

The Moli-Sani Project, a randomized, prospective cohort study in the Molise region in Italy; design, rationale and objectives

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Abstract

Background: Cardiovascular disease and malignancies account for more than 70% of all causes of mortality and morbidity in Italy.

There is a subtle balance between genetic determinants and lifestyle, that often defines the line between health and sickness. So far studies aiming at identifying risk factors have mainly come from Northern Europe and the USA. It was to understand this balance between genetics and environmental determinants better, and to tailor appropriate preventive strategies for Italian and other Southern European populations, that the Moli-sani project was launched, transforming a small Italian region into a large scientific laboratory: the “Molise lab”.

Methods: Each participant receives a thorough medical check-up at no cost to either him/her or the national health service, resulting in thousands of hours of free public health care. With a completely computerized system, Moli-sani is a “paperless” study, in which researchers and participants communicate using recently developed technologies such as mobile phone text messages (SMS). The newly established biological data bank (the “MoliBank”) will be one of the largest in Europe.

Results: Early results of 8000 participants so far show a slightly worrying pattern of risk factors for a Mediterranean population. The prevalence of obesity, hypertension and metabolic syndrome is growing at rates close to those of other Western countries. Hypertension and, to a lesser extent, high blood glucose levels, are under-diagnosed and underestimated by patients, and appropriate therapy appears under-used and not very successful. However, cholesterol management appears more efficient.

Conclusions: Paying particular attention towards innovation and new technologies, the Moli-sani project has placed itself at the cutting edge of a new paradigm crossing research and prevention.

Key words: cohort study, cancer, cardiovascular disease, biobank, prevention

Introduction

Cardiovascular disease and malignancies are leading causes of morbidity and mortality in the developed world [1-3]. In Italy cardiovascular disease accounts for about half of the deaths and 80% of chronic disabilities [4]. The incidence of malignancies is steadily rising, mainly because of changes in life-style and the spread of carcinogenic environmental factors [5-6]. Identifying major individual risk factors for atherothrombotic and malignant diseases is therefore a fundamental challenge for formulating strategies aiming at tailoring prevention and therapy [7-9]. Data are already available but cannot simply be transferred

or applied as such to Italian and - more in general - Mediterranean populations because:

1. The majority of studies on the identification of risk factors for athero-thrombotic diseases and malignancies have been conducted either in Northern Europe or in the United States.
2. Italy only appears in a few studies (such as MONICA, EPIC: 3,4,8,9) and usually only a small number of Northern Italian regions are included.
3. New therapies have been developed during the last ten years (statins, ACE-inhibitors) to manage specific risk factors (blood lipid disorders, hypertension) and prevent cardiovascular disease [10-11], but data are scant on their use and



- efficacy in the general Italian population [12-15]. Very little is known on which “new” risk factors explain the rest of risk in the general population.
4. Life-style habits vary largely within Europe and even between the northern and southern regions of Italy [16]. This difference might help explain differences in compliance to therapeutic regimens, whose efficacy has been mainly tested in Northern European populations.
 5. “New” risk factors, such as genetic risk factors, have rarely been considered in evaluations of risk for atherothrombosis and malignancies in the general population [17-20]. They too may vary within Italy and Europe as a whole [21-22].

Between two elements, genetics on one hand, lifestyle on the other, a complex equilibrium does exist, often setting the border between health and sickness. To know it better means to give new weapons, often crucial, in the hands of the doctors and their patients. It also means to adjust therapies, to find out which drug is good for a patient and which prevention strategy will work better for him/her. To better understand the equilibrium between genetics and environment, and its consequences on cardiovascular and cancer disease, the Moli-sani project is transforming an Italian region into a scientific laboratory. It is the “Molise Lab”.

The Moli-sani project was designed to overcome these difficulties by studying a “strategic” population, in this case people living in the Molise region. This is a privileged location within Italy and Europe: it is right in the heart of Italy and can be considered a homogeneous region, both culturally and genetically. In Molise, farms and small-medium enterprises are evenly represented throughout the region. The region boasts mountains and the seaside and they are all quite close. Lifestyles are those of the traditional areas of southern Italy: dietary habits in the Molise region can be considered “Mediterranean”. Finally, the rate of immigration remains at almost zero.

For all these reasons, the Molise region is an obvious candidate for a study of population epidemiology: in a relatively small area, all genetic/environmental factors can be evaluated as risk factors for both atherothrombotic diseases and malignancies. An integrated strategy like this, studying both cardiovascular and cancer risk factors in the light of their relationships and mutual influences, also offers a powerful tool for exploring the concept of “common soil” for these diseases [24,25], an approach that is attracting more and more attention worldwide.

A fundamental role in the Moli-sani project is the biological data bank (the “Moli-bank”). At the end of

recruitment, this state-of-the-art infrastructure will contain 700,000 biological samples preserved in liquid nitrogen. This “treasure chest” will serve as the basis not only for already planned tests and analyses; it will also give researchers, from our laboratories or other institutions, opportunities to develop new concepts and ideas.

From a practical point of view, prevention based on existing knowledge can already be put in place, with the potential for immediate effect, albeit moderate, on morbidity and mortality. However, it is often difficult to reach the general population effectively with early identification and intervention strategies, especially people with risk factors that have not yet translated into a disease. Lack of time during visits is a major factor limiting effective counseling by GPs [23]. In addition, GP practice can easily “miss” healthy people who very seldom go to see their doctor. Moreover, national health care systems could easily be overstretched by specific initiatives aimed at reaching a majority of the population with a strategy of early detection of risk factors and tailored interventions for changes in lifestyle [26].

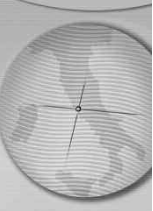
Against this backdrop, the Moli-sani project aims at becoming a new model of interaction between health and research. The time spent recruiting participants (about three hours in ambulatories for each one) is mainly spent collecting information and biological samples for research purposes, but is also useful for prevention. Indeed all participants receive at home, within 10 days from the recruitment, the results of clinical (Electrocardiogram, flow-volume spirometry, Body Mass Index, blood pressure, individual risk score for cardiovascular disease) and laboratory tests (lipid, glucose and CRP levels, hemocromocytometric examen) of current use. Moli-sani investigators avoid to directly discuss results (such as blood pressure levels) with the participants, but encourage all of them, irrespective of their risk situation, to refer themselves to their respective GP.

This is not only a strategy at the individual level: Moli-sani project findings will be very useful for helping regional and national health systems anticipate future healthcare needs in their populations and implement the necessary strategies for prevention and treatment.

Methods

Study population

The cohort of the Moli-sani Project is recruited from about 200,000 persons, aged ≥ 35 years, resident in the Molise region. The latter covers an area of 4,438 Km², including 136 towns. Participants are recruited from city-hall registries by a multistage



sampling. Firstly townships were sampled in three major areas of Molise region, starting from the main cities (Campobasso, Termoli, Isernia) by cluster sampling, then, within each township, participants are selected by simple random sampling, using electronically generated numbers. Exclusion criteria are pregnancy at the time of recruitment, disturbances in understanding or willingness, current poly-traumas or coma, refusal to sign the Informed Consent form. All subjects identified are sent a letter inviting them to participate in the project, followed a few days later by a phone call. The recruitment started in the area of Campobasso, then it will move to the areas of Termoli and Isernia.

Blood pressure and anthropometric measurements

Trained research personnel at our University centre took blood pressure (BP) and anthropometric measurements using methods standardized beforehand during preliminary training sessions. BP was measured by an automatic device (OMRON-HEM-705CP) [27] three times on the non-dominant arm and the last two values were taken as the BP [28,29]. Measurements were made in a quiet room with comfortable temperature with the participants lying down for at least 5 minutes. Body weight and height were measured on a standard beam balance scale with an attached ruler, in subjects wearing no shoes and only light indoor clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Waist circumferences were measured according to the National Institutes of Health, Heart, Lung, and Blood Guidelines [30].

Trained interviewers administered two structured questionnaires to collect personal and clinical information including socio-economic status, physical activity, dietary habits, physio/pathological medical history risk factors for CVD and/or tumor, family/personal history for CVD and/or tumor and drug use.

Definition of risk factors

Subjects were classified as non-smokers if they had never smoked cigarettes, ex-smokers if they had smoked cigarettes in the past and current smokers if they were currently smoking one or more cigarettes per day on a regular basis. Hypertension was defined as systolic BP ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg, or using pharmacological treatment for hypertension [31]. Hypercholesterolemia was considered as cholesterol ≥ 240 mg/dL or using pharmacological treatment to lower blood lipids [32]. Diabetes was defined as fasting glucose levels > 125 mg/dL or current treatment with antidiabetic drugs [33].

The risk of fatal cardiovascular events at ten years was calculated using the CUORE risk equation [34, 35], separately for men and women.

Metabolic syndrome was defined using the ATP III criteria [32]. Depending on CRP levels, participants were classified as at low (CRP < 1.0 mg/L), medium (CRP 1.0 to 3.0 mg/L), or high risk (CRP > 3.0 mg/L) [36].

Biochemical measurements

Blood samples were obtained between the hours of 7.00 and 9.00 a.m. from participants who had fasted overnight and had refrained from smoking for at least six hours. Blood samples were centrifuged for 20 min at 3000 rpm, within three hours. Biochemical analyses were done in the centralized Moli-sani laboratory on fresh samples, using commercial reagents and an automatic analyzer (Instrumentation Laboratory, Milan, Italy). Serum lipids and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350). LDL-cholesterol was calculated according to Friedewald [37].

Statistical analysis

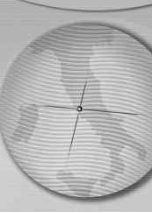
In general, follow-up studies of cardiovascular risk factors should include at least 400 cases to allow enough accuracy of estimation [38]. Estimated annual incidence for coronary and cerebrovascular events in a southern region of Italy is 0.35% and 0.20%, respectively [39,40]. From a cohort of 25,000 subjects, we expect approximately 137 coronary or cerebrovascular events each year of follow-up, and therefore a total of more than 540 events after four years. Altogether cancer has an annual estimated incidence in Italy of 0.60 % [41], therefore we expect 140 new cases/year, for a total of 560 new/year.

Continuous variables were reported as mean and standard deviation (SD). Categorical variables were reported as numbers and percentages.

All computations were carried out using the SAS statistical package (Version 8.2 for Windows. SAS Institute Inc. Cary, N.C.; SAS Institute Inc., 1989).

A paperless study

Moli-sani has been a "paperless" study from the very outset. Recruitment and booking visits, tracking samples, and strict privacy protection are managed by a specially designed software. Questionnaires are computerized too, with another tailor-made software allowing researchers to interview participants in a fully interactive way, including graphic representations of food choices (e.g. size of dishes).



Communication

Constant interaction with participants and the general population is a pillar of the whole project. All the information material, including informed consensus forms, was produced in close cooperation between researchers and our Communication Unit to ensure clear, understandable and comprehensive information. A large-scale communication campaign was organized to boost knowledge and create a positive attitude towards the project. This included newsletters, posters and thematic calendars (designed to provide health information as a "gift" to participants).

Randomly assigned participants received a letter providing full information, including "12 questions on Moli-sani". A couple of weeks later, members of our call centre made personal phone calls to ask whether the recipients agreed to enter the study, providing further information and answering any questions related to the project, then finally booking the visit.

Communication with participants has recently been made easier by a novel approach using mobile phone short text messages - SMS (kindly transmitted to each participant by Vodafone). More in detail, permission to use a personal mobile phone number is asked of each participant at time of the first phone contact. Then an automatic service starts, with a first message containing confirmation of the appointment and a second one, sent two days before the planned visit, reminding time and date. Finally, a third message, sent the day before the scheduled appointment, informs recruits about the requirements to follow in order to participate (fasting, carrying with them drugs they are taking etc.). This system is apparently resulting in a higher rate of compliance and a stricter adherence to the project requirements.

Biobank

The Biological Bank of the Moli-sani project will ultimately contain 700,000 biological samples, stored in liquid nitrogen and protected by the most sophisticated technologies, at the end of the recruitment phase. For each participant, a double batch of 14 "paillettes" each is stored, as follows: 4 paillettes containing EDTA-plasma, 3 citrated plasma, 4 serum, 3 buffy coats for DNA (to be extracted later-on). These samples will allow evaluation of biological and genetic markers linked to inflammation, haemostasis, antioxidant status. Moreover, a genome wide approach with SNPs maps [42,43] is also planned.

A local back-up of these samples is already being performed and a remote one will be

planned at the end of the recruitment for long-term storage. Liquid nitrogen in the tanks is constantly kept under control by an electronic system able to give the alarm to the biological bank staff and to the personnel of the company responsible for management and construction. In this way refurbishing is automatically activated. Electricity interruption might represent a serious danger for the Moli-Bank. That is why the whole Bank is linked to the electricity back-up system of the Catholic University of Campobasso that is able to continuously supply electricity. A batch of totally independent buffer batteries provides a further level of security. Access to the Biobank rooms is kept under strict surveillance; only the system operators are allowed to enter, but not before being identified by a biometric system. In practice, they are recognized by their fingerprint hedged in the memory of the surveillance computer. Finally, thanks to safe data transmission connections, operators are able to intervene from a distance to modify some characteristics of the system in order to immediately detect any anomalies that may occur.

The study was approved by the institutional ethics committee and is run under the supervision of both the Bioethics Institute of our University and the Istituto Superiore di Sanità, Rome. All participants enrolled provided written informed consent to giving blood samples for DNA analysis and biochemical measurements.

Results and Discussion

Since recruitment began, on March 2, 2005 (after a pilot study started on December 10, 2004), people's enthusiastic and widespread participation has been one of the most encouraging results of this study. On April 30, 2007, the Moli-sani project had recruited 10,313 participants, including its pilot phase. The rate of acceptance has so far been extremely high (around 80%).

An interim analysis of the first 8,020 participants showed a sample composition mirroring the Molise population, confirming the quality of the randomization. There are 47% men. Age distribution, education and occupations are shown in Table 1.

With regards to the main common risk factors, smokers account for 25% and 20% of the male and female study population respectively. The percentages of non-smokers and ex-smokers are encouraging (Figure 1). Obesity is relatively high, with 29% of women and 26% of men being overweight (Figure 2).

Other interesting findings relate to hypertension: after exclusion of subjects with previous

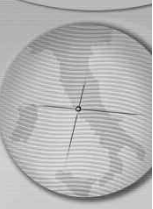


Table 1. Age, education and occupations of the Moli-sani population

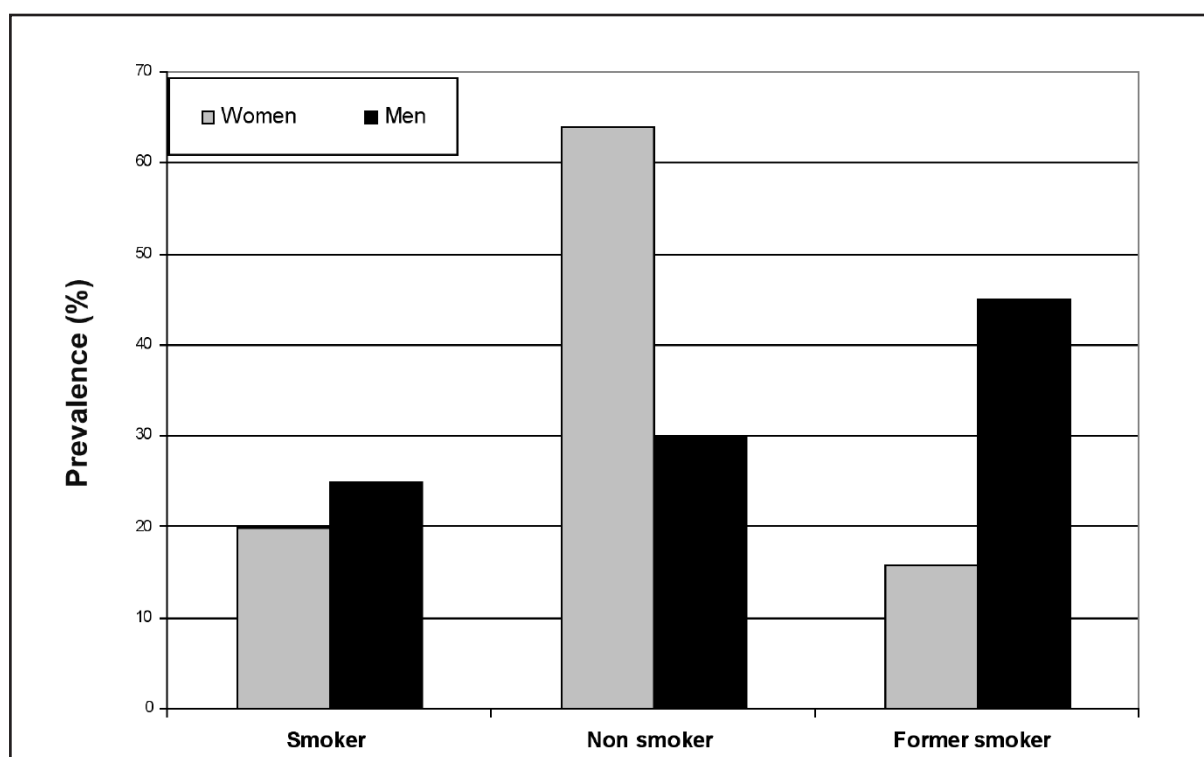
Type of data and unit of measure	WOMEN 4232 (53)	MEN 3 788 (47)
Age <i>years; mean (SD)</i>	55.4 (12.3)	56.1 (12.5)
Age range <i>frequency; n (%)</i>		
35-45	1032 (24)	912 (24)
46-55	1216 (29)	936 (25)
56-65	959 (23)	935 (25)
66-75	700 (16)	701 (18)
> 75	325 (8)	304 (8)
Education status <i>frequency; n (%)</i>		
Low	2074 (49)	1765 (47)
Middle	1524 (36)	1495 (39)
High	626 (15)	523 (14)
Occupation <i>frequency; n (%)</i>		
Manual	571 (13)	879 (23)
Not manual	1170 (28)	1438 (38)
Housewife	1486 (35)	2 (<0.5)
Retired	893 (21)	1335 (35)
Unemployed	38 (1)	60 (2)
Other	67 (2)	66 (2)

cardiovascular disease or diabetes, the prevalence of this condition is quite high (58%), but the most worrying aspect refers to awareness and treatment of hypertension. A considerable percentage of the participants screened were not fully aware of being hypertensive (Table 2). Among those receiving pharmacological treatment, only 17% reached the *gold standard* according to the British Society of

Hypertension [31]. This is very likely due to inadequate compliance with the treatment and some underassessment of the condition, resulting in treatment being less aggressive than is actually needed.

Data on cholesterol are somewhat better. Twenty-four percent of participants reported themselves as being hypercholesterolemic: 23%

Figure 1. Prevalence of smoking habits in men and women of the Moli-sani population



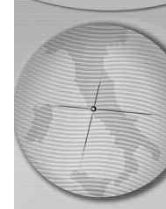


Figure 2. Body mass index distribution in men and women of the Moli-sani population

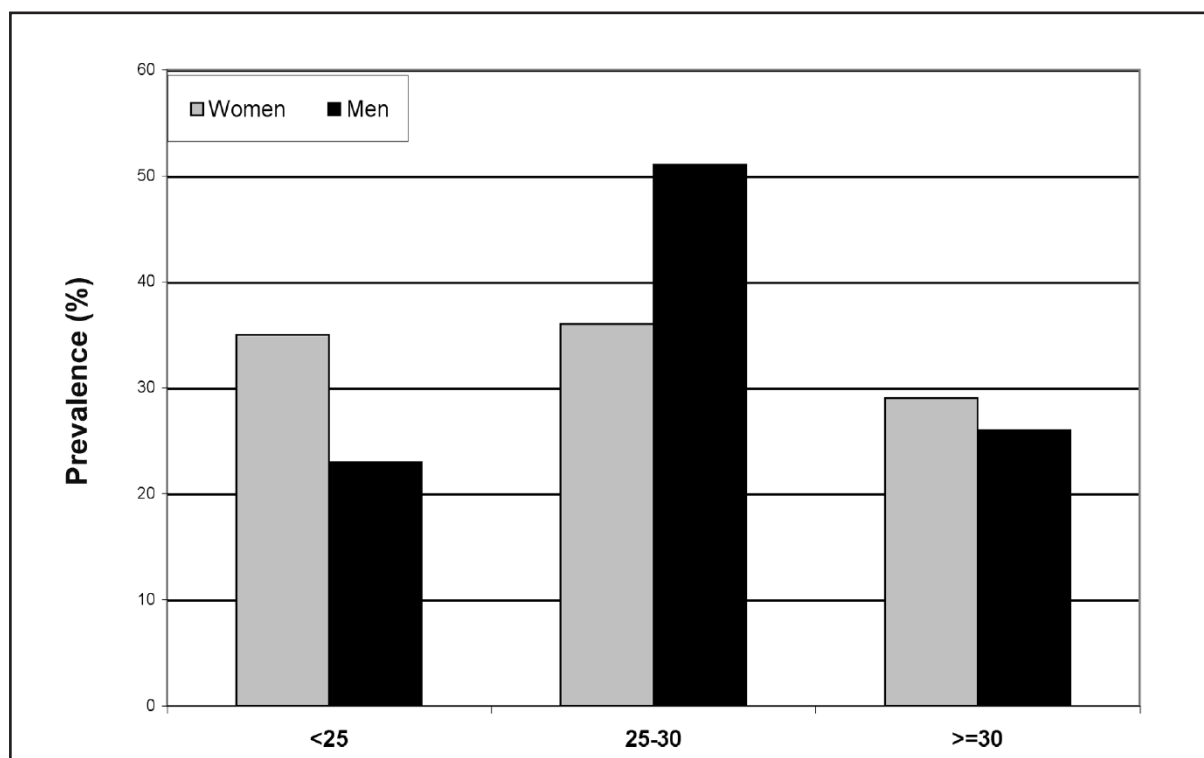


Table 2.

Prevalence, awareness and treatment of hypertension in men and women of the Moli-sani project		
	WOMEN 3920	MEN 3287
Prevalence of hypertension, no. (%)	2076 (53)	2075 (63)
Awareness of hypertension, no. (%)	1141 (29)	927 (28)
Treatment of hypertension, no. (%)	956 (24)	722 (22)
Awareness and treatment of hypertension in hypertensive subjects		
	WOMEN 2076	MEN 2075
Awareness of hypertension, no. (%)	1084 (52)	896 (43)
Treatment of hypertension, no. (%)	956 (46)	722 (35)

Participants with a history of cardiovascular disease or diabetes were excluded

are currently receiving therapy, and 43% are on a specific diet. Sixty percent of drug-treated subjects were under control, so overall management of hypercholesterolemia in this region was good.

Six percent of participants reported they were diabetic: 81% are currently following therapy and 69% are on a specific diet. A surprising proportion of people did not know their glucose levels, although laboratory analysis showed they were border-line or high (Table 3).

Metabolic syndrome had a prevalence of 28% in men and 26% in women.

The cardiovascular risk chart developed by the Italian Istituto Superiore di Sanità within the "Cuore" project [34] was measured in all eligible participants (79%) (Figure 3).

Finally, the most valuable result of the Moli-sani project lies in its deeply rooted interaction with the people of the Molise region - not only those recruited, but the population in general, communities like schools and social institutions. All of these are targets of communication work aiming at disseminating knowledge about a healthy lifestyle and disease prevention.

Conclusions

The interim analysis of the first observational data produced by the Moli-sani project has provided a clear though still incomplete picture of a population that can be considered representative of southern Italy and the Mediterranean area. The Mediterranean diet still appears to be widely followed, especially among

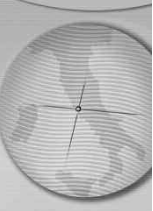
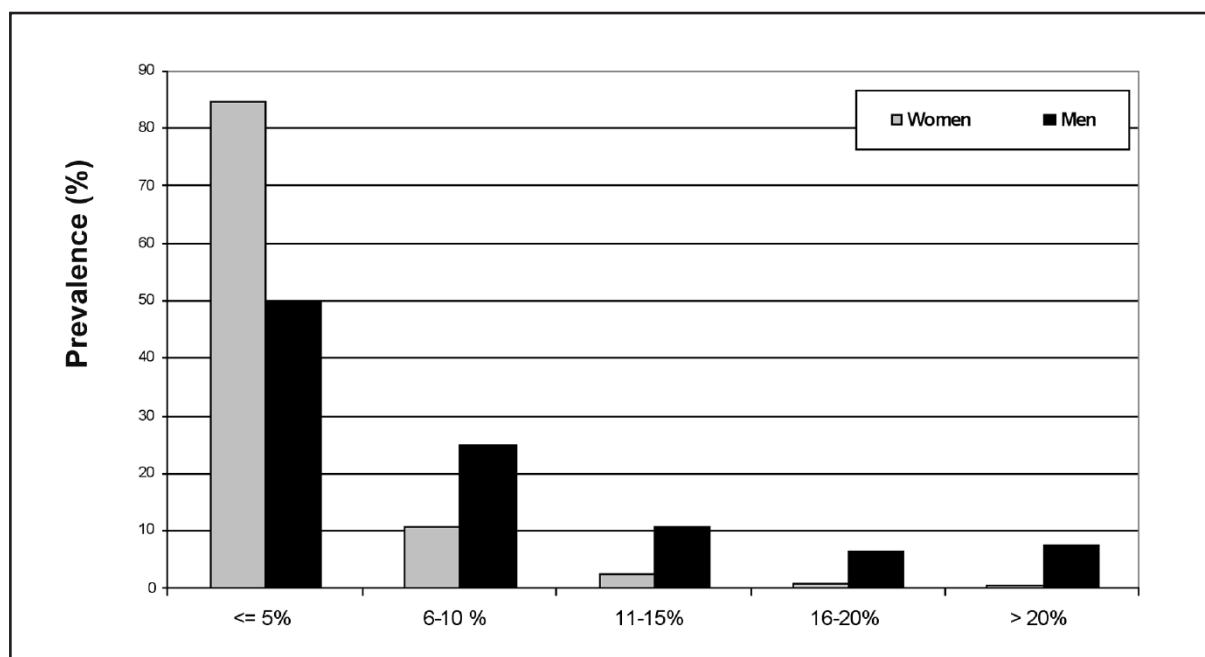


Table 3.

Prevalence and treatment of diabetes in men and women of the Moli-sani project			
		WOMEN 4201	MEN 3773
Prevalence of diabetes, no. (%)		216 (5)	453 (12)
Awareness of diabetes, no. (%)		170 (4)	304 (8)
Treatment of diabetes, no. (%)		132 (3)	240 (6)
Glucose levels in subjects without type 2 diabetes			
		WOMEN 39813	MEN 452
Glucose levels	< 100 mg/dL	3060 (77)	1968 (57)
	100-125 mg/dL	859 (22)	1320 (38)
	> 125 mg/dL	62 (1)	164 (5)

Figure 3. Global cardiovascular risk at ten years for men (2916) and women (3418) of the Moli-sani population



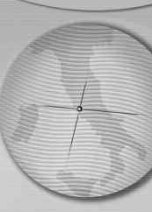
older people who are maybe more tied to the agricultural traditions of the region. But the reduction in manual work over the past few decades, with the consequent reduction in expenditure of calories, is currently causing a positive caloric balance leading to overweight or obesity. This clearly indicates a first, although not simple, suggestion: to maintain Mediterranean eating habits while reducing calorie intake.

While cholesterol appears to be controlled satisfactorily through dietary and therapeutic approaches, hypertension might become a greater health problem in coming years. Possibly for cholesterol an effective message, thanks also to widespread media attention and advertising, has reached the majority of people. Similar results have not yet been obtained for the control of hypertension. Thus, people at risk tend to pay less attention to regular checks, following a low-salt diet, and compliance with prescribed therapies.

This situation, very likely reflecting a nation-wide trend, calls for prompt, strong educational and informative intervention to raise awareness among the general public, GPs and the whole health system.

A similar observation is seen for blood glucose levels. Here too, raising awareness will lead to early diagnosis of impaired glucose tolerance, effectively preventing type 2 diabetes.

The Moli-sani study will reach its maximum potential with the start of the follow-up phase, which should begin within two years. Follow-up will include re-evaluation of health status, new interviews on lifestyle, and tracking intervening clinical events, offering new tools to explore the role of traditional risk factors and identify new ones, in relation to biochemical markers and genetic factors. The "common soil" hypothesis will also be put under scrutiny, looking for risk factors that might play a role in both heart disease and cancer.



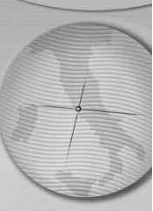
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