day, 47.1% between one and 5 cigarettes per day, 11.8% between 6 and 10 cigarettes per day, and only one subject stated to smoke 30 cigarettes per day. One male subject claimed to smoke only on weekends. Differences between the two groups and sexes were not significant.

5.2.2 Consumption of alcohol and caffeinated beverages

Frequencies of coffee, tea, energy drink and alcohol consumption have been evaluated. As far as coffee consumption is concerned, 18.1% of the sample (11.7% of IT and 25.0% of SP) stated not to drink coffee, 37.6% (36.4% of IT and 38.9% of SP) reported to consume a cup of coffee per day, 26.9% (32.5% of IT and 20.8% of SP) drank 2 cups per day, while 17.5% (19.5% of IT and 15.3% of SP) 3 or more cups of coffee per day. Coffee consumption per day is reported in figure 5.7.

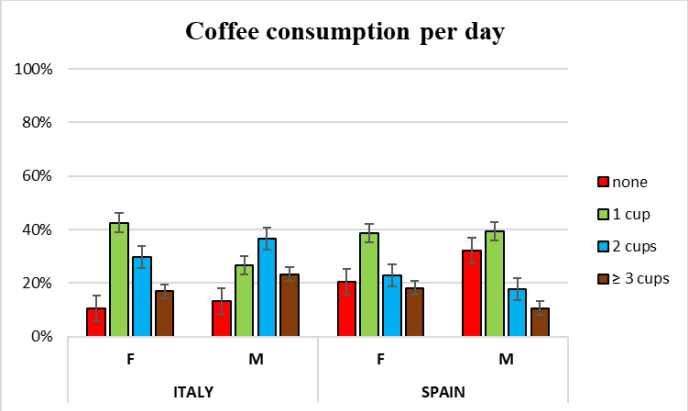

Figure 5.7. Coffee intake among Italian and Spanish subjects. F: females; M: males. Chi-square test not significant.

Regarding tea consumption (fig. 5.8), most of the sample (45.1%, 43.0% of IT and 48.4% of SP) stated not to drink tea, 3.1% (5.3% of IT and 6.3% of SP) reported to drink less than one cup per week, whereas 14.2 % (26.3% IT and 25.0% of SP) consumed at least one cup of tea per day. Only 0.6% of the sample, represented by only F, reported to consume 2 or more cups of tea per day, respectively. No significant differences were observed in the percentages of distribution.

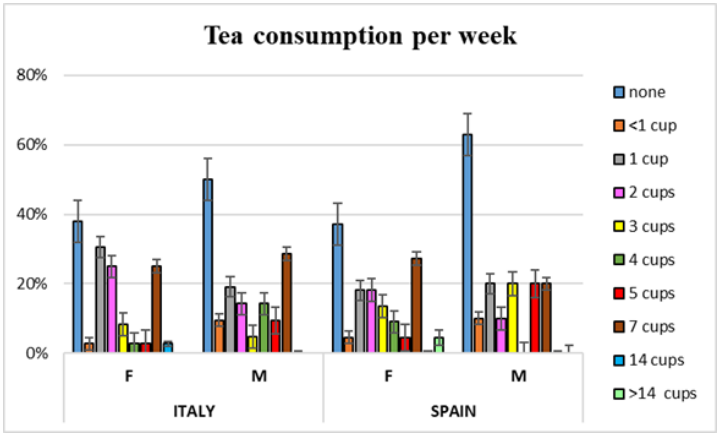

Figure 5.8. Tea consumption among Italian and Spanish students. F: females; M: males. Chi-square test not significant.

As regards energy drinks, 26.4% of IT and 42.9% of SP have declared to use it (P=0.0410). In particular, among Italian consumers 85.7% (90.0% of F and 83.3% of M) have reported to use them occasionally in the last 2 years, whereas 14.3% (10.0% of F and 16.7% of M) regularly. Concerning Spain, all the consumers stated to consume energy drinks occasionally in the last two years. In figure 5.9 the consumption frequency of energy drinks during the last year is reported. In particular, among the consumers, 71.4% of IT and 74.1% of SP declared to have used them 1-7 days during the last year, 21.4% of IT and 22.2% of SP have consumed them 8-30 days, 3.6% of IT have taken them for more than 30 days or daily, whereas 3.7% of SP have used energy drinks for more than 30 days during the last year, but no Spaniard have used them daily.

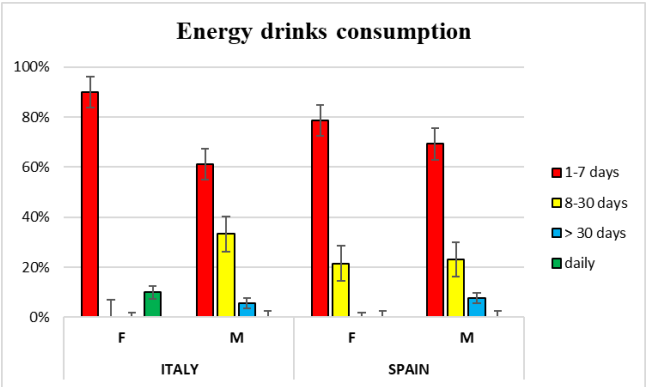

Figure 5.9. Frequency of energy drink consumption during the last 12 months. F: females; M: males. Chi-square test not significant.

Among the situations in which these drinks are used, subjects have reported to consume them mainly during study/exams periods (56.0% of IT and 48.0% of SP) and physical activity performance (76.0% of IT and 24.0% of SP). Moreover, 12.0% of SP have used energy drinks for recovery after sport. As regards the reason for use, 57.1% of IT and 48.0% of SP have stated to have used them to improve physical performance, 11.4% of IT and 8.0% of SP to improve the state of health/well-being, 11.4% and 2.9% of IT to stay awake and improve their cognitive functions, respectively. Among Spaniards, 4.0% and 8.0% have declared to use them to delay aging and as an alternative to using a drug, respectively.

Concerning positive effects, 48.0% and 44.0% of the Italian sample stated to have benefited enough or a little from the use of energy drinks, respectively, whereas most of the Spanish sample (40.7%) reported not to have benefited from their use, with 29.6% and 25.9% of the subjects who declared a sufficient or a little benefit, respectively. Both IT (88.9%) and SP (77.4%) have reported not to have experienced any side effects. Most of the IT (72.0%) was not directed by anyone to the use of energy drinks, 8.0% stated to have induced to use them by friends or relatives and advertising, respectively, whereas 4.0% by a pharmacist, a doctor or information programs or articles (radio, TV, press), respectively. As regards Spaniards, 69.6% have used energy drinks without being induced by others, 39.1% followed the advice of friends or relatives, 8.3% was induced by advertising, whereas 4.3% by a pharmacist and information programs, respectively.

Regarding alcohol intake, 86.8% of IT (35.9% of M and 50.9% of F) and 74.6% of SP (31.8% of M and 42.9% of F) stated to consume alcoholic beverages. Type and quantity of alcohol consumed on a weekday and Saturday night did not differ significantly (Table 5.2). Pattern of consumption during the week was mainly beer for both groups (60.9% IT, 78.7% SP), followed by wine (64.1% IT, 48.9% SP). It was followed by consumption of hard liquors (23.9% IT, 40.4% SP) and cocktails (31.5% IT, 31.9% SP).

Concerning alcohol consumption on Saturday night, 63.0% of Italian students consumed beer, 62.0% wine, 32.6% hard liquors, and 41.3% cocktails. On the contrary, 72.3% of Spanish students stated to consume beer, 34.0% wine, 42.6% hard liquors, and 44.7% cocktails.

Table 5.2. Percentages of drinkers for different alcoholic beverages.

Weekday	**IT-M** (N=38)	**IT-F** (N=54)	**SP-M** (N=20)	**SP-F** (N=27)
Beer (bottle 33 cl)	%	%	%	%
1, 2 portions	63.2, 5.3	51.9, 3.7	60.0, 5.0	48.2, 18.5
3, 4 portions	-	-	5.0, 5.0	11.1, -
8 portions	-	-	-	3.7
Total	68.5	55.6	75.0	81.5
Wine (glass 125 ml)	%	%	%	%
1, 2 portions	47.4, 7.9	57.4, 11.1	40.0, 5.0	40.7, 7.4
3, 4 portions	2.6, -	-	-	3.7, -
Total	57.9	68.5	45.0	51.8
Hard liquor (glass 40 ml)	%	%	%	%
1, 2 portions	21.1, 2.6	24.1, -	30.0, 5.0	22.2, 7.4
3, 4 portions	-	-	5.0, -	7.4, 3.7
Total	23.7	24.1	40.0	40.7
Cocktail (glass 40 ml)	%	%	%	%
1, 2 portions	21.1, 2.6	35.2, 1.9	20.0, 5.0	14.8, 11.1
8 portions	-	-	-	7.4, 3.7
T**otal**	23.7	37.1	25.0	37.0
Saturday night				
Beer (bottle 33 cl)	%	%	%	%
1, 2 portions	57.9, 18.4	35.2, 13.0	25.0, 10.0	14.8, 29.6
3, 4 portions	5.3, -	1.9, -	20.0, 5.0	25.9, 3.7
8 portions	-	-	5.0	3.7
Total	81.6	50.1	65.0	77.7
Wine (glass 125 ml)	%	%	%	%
1, 2 portions	29.0, 21.1	44.4, 14.8	30.0, 5.0	22.2, 3.7
3, 4 portions	5.3, 2.6	3.7, 1.9	-, -	7.4, -
Total	58.0	64.8	35	33.3

IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; N: number; %: percentage. Chi-square test: not significant.

Table 5.2. (*continued*)

Weekday	**IT-M** (N=38)	**IT-F** (N=54)	**SP-M** (N=20)	**SP-F** (N=27)
Hard liquor (glass 40 ml)	%	%	%	%
1, 2 portions	36.8, 7.9	24.1, -	15.0, 20.0	7.4, 11.1
3, 4 portions	-	-	15.0, 5.0	7.4, 7.4
Total	44.7	24.1	55.0	33.3
Cocktail (glass 40 ml)	%	%	%	%
1, 2 portions	29.0, 10.5	35.2, 7.4	25.0, 15.0	-, 22.2
3, 4 portions	-	-	5.0, -	11.1, 3.7
8, >8 portions	-	-	-	3.7, 3.7
Total	39.5	42.6	45.0	44.4

IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; N: number; %: percentage. Chi-square test: not significant.

On the other hand, when we stratified alcohol consumption, classifying it according to the place of residence of the university students, differences between Italian and Spanish resident students in the number of hard liquors and cocktails consumed, irrespective of the weekday or Saturday night pattern, were statistically significant (Table 5.3), as well as beer consumption on Saturday night.

Table 5.3. Alcohol consumption in resident and non-resident students

Beverage portions (N)	**Italians**		**Spaniards**	
Weekday	**Resident students** (N = 80)	**Non-resident students** (N = 12)	**Resident students** (N = 31)	**Non-resident students** (N = 16)
Beer (bottle 33 cl)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.5)	1.0 (1.0–2.8)
Wine (glass 125 ml)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.5)
Hard liquor (glass 40 ml)	1.0 (1.0–1.0)##	-	1.0 (1.0–3.0)	1.0 (1.0–2.0)
Cocktail (glass 40 ml)	1.0 (1.0–1.0)##	-	1.0 (1.0–3.5)	1.0 (1.0–2.0)
Saturday night				
Beer (bottle 33 cl)	1.0 (1.0–2.0)###	1.0 (1.0–1.0)	2.5 (1.3–3.0)	2.0 (1.0–3.0)
Wine (glass 125 ml)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.3)	1.0 (1.0–3.0)
Hard liquor (glass 40 ml)	1.0 (1.0–1.0)###	2.0 (2.0–2.0)	2.0 (2.0-3.0)	2.0 (1.0-3.0)
Cocktail (glass 40 ml)	1.0 (1.0–1.0)###	1.5 (1.0–2.0)	2.0 (1.0–3.0)	2.0 (1.0-2.3)

Beverage portions number (N) is expressed as median with interquartile range (Shapiro-Wilk Test failed). ##P <0.01; ###P <0.0001 (Italian vs Spanish resident students).

Despite the overall wine consumption was not significantly different among groups, non-resident Italian female students reported to consume only wine on Saturday night in quantity comparable to the resident female students (1.2 ± 0.4 and 1.5 ± 0.8, respectively); whereas, among SP-F the consumption of wine on weekends is higher in students who have moved away from home compared to the resident ones (wine: 1.8 ± 1.1 vs 1.2 ± 0.5). During a weekday, both female and male students consumed only wine and beer, in Italy, regardless their residence (around 1 portion), whereas SP-F who moved away from home tended to consume more wine than resident ones (1.4 ± 0.9 vs 1.2 ± 0.4), and male students consumed less wine (around 1 portion), regardless of residence.

5.2.3 Dietary habits, adherence to Mediterranean diet and dietary guidelines

As regards eating habits, most of the population studied (79.4%) stated to not follow any particular diet (86.8% of IT and 70.2% of SP), 4.6% stated to follow a lactose-free diet (5.7% of IT and 1.9% of SP), and 2.9% a gluten-free diet (2.8% of IT and 1.9% of SP). Among Italian students, there were subjects who followed a diet low in lipids and carbohydrates (0.9%), and a maintenance diet of 1700 kcal (0.9%); whereas, only among SP there were participants who went on a vegetarian (2.8%), vegan (2.8%), and protein (0.9%) diet. Consumption of meals out of home (fig. 5.10) and its frequency (fig. 5.11) has been evaluated. It emerged that not all IT and SP had breakfast in the morning, with most of the Italian sample (58.7%) who had it at bar, and 33.3% and 3.8% of Spanish subjects who had breakfast at bar and restaurant, respectively. While in Italy is not common to have dinner at bar, among Spanish subjects 16.7% (19.6% of F and 12.5% of M) reported to have it. Furthermore, a higher percentage of IT than SP went to the restaurant or pizzeria, whereas more SP were used to eat at fast food or street food.

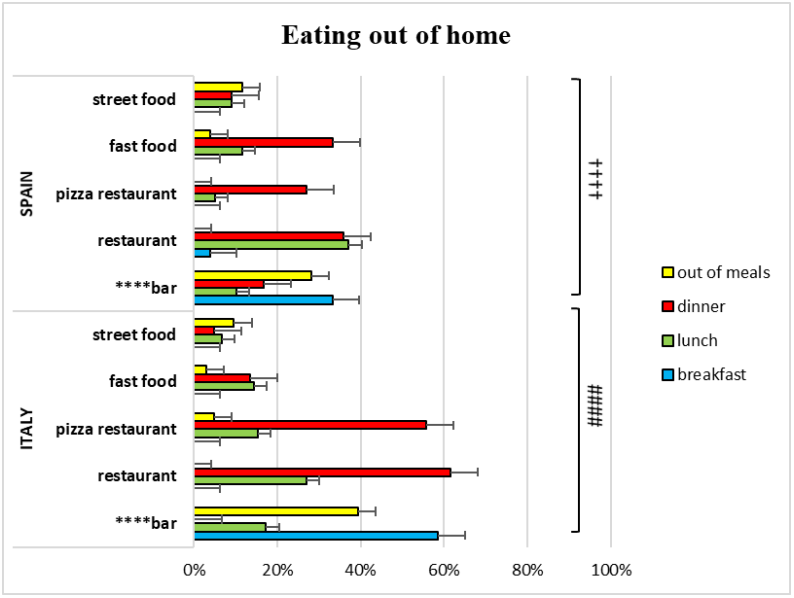

Figure 5.10. Percentages of students who consume meals out of home. ****P <0.0001 (Italians vs Spaniards); ††††P <0.0001; ####P <0.0001.

As regards the frequency of consumption of meals out of home (fig. 5.11), 34.0% of IT and 22.1% of SP were used to eat out of home rarely, whereas most of the Italian (55.0%) and Spanish (67.5%) students ate out once or twice a week; 5.0% of IT and 2.6% of SP consumed meals out of home 3 times a week, whereas a higher percentage of Spaniards (7.8%) than Italians (6.0%) ate out 4-5 times a week. Differences in the frequencies of meals consumption were significant (P <0.05).

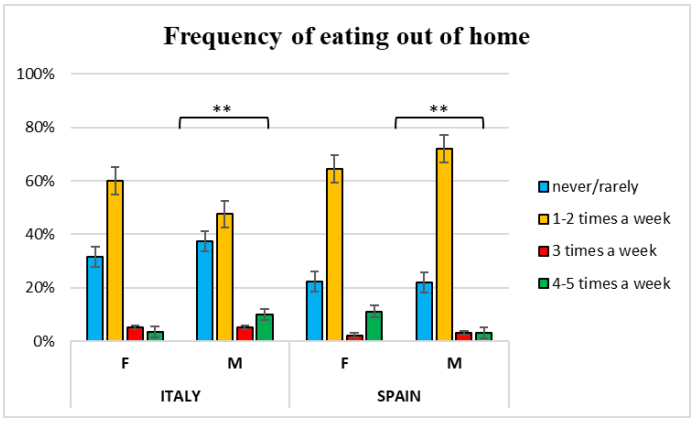

Figure 5.11. Frequencies of consumption of meals out of home. F: females; M: males. **P <0.01 (Italian vs Spanish males).

Figure 5.12 reports the average consumption of fruit, vegetable, red meat, fish and beans, grouped according to consumption of only wine or all alcoholic beverages. Differences between the two groups for Italian and Spanish subjects were not statistically significant.

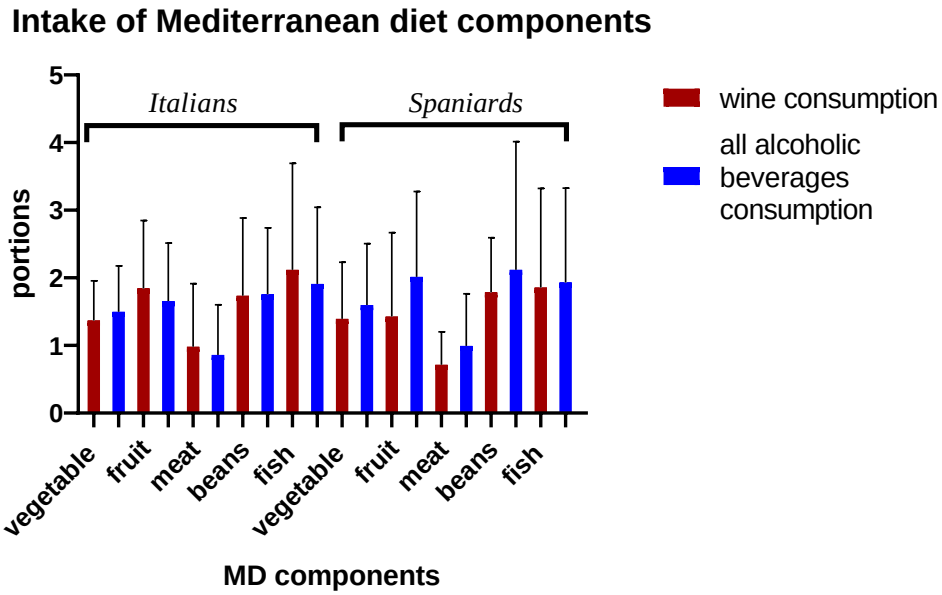

Figure 5.12. Consumption of the main components of Mediterranean diet (MD) among drinkers. For each category of food item, the average number of portions consumed by Italian and Spanish drinkers of only wine (red) or all alcoholic beverages (blue) are reported. Data are means with standard deviation.

Most of the students did not follow the MD pyramid recommendations for the consumption of vegetable (≥ 2/day), fruit (≥ 3/day), red meat (< 1/day), beans (≥ 3/week), and fish (≥ 3/week). In particular, 41.7% of the sample (39.3% of IT and 45.2% of SP) consumed two or more portions of vegetable per day, 23.3% (18.7% of IT and 30.1% of SP) consumed three or more portions of fruit per day, 35.6% (41.1% of IT and 27.4% of SP) consumed less than one portion of red meat per day, 25.6% (21.5% of IT and 31.5% of SP) consumed three or more portions of legumes per week, and 27.8% (26.2% of IT and 30.1% of SP) consumed three or more portions of fish per week. Differences between males and females, and Italian and Spanish subjects were not statistically significant; however, when data were stratified by place of residence, significant differences between IT and SP were observed within resident students (Table 5.4).

Table 5.4. Percentage of student who complied the recommended portion of Mediterranean diet components

	IT-M (N=45) %	**IT-F** (N=62) %	**SP-M** (N=25) %	**SP-F** (N=48) %
Vegetable	26.7	48.4	40.0	47.9
Fruit	22.2	16.1	36.0	27.1
Red meat	40.0	41.9	20.0	31.3
Beans	20.0	22.6	32.0	31.3
Fish	31.1	22.6	32.0	29.2
	Resident students (N = 80) %	**Non-resident students** (N = 12) %	**Resident students** (N = 31) %	**Non-resident students** (N = 16) %
Vegetable	39.0##	33.3	43.8	48.0
Fruit	19.0##	16.7	33.3	24.0
Red meat	42.1##	33.3	22.9	32.0
Beans	12.6##	8.3	39.6	36.0
Fish	21.1##	25.0	47.9	36.0

IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; N: number; %: percentage. ##P <0.01 (Italian vs Spanish resident students).

Although less than half of the Italian students who stayed with their family consumed the recommended portions of MD, they were more adherent to MD than those who moved away from home.

Related to Food Neophobia (FN), we hypothesized that it should be higher in students with higher MD adherence. However, no significant relationship was found among FNS and the different MD scores. Comparing countries, we observed that FNS was higher in IT compared to SP (Table 5.7). It emerged that 5.8% of the Italian sample had a maximum predisposition to taste new foods, and 26.2% (9.7% M, 16.5% F) had greater reluctance to try new foods. As regards Spanish students, 6.5% (1.6% M, 4.8% of F) had food neophilia, and 14.5% (3.2% M, 11.3% F) have reported a score higher than the cut-off point of 28. Differences between FNS scores of IT- and SP-F were statistically significant (Table 5.5).

Table 5.5. Standardized questionnaires results

	IT-M (N=46)	IT-F (N=62)	SP-M (N=35)	SP-F (N=51)
FNS**	22.0 (15.0–27.0)	22.0 (19.0–29.5)$^{\#\#}$	19.5 (14.3–22.8)	19.0 (14.0–23.5)
MDS-14	6.0 (5.0–7.0)	7.0 (5.0–8.0)	7.0 (4.0–8.0)†	7.0 (6.0–8.0)
MED-55	32.7 ± 4.5$^{\#}$	34.5 ± 4.2†	31.4 ± 5.8†	33.6 ± 7.3
QueMD	13.0 ± 3.1	13.4 ± 2.7	13.3 ± 2.8	12.9 ± 2.8
aMED*	3.0 (2.0–5.0)	5.0 (3.0–6.0)†††	3.0 (2.0–4.0)†	4.0 (3.0–5.0)$^{\#\#}$
ntMED	6.0 (4.0–8.0)	6.0 (4.8–8.0)	7.0 (5.8–8.0)	6.0 (5.0–8.0)
AUDIT**	3.0 (2.0–5.0)	3.0 (2.0–4.0)$^{\#\#}$	3.5 (0.8–8.0)	5.0 (2.5–7.0)

IT-M: Italian males; IT-F: Italian females; SP-M: Spanish males; SP-F: Spanish females; N: number; FNS: Food Neophobia Scale; MDS-14: Mediterranean Diet Score; MED-55: Mediterranean Score; QueMD: Questionnaire to measure Mediterranean Diet; aMED: alternate Mediterranean diet score; ntMED: non-typical MD foods score; AUDIT: Alcohol Use Disorders Identification Test. Continuous variables are expressed as mean with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). *P <0.05; **P <0.01 (Italian vs Spaniards); †P <0.05; †††P <0.001 (Male vs Female within country); $^{\#}$P <0.05; $^{\#\#}$P <0.01 (Italians vs Spaniards within gender).

Concerning the questionnaire for the evaluation of risk of alcohol consumption, medians of AUDIT scores (Table 5.7) were below those indicating hazardous or harmful alcohol use (cut-off points of 8 and 6 for men and women, respectively). However, 15.5% of IT and 30.7% of SP showed a hazardous alcohol use (14.3% of M and 25.5% of F) (P <0.001). Scores were significantly higher for Spaniards.

In Figure 5.13 are shown the percentages of students with low, medium and high MD adherence, assessed with different questionnaires: MDS-14, MED-55 and aMED. Among Italian and Spanish subjects only 4.9% and 6.5%, respectively, showed high MD adherence with MDS-14. Regarding MED-55 scores, none of the IT- and SP-M showed a score between 45 and 55, which represented the maximum level of adherence to MD. However, difference in MED-55 scores between Italian and Spanish students was statistically significant (Table 5.5).

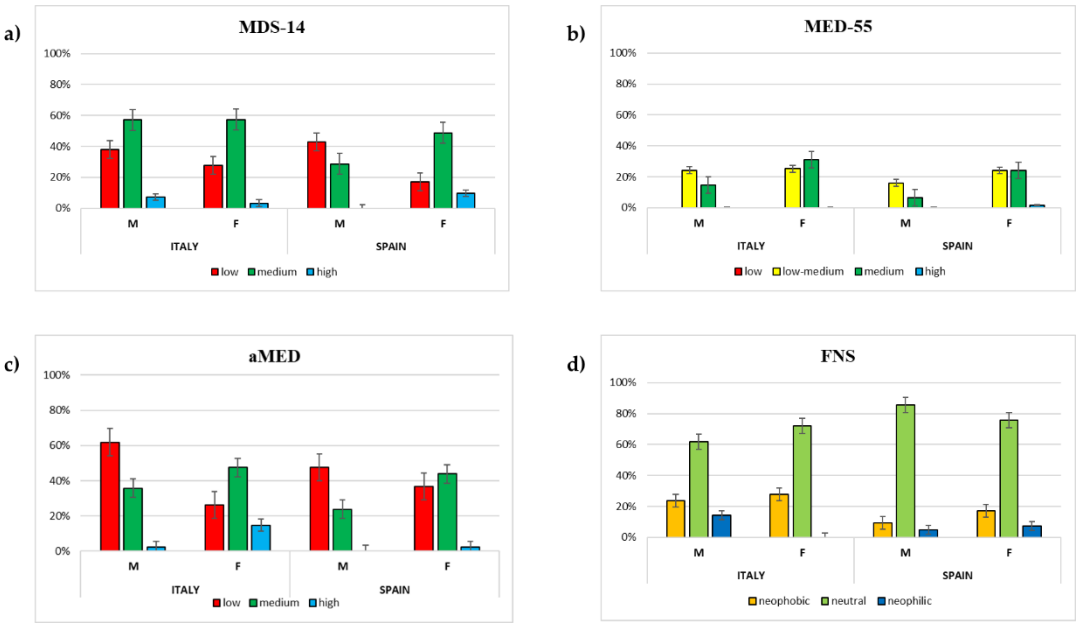

Figure 5.13. Distribution of the sample based on the adherence level to Mediterranean diet (MD) and the presence of food neophobia (FN). FNS: Food Neophobia Scale; MDS-14: Mediterranean Diet Score; MED-55: Mediterranean Score; aMED: alternate Mediterranean diet score. Percentages of Italians and Spaniards who presented a low, medium, and high adherence grade to MD according to the MDS-14 (a), MED-55 (b), and aMED (c) scores; d) Percentages of Italians and Spaniards who were neophobic, neutral and neophilic according to the FNS.

Regarding QueMD and ntMED, differences between Italian and Spanish students (both M and F) were not statistically significant. According to aMED scores, differences between Italian and Spanish subjects, and IT-M and IT-F were statistically significant (Table 5.5).

Comparing the results from the different questionnaires, linear regressions reported negative correlations both between AUDIT and QueMD and between AUDIT and ntMED in Spanish students (fig. 5.14).

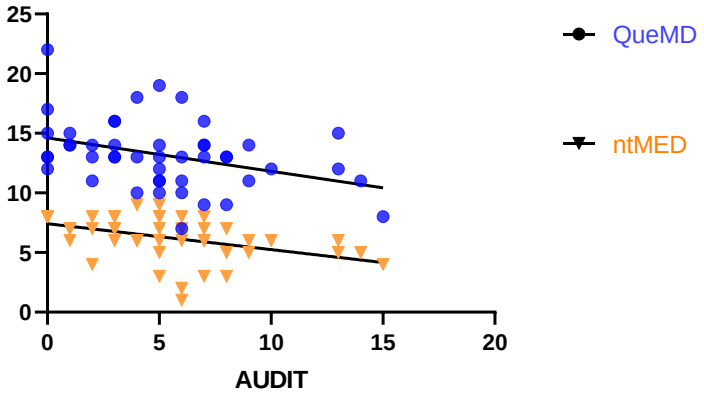

Figure 5.14. Linear regression between AUDIT score and adherence level to the Mediterranean diet according to QueMD questionnaire and the sub-score ntMED, in the Spanish sample. AUDIT: Alcohol Use Disorders Identification Test; QueMD: Questionnaire to measure Mediterranean Diet; ntMED: non-typical MD foods score. AUDIT vs QueMD: equation: y = -0.2789x + 14.612; R squared: 0.137; 95% CI: -0.489 to -0.069; P <0.05; AUDIT vs ntMED: equation: y = -0.2172x + 7.421; R squared: 0.201; 95% CI: -0.347 to -0.087; P <0.01.

The adherence to Italian and Spanish dietary guidelines has been evaluated and results are reported in figure 5.15. It has emerged that among Italian subjects there were satisfactory consumptions for cereals (pasta, bread), yogurt and cheese, beans, seasoning oil, and sweets, whereas adequate intakes among Spanish students have been reported for cereals, cheese, and fresh fruit. 95% of IT and 91.9% of SP consumed added saturated fat (e.g. butter). Moreover, only 31.7% of IT and 16.2% of SP drank wine with moderation. Among the excesses, the habit of drinking carbonated beverages was very marked, with 82.2% of IT and 82.4% of SP who consumed them, whereas 90.1% of IT and 87.8% of SP exceeded with sugar. If on one hand half of the sample showed an adequate intake of red meat, on the other hand 51.5% of IT and 62.2% of SP exceeded with the portions of white meat. Both Italian and Spanish participants did not have an adequate intake of fish, eggs, vegetables and dried fruit. Differences between Italian and Spanish volunteers were significant (P <0.0001).

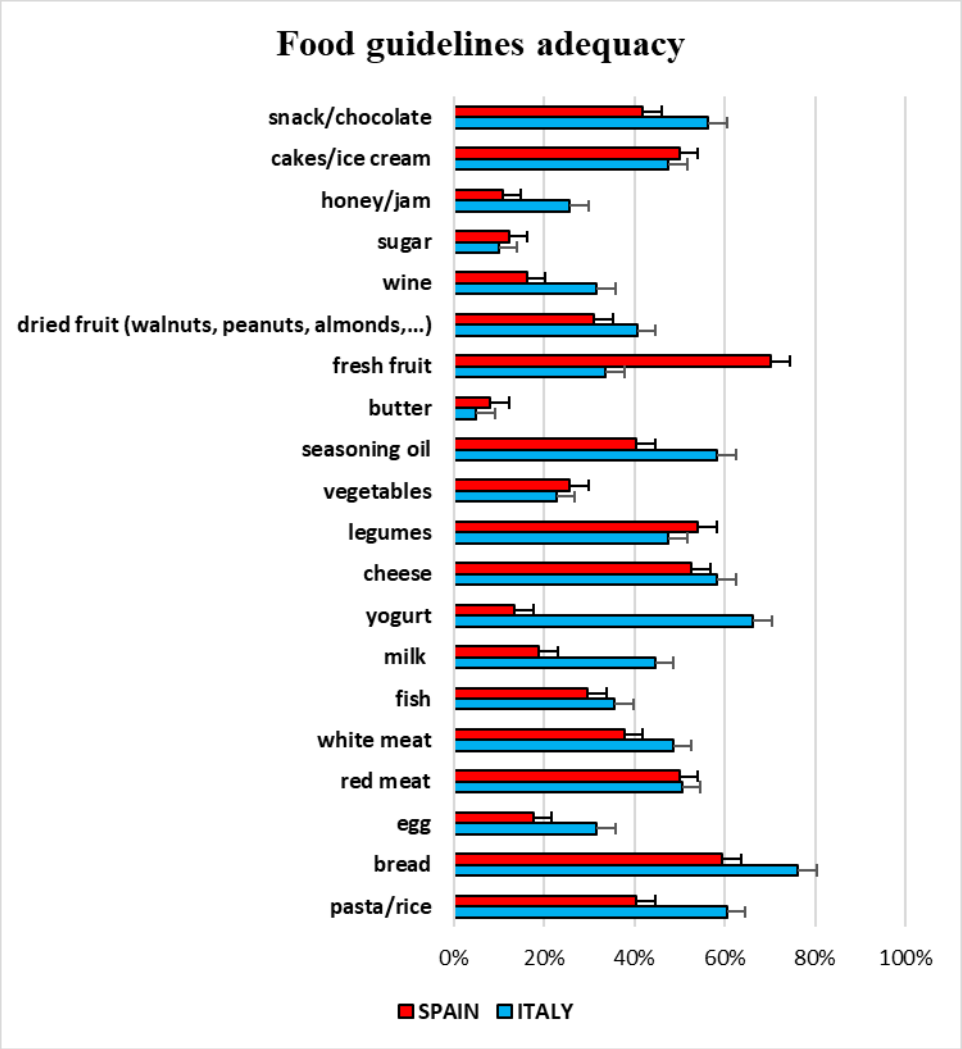

Figure 5.15. Percentages of Italians and Spaniards who adhere to Italian (LARNs) and Spanish (SENC) dietary guidelines. Recommended portions for Italian and Spanish population are reported in Appendix 12.

The nutritional profile of the subjects in terms of macronutrients was evaluated (Table 5.6) through processing and analysis of the 7-day food diaries drawn up by a subgroup of the sample (N=28). The average daily energy intake was significantly higher in IT than SP. As regards macronutrients, in particular proteins, it was evident that in the Spanish sample the quantity of proteins expressed as kg/body weight was greater. The general dietary recommendations for the adult and healthy population provide for a percentage of energy from proteins between 12 and 18% and, in any case, never more than double the minimum requirement, or

0.9 g/kg body weight (SINU, 2019a). The difference in protein intake is mainly due to foods of animal origin. The daily energy intake of lipids, expressed as a percentage, was suitable for the Italian group, in line with the indications from the SINU which recommends between 20 and 35% in adults (SINU, 2019b). The consumption of carbohydrates was adequate only in the Italian group. In fact, dietary guidelines recommend a carbohydrate intake corresponding to 50-60% of the total calories. Dietary fiber intake was low, in fact a consumption of at least 25 g per day is suggested in adult population, quantity not reached by the two groups.

Table 5.6. Average daily macronutrient intake

	IT (N = 22)	**SP** (N = 6)
Protein		
% energy	12.5 (10.0 – 15.5)	13.5 (11.8 – 17.3)
g/kg bw**	0.5 (0.0 – 1.0)	1.5 (1.0 – 2.3)
Animal (g)	43.5 (33.8 – 55.3)	46.0 (33.0 – 72.8)
Vegetal (g)	27.1 ± 10.36	26.0 ± 15.8
Lipids		
% energy**	33.0 (29.0 – 37.8)	50.5 (44.8 – 65.0)
Animal (g)	37.2 ± 12.4	34.5 ± 18.6
Vegetal (g)	34.0 (26.8 – 43.0)	115.0 (73.5 – 155.8)
Carbohydrates		
% energy**	49.7 ± 9.9	33.5 ± 12.4
Fiber (g)	16.0 ± 5.8	20.7 ± 5.6
Energy (Kcal)*	2156 (1828 – 2614)	3118 (2695 – 3333)

IT: Italians; SP: Spaniards; bw: body weight. Continuous variables are expressed as mean with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). *P <0.05; **P <0.01.

5.2.4 Physical activity and use of supplements

Concerning physical activity (PA, fig. 5.16), IT have shown to be more active than SP (P <0.05). However, IT-M spent more time sitting than IT-F, both during the week and on weekends, whereas among Spanish students, F were the most inactive. Differences between IT-F and SP-F in sedentary activity were significant (P <0.05).

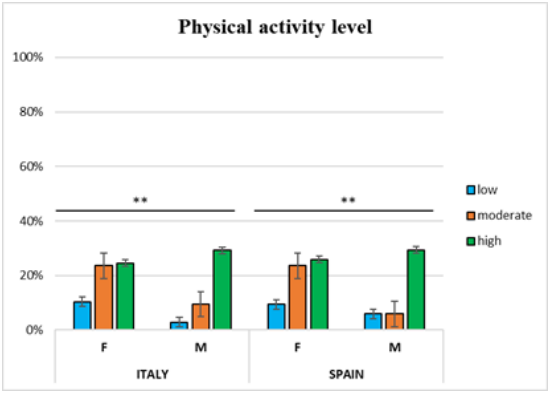

Figure 5.16. Level of physical activity among Italian and Spanish subjects according to IPAQ. F: females; M: males. Low: total METs<700; Moderate: total METs between 700 and 2519; High: total METs≥2520. **P <0.05.

Sports most practiced were gym (47.4% of IT and 28.1% of SP), swimming (7.7% of IT and 5.3% of SP), football (3.8% of IT and 7.0% of SP), and running (5.1% of IT and 8.8% of SP), and differences in the sample were significant (P <0.001). The weekly frequency of PA stated by Italian and Spanish students (fig. 5.17) reflects the level of PA reported by the sample.

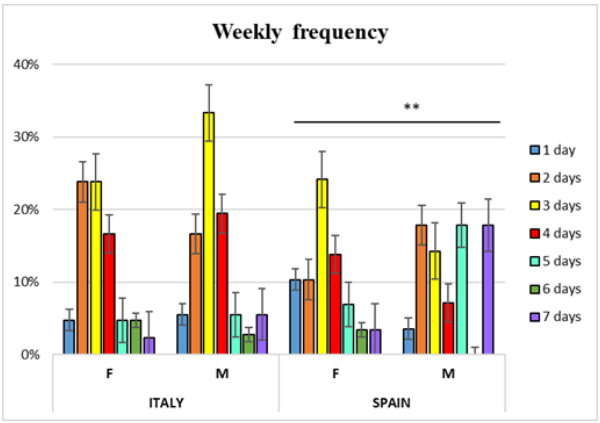

Figure 5.17. Weekly frequency of physical activity among Italian and Spanish students. F: females; M: males. P <0.0001.

In particular, SP-M tended to train more times a week for one or two hours than IT-M (P <0.0001), whereas most of the IT-F and SP-F has declared to do sport at least 3 times a week for an hour, with an overall frequency higher among IT-F. Differences emerged in the sample were significant (P <0.0001).

As regards the use of supplements, among Italian students 51.7% of F and 48.8% of M stated to consume them in the last 12 months, whereas among Spanish

subjects, 38.2% of F and 85.2% of M have used them, with significant differences among Spanish students and between IT-M and SP-M (P=0.0002 and P=0.0024, respectively). Most of the sample (58.5% of IT and 63.6% of SP) reported to use supplements occasionally (8-30 days in the last 12 months), with only 10.7% of IT and 11.5% of SP that consume them daily. Among supplements users, most of them (64.0%) consumed vitamins, followed by mineral salts (38.0%), sports supplements (37.0%), probiotics (28.0%), and enriched/fortified supplements (20.0%), represented by common foods (drinks, yogurt, cereals, etc) that declare the addition of specific substances on the label, such as minerals, vitamins, fibers, etc (fig. 5.18). Differences between Italian and Spanish students were significant (P <0.0001).

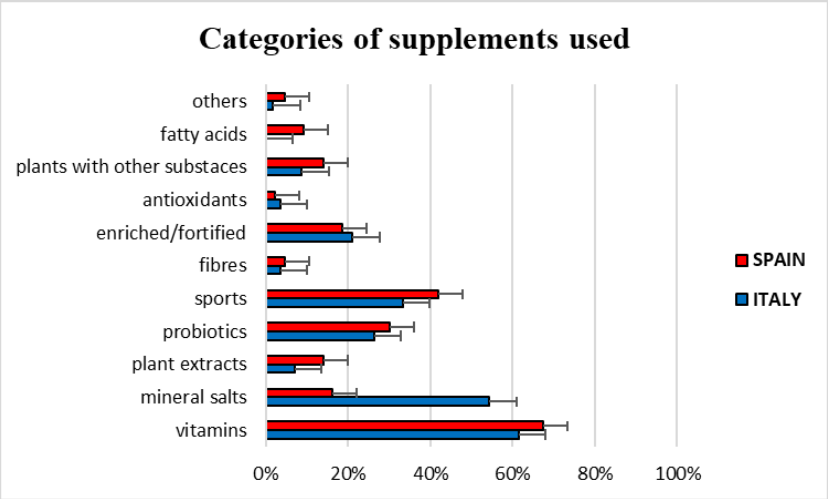

Figure 5.18. Supplements used by Italian and Spanish subjects in the last 12 months.

Most of the sample (66.7% of IT and 69.8% of SP) consumed supplements to improve their well-being and health, whereas 31.6% to improve physical performance, with a higher prevalence among M than F. Furthermore, 10.5% of IT and 11.6% of SP have declared to use supplements for dietary needs. Only 5.7% of IT-F and 15.0% of SP-F have consumed supplements to gain or lose weight, whereas only SP-M have used them to try the flavour (8.7%) or because considered convenient when hungry or thirsty (4.3%).

Among Spanish students most of them (32.6%) used supplements without asking/receiving guidance from healthcare professionals, whereas 25.6% and 23.3%

have been advised by a nutritionist or a friend/relative, respectively. On the contrary, Italian students have declared to make use of supplements following the indication of a pharmacist or a doctor, whereas only 14.0% used them without the advice of a healthcare professional. Differences between Italian and Spanish F and M were significant (P=0.0401 and P=0.0350, respectively). In particular, as regards female students, while IT-F used supplements on the advice of a pharmacist (45.7%) or a doctor (42.9%), SP-F tended to manage the intake of supplements autonomously (35.0%), or on the advice of a doctor (25.0%), a nutritionist or friend/relative (20.0%), a pharmacist (15.0%), or on the basis of the products labels, internet information or natural food stores (10.0%). As regards male students, 54.5% of IT-M followed the advice of a pharmacist, 27.3% and 22.7% took supplements on the guidance of a nutritionist or a doctor, and 13.6% have been advised by the personal trainer or a friend/relative, whereas among the Spanish ones, 30.4% consumed them autonomously or on the advice of a nutritionist, 26.1% and 21.7% followed the recommendation of a friend/relative or a personal trainer, respectively, whereas 17.4% and 13% followed internet information or the products labels, respectively.

Among Spanish users of supplements, 53.8% stopped taking them for the excessive cost, 38.5% for their ineffectiveness, and 15.4% for side effects. On the contrary, 20.0% of Italian supplements users have stopped the intake of supplements for ineffectiveness, high cost and side effects.

5.2.5 Drugs and vaccines

As regards medicines use, 93.2% of IT (38.8% M, 54.4% F) and 86.9% of SP (34.4% M, 52.5% F) has declared to have used medicines in the last 12 months. Of them, 78.1% of IT (35.4% M, 42.7% F) and 84.9% of SP (34.0% M, 50.9% F) used it occasionally, whereas 22.9% of IT (7.3% M, 15.6% F) and 15.1% of SP (5.7% M, 9.4% F) regularly. Most of them have used drugs for a week (35.9% of IT and 49.25% of SP) or a month (34.0% of IT and 24.6% of SP), whereas 14.6% of IT and 4.9% of SP have taken drugs for more than a month, and 9.7% of IT and 11.5% of SP have used them every day. 47.6% of IT and 31.1% of SP read the leaflet before taking a drug (P=0.0482). Most of the sample (78.6% of IT and 53.3% of SP) considered medicines both useful and harmful, 20.4% of IT and 25.0% of SP deemed medicines more

useful and less harmful (P <0.0001), with 95.1% of IT and 77.0% of SP who thought that drugs can cause poisoning (P=0.0018).

Only 27.2% of IT and 37.7% of SP used medicines with medical prescription, and in case of the same symptoms accused by a friend/family member only 9.7% of IT and 16.4% of SP would take the same drugs without consulting the doctor. In the event that the same symptoms returned, before taking the same drugs used on the last occasion, 71.8% of IT and 67.2% of SP would ask a doctor for advice, 22.3% of IT and 14.8% of SP would address a pharmacist, 8.2% of SP a friend, whereas 5.8% of IT and 6.6% of SP would not ask for help. Almost the whole sample (94.2% of IT and 80.3% of SP) used antibiotics to treat bacterial infections, 4.9% of IT and 8.2% of SP for flu, and 1.0% of IT and 11.5% of SP for viral infections. Most of the sample (98.1% of IT and 90.2% of SP) deemed that psychiatric drugs could be taken when doctor prescribed them, since were considered dangerous by 95.1% of IT and 86.9% of SP. 96.1% of IT and 90.2% of SP were aware that tranquilizers and sleeping pills, if taken for a long time, could be addictive. Only 9.7% of IT and 23.0% of SP took drugs with alcohol (P=0.0241).

The most used drugs by Italians were Non-Steroidal Anti-Inflammatory Drugs (NSAIDs, 68.4%), antihistamine (11.2%) and antibiotics (10.2%), whereas among Spaniards 81.3% took NSAODs, 10.4% hormonal contraceptives, and 8.3% antibiotics, whereas only 4.2% used antihistamine. 38.8% of IT and 27.9% of SP used pomades, patches, and anti-pain bands for muscle and join pain, wounds and dermatitis, whereas only 4.9% of IT and SP took homeopathic medicines to treat anxiety, stress, and eliminate toxins, and 12.6% of IT and 23.0% of SP consumed enriched foods. In figure 5.19 are reported the percentages of subjects who turned to different professional and non-professional figures to take drugs.

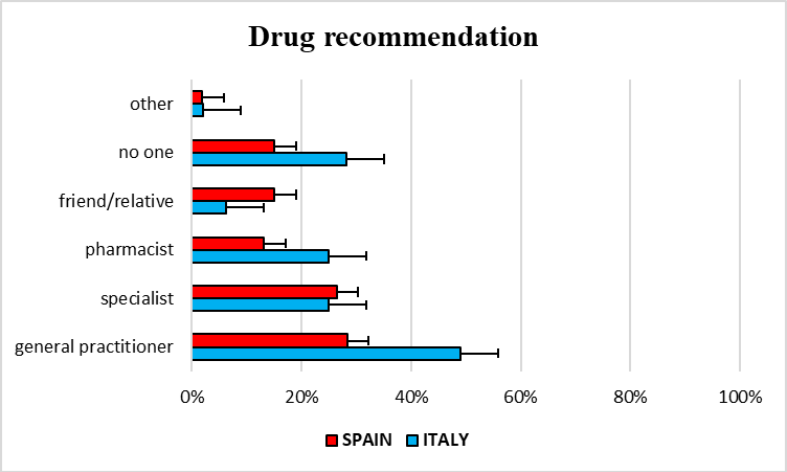

Figure 5.19. Drug recommendation for intake of drugs by different professional and non-professional figures.

Concerning the popular beliefs, most of the volunteers believed that: aspirin does not treat the flu (54.4% of IT and 65.6% of SP), in spring it is not important make a restorative cure (48.5% of IT and 47.5% of SP, P=0.0078), drugs that are good for one person might not be also good for another (93.2% of IT and 93.4% of SP), medicines of natural origin and herbal remedies have side effects (86.4% of IT and 85.2% of SP), drugs are addictive (98.1% of IT and 86.9% of SP, P=0.0065), not any drug is beneficial to health (95.1% of IT and 86.9% of SP, P=0.0127), students do not need to take memory drugs (89.3% of IT and 95.1% of SP), when the bowel is sluggish, a laxative does not have to be taken (74.8% of IT and 80.3% of SP), increase the dose of the medicines does not increase the beneficial effects (94.2% of IT and 96.7% of SP), when you have a fever it is better not to take an antibiotic right away (95.1% of IT and 93.4% of SP). Furthermore, they deemed correct taking the appropriate medication (99.0% of IT and 96.7% of SP), taking the right dose at the right time intervals (99.0% of IT and 98.4% of SP), checking the contraindications on the package insert (94.2% of IT and 91.8% of SP), change treatment periods and taking medications after consulting a doctor/pharmacist (95.1% of IT and 90.2% of SP), not taking multiple medications at the same time without consulting a doctor/pharmacist (99.0% of IT and 96.7% of SP), not taking a drug on the advice of people not authorized to prescribe it (98.1% of IT and 93.4% of SP), not drinking alcohol along with certain medications (e.g. tranquilizers) (99.0% of IT and 96.7% of SP), not taking expired medications (89.3% of IT and 96.7% of SP), not accepting

drugs from unknown people (98.1% of IT and 98.4% of SP). Moreover, only 20.4% of IT and 26.2% of SP have made changes to a medical prescription (dose, methods, and timing of administration), 50.5% of IT and 54.1% of SP did not check sometimes the contraindications on the package insert, but 93.2% of IT and 93.4% of SP did it generally, 58.3% of IT and 49.2% of SP did not take drugs without consulting a doctor/pharmacist, whereas 85.4% of IT and 83.6% of SP did not used multiple medications at the same time without consulting a doctor/pharmacist. Furthermore, only 19.4% of IT, contrary to 44.3% of SP, have taken a drug on the advice of nurses (P=0.0012), whereas no Italians and 8.2% of Spaniards would take a drug on the advice of managers of wellness centers or gyms (P=0.0064). 15.5% of IT and 32.8% of SP have drunk alcohol close to taking a drug (P=0.0118), 37.9% of IT and 23.0% of SP have taken medications expired for a few days, whereas only 5.8% of IT and 4.9% of SP have used a long-term expired drugs.

45.7% of IT and 44.7% of SP underwent clinical analysis at least one a year, 53.3% of IT and 59.2% of SP went to the doctor as needed, and most of the Italian subjects (55.2%) only went to the doctor for a check-up, whereas most of the Spanish sample (63.2%) went only if they were sick.

As regards vaccines, in figure 5.20 are reported the percentages of students who were vaccinated against the most common diseases. Differences in the percentage of vaccinated among IT and SP for the different diseases were significant (P <0.0001, respectively). Most of the sample (97.1% of IT and 86.7% of SP) agreed in believing that vaccines are important in reducing or eliminating serious diseases, 90.5% of IT and 82.7% of SP believed that vaccines are useful in certain situations (i.e., in developing countries). Only 2.0% of IT believed more in natural immunity, acquired through disease, than in vaccines, contrary to 21.3% of SP (P=0.0003), 94.3% of IT and 84.0% of SP believed in vaccinations deeming that they did more good than harm, 3.8% of IT and 12.0% of SP thought to not be at risk of contracting any infectious disease, only 5.7% of IT were afraid of getting sick after getting vaccinated, contrary to 14.7% of SP; only 2.0% of IT and 5.4% of SP believed that vaccines were not effective, with only 2.9% of IT who were wary of the long-term health effects of vaccinations, contrary to 17.4% of SP (P <0.0001). Differences emerged among IT and SP on their opinion on different aspects of vaccines were significant (P <0.0001, respectively).

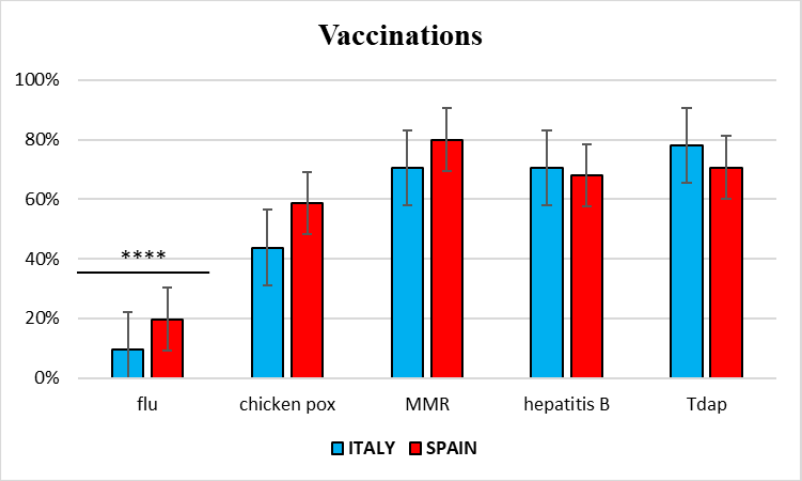

Figure 5.20. Vaccinated subjects among Italians and Spaniards. MMR: measles, mumps, rubella; Tdap: tetanus, diphtheria, pertussis. ****P <0.0001.

5.3 ORTHOREXIA NERVOSA AND OTHER PSYCHOMETRIC QUESTIONNAIRES: OVERLAPS AND CORRELATIONS

The prevalence of Orthorexia Nervosa (ON) and its correlation with other psychometric questionnaires was assessed in a subgroup of the sample (N=160, 100 IT and 60 SP). Characteristics of BMI, Physical Activity, Smoking habits and level of Psychological Distress are reported in Table 5.7. Only in Italy, IT-F had a significant lower BMI than IT-M, whereas the prevalence of underweight (UW), overweight (OW) and obese (OB) students, as well as of volunteers who practice Low, Moderate or High physical activity (PA), did not reach significant differences within and between countries (Table 5.7). However, SP-M on average practiced more PA than SP-F, and gender differences were found in IPAQ intense (MET-min/week from intense activities), in both countries (Table 5.7). No differences were found in smoking habits, whereas a high percentage of SP-M reported mild psychological distress (assessed by K10) than IT-M (Table 5.7).

Table 5.7. Characteristics of the subgroup of students

	IT-F (N = 59)	**IT-M** (N = 41)	**SP-F** (N = 33)	**SP-M** (N = 27)
BMI (kg/m²)	20.7 (19.4-22.4)†	24.5 (22.1-27.1)†	23.3 (20.2–24.5)	24.1 (21.8-25.5)
Underweight (%)	11.9	0.0	9.1	3.7
Overweight (%)	6.8	31.7	15.1	25.9
Obese (%)	5.1	9.7	3.0	3.7
IPAQ				
MET-min/week	2232 (1080 - 4986)	4380 (2305 – 6277)	2100 (1202 – 4395)†	4970 (2575 – 6780)†
IPAQ walking	630 (315-1260)	700 (488-1323)	525 (244-1230)	600 (240-1470)
IPAQ moderate	720 (160-1680)	600 (240-1440)	600 (240-1440)	720 (120-1440)
IPAQ intense	480 (0-1920)†	2160 (400-3840)†	800 (0-1680)†	2400 (960-4320)†
PA Low (%)	16.9	7.3	18.2	7.4
PA Moderate (%)	40.7	19.5	36.4	14.8
PA High (%)	42.4	73.2	45.5	77.8
Smokers (%)				
Habitual	15.3	19.5	9.1	7.7
Occasional	16.9	19.5	15.2	15.4
Ex-smokers	8.5	9.8	9.1	3.8
K10	15.0 (13.0-19.0)	14.0 (12.0-17.0)	19.0 (15.0-27.5)	17.0 (14.0-20.0)
Mild (%)	11.9	0.0#	15.2	25.9#
Moderate (%)	6.9	9.8	18.2	3.7
Severe (%)	5.1	4.9	15.2	3.7

Categorical variables are expressed as percentages. Continuous variables are expressed as means with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). IT-F: Italian females; IT-M: Italian males; SP-F: Spanish females; SP-M: Spanish males; BMI: body mass index; IPAQ: International Physical Activity Questionnaire; PA: physical activity; K10: Kessler Psychological Distress Scale. #P <0.05 (Italians vs Spaniards within gender); †P <0.05 (Male vs Female within country).

SP-M were also those with higher total scores in tests related to body-self relations and appearance concerns, as MBSRQ, MBSRQ-AO, -FO, -HOr, -AS and -BASS, whereas no differences were found in MBSRQ-IO and -OP (Table 5.8). On the contrary, IT-F had higher BUT-A–BIC and -WP than IT-M, but lower compared to SP-F.

Table 5.8. Body image concerns

	IT-F (N = 59)	**IT-M** (N = 41)	**SP-F** (N = 33)	**SP-M** (N = 27)
MBSRQ	227.0 ± 21.7	231.6 ± 27.6[#]	227.9 ± 24.3[†]	249.3 ± 27.5[†#]
MBSRQ-AO	3.3 ± 0.9	3.2 ± 0.5[#]	3.5 ± 0.5	3.5 ± 0.5[#]
MBSRQ-FO	3.4 (3.0-3.9)	3.5 (3.2-4.2)	3.5 (3.0-4.0)[†]	4.2 (3.6-4.4)[†]
MBSRQ-HOr	3.4 ± 0.5	3.4 ± 0.6	3.2 ± 0.6[†]	3.6 ± 0.6[†]
MBSRQ-IO	3.3 ± 0.6	3.3 ± 0.5	3.4 ± 0.5	3.6 ± 0.6
MBSRQ-AS	3.1 ± 0.3	3.1 ± 0.4[#]	3.1 ± 0.4[†]	3.4 ± 0.4[†#]
MBSRQ-BASS	3.2 ± 0.7	3.4 ± 0.6	3.1 ± 0.8†	3.5 ± 0.7†
MBSRQ-OP	2.0 (1.8-2.8)	2.5 (1.8-3.0)	2.5 (2.1-3.0)	2.5 (2.0-3.3)
BUT	33.0 (19.0-61.0)	16.0 (6.0-39.0)	60.0 (30.5-82.0)	35.0 (21.0-74.0)
BUT-A	16.0 (9.0-36.0)	10.0 (2.0-20.0)	33.0 (12.5-51.0)	18.0 (13.0-42.0)
BUT-A-WP	0.9 (0.4-1.6)[#]	0.5 (0.1-1.0)	1.8 (0.8-2.6)[#]	1.1 (0.6-1.8)
BUT-A-BIC	0.7 (0.2-1.3)[†]	0.2 (0.0-0.9)[†]	0.9 (0.5-1.6)	0.6 (0.2-1.6)
BUT-B	15.0 (7.0-31.0)	11.0 (3.5-21.0)	25.5 (14.0-42.5)	21.0 (8.0-36.0)

Categorical variables are expressed as percentages. Continuous variables are expressed as means with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). IT-F: Italian females; IT-M: Italian males; SP-F: Spanish females; SP-M: Spanish males. MBSRQ: Multidimensional Body-Self Relations Questionnaire (AO: appearance orientation, FO: fitness orientation, HOr: health orientation, IO: illness orientation, AS: appearance scales, BASS: body areas satisfaction scale, OP: overweight preoccupation); BUT: Body Uneasiness Test (WP: Weight phobia, BIC: Body image concerns). [#]P <0.05 (Italians vs Spaniards within gender); [†]P <0.05 (Male vs Female within country).

Concerning the results from ORTO-15 questionnaire for ON, lower values (indicating high ON) were observed in Spanish students compared to Italians, when using the first proposed cut-off point of 40 (Donini *et al*., 2005). Similar results were observed with ORTO-12, -11 and -9 questionnaires (Table 5.9).

Table 5.9. Orthorexia, eating attitude and malnutrition

	IT-F (N=59)	**IT-M** (N=41)	**SP-F** (N=33)	**SP-M** (N=27)
ORTO-15	36.8 ± 3.4#	36.4 ± 34.2#	34.2 ± 3.6#	33.8 ± 3.4#
cut-off 40 (%)	76.3#	70.7#	97.0#	96.3#
cut-off 35 (%)	23.7#	36.5	48.5#	55.5
ORTO-12	30.7 ± 3.0#	30.4 ± 3.4#	27.5 ± 2.9#	27.2 ± 2.7#
ORTO-11	27.8 ± 2.8#	27.6 ± 3.4#	25.2 ± 2.9#	25.4 ± 3.0#
cut-off 25 (%)	11.9#	19.5#	36.4#	25.9#
ORTO-9	30.4 ± 3.4#	30.0 ± 3.7#	27.8 ± 3.2#	27.1 ± 3.2#
cut-off 26.7 (%)	13.6#	19.5#	30.3#	40.7#
ORTO-7	20.0 (18.0-22.0)#	19.0 (18.0-21.0)	19.0 (15.5-19.5)#	19.0 (16.0-19.0)
cut-off 19 (%)	32.2	29.3	48.5	48.1
EAT-26	6.0 (3.0-10.0)#	5.0 (3.0-8.5)#	11.0 (5.0-17.5)#	13.0 (7.0-17.0)#
cut-off 20 (%)	8.5	4.9	21.2	11.1
Dieting	2.0 (0.0-5.0)	2.0 (0.0-4.5)	4.0 (0.5-9.5)	6.0 (3.0-9.0)
Bulimia	3.0 (3.0-3.0)	3.0 (3.0-3.0)	3.0 (3.0-5.5)	3.0 (3.0-5.0)
SSI	14.0 (8.0-22.0)#	10.0 (4.5-16.0)#	24.0 (20.0-43.0)#	18.0 (12.0-30.0)#

Categorical variables are expressed as percentages. Continuous variables are expressed as means with standard deviation (Shapiro-Wilk Test passed), or as median with interquartile range (Shapiro-Wilk Test failed). IT-F: Italian females; IT-M: Italian males; SP-F: Spanish females; SP-M: Spanish males. ORTO: scores for ON (reverse scores, lower values indicate high ON); EAT-26: Eating Attitudes Test; SSI: Starvation Symptom Inventory. #P <0.05 (Italians and Spaniards within gender).

By using the lower cut-off point of 35 for ORTO-15 or the ORTO-11, -9, and -7, IT-F resulted with less tracts of ON than SP-F (Table 5.9).

Both SP-F and SP-M had higher EAT-26 total score than Italian counterparts, despite dieting and bulimia components did not reach statistical significance (Table 5.9). Similar results came from SSI, suggesting more starvation symptoms in SP compared to IT.

Orthorexia nervosa (ON) is a complex condition that may include symptoms shared with other eating disorders.

Figures 5.21 and 5.22 show the relationships between the prevalence of ON and the eating attitudes and psychological distress.

Figure 5.21a illustrates the prevalence of ON, by using different cut-off points and scores for ORTO questionnaire, among students who presented ED risk (EAT-26 positive), mild, moderate, or severe psychological distress (K10).

Psychological distress was observed both in students presenting ED risk (EAT-26 positive) as well as in those who did not (resulting negative to the EAT-26 test), without gender differences (Figure 5.21b).

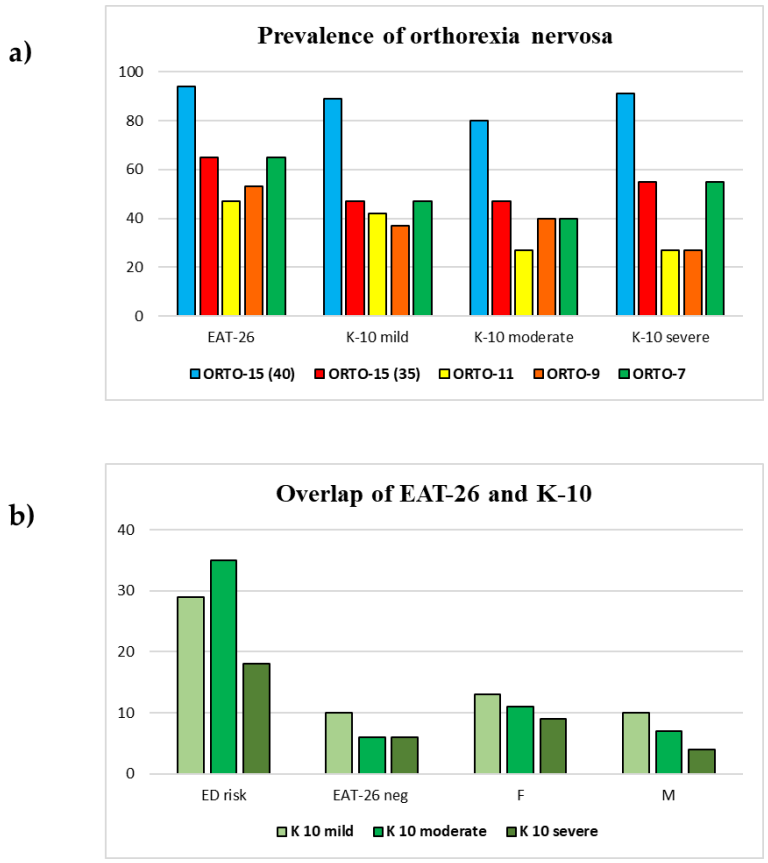

Figure 5.21. Relationships between psychometric questionnaires. a) Prevalence of ON among students who presented ED risk (EAT-26) or psychological distress (K10); b) overlap of EAT-26 and K10. ORTO: scores for orthorexia nervosa; K10: Kessler Psychological Distress Scale; EAT-26: Eating Attitude Test; F: Italian and Spanish females; M: Italian and Spanish males.

On the other hand, in Figure 5.22 are presented the prevalence of ON among different BMI classes and lifestyle factors, such as physical activity (PA) level and smoking habits.

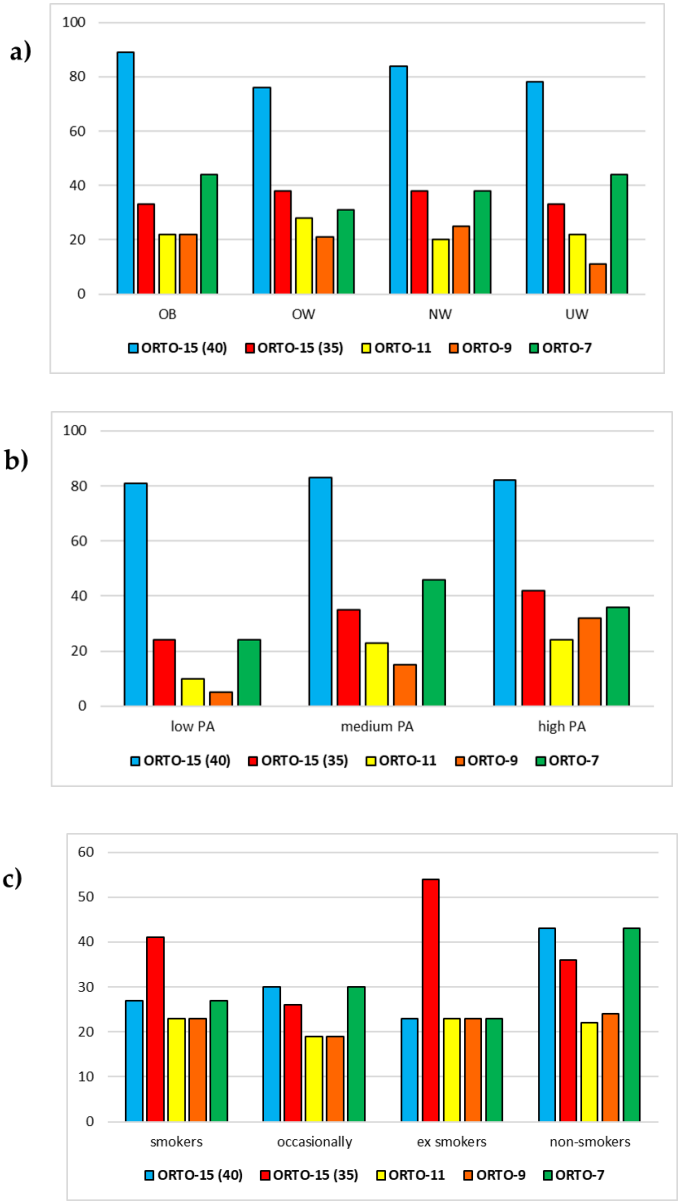

Figure 5.22. Prevalence of ON among different a) BMI classes, b) physical activity and c) smoking habits. ORTO: scores for orthorexia nervosa; OB: Obese; OW: Overweight; NW: Normal weight; UW: Underweight; PA: Physical activity.

Among students with ON assessed with ORTO-15 (40 cut-off), the percentage of non-smokers (60.6%) was higher ($P < 0.05$) compared to those of ex-smokers (7.6%) and smokers (13.6%). Similar results came when using ORTO-15 (35 cut-off point) (prevalence of ON: non-smokers 60.0 %, smokers 16.0%), as well as for

ORTO-11 (prevalence of ON: non-smokers 66.7%, smokers 16.7%), ORTO-9 (prevalence of ON: non-smokers 66.7%, smokers 13.3%) and ORTO-7 (prevalence of ON: non-smokers 74.1%, smokers 7.4%).

Among the students with ON the percentage of those practicing high PA was higher ($p<0.05$) compared to low PA for ORTO-15 (cut-off 40: high PA 54.4% and low PA 15.2%; cut-off 35: high PA 68.0% and low PA 8.0%), ORTO-11 (high PA 75.0% and low PA 0%), ORTO-9 (high PA 80.0% and low PA 0.0%) and ORTO-7 (high PA 59.3% and low PA 11.1%).

In order to reduce the potential confounder as being on caloric restriction (Roncero *et al*., 2017), it has been evaluated the overlap of ON with other questionnaires, to establish which individuals actually suffered from ORTO, hence excluding confounders. It has been assessed if subjects who were positive on the ORTO-test were also positive on the EAT-26 and the other questionnaires. Therefore, Spearman correlations were evaluated in both the total sample (N=160) and in a subgroup of students (N=66) with normal BMI, excluding those who suffered from mild, moderate and severe distress, or potentially at ED risk (NW-K10^{neg}-EAT-26^{neg} group) (Table 5.10).

As regard the NW-K10^{neg}-EAT-26^{neg} group, all subjects resulted with ON when ORTO-15 (40 cut-off point) was applied, whereas a prevalence of 37.9% was observed applying ORTO-15 (35 cut-off point), 18.2% considering ORTO-11, 22.7% with ORTO-9 and 40.0% with ORTO-7.

Considering the overall sample, a relationship between ORTO-9 and BMI was found. All ORTO scores were inversely correlated to SSI (Table 5.10) which indicates that high ON corresponds to more starvation symptoms, since ORTO has reverse scores. Similarly, all ORTO scores were inversely correlated to EAT-26 and its components (Table 5.10).

Weight phobia (BUT A–WP) was inversely correlated with ORTO-12, ORTO-9 and ORTO-7. The latter resulted the only one not related to MBRSQ-AS, MBSRQ and to its health component (MBSRQ-HOr), as well as to the MBSRQ-OP (Table 5.10). Moreover, the coefficient of correlations between ORTO scores and MBSRQ-AO and -FO, were lower for ORTO-7 compared to the other scores (Table 5.10). On the other hand, ORTO-7 resulted the only one that is related to the MBSRQ-BASS,

and ORTO-9 was the only score related to IPAQ and to its intense activities and walking components (Table 5.10).

Table 5.10. Spearman correlations

	ORTO-15	ORTO-12	ORTO-11	ORTO-9	ORTO-7
BMI (N=160)	ns	ns	ns	-0.184*	ns
NW-K10^{neg}-EAT-26^{neg} (N=66)	-0.315*	-0.304*	-0.284*	-0.432***	ns
SSI (N=160)	-0.169*	-0.264***	-0.223**	-0.244**	-0.228**
EAT-26 (N=160)	-0.363***	-0.414***	-0.349***	-0.432***	-0.357***
Dieting (N=160)	-0.429***	-0.444***	-0.387***	-0.516***	-0.307***
Bulimia (N=160)	-0.188*	-0.263***	-0.180*	-0.304***	-0.223**
BUT (N=160)	ns	ns	ns	ns	-0.160*
BUT A–WP (N=160)	ns	-0.187*	ns	-0.181*	-0.166*
BUT B (N=160)	ns	ns	ns	ns	-0.165*
MBSRQ (N=160)	-0.354***	-0.279***	-0.341***	-0.414***	ns
MBSRQ-AO (N=160)	-0.333***	-0.296***	-0.326***	-0.359***	-0.177*
NW-K10^{neg}-EAT-26^{neg} (N=66)	ns	-0.285*	ns	-0.289*	ns
MBSRQ-FO (N=160)	-0.361***	-0.310***	-0.358***	-0.414***	-0.180*
NW-K10^{neg}-EAT-26^{neg} (N=66)	-0.304*	-0.371*	-0.373*	-0.450***	ns
MBSRQ-HOr (N=160)	-0.341***	-0.248**	-0.333***	-0.402***	ns
NW-K10^{neg}-EAT-26^{neg} (N=66)	-0.358**	-0.408***	-0.433***	-0.491***	ns
MBSRQ-IO (N=160)	-0.162*	ns	-0.181*	ns	ns
MBSRQ-BASS (n=160)	ns	ns	ns	ns	-0.169*
MBSRQ-OP (N=160)	-0.177*	-0.216**	-0.165*	-0.302***	ns
NW-K10^{neg}-EAT-26^{neg} (N=66)	ns	-0.366**	ns	-0.357**	ns
MBRSQ-AS (N=160)	-0.195*	-0.155*	-0.161*	-0.265***	ns
NW-K10^{neg}-EAT-26^{neg} (N=66)	ns	-0.244*	ns	-0.306*	ns
IPAQ (N=160)	ns	ns	ns	-0.183*	ns
Walking (N=160)	ns	ns	ns	-0.162*	ns
Intense activity (N=160)	ns	ns	ns	-0.178*	ns
NW-K10^{neg}-EAT-26^{neg} (N=66)	-0.404***	-0.330**	-0.273*	-0.461***	ns

ORTO: scores for orthorexia nervosa; BMI: body mass index, NW-K10^{neg}-EAT-26^{neg} : normal weight students excluded those with K10 or EAT-26 positive test; SSI: Starvation Symptom Inventory; EAT-26: Eating Attitudes Test; BUT: Body Uneasiness Test (WP: Weight phobia); MBSRQ: Multidimensional Body-Self Relations Questionnaire (AO: appearance orientation, FO: fitness orientation, HOr: health orientation, IO: illness orientation, AS: appearance scales, BASS: body areas satisfaction scale, OP: overweight preoccupation); IPAQ: International Physical Activity Questionnaire. *P <0.05, **P <0.01, ***P <0.001. ns: not significant.

Although no relationship was found among ORTO score and SSI in the NW-K10^{neg}-EAT-26^{neg} subgroup, SSI was correlated with MBSRQ-OP (0.291, P <0.05). The latter, as well as MBSRQ-AO and -AS, was correlated with ORTO-12 and ORTO-9 (Table 5.10), but also with BMI (0.260, P <0.05).

On the other hand, in the NW-K10^{neg}-EAT-26^{neg} subgroup, MET-min/week from intense activities correlated with ORTO-15, -12, -11 and -9 (Table 5.10), as well as with BMI (0.442, P <0.001), MBSRQ-FO (0.629, P <0.001), -HOr (0.387, P <0.01), that were highly related (coefficient of correlation MBSRQ-FO versus MBSRQ-HOr: 0.629, P <0.001) and, to a lesser extent, IPAQ- intense activity was related to MBSRQ-OP (0.253, P <0.05). MBSRQ-IO correlated with both MBSRQ-HOr (0.447, P <0.001) and -AO (0.256, P <0.05), that was related to MBSRQ-FO (0.329, P <0.01). Of interest, ORTO-7 resulted the only score unrelated neither with BMI nor with the other evaluated outcomes in the NW-K10^{neg}-EAT-26^{neg} subgroup (Table 5.10). The prevalence of ON from ORTO-7 in students included in the NW-K10^{neg}-EAT-26^{neg} subgroup on the overall sample resulted to be 16.9%, 12.2%, 15.2% and 25.9% for IT-F, IT-M, SP-F and SP-M, respectively.

5.4 METABOLIC STATUS ASSESSMENT

Once the questionnaires were performed by all volunteers**,** in a subgroup of 45 Italians and 73 Spaniards (N=118), the biochemical nutritional status has been evaluated by capillary sampling to determine glucose (Glu), total cholesterol (TC), triglycerides (TG), and ketones (Ket) plasma concentrations (Table 5.11). A 20.0% of IT and 44.1% of SP had glucose concentrations between 101 and 125 mg/dl indicating a prediabetes condition (glucose intolerance), worthy of long-term monitoring, whereas 10.0% of IT and 8.2% of SP reported cholesterol values ≥200 mg/dl, related to a moderate risk of experiencing cardiovascular diseases. Furthermore, as regards TG concentration, a high risk for health, in particular for cardiovascular system, was reported by only 2.3% of Spanish students (only F) with values ≥200 mg/dl. High TG and TC values indicate dyslipidemia and are risk factors for cardiovascular diseases, the same for high fasting glucose levels, whose alterations are the result of possible insulin resistance that could precede the type 2 diabetes mellitus with advancing age. As regards ketones concentrations, only 2.2% of Spanish sample (only males) reported a ketosis condition, with blood concentrations between 0.6 and 1.5 mmol/l.

Table 5.11. Metabolic status parameters

	IT-F (N=26)	**IT-M** (N=19)	**SP-F** (N=57)	**SP-M** (N=16)
Glu (mg/dl)****	91.5 ± 11.1##	90.5 ± 11.2###	102.4 ± 12.6##	103.8 ± 9.9###
Hypoglycemic (%)	0.0	0.0	0.0	0.0
Normal (%)	80.8	78.9	31.6	86.7
Prediabetes (%)	19.2	21.1	68.4	13.3
Diabetes (%)	0.0	0.0	0.0	0.0
TC (mg/dl)	160.0 ± 25.1	158.7 ± 25.3	168.5 ± 34.2	154.1 ± 26.6
Desired values (%)	86.4	94.4	91.2	93.8
Moderate risk (%)	13.6	5.6	8.8	6.3
High risk (%)	0.0	0.0	0.0	0.0
TG (mg/dl)	81.4 ± 22.0	78.0 (64.3 – 89.5)	66.0 (53.0 – 109.5)	75.0 (58.8 – 88.8)
Normal (%)	100.0	93.8	85.7	95.5
Moderate risk (%)	0.0	6.3	9.5	4.5
High risk (%)	0.0	0.0	4.8	0.0
Ket (mmol/l)**	0.2 (0.1 – 0.2)	0.1 (0.1 – 0.2)	0.2 (0.2 – 0.2)†	0.2 (0.2 – 0.3)†
Normal (%)	100.0	100.0	100.0	95.7
Ketosis (%)	0.0	0.0	0.0	4.3
Ketoacidosis (%)	0.0	0.0	0.0	0.0

Categorical variables are expressed as percentages. Continuous variables are expressed as means with standard deviation (Shapiro-Wilk Test passed). IT-F: Italian females; IT-M: Italian males; SP-F: Spanish females; SP-M: Spanish males. Glu: glucose (hypoglicemic, <60 mg/dl; normal, between 60 and 100 mg/dl; prediabetes, between 101 and 125 mg/dl; diabetes, ≥126 mg/dl); TC: total cholesterol (desired values, <200 mg/dl; moderate risk, between 200 and 239 mg/dl; high risk, ≥240 mg/dl); TG: triglycerides (normal, <150; moderate risk, between 150 and 199; high risk, between 200 and 499); Ket: ketones (normal, <0.6 mmol/l; ketosis, between 0.6 and 1.5 mmol/l; ketoacidosis, >1.5 mmol/l). **P <0.01; ****P <0.0001 (Italians vs Spaniards); †P <0.05 (Males vs Females within country); ##P <0.01; ###P <0.001 (Italians vs Spaniards within gender).

VI - DISCUSSION

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6.1 EVALUATION OF PHYSICAL CONDITION AND LIFESTYLE

This study aimed to compare the different lifestyle in two countries of the Mediterranean basin, Italy and Spain, among university students. Since respondents were from central Italy and Southern Spain because of the universities location, the results of this study may not be generalizable to all Italian and Spanish university students. 17.4% of IT-M and 17.1% of SP-M were overweight according to the classification with BMI, but from BIA they had a percentage of muscle mass higher than fat mass. Often anthropometric measurements can misclassify when it comes to sports subjects, in whom the percentage of muscle tissue, affecting the weight, could alter the classification (Kungland Torstveit & Sundgot-Borgen, 2012). For this reason, BIA reduces the margin of error with a more detailed description of body composition. Therefore, although BMI can be used as a general obesity indicator, it shows limitations when applied to certain groups, such as sports subjects; whereas, FM percentage is directly correlated with increased health risk, especially for metabolic and cardiovascular diseases (Chuang *et al*., 2012). In our sample 32.6% of IT and 48.8% of SP reported a high percentage of FM. It is important to consider also the level of visceral fat, whose excessive presence is correlated with a greater risk of undergoing cardiovascular pathologies. In our sample a high visceral fat level has emerged among both Italian and Spanish males, whereas very high levels have been observed only among Italians.

WHtR and WC are supposed to have greater discriminatory power compared to BMI (Ashwell *et al*., 2012), and are more sensitive than BMI as an early predictor of health-related risks (Ashwell & Hsieh, 2005). In particular, WHtR is probably the most sensitive anthropometric index for the screening of the metabolic syndrome (MetS) in Mediterranean populations, compared to both BMI and WC (Mombelli *et al*., 2009). WHR data showed an approximately 20% of individuals in both countries with levels surpassing the 0.9 cut-off, indicating higher cardiovascular risk.

A problem that is manifesting itself more and more in university students is linked to the frequent consumption of fast food. In a cross-sectional study carried out among medical students to evaluate the consumption of soft drinks and fast food, in relation to adiposity and PA, has emerged that about 38% of the sample were overweight or obese, and 56.2% had consumed fast food in the past 24 hours. The main reasons for the consumption of fast food were the lack of time to cook healthy food, the pleasant taste and the influence of family and friends (Ahmed *et al*., 2019). In this study overweight or obesity were more prevalent among males (Ahmed e*t al*., 2019), whereas in our sample overweight was more prevalent in males and obesity in females. In line with this study, overweight and obese participants also had more moderate PA than those normal weight and underweight. PA has been suggested as a means to reduce and control body fatness. More in general, regular PA has proved to effectively reduce diverse health risk factors, especially those related to CVD and the MS (Reimers *et al*., 2012; Wagner *et al*., 2012).

Another aspect taken into consideration in this study is the tendency to eat out of home. A great frequency of meals, especially dinner and lunch, consumed out of home could be the result of their frenzied life, that can lead to an unhealthy diet (Teleman *et al*., 2015). In fact, eating out has been associated with weight gain, since in this occasion food choices are usually high in energy content, which contributes to excessive energy intake (Bezerra *et al*., 2021). In the population under examination it emerged that 49.5%, 77.5% and 100.0% of the sample had breakfast, lunch and dinner out of home, respectively. Snacks between meals are often consumed to be able to sustain the rhythm of the university day, and it has emerged that 51.1% of students went to the bar, but with a certain frequency also to pizzeria, fast food or street food. As regards the frequency of eating out of home, 58.8% of the sample have declared to consume meals out of home at least 1-2 times a week.

Since students, especially who move away from home, assume the new responsibilities in the organization of meals and preparation of their menus, the lack of practice can lead to including foods that do not require complex processing or time prolonged cooking, such as frozen, pizzas, sausages or precooked meats (Bárbara & Ferreira-Pêgo, 2020; Ortiz-Moncada *et al*., 2012). In line with previous studies (Poscia *et al*., 2017; Iglesias López *et al*., 2014; Sánchez Socarrás *et al*., 2014; Lazzeri *et al*., 2013; Ortiz-Moncada *et al*., 2012; Bach-Faig *et al*., 2011; Ledo-Varela *et*

al., 2011), more than half of the sample have reported a high consumption of red meat and an intake of milk lower than the recommended portions, 59.3% showed an inadequate intake of fruit, with the tendency of men to eat less fruit than women, and no student made a consumption in line with the recommendations of the MD pyramid for all the food groups.

6.2 ALCOHOL CONSUMPTION IN RELATION TO ADHERENCE TO THE MEDITERRANEAN DIET AND TO THE FOOD NEOPHOBIA

In both countries, the average AUDIT scores were below those indicating hazardous or harmful alcohol use (cut-off points of 8 and 6 for men and women, respectively). However, 15.5% of IT and 30.7% of SP showed a hazardous alcohol use. A recent study conducted in Spain reported that 26.2% of participants were categorized as high-risk drinkers, according to the AUDIT questionnaire (López-Moreno *et al*., 2021). It has been found that 17.6% of the recruited Spanish university students may be considered at risk of developing alcohol dependency, and 2.4% can be considered as having alcohol dependence syndrome (ADS) (Carlos *et al*., 2020). López-Moreno *et al*., according to the AUDIT score, categorized 26.2% of Spanish university students as high-risk drinkers, and 7.7% as suffering from ADS (López-Moreno *et al*., 2021). In our study, the safe consumption of alcohol can be due to the habits of our sample, since 77.4% of IT and 73.0% of SP stated to generally stay at home in the evening, while 22.6% of IT and 27.0% of SP went out. It was customary to have an aperitif before dinner (62.3% of IT and 57.1% of SP). Worldwide, most of alcohol is consumed in the form of spirits, while in Italy the portion of liters of beer has increased and that of wine has decreased (Ministero della Salute, 2016). In our sample the main alcoholic beverage used was beer, consumed by 60.9% of IT and 76.6% of SP.

Participants were asked to specify the type of alcoholic beverage consumed and the frequency of consumption in a day, to correlate the consumption of alcohol, especially red wine, with the adherence to MD. Previous studies have demonstrated that young adults did not follow national recommendations (Teleman *et al*., 2015; Durá Travé & Castroviejo Gandarias, 2011). In line with what has been reported by OECD (OECD, 2014), female students had better dietary habits than male ones. We observed that Italian university students who live with

their parents eat much more fruit, vegetables, fish and legumes, whereas the departure from the ideal MD model seems much more pronounced among the non-resident students. This choice can be attributed not only to their greater independence, but also to the economic limitations that push them to spend less on the purchase of food (Lupi *et al*., 2015). In fact, they tend to consume high energy density nutrient-free foods, that are cheaper than fruit and vegetable (Rehm *et al*., 2015; Rao *et al*., 2013). In our study, IT-F had a higher FNS and lower AUDIT score, compared to SP-F. It has been recently reported that familiarity with coffee/tea characterized by a higher bitterness and astringency was lower in individuals high in FN (De Toffoli *et al*., 2019). The latter was associated with both low adherence to MD (Predieri *et al*., 2020) and low familiarity with vegetables, irrespective of their sensory properties, in Italian adults (De Toffoli *et al*., 2019). Nonetheless, FN correlated with a low vegetable consumption in Spanish schoolchildren (Rodriguez-Tadeo *et al*., 2018).

Contrary to Scholz *et al*. (Scholz *et al*., 2016), in the present study university students who consumed only wine did not have a greater adherence to MD than other volunteers. Although Minzer *et al*. suggested that moderate wine consumption with meals is a positive item in the MD score (Minzer *et al*., 2020), among the three different used validated questionnaires, only aMED assigned a gender specific moderate wine consumption (Gnagnarella *et al*., 2018; Bach-Faig *et al*., 2011). MDS-14 assigns a greater score to higher quantity of wine consumed (Martínez-González *et al*., 2012), whereas MED-55 assigns a higher score to a lower consumption of alcoholic beverages, regardless of type (Panagiotakos *et al*., 2006; Panagiotakos *et al*., 2002).

In our study only two IT and none of the SP stated to consume at least a glass of wine per day (the minimum required to comply MSD-14 score). However, 86 IT and 53 SP have reported an intake of less than 300 ml of alcoholic beverages per day, which is the maximum required to be considered adherent to MD, according to the MED-55 score (Panagiotakos *et al*., 2006). In the study conducted by López-Moreno *et al.* the questionnaire from the PREDIMED study (MDS-14) was used, and it was reported that a high percentage of the students did not reach the intake of wine for the assignment of 1 point (López-Moreno *et al*., 2021). On the other hand, other studies used the Mediterranean diet quality index for children and adolescents (KIDMED), not including alcoholic beverages, to evaluate the

relationship between hazardous alcohol use and MD adherences in Spanish university students (Rodríguez-Muñoz *et al.*, 2021; Carlos *et al.*, 2020). Rodríguez-Muñoz *et al.* reported that the students who declared eating fruit or drinking fruit juice every day, appreciating pulses, and using olive oil, had a lower tendency to drink heavily, assessed with AUDIT (Rodríguez-Muñoz *et al.*, 2021). Carlos *et al.* found no relationship between the level of alcohol consumption and the degree of adherence to MD (Carlos *et al*., 2020). Accordingly, we found no relevant differences between the preference for alcoholic beverages and adherence to MD with MDS-14 and MED-55. The low relationship may be due to the consumption of other alcoholic beverages. In particular, 56.5% of IT and 53.2% of SP have reported to drink a bottle of beer (33 cl) within a day, 4.4% of IT and 12.8% of SP consumed two bottles within a day, whereas only one SP-F declared to consume 8 bottles of beer within a day. 22.8% of IT and 22.5% of SP consumed a hard liquor (40 ml), while 29.35% of IT and 17.0% of SP consumed an alcoholic cocktail (40 ml). There is an inconsistency between the MED-55 and the food pyramid proposed by Vitiello *et al*, regarding the score assigned to the intake of red meat and dairy products (Vitiello *et al*., 2016). Furthermore, the MDS-14 includes the negative consumption of butter, which is not contemplated in the MED-55, that takes into consideration the consumption of milk and derivatives, assigning a lower score for a high frequency of consumption. However, due to the low score of MD adherence assigned to alcohol consumption, regardless of type of beverage, MED-55 can be considered healthier according to the latest Italian national guidelines for nutrition (CREA, 2018) and to the "Healthy Hydration Pyramid" of the SENC (Aranceta-Bartrina *et al*., 2019).

In the present study, on average IT had moderate MD adherence assessed as MED-55 and aMED. From the aMED (including vegetables, fresh fruits, dried fruits, wholegrain cereals, pulses, fish, and olive oil) it emerged that 37.6% of the sample had a poor adherence to MD, whereas the analysis of MED-55 has revealed that no subject had a low adherence to this dietary pattern. Significant differences in ntMED (including carbonated and/or sugar sweetened beverages (soft drinks), butter, margarine or cooking cream, and manufactured sweets, pastries and cakes), between IT and SP, were not found. However, there were negative correlations between AUDIT and QueMD ($P < 0.05$), and AUDIT and ntMED ($P < 0.01$), the score that includes foods not typical of MD (usually consumed on Saturday night among

SP), but no relationship between AUDIT and others MD scores, including aMED. Therefore, not typical MD foods had the greatest impact in our sample, especially among SP. In agreement, in the study conducted by Rodríguez-Muñoz *et al.* the Spanish university students who declared going to a fast-food restaurant more than once a week, and eating sweets and candy several times every day, had a higher tendency to be at risk of excessive alcohol consumption (Rodríguez-Muñoz *et al*., 2021).

The major strength is that we used different standardized questionnaires for the evaluation of MD adherence. Another point of strength is that we found a relationship only between ntMED and AUDIT in SP, whereas IT presented a high FN, suggesting a higher attachment to traditions. The study points to differences in dietary patterns at the household level between the two countries, which calls for further studies to elucidate.

Despite of the limited sample size, moderate adherence to MD was observed, and females had a higher adherence to MD (assessed with aMED) than males, in accordance with other studies (La Fauci *et al*., 2020; Wansink *et al*., 2013; El Ansari *et al*., 2012; Moreno-Gómez *et al*., 2012). Furthermore, students who presented more symptoms of depression and anxiety during the lockdown for COVID-19 pandemic have reported an increased alcohol consumption (Huber *et al*., 2021; Dumas *et al*., 2020; Lechner *et al*., 2020; Rehm *et al*., 2020; Rodriguez *et al*., 2020). Therefore, further research is needed to demonstrate the association between alcohol intake and specific dietary patterns.

6.3 SELF-MEDICATION, USE OF SUPPLEMENTS AND PHYSICAL ACTIVITY LEVEL

Self-medication can be defined as the selection and use of medicines by individuals to treat self-recognised illnesses or symptoms, with a prevalence of 68% in European countries and much higher in the developing ones (Alshogran *et al*., 2018; Helal & Abou-ElWafa, 2017; Gyawali *et al*., 2015; Ehigiator *et al*., 2013; Parikh *et al*., 2013), with rates going as high as 92% in the young people of Kuwait. It has been estimated that over 50% of antibiotics worldwide are commonly purchased without a prescription (Alhomoud *et al*., 2017). Most of our sample (72.8% of IT and 62.3% of SP) used medicines without medical prescription, with a higher

tendency to self-medication among IT-F and SP-M. In line with previous studies (Scuri *et al*., 2019; Alshogran *et al*., 2018; Fadare & Tamuno, 2018; Kanwal *et al*., 2018; Azhar *et al*., 2013; Kumari *et al*., 2012), the primary conditions were found to be headache, common cold, cough, fever and abdominal pain, all illnesses of mild nature. Self-medication is more common among healthcare professionals like physicians, nurses and pharmacists than the general public. However, apart from age, gender, self-care orientation and medical knowledge, other factors, such as a prior experience of treating similar illness, advice of family or friends, time saving, and economic considerations, can influence the decision (Abdi *et al*., 2018; Gillani *et al*., 2017; Rathish *et al*., 2017; Alkhatatbeh *et al*., 2016; Shah *et al*., 2014b; Badiger *et al*., 2012; Banerjee & Bhadury, 2012; Osemene & Lamikanra, 2012; Pan *et al*., 2012). Generally, the most used drugs are the over-the-counter (OTC) ones (Afridi *et al*., 2015; Shehnaz *et al*., 2014). A study conducted in Malaysia stated that 75% of the studied population used OTC drugs (Azhar *et al*., 2013). As regards our sample, 92.9% of IT and 93.8% of SP used OTC drugs, and in line with previous studies the common OTC drugs used were analgesics, antipyretics and antibiotics, along with cough remedies and supplements (Scuri *et al*., 2019; Azhar *et al*., 2013). Self-medication with antibiotics is a serious public health problem worldwide (Xu *et al*., 2019; Morgan *et al*., 2011), with high prevalence among different populations and countries (Alhomoud *et al*., 2017; Ocan *et al*., 2015). A study has reported that increasing education level presented a higher prevalence for undergraduates, master and PhD students (Xu *et al*., 2019), contrary to the previous evidence that illiteracy is a high risk for self-medication with antibiotics (Ocan *et al*., 2015). In line with a previous study (Alshogran *et* al., 2018), most of the sample is dependent on physicians or pharmacists' advice. The use of antibiotics without professional advice, prescriptions, or medical supervision, can lead to inappropriate use, such as change of antibiotic dosage during the course of treatment, change of antibiotic during the course of treatment or discontinuation of antibiotic use. This may not be inherently dangerous, but may become so when the person does not know how the drug should be used, for example, when antibiotics are used to treat viral infections or the flu (Scuri *et al*., 2019; Albatti *et al*., 2017; Lee *et al*., 2017). In our study 6.3% of the sample used antibiotics to treat a flu, whereas 5.0% for viral infections. Furthermore, most of the sample (95.1% of IT and 96.7% of SP) changed the antibiotic treatment period only after consulting a doctor. The possible

consequences include not only the emergence of antibiotic resistance, but also masking symptoms, treatment failure, drug toxicity, adverse drug events, and even death (Scuri *et al*., 2019; Grappasonni *et al*., 2018).

50.5% of IT and 50.9% of SP used food supplements, and among them 35.1% of IT and 11.6% of SP took them on medical advice, whereas 64.9% of IT and 39.6% of SP on a specialist. Contrary to a previous work that reported more frequent use of food supplementation by women (Dickinson *et al*., 2014), our study did not reveal any significant difference between sexes. The most consumed were vitamins (64.0%), followed by mineral salts (38.0%), sports supplements (37.0%), probiotics (28.0%), and enriched supplements (20.0%). Most of the sample has justified their use to improve their health (66.0%) and physical performance (35.0%). There might be a link on the use of supplements with a health effect, that although are not used in deficient conditions, they bring a source of micronutrients and macronutrients anyway useful for physiological processes or with nourishing functions for the body. In fact, EFSA recognizes these beneficial activities of supplements a priori (EFSA, 2013). Different is the case for those who have motivated the use of supplements with the aim of improving sports performance, for which EFSA dissociates from any supplement that is credited with this ability. Surely, it is a more controversial topic in which, however, many scientific studies have shown the validity of these products as ergogenic aids. Branched chain amino acids, taurine, and creatine are the most used in sports (Jagim & Kerksick, 2021; Kreider & Stout, 2021; Kurtz *et al*., 2021; Plotkin *et al*., 2021; Almeida *et al*., 2020; Pickering & Grgic, 2019; EFSA, 2013).

As regards energy drinks, a good percentage of the sample have used them to improve physical performance; in fact, the common Red Bull contains caffeine, taurine, B vitamins, and glucuronolactone, to which a stimulatory activity, and consequent ergogenic aid, can be attributed. An interesting finding was the significantly higher percentage of SP consuming energy drinks compared to IT. Some authors have reported a higher prevalence of energy drinks consumption among SP-M university students, related to exams periods as well as in combination with alcoholic drinks (Pinedo et al, 2019). The growth in their popularity may be partly attributed to their availability in bars and clubs, where energy drinks are used as mixers, and at any local supermarket or grocery store, where consumers can find pre-mixed alcoholic energy drinks.

Nowadays, among generations of students, it is common the use of brain stimulants, food supplements, drugs subject to medical prescription, amphetamines and energy drinks in order to enhance their mental faculties, namely memory, concentration, and reasoning (Hildt *et al*., 2015; Sattler *et al*., 2014; Maier *et al*., 2013; Webb *et al*., 2013; Arria *et al*., 2010). The main substances used for this purpose are caffeine, methylphenidate, modafinil, piracetam, energy drinks and amphetamines, with a significant risk of dependence (Hildt *et al*., 2015). It has been estimated that 5% to 35% of university students use these drugs to improve their academic performance, stimulate cognitive function and decrease physical and mental fatigue (Nessler *et al*., 2020; Boclin *et al*., 2020; Hildt *et al*., 2015; Arria *et al*., 2010; Wilens *et al*., 2008). In line with a previous study (Majori *et al*., 2018), in our sample only among Italian students, 11.8% used energy drinks to decrease the mental fatigue and improve their academic performance.

MD pyramid gives importance not only to proper nutrition, but generally also to sport and PA (Serra-Majem & Ortiz-Andrellucchi, 2018). In line with a previous study (Sánchez Socarrás & Aguilar Martínez, 2014), 25.7% of IT and 32.9% of SP did not do PA. Furthermore, 14.1%, 31.4%, 54.5% had a low, moderate, and high PA level, respectively. Due to COVID-19 pandemic, students have had to experience a myriad of changes, such as remote learning, home confinement, reduced mobility both by foot and vehicle, halted access to recreation and PA facilities, and disruptions in their access to food (Bertrand *et al*., 2021). From a multicontinental survey among adults has emerged that during the COVID-19 pandemic daily sitting time has increased by 28.6%, and frequency and duration of PA has decreased by 24% and 33.5%, respectively (Ammar *et al*., 2020). The study also reported significant increases in consumption of unhealthy food, eating out of control, and snacking between meals (Ammar *et al*., 2020). Even other studies have reported an increase in sedentary time (Sidebottom *et al*., 2021; Alomari *et al*., 2020; Barkley *et al*., 2020; Castañeda-Babarro *et al*., 2020; Deschasaux-Tanguy *et al*., 2021; Rodríguez-Pérez *et al*., 2020; Romero-Blanco *et al*., 2020; Sidor & Rzymski, 2020; Wathelet *et al*., 2020) and PA level in F during lockdown, whereas no change has been observed in M (Romero-Blanco *et al*., 2020; Wathelet *et al*., 2020). As reported by another study carried out among students (Bertrand *et al*., 2021), F had greater PA levels. In our study these results could be explained by the disproportionate ratio of F (69.0%) to M (31.0%) in our sample, or by the different motivation (peer

pressure, weight loss, or maintenance) for PA that led females to higher PA levels, particularly during university enrollment when body weight is likely to increase.

6.4 OVERLAP OF ORTHOREXIA, EATING ATTITUDES AND PSYCHOLOGICAL DISTRESS

In light of the reported overlaps of ON with other conditions and of the criticism highlighted from literature about ORTO-15 (Guglielmetti *et al*., 2022; Niedzielski & Kaźmierczak-Wojtaś, 2021; Uriegas *et al*., 2021; Abdullah *et al*., 2020; Babeau *et al*., 2020; Gorrasi *et al*., 2020; Plichta & Jezewska-Zychowicz, 2020; Clifford & Blyth, 2019; Farchakh *et al*., 2019; Gramaglia *et al*., 2019; Lucka *et al*., 2019; Moller *et al*., 2019; Parra-Fernandez *et al*., 2018; Barnes & Caltabiano, 2017; Malmborg *et al*., 2017; Roncero *et al*., 2017; Missbach *et al*., 2015), we have evaluated the prevalence of ON among 160 Italian and Spanish university students, from the 194 recruited, who agreed to fill the standardized questionnaires: EAT-26, K10, BUT, MBSRQ, and IPAQ. In this way we wanted to discern between the pathological condition of ON and healthy orthorexia. The sample had, on average, a medium adherence to the MD and a low risk of excessive alcohol consumption (Aiello *et al*., 2022).

Higher SSI, EAT-26 and ON, by using ORTO-15 (40 cut-off), -12, -11 and -9, were observed in Spaniards compared to Italians, regardless of gender. No significant difference between females and males in ORTO-15 have been reported in university students, in accordance with other authors (Guglielmetti *et al*., 2022; Brytek-Matera *et al*., 2017; Oberle *et al*., 2017).

Among normal weight Polish university students, ORTO-15 correlated with MBSRQ-OP, -AO, -FO, -HOr and –BASS in females, whereas body image concerns were not associated with ON in males (Brytek-Matera *et al*., 2015). The Spearman correlations confirmed the previously reported relationship between ON and MBSRQ-OP, -AO (Barnes *et al*., 2017), and EAT-26 (Farchakh *et al*., 2019; Gramaglia *et al*., 2019), and we have observed overlaps among ORTO scores, EAT-26 and K10.

In order to reduce the potential confounder as being on a diet (Roncero *et al*., 2017), we evaluated a subgroup of normal weight students. We also excluded volunteers presenting distress or ED risk (NW-K10^{neg}-EAT-26^{neg}). From this sample, all students showed ON, when ORTO-15 with the 40 cut-off was applied, whereas the percentage of ON varied with ORTO-15 (35 cut-off), -11, -9 and -7. In this

context, it has been proposed to distinguish between orthorexia nervosa and healthy orthorexia, the non-pathological tendency to follow a healthy diet (Depa *et al*., 2019). Healthy orthorexia can be the successful result of dissemination campaigns, from WHO and National recommendations, aimed to increase nutrition knowledge, but the proposed etiology of ON includes high level of education, pseudoscientific nutritional news on social media, and psychological factors (Douma *et al*., 2021). From a study carried out in nutrition and dietetics university students emerged that Instagram use might be considered as ON risk factor (Villa *et al*., 2022). Besides, K10 median of the scores of students enrolled in health-related courses was higher than those of non-health-related degree courses (Pehlivan *et al*., 2021). Furthermore, it has been suggested that obsessive healthy eating fixations may increase the risk for eating disorders in athletes, and that more education and awareness are warranted to minimize the risk for ON and eating disorders in student-athletes (Uriegas *et al*., 2021). High level of physical activity in association with ON, assessed with ORTO-15 using cut-off scores of 35, was more often seen in men from sports science and less often in women from business course (Malmborg *et al*., 2017). With a cut-off of 40, ORTO-15 resulted lower among students who performed more than ten hours/week of exercise, regardless the engagement in university sport teams, including athletes competing in aesthetic and weight dependent sports (Clifford & Blyth, 2019). In our sample, gender differences were found in MET-min/week from intense activities, in both countries. In the NW-K10^{neg}-EAT-26^{neg} group, among the students with ON the percentage of those practicing high PA was higher compared to those having low PA.

A pilot study that reported high levels of disparity among psychometric scores, including ORTO-15, EAT-26 and MBSRQ, and dietary intakes of 10 individuals (assessed using 24-h recall), failed to meet the guidelines for several nutrients and authors recommended the use of multiple psychometric instruments for ON diagnosis (Mitrofanova *et al*., 2021). As a point of strength, we have used different standardized questionnaires in order to exclude students presenting overlaps. The relationships between SSI and all ORTO scores observed in the whole sample, but not in the NW-K10^{neg}-EAT-26^{neg} subgroup, suggest that students with more starvation symptoms were excluded when we applied exclusion criteria. In fact, the greatest strength is that ORTO-7 resulted the only score unrelated neither with BMI nor with the other evaluated outcomes in the NW-K10^{neg}-EAT-26^{neg} subgroup.

The prevalence of ON from ORTO-7 after the "exclusion diagnosis" in the NW-K10^{neg}-EAT-26^{neg} subgroup ranged between 12.2% and 25.9%, values lower than those reported in the whole sample. However, it was higher than some observed with other sub-scores of ORTO test. This finding suggests that we did not include in the "exclusion diagnosis" the obsessive compulsive disorder (OCD) (Brytek-Matera *et al*., 2021). In this sense, Łucka *et al*. found that individuals with suspected ON (ORTO-15, score of 35) had higher BMI and EAT-26 score, whereas Maudsley Obsessive Compulsive Inventory (MOCI) did not differ from ORTO-15 negative group (Lucka *et al*., 2019). From that, authors suggested that ON meets the criteria of eating disorder and not of OCD (Lucka *et al*., 2019). On the other hand, considering the relationship among ORTO scores, body concerns and high physical activity component of IPAQ, questionnaires evaluating exercise addiction (Trott *et al*., 2020; Oberle *et al*., 2018) and muscle dysmorphia (Gorrasi *et al*., 2020) should also be included in the "exclusion diagnosis".

Among exclusion diagnosis (gastrointestinal disorders) there are functional dyspepsia (Mounsey *et al*., 2020), non-celiac gluten sensitivity (NCGS) (Ahmed *et al*., 2021; Tanveer & Ahmed, 2019) and irritable bowel syndrome (IBS) (Ahmed *et al*., 2021). Both NCGS and IBS are more prevalent in young women compared to men (Ahmed *et al*., 2021), and up to 90% of patients with IBS exclude certain foods to improve their gastrointestinal symptoms (McGowan & Harer, 2021). Besides, negative effects of prescribing restrictive diets can be observed due to the association between eating disorders and gastrointestinal symptoms (McGowan & Harer, 2021). In this context, the differential diagnosis and assessment of anorexia nervosa already began with the exclusion of diseases, including IBD (Crohn's disease or ulcerative colitis), malignancies, thyrotoxicosis, diabetes, cerebral tumour, major depressive or schizophrenic illnesses (Treasure *et al*., 2020).

Although the scientific community is divided into those who consider ON as a separate eating disorder and those who do not (Gramaglia *et al*., 2022), agreement exists on the needs of prevention (primary and secondary) and some diagnostic criteria, as reviewed by Atzeni *et al*., including: obsessive concern for healthy eating, fear anxiety and avoidance of certain foods components (additives, preservatives, fats or other elements considered unhealthy) (Atzeni *et al*., 2020). Furthermore, there is broad consensus on the induction of malnutrition by ON that impacts on social and professional functioning. Other suggested criteria (not

endorsed by all experts) included differences between ON and OCD or from schizophrenia, excessive time spent or rituals in preparing meals, excessive spending money for buying healthy foods; anxieties and fears concerning transgressions, and the exclusion of individuals who observe a religious practice or have medical problems (Atzeni *et al*., 2020). Our work suggests including eating disorders and psychological distress among the medical problems for the exclusion diagnosis of ON in healthy normal weight individuals. Otherwise, we suggest considering ON as a symptom of other diseases or a disease-induced comorbidity.

6.5 NUTRITIONAL AND METABOLIC STATUS

From the analysis of the nutritional profile it has been observed a higher intake of lipids and a lower consumption of carbohydrates among SP than IT, with an adequate intake of protein for both groups. Recent studies have reported that stress increases the intake of high-fat foods (Vidal *et al*., 2018; Yau & Potenza, 2013). A study that enrolled 40 female subjects reported that women under severe stress preferred sweet, high-fat foods over others (Vidal *et al*., 2018). Although the dietary intake is difficult to measure, dietary record is considered one of the most reliable methods of diet evaluation (Ortega *et al*., 2015). However, it is subject to errors derived mainly from the tendency of the respondent to declare consumption of food close to what he considers correct, the possible induction of modifications in the diet of the subjects or difficulties in describing the foods and/or portions consumed. We have tried to tackle some of these problems with prior training.

As regards the metabolic status, higher levels of glycaemia and Ket have been reported among SP. Prolonged fasting, alcoholism, diets high in fat and low in carbohydrates can affect the values obtained (Weber *et al*., 2009). 10.0% of IT and 8.2% of SP had TC values higher than the desired ones, representing a moderate risk condition. High TG or TC values are a signal of dysplidemia, which are risk factors for cardiovascular diseases. A study carried out at Veracruz University reported that young people aged 17-24 adopt an improper lifestyle, with 37% of students who are OW or OB. They stopped exercising and skipped breakfast, with a consequent increase in insulin resistance, associated with the development of T2DM (Romero-Blanco *et al*., 2020).

People's lifestyles in developed countries have changed considerably over recent decades (Gersh *et al*., 2010). As a consequence, overweight and obesity, sedentarism, and dyslipidemia occur more frequently during childhood and adolescence, producing a higher prevalence of cardiovascular diseases in adults (Al Majed *et al*., 2011; Taylor *et al*., 2010). Individuals with obesity, particularly abdominal obesity, commonly exhibit an altered plasma lipid profile known as dyslipidemia. The transition from adolescence to young adulthood commonly involves major lifestyle changes. In particular, young adults may develop habits during their university years that can alter their risk of later developing chronic diseases including CVD (Shawar *et al*., 2012; Nelson *et al*., 2008). Among university students, those enrolled in health science studies represent an important target group for health-promotion and disease-prevention programs, which include nutrition and physical-activity education because these subjects will eventually be responsible for health promotion in the general population. In this way, their lifestyle choices can not only affect their personal lives but also the lifestyles and behaviours of other groups with whom they interact (Tol *et al*., 2013).

In addition to the lifestyle changes occurred over the last decades, COVID-19 pandemic has enforced social distancing by closure of restaurants, sports and cultural facilities, as well as by prohibiting interaction of people from different households. Food acquisition was restricted to supermarkets, grocery stores and delivery services (Huber *et al*., 2021). Eating behaviour is strongly influenced by cultural and social aspects: sharing a meal with friends, family or colleagues is a common habit, which is deeply rooted in our culture and society (Cruwys *et al*., 2015; Oh *et al*., 2014). It is well known that eating behaviour is different when eating alone compared to eating with other people (Herman *et al*., 2003). On the other hand, eating behaviour has a major impact on health condition and the development of various diseases. Dietary habits are crucial for the development of CVD and even for all-cause mortality. Therefore, a so-called healthy diet plays a key role in both primary and secondary cardiovascular prevention, and a class I recommendation is given in current guidelines (Piepoli *et al*., 2016). Further, unhealthy diet patterns may have pro-inflammatory properties with the risk of the development and aggravation of inflammatory diseases (Messina *et al*., 2020).

VII - CONCLUSIONS

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- There is a medium adherence to MD and a low risk of excessive alcohol consumption, both in Italian and Spanish university students.

- Saturday night consumptions and being far from home can be risk factors for excessive alcohol consumption

- Questionnaires for the evaluation of MD adherence considered and/or assigned scores to wine consumption in a different way, and only MED-55 assigned a low score to alcohol consumption, regardless of type of beverage. This must be taken in mind when comparing data from different studies, and the use of more than one score could be useful.

- Italian and Spanish university students show deviations from dietary guidelines, such as a low consumption of fruits and vegetables, and an increase in the consumption of dairy products. It is recommended that health strategies aimed at this population insist on the importance of not omitting breakfast and ensuring that it is of quality.

- Results are consistent with the view that in modern Western Societies women generally show a tendency to perform healthier food choices and are much more concerned about the importance of food choice and eating behaviour to stay in a good physical shape than men.

- Although the sample has showed to have sufficiently correct eating habits, it would be desirable to implement information and prevention strategies in order to educate students to a reduced and conscious consumption of alcohol and a healthy and balanced diet, as a protective factor for numerous

pathologies, stressing the importance of considering a gender specific approach, both in terms of behaviour and of physiology.

- In order to reduce self-medication practices, various educational programmes should be initiated in medical institutions to raise awareness among students on the pros and cons of responsible self-medication to eventually improve their attitudes towards it. Meetings and promotional activities for rational use of antibiotics and other drugs should be conducted regularly in non-medical and medical universities.

- Orthorexia nervosa is an indicator/symptom of other problems related to body image perception, as well as high physical activity, psychological distress, appearance, fitness, health, or illness orientation.

- ORTO-7 was found to be independent from these confounders, after the exclusion of underweight, overweight, obese and EAT-26 and K10 positive students, suggesting the possibility of defining subjects with ON.

- Considering the overlap conditions, we suggest including flowcharts, diagnostic algorithms and decision tree for differential/exclusion diagnosis and management of ON, guidelines and consensus statements of experts in the future.

VIII – LIMITATIONS AND FUTURE INVESTIGATIONS

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The study had several limitations and our results should be interpreted carefully. Participants were predominantly women and students of scientific area; however, similar limitations were reported in a larger study (López-Moreno *et al*., 2021). The major limitation of our study is the limited sample size. Therefore, the generalization to the whole population of university students cannot be accomplished.

Unfortunately, COVID-19 pandemic has represented a limitation to this study since it has induced strong changes in the lifestyle and eating habits of university students and limited the recruitment. Furthermore, due to COVID-19 pandemic students have reduced their quantity of drinking owing to the closing of the pubs and returning to live with their parents may have been a protective factor for heavy drinking (Jaffe *et al*., 2021; White *et al*., 2020).

Furthermore, we have not evaluated the nutritional status of orthorexic subjects. The prevalence of ON from ORTO-7 after the "exclusion diagnosis" in the NW-K10^{neg}-EAT-26^{neg} subgroup on the overall sample ranged between 12.2% and 25.9%, values lower than those reported in the whole sample. However, it was higher than some observed with other sub-scores of ORTO test. This finding suggests among the limitations that we have not included in the "exclusion diagnosis" the OCD (Brytek-Matera *et al*., 2022).

Different types of diet and bioactive compounds taken with the diet can also have an impact on the composition of the intestinal microbiota. In particular, the MD has been associated with a greater abundance of Bacteroidetes, Prevotellacea and Prevotella, and a lower concentration of Firmicutes and Lachnospiraceae (Gutiérrez-Díaz *et al*., 2016). Although several studies have investigated the effect of the MD on intestinal microbiota (Garcia-Mantrana *et al*., 2018; Mitsou *et al*., 2017; Gutiérrez-Díaz *et al*., 2016), little attention has been paid to the effect of this diet on the oral microbiota, despite the oral cavity being one of the most relevant microbial habitats from the clinical point of view and has been shown to be a significant risk

factor for cardiovascular diseases, diabetes mellitus, bacteraemia and tumors (He *et al*., 2015).

It would be therefore interesting to analyze and investigate these aspects in the future.

IX - REFERENCES

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X - APPENDICES

APPENDIX 1: Mediterranean Diet Score Questionnaire (MDS-14).

Questions	*Criteria for 1 point*
1. Do you use olive oil as main culinary fat	Yes
2. How much olive oil do you consume in a given day (including oil used for frying, salads, out-of-house meals, etc)?	≥ 4 tbsp
3. How many vegetable servings do you consume per day? (1 serving: 200 g [consider side dishes as half a serving])	≥ 2 (≥ 1 portion raw or as a salad)
4. How many fruit units (including natural fruit juices) do you consume per day?	≥ 3
5. How many servings of red meat, hamburger, or meat products (ham, sausage, etc.) do you consume per day? (1 serving: 100-150 g)	< 1
6. How many servings of butter, margarine, or cream do you consume per day? (1 serving: 12 g)	< 1
7. How many sweet or carbonated beverages do you drink per day?	< 1
8. How much wine do you drink per week?	≥ 7 glasses
9. How many servings of legumes do you consume per week? (1 serving: 150 g)	≥ 3
10. How many servings of fish or shellfish do you consume per week? (1 serving 100-150 g of fish or 4-5 units or 200 g of shellfish)	≥ 3
11. How many times per week do you consume commercial sweets or pastries (not homemade), such as cakes, cookies, biscuits, or custard)	<3
12. How many servings of nuts (including peanuts) do you consume per week? (1 serving 30 g)	≥ 3
13. Do you preferentially consume chicken, turkey, or rabbit meat instead of veal, pork, hamburger, or sausage?	Yes
14. How many times per week do you consume vegetables, pasta, rice, or other dishes seasoned with sofrito (sauce made with tomato and onion, leek, or garlic and simmered with olive oil)?	≥ 2

Abbreviation: tbsp, tablespoon (Martínez-González *et al*., 2012).

APPENDIX 2: Mediterranean Score MED-55.

How often do you consume	**Frequency of consumption (servings/month)**					
	Never	***1-4***	***5-8***	***9-12***	***13-18***	***>18***
Non-refined cereals (wholegrain bread, pasta, rice, etc.)	0	1	2	3	4	5
Potatoes	0	1	2	3	4	5
Fruits	0	1	2	3	4	5
Vegetables	0	1	2	3	4	5
Legumes	0	1	2	3	4	5
Fish	0	1	2	3	4	5
Red meat and products	5	4	3	2	1	0
Poultry	5	4	3	2	1	0
Full fat dairy products (cheese, yoghurt, milk)	5	4	3	2	1	0
Use of olive oil in cooking (times/week)	***Never***	***Rare***	***<1***	***1-3***	***3-5***	***Daily***
	0	1	2	3	4	5
Alcoholic beverages (ml/day, 100 ml = 12 g ethanol)	***<300***	***300***	***400***	***500***	***600***	***>700 or 0***
	5	4	3	2	1	0

(Panagiotakos *et al*., 2006)

APPENDIX 3: Questionnaire to measure Mediterranean diet (QueMD).

		Daily frequency of consumption				
Food items	***Reference portions***	***Never or seldom***	***<1 per day***	***1 per day***	***2 per day***	***≥3 per day***
1. Wholegrain pasta or rice	80 g	0	0	1	1	1
2. Vegetables, all type (raw and cooked)	200 g 80 g (salad)	0	0	0	1	1
3. Fruits, all types fresh and fresh juices	150 g	0	0	0	1	1
4. Milk and yoghurt	125 g	0	0	2	3	4
		Never or seldom	***<1 per day***	***1-2 per day***	***3-4 per day***	***≥5 per day***
5. Wholegrain bread and substitutes	50 g (1-2 slices)	0	1	1	1	1
6. Olive oil to cook and to dress	10 ml (1 spoon)	0	0	0	1	1
7. Butter, margarine or cooking cream	10 g (1 spoon)	0	1	2	3	4
8. Wine (white and red)	125 ml (1 glass)	0	0	1	0	0
		Weekly frequency of consumption				
		Never or seldom	***<1 per week***	***1-3 per week***	***4-6 per week***	***≥7 per week***
9. Red meat (beef, veal, pork), meat products	100 g (raw meat) 50 g (meat product)	0	0	0	1	1
10. White meat (chicken, turkey, rabbit)	100 g	4	3	2	1	0
11. Carbonated and/or sugar-sweetened beverages	200 ml (1 glass)	4	3	2	1	0
12. Manufactured sweets, pastries, biscuits, creams...	100 g	4	3	2	1	0
		Never or seldom	***<1 per week***	***1 per week***	***2-3 per week***	***≥4 per week***
13. Fish (fresh or frozen) or sea foods	150 g (fish) 50 g (fish products)	0	0	0	1	1
14. Dried fruits (nuts, almonds, hazelnuts)	30 g (1 fist)	0	0	0	1	1
15. Pulses (chickpeas, lentils, peas, beans)	50 g (dried) 150 g (canned/raw)	0	0	0	1	1

(Gnagnarella *et al*., 2018)

APPENDIX 4: ORTO-15 questionnaire .

Questions	*Always*	*Often*	*Sometimes*	*Never*
1) When eating, do you pay attention to the calories of the food?	2	4	3	1
2) When you go in a food shop do you feel confused?	4	3	2	1
3) In the last 3 months, did the thought of food worry you?	1	2	3	4
4) Are your eating choices conditioned by your worry about your health status?	1	2	3	4
5) Is the taste of food more important than the quality when you evaluate food?	4	3	2	1
6) Are you willing to spend more money to have healthier food?	1	2	3	4
7) Does the thought about food worry you for more than three hours a day?	1	2	3	4
8) Do you allow yourself any eating transgressions?	4	3	2	1
9) Do you think your mood affects your eating behaviour?	4	3	2	1
10) Do you think that the conviction to eat only healthy food increases self-esteem?	1	2	3	4
11) Do you think that eating healthy food changes your lifestyle (frequency of eating out, friends,...)?	1	2	3	4
12)Do you think that consuming healthy food may improve your appearance?	1	2	3	4
13) Do you feel guilty when transgressing?	2	4	3	1
14) Do you think that on the market there is also unhealthy food?	1	2	3	4
15) At present, are you alone when heaving meals?	1	2	3	4

(Donini *et al*., 2005)

APPENDIX 5: Food Neophobia Scale (FNS)

Items	***Not at all***	***Little***	***Sufficiently***	***Very***	***Strongly***
1. I am constantly sampling new and different foods	5	4	3	2	1
2. I don't trust new foods	1	2	3	4	5
3. If I don't know what a food is, I won't try it	1	2	3	4	5
4. I like foods from different cultures	5	4	3	2	1
5. Ethnic food looks too weird to eat	1	2	3	4	5
6. At dinner parties, I will try new foods	5	4	3	2	1
7. I am afraid to eat things I have never had before	1	2	3	4	5
8. I am very particular about the foods I eat	1	2	3	4	5
9. I will eat almost anything	5	4	3	2	1
10. I like to try new ethnic restaurants	5	4	3	2	1

(Guidetti *et al.*, 2018)

APPENDIX 6: Alcohol Use Disorders Identification Test (AUDIT)

Questions	***0***	***1***	***2***	***3***	***4***
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2-4 times a month	2-3 times a week	4 or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 or 9	10 or more
3. How often do you have six or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
4. How often during the last year have you found that you were not able to stop drinking once you have started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
5. How often during the last year have you failed to do what was normally expected from you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
7. How often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily
9. Have you or someone else been injured as a result of your drinking?	No		Yes, but not in the last year		Yes, during the last year
10. Has a relative or friend or a doctor or another health worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year

(Allen *et al*., 1997)

APPENDIX 7: MBSRQ and item numbers for each MBSRQ subscale

Items	*Strongly disagree*	*Mostly disagree*	*Neither agree or disagree*	*Mostly agree*	*Strongly agree*
1. Before going out in public, I always notice how I look	1	2	3	4	5
2. I am careful to buy clothes that will make me look at my best	1	2	3	4	5
3. I would pass most physical – fitness tests	1	2	3	4	5
4. It is important that I have superior physical strength	1	2	3	4	5
5. My body is sexually appealing	1	2	3	4	5
6. I am not involved on a regular exercise program	5	4	3	2	1
7. I am in control of my health	1	2	3	4	5
8. I know a lot about things that affect my physical health	1	2	3	4	5
9. I have deliberately developed a healthy lifestyle	1	2	3	4	5
10. I constantly worry about becoming fat	1	2	3	4	5
11. I like my looks just the way they are	1	2	3	4	5
12. I check my appearance on the mirror whenever I can	1	2	3	4	5
13. My physical endurance is good	1	2	3	4	5
14. Before going out, I usually spend a lot of time getting ready	1	2	3	4	5
15. Participating in a sport is unimportant to me	5	4	3	2	1
16. I do not actively do things to keep physically fit	5	4	3	2	1
17. My health is a matter of unexpected ups and downs	5	4	3	2	1
18. Good health is one of the most important things in my life	1	2	3	4	5
19. I don't do anything that I know might threaten my health	1	2	3	4	5
20. I am very conscious of even small changes of my weight	1	2	3	4	5
21. Most people would consider me good looking	1	2	3	4	5

22. It is important that I always look good	1	2	3	4	5
23. I use very few grooming products	5	4	3	2	1
24. I easily learn physical skills	1	2	3	4	5
25. Being physically fit is not a strong priority in life	5	4	3	2	1
26. I do things to increase my physical strength	1	2	3	4	5
27. I am seldom physically ill	1	2	3	4	5
28. I take my health for granted	5	4	3	2	1
29. I often read books and magazines that pertain to health	1	2	3	4	5
30. I like the way I look without my clothes on	1	2	3	4	5
31. I am self-conscious if my grooming isn't right	1	2	3	4	5
32. I usually whatever is handy without caring how it looks	5	4	3	2	1
33. I do poorly in physical sports and games	5	4	3	2	1
34. I seldom think about my athletic skills	5	4	3	2	1
35. I work to improve my physical stamina	1	2	3	4	5
36. From day to day, I never know how my body will feel	5	4	3	2	1
37. If I am sick, I don't pay much attention to my symptoms	5	4	3	2	1
38. I make no special efforts to eat a balanced and nutritious diet	5	4	3	2	1
39. I like the way my clothes fit me	1	2	3	4	5
40. I don't care what people think about my appearance.	5	4	3	2	1
41. I take special care with my hair grooming	1	2	3	4	5
42. I dislike my physique	5	4	3	2	1
43. I don't care to improve my abilities in physical activity	5	4	3	2	1
44. I try to be physically active	1	2	3	4	5
45. I often feel vulnerable to sickness	5	4	3	2	1
46. I pay close attention for my body for any signs of illness	1	2	3	4	5
47. If I'm coming down with cold and flu, I just ignore it and go on as usual	5	4	3	2	1
48. I am physically unattractive	5	4	3	2	1
49. I never think about my appearance	5	4	3	2	1

50. I am always trying to improve my physical appearance	1	2	3	4	5
51. I am very well coordinated	1	2	3	4	5
52. I know a lot about physical fitness	1	2	3	4	5
53. I play sports regularly throughout the year	1	2	3	4	5
54. I am a physically healthy person	1	2	3	4	5
55. I am very aware of small changes in my physical health	1	2	3	4	5
56. At first sign of illness, I seek medical advice	1	2	3	4	5
57. I am on a weight loss diet	1	2	3	4	5
	Never	***Rarely***	***Sometimes***	***Often***	***Very often***
58. I have tried to lose weight by fasting or going on crash diet	1	2	3	4	5
	Very underweight	***Somewhat underweight***	***Normal weight***	***Somewhat overweight***	***Very overweight***
59. I think I am:	1	2	3	4	5
60. From looking at me, most people think I am:	1	2	3	4	5
For questions 61-68, choose 1-5 scale to choose how satisfied or dissatisfied you are with each of the following areas of your body.					
61. Face (features and complexions)	1	2	3	4	5
62. Hair (color, thickness, texture)	1	2	3	4	5
63. Lower torso (buttocks, hips, thighs, legs)	1	2	3	4	5
64. Mid torso (waist, stomach)	1	2	3	4	5
65. Upper torso (chest or breasts, shoulders, arms)	1	2	3	4	5
66. Muscle tone	1	2	3	4	5
67. Weight	1	2	3	4	5
68. Height	1	2	3	4	5

69. Overall appearance	1	2	3	4	5

(Chase, 2000)

Item numbers for each MBSRQ subscale (*reverse-scored items) (Cash, 2000)

APPEARANCE EVALUATION 5**,** 11, 21, 30, 39, 42*, 48*

APPEARANCE ORIENTATION 1, 2, 12, 13, 22, 23*, 31, 32*, 40*, 41, 49*, 50

FITNESS EVALUATION 24, 33*, 51

FITNESS ORIENTATION 3, 4, 6*, 14, 15*, 16*, 25*, 26, 34*, 35, 43*, 44, 53

HEALTH EVALUATION 7, 17*, 27, 36*, 45*, 54

HEALTH ORIENTATION 8, 9, 18, 19, 28*, 29, 38*, 52

ILLNESS ORIENTATION 37*, 46, 47*, 55, 56

BODY AREAS SATISFACTION 61, 62, 63, 64, 65, 66, 67, 68, 69

OVERWEIGHT PREOCCUPATION 10, 20, 57, 58

SELF-CLASSIFIED WEIGHT 59, 60

APPENDIX 8: Eating Attitudes Test (EAT-26)

Please indicate the answer which applies best to your current experience	*Always*	*Very often*	*Often*	*Sometimes*	*Rarely*	*Never*
1. Am terrified about being overweight	3	2	1	0	0	0
2. Avoid eating when I am hungry	3	2	1	0	0	0
3. Find myself preoccupied with food	3	2	1	0	0	0
4. Have gone on eating binges where I feel that I may not be able to stop	3	2	1	0	0	0
5. Cut my food into small pieces	3	2	1	0	0	0
6. Aware of the calorie content of foods that I eat	3	2	1	0	0	0
7. Particularly avoid foods with a high carbohydrate content (*e.g.* bread, potatoes, rice, etc.)	3	2	1	0	0	0
8. Feel that others would prefer if I ate more	3	2	1	0	0	0
9. Vomit after I have eaten	3	2	1	0	0	0
10. Feel extremely guilty after eating	3	2	1	0	0	0
11. Am preoccupied with a desire to be thinner	3	2	1	0	0	0
12. Exercise strenuously to burn off calories	3	2	1	0	0	0
13. Other people think that I am too thin	3	2	1	0	0	0
14. Am preoccupied with the thought of having fat on my body	3	2	1	0	0	0
15. Take longer than others to eat my meals	3	2	1	0	0	0
16. Avoid foods with sugar in them	3	2	1	0	0	0
17. Eat diet foods	3	2	1	0	0	0
18. Feel that food controls my life	3	2	1	0	0	0
19. Display self-control around food	3	2	1	0	0	0
20. Feel that others pressure me to eat	3	2	1	0	0	0
21. Give too much time and thought to food	3	2	1	0	0	0
22. Feel uncomfortable after eating sweets	3	2	1	0	0	0
23. Engage in dieting behaviour	3	2	1	0	0	0
24. Like my stomach to be empty	3	2	1	0	0	0
25. Have the impulse to vomit after meals	0	0	0	1	2	3
26. Enjoy trying new rich foods	3	2	1	0	0	0

(Garner & Garfinkel, 1997)

APPENDIX 9: Body Uneasiness Test (BUT) and scoring

BUT A	*Never*	*Seldom*	*Sometimes*	*Often*	*Very often*	*always*
1. I spend a lot of time in front of the mirror	0	1	2	3	4	5
2. I don't trust my appearance: I'm afraid it will change suddenly	0	1	2	3	4	5
3. I like those clothes which hide my body	0	1	2	3	4	5
4. I spend a lot of time thinking about some defects of my physical appearance	0	1	2	3	4	5
5. When I undress, I avoid looking at myself	0	1	2	3	4	5
6. I think my life would change significantly if I could correct some of my aesthetic defects	0	1	2	3	4	5
7. Eating with others causes me anxiety	0	1	2	3	4	5
8. The thought of some defects of my body torments me so much that it prevents me being with others	0	1	2	3	4	5
9. I'm terrified of putting on weight	0	1	2	3	4	5
10. I make detailed comparisons between my appearance and that of others	0	1	2	3	4	5
11. If I begin to look at myself, I find it difficult to stop	0	1	2	3	4	5
12. I would do anything to change some parts of my body	0	1	2	3	4	5
13. I stay at home and avoid others seeing me	0	1	2	3	4	5
14. I am ashamed of the physical needs of my body	0	1	2	3	4	5
15. I feel I am laughed at because of my appearance	0	1	2	3	4	5
16. The thought of some defects of my body torments me so much that it prevents me studying or working	0	1	2	3	4	5
17. I look in the mirror for an image of myself which satisfies me and I continue to search until I am sure I have found it	0	1	2	3	4	5
18. I feel I am fatter than others tell me	0	1	2	3	4	5
19. I avoid mirrors	0	1	2	3	4	5

20. I have the impression that my image is always different	0	1	2	3	4	5
21. I would like to have a thin and bony body	0	1	2	3	4	5
22. I am dissatisfied with my appearance	0	1	2	3	4	5
23. My physical appearance is disappointing compared to my ideal image	0	1	2	3	4	5
24. I would like to undergo plastic surgery	0	1	2	3	4	5
25. I can't stand the idea of living with the appearance I have	0	1	2	3	4	5
26. I look at myself in the mirror and have a sensation of uneasiness and strangeness	0	1	2	3	4	5
27. I am afraid that my body will change against my will, in a way I don't like	0	1	2	3	4	5
28. I feel detached from my body	0	1	2	3	4	5
29. I have the sensation that my body does not belong to me	0	1	2	3	4	5
30. The thought of some defects of my body torments me so much that it prevents me having a sexual life	0	1	2	3	4	5
31. I observe myself in what I do and ask myself how I seem to others	0	1	2	3	4	5
32. I would like to decide what appearance to have	0	1	2	3	4	5
33. I feel different to how others see me	0	1	2	3	4	5
34. I am ashamed of my body	0	1	2	3	4	5
BUT B						
1. height	0	1	2	3	4	5
2. the shape of my head	0	1	2	3	4	5
3. the shape of my face	0	1	2	3	4	5
4. skin	0	1	2	3	4	5
5. hair	0	1	2	3	4	5
6. forehead	0	1	2	3	4	5
7. eyebrows	0	1	2	3	4	5
8. eyes	0	1	2	3	4	5
9. nose	0	1	2	3	4	5

10. lips	0	1	2	3	4	5
11. mouth	0	1	2	3	4	5
12. teeth	0	1	2	3	4	5
13. ears	0	1	2	3	4	5
14. neck	0	1	2	3	4	5
15. chin	0	1	2	3	4	5
16. moustache	0	1	2	3	4	5
17. beard	0	1	2	3	4	5
18. hairs	0	1	2	3	4	5
19. shoulders	0	1	2	3	4	5
20. arms	0	1	2	3	4	5
21. hands	0	1	2	3	4	5
22. chest	0	1	2	3	4	5
23. breasts	0	1	2	3	4	5
24. stomach	0	1	2	3	4	5
25. abdomen	0	1	2	3	4	5
26. genitals	0	1	2	3	4	5
27. buttocks	0	1	2	3	4	5
28. hips	0	1	2	3	4	5
29. thighs	0	1	2	3	4	5
30. knees	0	1	2	3	4	5
31. legs	0	1	2	3	4	5
32. ankles	0	1	2	3	4	5
33. feet	0	1	2	3	4	5
34. odour	0	1	2	3	4	5
35. noises	0	1	2	3	4	5
36. sweat	0	1	2	3	4	5
37. blushing	0	1	2	3	4	5

(Cuzzolaro *et al.,* 2006)

SCORING BUT A (items 1-34)

Global measure

Global Severity Index (GSI): the average rating of all 34 items constituting the BUT A

Subscales

Weight Phobia (WP): average (9+10+18+21+24+31+32+33)

Body Image Concerns (BIC): average (3+4+6+12+15+22+23+25+34)

Avoidance (A): average (5+8+13+16+19+30)

Compulsive Self-Monitoring (CSM): average (1+11+17+20+27)

Depersonalization (D): average (2+7+14+26+28+29)

SCORING BUT B (items 1-37)

Global measures

Positive Symptom Total (PST): the number of symptoms rated higher than zero

Positive Symptom Distress Index (PSDI): the average rating of those items constituting the PST

Subscales

BUT B I *Mouth*: average (7+8+9+10+11+12)

BUT B II *Face Shape*: average (2+3+6+13+14+15)

BUT B III *Thighs*: average (24+25+28+29+30)

BUT B IV *Legs*: average (1+21+31+32+33)

BUT B V *Harms*: average (19+20+22+23+26)

BUT B VI *Moustache*: average (16+17+18)

BUT B VII *Skin*: average (4+5)

BUT B VIII *Blushing*: average (27+34+35+36+37)

APPENDIX 10: Kessler Psychological Distress Scale (K10)

Questions	*None of the time*	*A little of the time*	*Some of the time*	*Most of the time*	*All of the time*
1. In the past 4 weeks, about how often did you feel tired out for no good reason?	1	2	3	4	5
2. In the past 4 weeks, about how often did you feel nervous?	1	2	3	4	5
3. In the past 4 weeks, about how often did you feel so nervous that nothing could calm you down?	1	2	3	4	5
4. In the past 4 weeks, about how often did you feel hopeless?	1	2	3	4	5
5. In the past 4 weeks, about how often did you feel restless or fidgety?	1	2	3	4	5
6. In the past 4 weeks, about how often did you feel so restless you could not sit still?	1	2	3	4	5
7. In the past 4 weeks, about how often did you feel depressed?	1	2	3	4	5
8. In the past 4 weeks, about how often did you feel that everything was an effort?	1	2	3	4	5
9. In the past 4 weeks, about how often did you feel so sad that nothing could cheer you up?	1	2	3	4	5
10. In the past 4 weeks, about how often did you feel worthless?	1	2	3	4	5

(Kessler *et al*., 2003)

APPENDIX 11: Questions with score of AQUA

Questions	*Yes*	*Mother and father*	*Mather or father*	*Other relatives*
4. Did any doctor diagnose you an allergic disease?	4			
5. Do you suspect to suffer from allergy, independently from any medical diagnosis?	4			
6. Did you ever use anti-allergic drugs (antihistamines, topical steroids, ''allergy vaccines'')?	3			
7. Is there any allergic subject in your family?		3	2	1
8. Have you frequently red eyes with tearing and itching?	2			
9. Do you frequently sneeze, have a running, itchy nose (apart from colds)?	5			
10. Did you ever feel tightness of your chest and/or wheeze?	2			
11. Have you ever had itchy skin eruptions?	2			
12. Have you ever had severe allergic or anaphylactic reactions?	1			
13. Have you ever had shortness of breath, cough and/or itching of the throat following exercise?	2			
15. Have you ever had allergic reactions to foods?	2			

(Bonini *et al*., 2009)

APPENDIX 12: Recommended portions for Italian and Spanish population according to their dietary guidelines

Food group	*LARNs*	*SENC*
Pasta/rice	80 g	60-80 g
Bread	50 g	40-60 g
Egg	1 (50 g)	1 (50 g)
Red and white meat	100 g	100 - 125 g
Cheese	100 g	80 – 125 g
Legumes	50 g	60-80 g
Vegetables	200 g	150-200 g
Seasoning oil	10 ml	10 ml
Butter	10 g	Occasional and moderate consumption
Fresh fruit	150 g	120 - 200 g
Dried fruit	30 g	20 – 30 g
Wine	125 ml	100 ml
Sugar	5 g	Occasional and moderate consumption
Honey/jam	20 g	Occasional and moderate consumption
Snack/chocolate	30 g	Occasional and moderate consumption

LARNs: Reference Intake Levels of Nutrients and Energy for Italian population; SENC: Spanish Society of Community Nutrition