Nutrition, Metabolism and Cardiovascular Diseases

CA.ME.LI.A. An epidemiological study on the prevalence of cardiovascular, metabolic, liver and autoimmune diseases in Northern Italy

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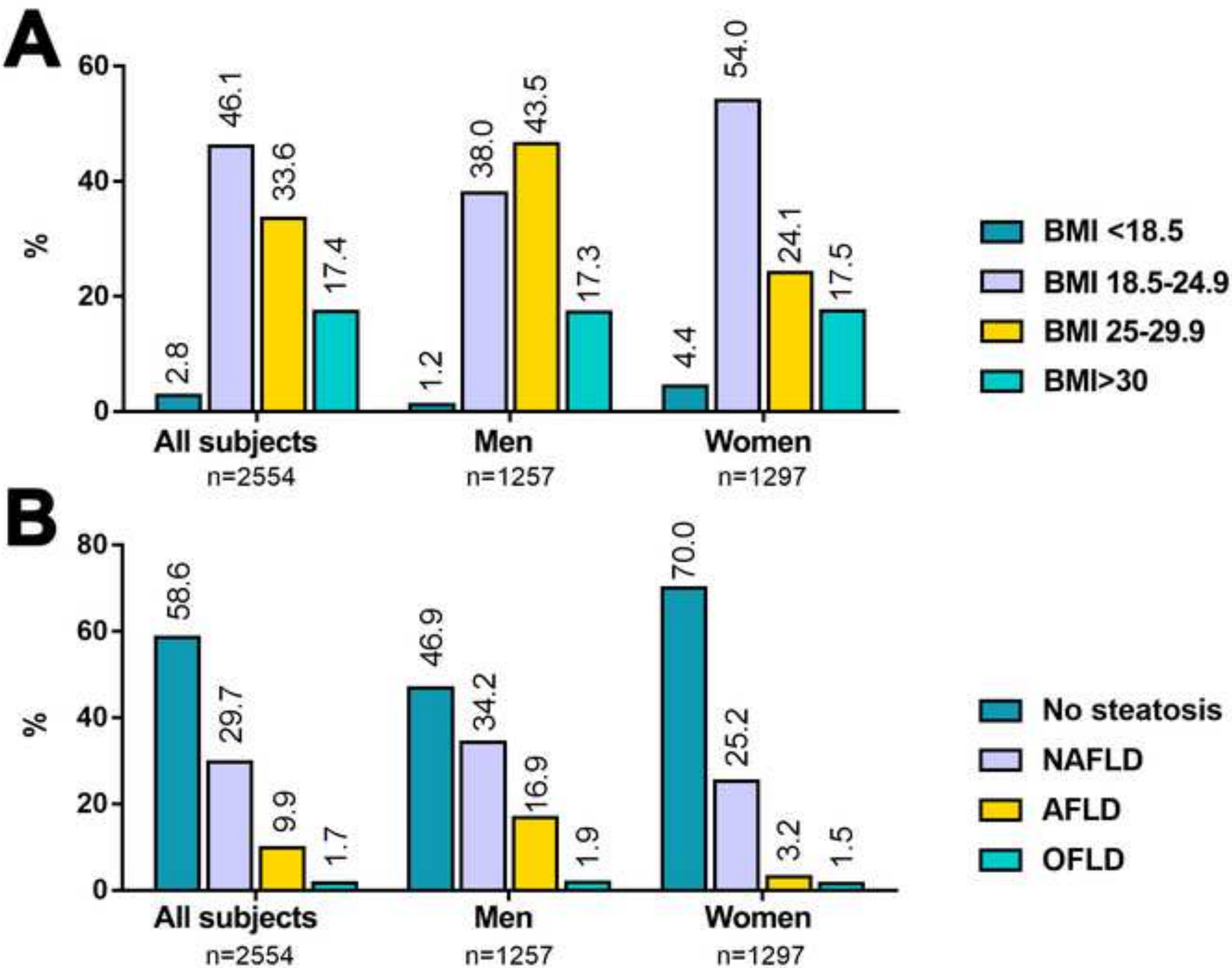
Abstract:	Background and Aims. CA.ME.LI.A (CA rdiovascular risks, ME tabolic syndrome, LI ver and A utoimmune disease) is a cross-sectional, epidemiological study performed 2009-2011 Abbiategrasso (Milan, Italy), to estimate the prevalence of cardiovascular risk factors, metabolic syndrome, liver and autoimmune diseases in the general adult population. This report focuses on the description and presentation of baseline characteristics of the population. Methods and Results. Citizens were randomly selected from the city electoral registers (n=30903), yielding a sample of 2554 subjects (M=1257, F=1297; age, 47±15 yrs; range 18-77 yrs). Men had higher prevalence of overweight or obesity (60.8% vs 41.6%; p<0.0001) and greater thickness of visceral adipose tissue (40±19 vs 27±17 mm; p<0.0001); no gender difference was found in subcutaneous adipose tissue thickness. Men also showed higher levels of serum triglycerides, g-GT, fasting blood glucose, insulin and Homa-IR Index, while HDL, CRP, and prevalence of elevated (>5.0 mg/L) CRP were lower. Compared to normal weight men, risk-ratio (RR) of CRP elevation was 1.32 (ns) in overweight and 2.68 (p<0.0001) in obese subjects. The corresponding figures in females were 2.68 (p<0.0001) and 5.18 (p<0.0001). Metabolic syndrome was more frequent in men (32.7% vs. 14.5%; RR: 2.24, p<0.0001). Interadventitia common carotid artery diameter was higher in men and increased with age and BMI. Conclusions. The present study reports on the overall characteristics of a large population from Northern Italy. It aims to identify the associations among cardiovascular risk factors to prevent their development and progression, improve

	healthy lifestyle, identify subjects liable to pharmacological interventions.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n=1257
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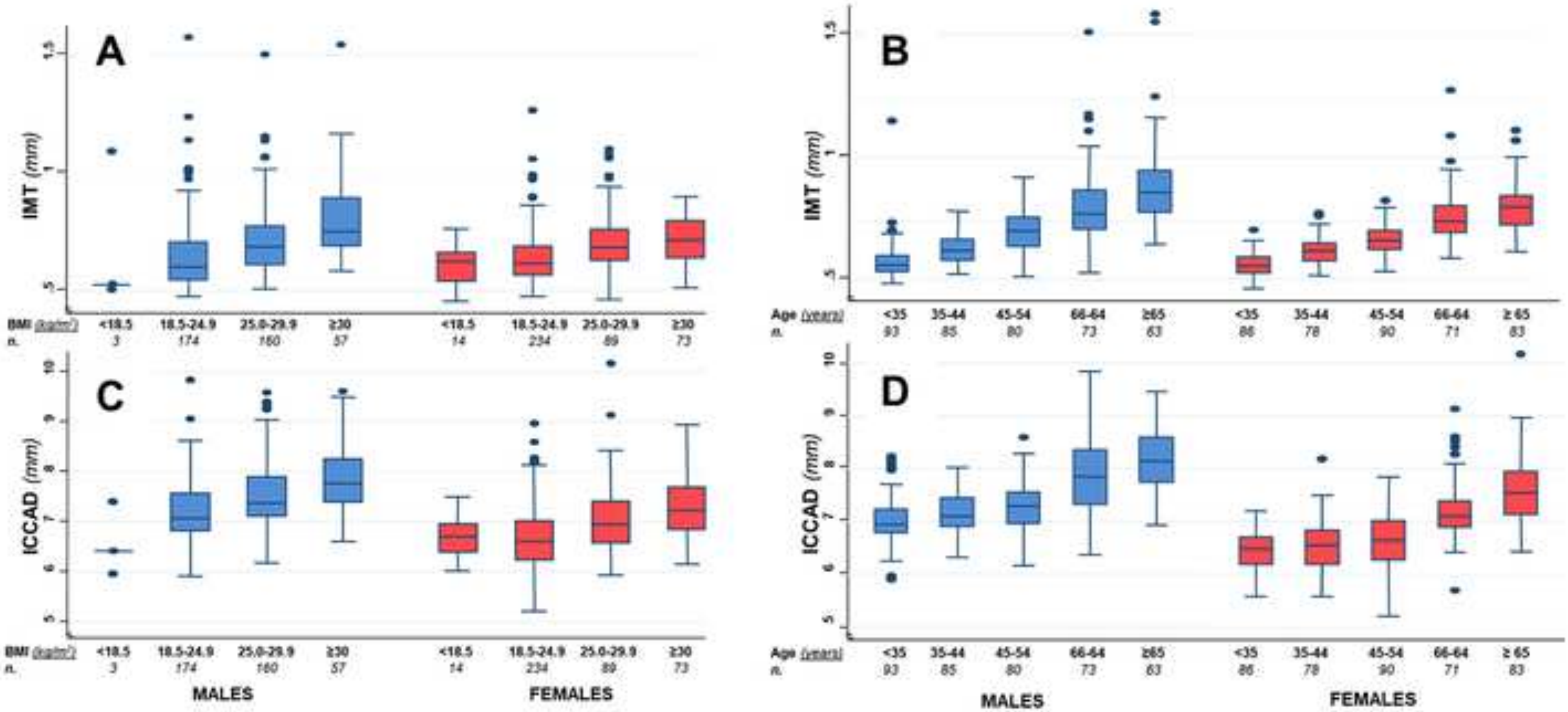
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IMT (mm)
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Age (years)
<35
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≥65
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MALES
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ICCAD
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UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI
SCIENZE DELLA SALUTE

Milan, 29/01/2021

Giovanni Targher, MD, PhD
Professor of Endocrinology and Diabetology
University of Verona, Dpt. of Medicine
Co- Editor
NUTRITION, METABOLISM & CARDIOVASCULAR DISEASES

RE: manuscript # NMCD-D-20-01282

Dear Prof Targher,

Please find here enclosed the revised version of the manuscript "CA.ME.LI.A. An epidemiological study on the prevalence of cardiovascular, metabolic, liver and autoimmune diseases in Northern Italy Nutrition, Metabolism and Cardiovascular Diseases" ref. NMCD-D-20-01282.

We would like to express our gratitude to you and the reviewer for the extremely helpful comments and for your guidance in the revision. We think that we have addressed all concerns of the reviewer, and we believe that you will appreciate the changes that we have made in response to the comments.

Moreover, as you kindly suggested, we reformatted the highlights with no more than 85 characters and each authors signed the ICMJE Conflict of Interest form.

Now, we hope that our revised manuscript could be accepted for publication in Nutrition, Metabolism and Cardiovascular Diseases.

Thank you again for consideration of our revised manuscript and I look forward to hearing from you at your earliest convenience.

Sincerely yours,

--

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Italia

CA.ME.LI.A. An epidemiological study on the prevalence of cardiovascular, metabolic, liver and autoimmune diseases in Northern Italy

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Keywords: Study population**;** Diabetes; Cardiovascular risk; Metabolic Syndrome; Obesity; Liver disease; epidemiology

Abbreviations: fatty liver disease, FLD; non-alcoholic liver disease, NAFLD; alcoholic steatosis, AFLD; steatosis due to other causes, OFLD; C-reactive protein, CRP; thyroid-stimulating hormone, homocysteine, hCys; TSH; carotid intima-media thickness, C-IMT; confidence intervals, CI; Body mass index, BMI; underweight, UW; normal weight, NW; overweight, OW; Obese, O; interadventitia common carotid artery diameter, ICCAD;

Abstract

Background and Aims. CA.ME.LI.A (CArdiovascular risks, MEtabolic syndrome, LIver and Autoimmune disease) is a cross-sectional, epidemiological study performed 2009-2011 Abbiategrasso (Milan, Italy) to estimate the prevalence of cardiovascular risk factors, metabolic syndrome, liver and autoimmune diseases in the general adult population. This report focuses on the description and presentation of baseline characteristics of the population. **Methods and Results.** Citizens were randomly selected from the city electoral registers (n=30903), yielding a sample of 2554 subjects (M=1257, F=1297; age, 47±15 yrs; range 18-77 yrs). Men had higher prevalence of overweight or obesity (60.8% *vs* 41.6%; p<0.0001) and greater thickness of visceral adipose tissue (40±19 vs 27±17 mm; p<0.0001); no gender difference was found in subcutaneous adipose tissue thickness. Men also showed higher levels of serum triglycerides, γ-GT, fasting blood glucose, insulin and Homa-IR Index, while HDL, CRP, and prevalence of elevated (>5.0 mg/L) CRP were lower. Compared to normal weight men, risk-ratio (RR) of CRP elevation was 1.32 (ns) in overweight and 2.68 (p<0.0001) in obese subjects. The corresponding figures in females were 2.68 (p<0.0001) and 5.18 (p<0.0001). Metabolic syndrome was more frequent in men (32.7% vs. 14.5%; RR: 2.24, p<0.0001). Interadventitia common carotid artery diameter was higher in men and increased with age and BMI. **Conclusions.** The present study reports on the overall characteristics of a large population from Northern Italy. It aims to identify the associations among cardiovascular risk factors to prevent their development and progression, improve healthy lifestyle and identify subjects liable to pharmacological interventions.

1. INTRODUCTION

Over the last six decades, the study of cardiovascular epidemiology has improved our understanding of the pathogenesis of cardiovascular diseases with the identification of several major risks and the development of strategies for their prevention and treatment [1]. While Framingham Heart Study remains the most popular one, many other studies followed in the United States [2], North and South Europe [3,4], Asia [5], Middle East [6], Latin America [7] and Italy [8–10]. Some Italian population studies estimated the prevalence of diabetes in specific areas of Northern Italian regions [11–13], or the correlation between glucose tolerance and non-alcoholic liver disease (NAFLD) [14]. Another one investigated the correlation between liver enymes and metabolic syndrome [15]. CA.ME.LI.A (CArdiovascular risk, MEtabolic syndrome, LIver disease, Autoimmunity) is a population study specifically designed to identify a cohort representative of a Northern Italy population the metabolic and clinical risk factors for cardiovascular and liver diseases. This paper describes the study design and presents the baseline characteristics of the study population.

2. METHODS

2.1 Description of the CA.ME.LI.A Study

CA.ME.LI.A is an epidemiological study that took place between 2009 and 2011. It was carried out under the patronage of the Municipality of Abbiategrasso, and the financial support of Istituto Superiore di Sanità, Rome, Italy, and Regione Lombardia. Its main goal was to investigate the associations between cardiovascular, metabolic, hepatobiliary, and autoimmune diseases in a relatively large sample population. This population was specifically selected to be representative of Northern Italy based on economic, social, and cultural characteristics. The cohort consisted of adult inhabitants (age 18-77 years) chosen in the Municipality of Abbiategrasso (MI, Lombardy region), a medium-sized town that on December 31, 2006 counted 30120 residents. The characteristic of this community is an economy based predominantly on agriculture, manufacturing, and service. Our choice fell on the basis of the relative demographic stability of the town, as assessed through the migration flows (immigration rate about 14%) in the previous decade.

The CA.ME.LI.A project consisted of two phases: a cross-sectional study (CA.ME.LI.A 1) and a longitudinal study (CA.ME.LI.A 2). CA.ME.LI.A 1 lasted about 28 months (from May 5th, 2009 to September 30th, 2011) after an information campaign for the population, with a 1st level survey, followed by a 2nd level survey when clinically indicated. “CA.ME.LI.A 1 comprises four different sections aimed at different objectives. **Supplementary Table S1** describes in details the sections of the project and the related investigations, and they were: (1) the cardiovascular (CV) risk factors caused by inflammatory, autoimmune and metabolic factors; (2) the sleep disorders correlated with CV risk; (3) presence of liver diseases and association with metabolic risk factors and (4) the establishment of a biobank to store the serum and plasma samples of people recruited to allow future researches on elements of emerging relevance in the longitudinal phase of the study.

The CA.ME.LI.A 2 study aims to obtain follow-up data on the patients enrolled in CA.ME.LI.A 1 cross-sectional study. All the general practitioners of Abbiategrasso were preliminarily informed of the nature of the CA.ME.LI.A studies during specific meetings. They agreed to provide information concerning all cardiovascular, autoimmune, and neoplastic events in the subjects enrolled during CA.ME.LI.A 1.

We also took advantage of the administrative database of the inpatient population in Lombardia that includes information on all patients discharged from any hospital in the region: sex, date of birth, discharge diagnoses based on the WHO International Classification of Diseases 9th Edition, Clinical Modification (ICD-9-CM, **Supplementary Table S2**), dates of hospitalization and discharge, date and cause of death of participants who died in hospital.

Italian citizens enjoy universal income tax-financed healthcare, enabling access to diagnostic and therapeutic procedures in public hospitals after the charge of a small co-payment, both in public hospitals and in those operating within the national or regional healthcare service. Subjects affected by chronic conditions, including CV diseases, can obtain a disease-specific exemption code that frees them from any co-payment. Thus, the number of affected patients who did not request the exemption code was almost nil. Therefore, we used the exemption code to supplement the inpatient registry since it allowed the identification of patients with a specific disease, which never required hospitalization.

Finally, data tracking the vital status of residents are continuously updated by the Central Registry Office of the Lombardia Region: for the purposes of the CA.ME.LI.A 2 study, such data were obtained relatively to the period between enrollment of each subject (May 5th, 2009 - September 30th, 2011) and the date of study termination, i.e., after ten years from the enrollment of the last patient August 31st, 2017. Results of the CA.ME.LI.A 2 study will be the object of a separate report. The details on the location and the staff involved in the study are provided in **Supplementary Materials**.

2.2 Organizational Planning and Subjects

An information campaign aimed at the local population was carried out in the three months preceding the beginning of the project. Epidemiologists of the Italian Istituto Superiore di Sanità, made the enrolment based on the electoral lists and convocation of patients in Abbiategrasso. At the time of enrollment, the number of residents in the Municipality of Abbiategrasso was 30903 (data on December 31, 2006), and 20731 were aged 18-77 years. According to a stratified randomization procedure, one out of six was randomly selected according to age (5-year classes) and sex. They were contacted through two letters delivered at home (30 and 7 days before the convocation). Each letter reported the aims of the study and the day of convocation. The selected subjects were asked to attend in fasting conditions, without smoking or taking coffee in the previous hours, and were asked to bring a list of the medications in use with them. This way, 3650 inhabitants aged 18-77 were selected, and 2554 (1257 men and 1297 women) gave written informed consent to participate in the study and underwent first level exams (**Figure S1**). Both healthy subjects and patients suffering from diseases such as diabetes, hypertension, and metabolic syndrome were enrolled. Patients with liver disease at the time of recruitment or subjects receiving a diagnosis of alcoholic steatosis during first level investigations were excluded from the CA.ME.LI.A 2, longitudinal study. Recruitment took place between May 2009 and September 2011. Among those who agreed to participate in the study, about 50 subjects per week were contacted for the medical examination. The follow-up period, which ended in August 2017, had a median duration of 7.4 years. The maximum duration of follow-up and total follow-up were 8.3 years and 15568 person-years, respectively. During the follow-up, 32 people (1.25%) were lost.

2.3 Medical Examination

2.3.1 First level screening exams

A food questionnaire (about 90 questions on the subject's eating habits) and a general questionnaire (240 questions regarding social status, work activity, known diseases, and family history, risk factors for cardiovascular and metabolic diseases, physical activity, sleep disorders, physiological history, pharmacological history, description of frequency, type and quantity of alcoholic beverages eventually taken, number of cigarettes smoked) were administered.

After this first phase, subjects moved on to undergo the following investigations (**details in Supplementary materials**):

1. abdomen ultrasound scan for (1) evaluating the presence of hepatic steatosis, or any areas of focal steatosis; (2) measurement of the transverse diameter of the aorta in the supra and sub-renal area along with hepatic and portal veins; (3) measurement of visceral abdominal fat thickness; (4) measurement of the subcutaneous and antero-peritoneal fat thickness in the sub-xiphoid and supra-umbilical area [16];
2. measurement of arterial blood pressure and anthropometric characteristics;
3. blood tests: complete blood count with leukocyte formula, liver and kidney function, glycaemic and lipid profile, insulinemia, C-reactive protein (CRP, not evaluated by the high-sensitivity assay), homocysteine, iron metabolism, urate, thyroid-stimulating hormone (TSH), anti-HB core, and anti-HCV antibodies. In the case of positive results to the last two examinations, the viral load and genotype in the case of HCV and the complete antibody and antigenic profile in the case of HBV were automatically determined, as well as the search for HDV-Ab and the amount of HBV-DNA.
4. at the same time, after acquiring the informed consent, an aliquot of whole blood was drawn and kept at -80°C with additional aliquots of serum and plasma;
5. carotid arteries B-mode ultrasonography (on 1/3 subjects by randomization);
6. complete urine test (on 1/3 of subjects by randomization).

Laboratory analyses were performed within one hour of blood collection, in the same location. The reading and analysis of carotid intima-media thickness (C-IMT) data were performed in the Doppler echocardiography Unit of the Monzino Cardiology Center in Milan (Prof. D. Baldassarre, see **Supplementary materials**) [17]. The communication of the first level survey results was sent to the respective general practitioners. In the presence of any clinical or laboratory alteration, even if unrelated to the study, an explanation letter was attached to the investigation results.

2.3.2 Second level screening

The second level medical examinations were conducted on all individuals who, in the first phase of the study (screening phase), reported clinical, instrumental, and hematochemical features requiring further investigations. According to National and International Guidelines, communications and subsequent diagnostic-therapeutic procedures were provided to these subjects by their general practitioners. To this purpose, the hospital of Abbiategrasso was made available to carry out the investigations at the Hepatology/Internal Medicine Day Hospital facilities. The results of second level medical tests were also sent to the general practitioners with a cover letter. The new diagnostic classification of the participants was recorded in the database of the CA.ME.LI.A Project, which could be available for the follow-up phase of the study.

2.4 Statistical Analysis

The results obtained in the present study originated from a database devised explicitly for the CA.ME.LI.A project. Such data were subsequently exported to the Stata statistical analysis software (version 13.0. The STATA Corporation, College Station, Tx. USA). The first clinical events occurring from the date of enrollment to August 2017 and requiring hospitalization were recorded in the computer system of District Milan 1 based on the coding of Hospital Discharge Records (Scheda di Dimissione Ospedaliera, SDO). The compilation and categorization of the SDOs took place based on the ICD-9-CM codes listed in **Supplementary Table S2**. In addition, mortality data were obtained from the Central Registry Office of the Lombardia region. The data concerning clinical events were obtained by one of the Authors, AR, Head of the Epidemiology Unit, Agency for Health Protection of Milan.

Data were expressed as raw numbers and percentages, prevalence ratios, and 95% confidence intervals. Continuous variables were presented as mean±SD, median and range. Differences were assessed using the Mann-Whitney test to compare two groups, or Kruskal-Wallis test, to compare more than two groups. Significant results ($p<0.05$) from multi-group comparisons were further investigated using Mann-Whitney tests to assess which group differed from the others. For each comparison, the significance value was multiplied by the number of comparisons made (Bonferroni inequality method). Differences in proportions were tested using Fisher's exact test or chi-square statistics. Prevalence rate ratios and their 95% confidence intervals (CI) were used to describe the prevalence of a specific finding relative to a reference category.

To estimate risk ratios (RR) or prevalence ratios and simultaneously adjust for the effect of other variables under study, relative risk regression analyses were carried out using a Poisson working model with a robust error variance.

For the analysis of CA.ME.LI.A 2 study data, cumulative proportions of subjects developing a cardiovascular event will be estimated through the Kaplan-Meier approach using the date of enrollment in the CA.ME.LI.A study as the starting point. The final observation time will be considered the first event occurring among the following: *a)* the first cardiovascular event, *b)* the date of death due to cardiovascular causes, *c)* the loss of the subject to follow-up, or *d)* the date of study termination, i.e. 10 years after enrollment of the last patient, for subjects who will complete the entire follow up period. The log-rank test will be used to assess differences in the incidence of events among groups of subjects. To compare subjects among levels of a continuous variable, they will be typically categorized into quartiles. In any case, the association with cardiovascular events will also be carried out by univariate analysis, introducing it as a continuous variable in Cox time-dependent models. The variables significantly associated by either the log-rank test (categorical variables) or a time-dependent Cox model with a single variable (continuous variables) will be introduced in a multivariate Cox time-dependent model to identify those having an independent prognostic value.

The study protocol established that the analyses should be carried out separately in men and women, given the multiple associations between the studied variables and genders.

When prospectively planning the study, it was assumed an incidence of cardiovascular events, or death, equal to 10% after seven years from enrollment. It was calculated that at least 1189 subjects in each group would be necessary. This would give a power of at least 90% to detect a 5% difference in the incidence of events between two groups using the two-sided log-rank test [18]. All analyses were two-sided. Differences with $p < 0.05$ were considered statistically significant.

3. RESULTS

Of the 2554 participating subjects, 1257 (49.2%) were men, and 1297(51.8%) were women. Their age and sex distribution did not differ from that of the general population of Abbiategrasso. The anthropometric characteristics of the study population are reported in **Table 1**. Except for age and IMT_{mean}, substantial gender differences were observed in the distribution of most variables considered.

According to the WHO classification, subjects were classified as underweight (UW), BMI<18.5 kg/m^2; normal weight (NW), 18.5-24.9 kg/m^2; overweight (OW), 25-29.9 kg/m^2; obese (O), ≥30 kg/m^2. The prevalence of OW or O subjects was 60.9% among men and 41.6% among women (p<0.0001) (**Figure 1A and Supplementary Table S3**)**.** Visceral Adipose Tissue (VAT) and Subcutaneous Adipose Tissue (SAT) thickness were greater in men than in women (p<0.0001, **Table 1**).

According to the study protocol, ultrasound (US) measurements of the carotid artery intima-media thickness (C-IMT) and interadventitia common carotid artery diameter (ICCAD) were performed as non-invasive indicators of subclinical atherosclerosis in 1:3 subjects were selected according to a random assignment procedure. Results are reported in **Table 1**. **Figure 2** shows the values of the two measurements in the male and female populations stratified according to BMI **(Figure 2 panel A,C)** or age classes **(Figure 2 panel B,D)**. As expected, both IMT measures were significantly higher in men than in women (p<0.0001). In men, ICCAD showed a progressive increase with age (+16% difference between subjects younger than 34 and those older than 65 years) and BMI (+22% difference between subjects with BMI <18.5 and those with BMI >30). Milder increases were found in women (**Supplementary Table S4 and S5, Figure 2**).

Table 2 reports the baseline biochemical and haematological characteristics. Men showed higher values of serum triglycerides and Gamma-GT (both p<0.0001). Serum liver enzyme levels, except alkaline phosphatase (ALP), were higher in men than in women as were fasting blood glucose, total homocysteine (hCys), insulin levels and Homa-IR Index. Conversely, total cholesterol, HDL cholesterol, and C-reactive protein (CRP) levels (all p<0.0001) were higher in women.

Elevated (>5.0 mg/L) CRP values were found in 11.4% of the overall population, with a higher prevalence in women (13.5% vs 9.3%, p=0.001). In the male population, the prevalence of abnormal CRP was associated with increased body weight: compared to subjects with BMI<25 kg/m^2, the risk ratio (RR) of having an elevated CRP level was 1.32 (0.86-2.04; ns) in overweight males and 2.68 (1.72-4.17; p<0.0001) in obese men. Stronger associations with inflammation markers were found in women, RR being 2.68 (1.86-3.87; p<0.0001) in overweight, and 5.18 (3.71-7.20; p<0.0001) in obese women.

Smoking and drinking habits along with the personal and family health history in the CA.ME.LI.A population, are reported in **Table 3**. Smokers or former smokers accounted for 49% of the whole population, and the prevalence of former smokers was significantly higher in men (p<0.0001). About 60% of the subjects were alcohol consumers, more frequently men (75% vs 43%, p<0.0001). Among alcohol consumers, 73% of subjects (56% men and 89% women) drank less than 15 g of alcohol per day and 27% (44% men and 11% women) drank more than 15 g/day. About 43% of the population reported a sedentary lifestyle (**Table 3**) and 7% reported a previous diagnosis of diabetes (9% men and 5% women; p<0.0001). Cardiovascular disease had been diagnosed in 8% of the population: the overall prevalence of myocardial infarction was 3% and was higher among men (p=0.0006). Ischemic stroke had been diagnosed in 1% of the whole population. The prevalence of hypertension, defined as a systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or anti-hypertensive medication was 23% in the whole population with no gender difference. Women more frequently reported a family history of cardiovascular diseases and hypertension.

Table 4 reports the prevalence of metabolic syndrome and its determinants according to the National Cholesterol Education Program Adult Treatment Panel III criteria [19]. Metabolic syndrome was detected in 23.3% of the subjects and was more prevalent in men (32.7% vs, 14.5%; p<0.0001) with a male to female RR of 2.24 (1.92-2.610; p<0.001). In the 2554 subjects who underwent upper US examination, the detection rate of fatty liver disease (FLD) was 41.3% (**Figure 1B**, **Supplementary Table S6**) and was significantly higher in men (53% vs. 30%; *p*<0.0001). Among the 1056 subjects with FLD, 71.9% had non-alcoholic steatosis (NAFLD), 23.9% alcoholic steatosis (AFLD), while 4.2% of steatosis was ascribed to other causes, including HBV or HCV infection, hemochromatosis, and Wilson disease (OFLD).

Treatment with oral medications was quite limited in the population, with the exception of anti-hypertensive drugs, the use of which was reported by 21.3% of the population (21% of men and 22% of women; ns) (**Supplementary Table S7**).

4. DISCUSSION

This paper describes the general structure of the CA.ME.LI.A project and gives a metabolic snapshot of the Abbiategrasso population, providing the baseline characteristics and identifying sex differences in the main variables associated with cardiovascular risk and metabolic syndrome. So far, no study has yet presented the general characteristics of the project, even if other sector-specific papers based on the CA.ME.LI.A database have been published [20–22].

This paper presents only the cross-sectional data characterizing the population of the study at the baseline. We do not present longitudinal data because the follow-up is still ongoing with the expectation to reach the 10-years observation period in line with other epidemiological studies [23–25].

Smoking and drinking habits of Abbiategrasso population were very close to those reported by the Italian Istituto Superiore di Sanità in 2011 at national level [26,27]. Interestingly, the number of subjects claiming not to drink at all (teetotalers) in our cohort was about 39% (prevalently women), while in Italy it was 29.3% [28].

The prevalence of chronic diseases in Abbiategrasso was in line with the national values: diabetes accounting for 7% in Abbiategrasso (9% men, 5% women) vs. 5.3% in Italy; chronic renal failure was estimated at 8% (9% men, 7% women) vs. 8.3% in Italy (6.6% men and 6.3% women) [29]. About hypertension, data obtained in Abbiategrasso (tot. 23%, 24% men, 22% women) are slightly different from those in the registry of Italian General Practitioners (GPs). In the latter, the prevalence of hypertension shows a growing trend (from 21.0-26.7% between 2005-2013) and it is higher in women (27.4% in 2013) than in men (26% in 2013) [30]. The cardiovascular risk was equal to 24% (27% in men, 22% in women), while in the whole country it was around 23%. The prevalence of subjects who admitted a sedentary lifestyle among Abbiategrasso residents was relatively higher: 43% vs. 30% in Italy [26].

Men, compared to women, displayed higher serum levels of almost all the biochemical indices, except HDL and CRP. In line with the literature [31–33] also in our study women displayed a higher prevalence of altered CRP values (≥5 mg/L) compared to men (13.5% vs 9.3%, p<0.0001), with a strong association with inflammation. CRP is a useful inflammatory biomarker of incident clinical cardiovascular disease, although there is a debate over its role as a causal factor; for example, in the Dallas Hearth Study on 2749 subjects participating the investigation [32], significant race and gender differences were found in the distribution of CRP. Further analysis on our data will allow not only to confirm the differences observed in the Dallas Study, based both on gender and ethnic difference, but also to evaluate the correlation between CRP and BMI, which appears to be stronger than other continuous variables, including age and all measured lipid risk factors. Prospective studies are recommended to better assess the usefulness of CRP as a marker of atherosclerotic cardiovascular disease and cardiovascular risk predictor in women and particularly in type 2 diabetes [33]. Since the end of the CA.ME.LI.A study many novel biomarkers involved, demonstrating the connection among diabetes, inflammation and cardiovascular diseases, have been proposed and validated [34–39]. Many of these will be possibily included in our future studies on the CA.ME.LI.A cohort.

Another important aspect, which will be the subject of further investigation on the data of the CA.ME.LI.A project, is the role of hyperinsulinemia and insulin resistance. In fact, higher plasma glucose and triglycerides, lower HDL cholesterol, increase in both systolic and diastolic blood pressure are closely linked to augmented risk of coronary heart disease, also in nondiabetic individuals [40,41].

Regarding BMI data, those reported in the Istat surveys on the Italian population reported a mean BMI of 25 kg/m^2, borderline and overweight 46.5%, while 10.5% trespass obesity. In Southern Italy, the overweight percentage concerns 60-65% of the population, and obesity 30% [42]. "Mediterranean dietary style" [43,44], together with the absence of a systematic increase in portion size [27], may have been important factors that contributed to counteract the epidemic of obesity in Italy, in comparison with United States (about 35% on the whole population) [46].

The results coming from CA.ME.LI.A are consistent with those mentioned, where the mean BMI in both sexes was borderline (25.8 kg/m^2) with the overall prevalence of overweight and obesity (≥25 kg/m^2) of 51%, 60.9% in men and 41.6% in women (p<0.0001, **Figure 1A, and Supplementary Table S3**). The percentages regarding Italy for gender differences of overweight and obesity (55.6% men vs. 36.8% women) are consistent with the ones detected in Abbiategrasso (ns, p=0.56) and shown in **Table S3** [47]. Overweight is much more common in men than in women, as well as obesity, although to a lesser degree.

Furthermore, overweight/obesity are heterogeneous conditions, and their definition based on BMI alone is insufficient to explain the variability in the onset of cardiovascular and metabolic clinical diseases [48]. In fact, VAT and SAT were also measured in order to better understand if a different distribution of body fat could be associated with differences in morbidity and mortality. VAT, which is a more pathogenic fat depot and generally associated with increased cardiometabolic risk, was higher in men than in women. On the other hand, no gender differences were found in SAT thickness. VAT thickness is more significantly associated with metabolic and cardiovascular risk factors than BMI and high waist circumference [49].

Interesting data also came from the evaluation of the liver and abdomen ultrasound, which allowed to identify that the 41% of participants presented a prevalence of FLD, particularly 53% men and 30% women, while in Italy FLD prevalence ranges are between 20% and 30% [50]. The FLD is generally a manifestation of alcohol consumption but, if present in abstemious subjects (non-alcoholic fatty liver disease; NAFLD), it may constitute an early indicator of the risk of incipient liver diseases and non-hepatic diseases (e.g. diabetes and lipid dysmetabolism, cardiovascular diseases, problems in terms of metabolism) [51–53]. In Abbiategrasso, the FLD was identified in 1/3 of subjects who stated that they were not alcohol users and it was present in 45% of overweight subjects and in 60-70% of obese people. FLD, in fact, is commonly associated with visceral obesity, type 2 diabetes mellitus, dyslipidemia, and hypertension, all components of the metabolic syndrome, so that FLD might be considered an additional component of the metabolic syndrome itself [54].

The ICCAD and IMT values, obtained from carotid ultrasound, complete the scenario of the critical variables for cardiovascular risk. Carotid IMT reveals a structural deterioration of the arterial wall, it is considered a significant predictive marker of generalized atherosclerosis and cardiovascular events in adults, and for this reason, it is often used as a risk predictor for cardiovascular complications in epidemiological studies [55]. ICCAD values could add strength to IMT measurements and may improve risk assessment in asymptomatic individuals despite not many studies consider the prognostic value of ICCAD [56].

In the CA.ME.LI.A population, almost all IMT measurements are significantly different between sex (**Table 1**). Moreover, there is a close-proportionate increase of IMT_{mean} from 18 to 77 yrs (the regular ultrasound aspect of the arterial parade changes: it thickens uniformly, especially in the straight vascular segments) in both sexes (**Figure 2B and Table S5**). This increase is not synonymous with subclinical atherosclerosis but is related to it [57]. The same considerations apply to the relationship between IMT_{mean} and BMI (**Figure 2A, Table S4**).

In our population, ICCAD confirms the information coming from IMT data and shows increased values, about +15% from 18 to 77 yrs, both in men and in women (**Figure 2D, Table S5**), even if the most meaningful variable is the relationship with the increase in BMI (18.5-30 kg/m^2) that accounts for +21.8% in men and +7.4% in women (**Figure 2C, Table S4**).

ICCAD correlates with age, but it is accelerated by the presence of the same risk factors known for atherosclerotic disease. The examination, in fact, allows us to identify the onset of early atherosclerosis: if this will be found in subjects suffering from steatosis, we will have taken a step forward in demonstrating the connection between the two.

A concrete effect of the CA.ME.LI.A project has emerged in several cases. Indeed, for many subjects enrolled in the study medical tests have highlighted the need for more detailed clinical examinations. This was done by developing a personalized diagnostic path, in agreement with the patient's general practitioner. The second level tests involved 14% of the citizens who participated in the study, making it possible to identify pathologies that had not previously been diagnosed.

Strengths

The strengths of the project are multiple: high participation of citizens guarantees an accurate statistical analysis (71% of the enrolled), the subjects were recruited through a randomization process that allowed to have a sample that reflected the entire population, homogeneously distributed by gender and age. Both healthy subjects and patients affected by diabetes, hypertension, metabolic syndrome, and others were enrolled. The data collected during the enrollment of the subjects were complete, precise and accurate, and more importantly, consistent with the larger frame because of little relevance of bias within the findings. Finally, it is worth noting that this study recorded a low number of patients lost during the follow-up.

Limitations

The survival study could be affected by the presence of unregistered alcohol abuse, as it was not possible to determine with certainty what the total intake of alcohol was by the people enrolled. The selected population was purified by all subjects diagnosed with AFLD (alcoholic steatosis) to minimize the possible effect of alcohol abuse on survival estimation,

In 20% of the registered deaths, it was impossible to trace the cause, so they were categorized as "deaths from another cause". It is possible that the hospital discharge card reported the main disease of the subject, without considering that a possible cardiovascular event may have been contributed to the death. It is also possible that some subjects went through cerebral or cardiovascular events but did not go to the hospital, therefore they were not registered in the database. These represent limits to the study because, this way, the number of deaths or cardiovascular events could be underestimated.

Conclusions

In conclusion, with the snapshot of Abbiategrasso, we believe we may provide a good picture of Northern Italy, without taking into account the variability in regional habits such as lifestyle and diet. This study should be considered a starting point for prevention programs aimed at the diseases investigated.

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Tables.

Table 1. Baseline anthropometric and ultrasonographic characteristics in the CA.ME.LI.A study population

	All subjects (n=2554)			**Men** (n=1257)			**Women** (n=1297)			
	Mean ±SD	**Median**	**Range (min-max)**	**Mean ±SD**	**Median**	**Range (min-max)**	**Mean ±SD**	**Median**	**Range (min-max)**	***p***
Age (years)	47±15	47.0	18-77	47±15	46	19-77	48±15	47	18-77	ns
Weight (Kg)	72±16	71	37-142	80±13	78	42-142	64±14	61	37-130	<0.0001
Height (cm)	167±10	167	134-198	174±7	174	151-198	160±7	160	134-182	<0.0001
BMI (kg/m^2)	25.8±5	25	16-53	26±4	26	16-45	25±6	24	16-53	<0.0001
Waist circumference (cm)	92±13	91	43-174	95±12	95	43-174	88±13	86	60-150	<0.0001
SBP (mmHg)	123±18	120	80-230	125±17	122	87-210	120±19	117	80-230	<0.0001
DBP (mmHg)	77±11	78	48-130	79±10	80	50-130	74±11	74	48-120	<0.0001
VAT (mm)	34±19	30	0-100	40±19	38	3-100	27±17	23	0-100	<0.0001
SAT (mm)	13±7	12.5	1-91	12±6	12	1-91	14±7	13	1-83	<0.0001
1stCC-IMT$_{mean}$ (mm) [(a)]	0.71±0.16	0.69	0.45-2.11	0.73±0.16	0.70	0.45-1.70	0.69±0.15	0.67	0.47-2.11	0.003
IMT $_{mean}$ (mm)[(a)]	0.68±0.13	0.65	0.46-1.58	0.69±0.16	0.67	0.48-1.57	0.67±0.12	0.64	0.46-1.27	ns
IMT $_{max}$ (mm) [(a)]	0.98±0.30	0.91	0.57-4.31	1.01±0.33	0.93	0.60-2.96	0.94±0.28	0.90	0.57-4.31	0.006
IMT $_{mean-max}$ (mm) [(a)]	1.10±0.35	0.99	0.63-3.46	1.14±0.37	1.02	0.63-2.69	1.07±0.33	0.98	0.66-3.46	0.004
ICCAD (mm) [(a)]	7.15±0.75	7.07	5.20-10.2	7.45±0.70	7.30	5.90-9.82	6.87±0.68	6.82	5.20-10.2	<0.0001

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; VAT: Visceral Adipose Tissue; SAT: Subcutaneous Adipose Tissue, IMT: intima-media caritud artery thickness; ICCAD: interadventitia common carotid artery diameter.

[(a)] These measurements were carried out by US in 804 subjects (394 men, 410 women) who were selected according to a 1:3 random assignment procedure, according to the study protocol

Table 2. Baseline biochemical and haematological characteristics of the CA.ME.LI.A study population

	All subjects (n=2554)			**Men** (n=1257)			**Women** (n=1297)			
	Mean±SD	**Median**	**Range (min-max)**	**Mean±SD**	**Median**	**Range (min-max)**	**Mean±SD**	**Median**	**Range (min-max)**	***p***
Triglycerides (mg/dL)	108±69	92	25-1079	119±77.6	100	25-815	97± 56.7	85	27-1079	*<0.0001*
Total cholesterol (mg/dL)	205±40	202	80-387	201±39	199	80-369	209±41	206	110-387	*<0.0001*
LDL cholesterol (mg/dL)	136±38	133	40-269	137±32	136	40-268	136±33	131	54-269	*ns*
HDL cholesterol (mg/dL)	55±14	54	24-141	49±11	48	24-98	60±13	59	29-141	*<0.0001*
Gamma-GT (U/L)	29±41	20	5.0-1231	38±51	27	7-1231	21±25	15	5-522	*<0.0001*
ALT (U/L)	27±19	22	2.0-398	32±20	27	5-398	22±17	18	2-259	*<0.0001*
AST (U/L)	25±13	23	10-373	27±14	25	11-373	23±11.96	21	10-237	*<0.0001*
ALP (U/L)	55±20	52	14-475	55±17	53	20-176	54±22	51	14-475	*ns*
Glucose (mg/dL)	97.8±22.3	94	49-372	102±23	96	61-372	94±21	91	49-361	*<0.0001*
Insulin (U/L)	6.4±5.1	5.2	0.2-112.6	7.0±6.0	5.0	1-113	6.0±4	5.0	0.2-40	*<0.0001*
HOMA index	1.7±2.0	1.2	0.1-57	2.0±2.4	1.0	0.2-57	1.5±1.5	1.0	0.1-32	*<0.0001*
Haematocrit (%)	42.3±3.8	42.4	28.1-54.1	44.7±2.8	44.7	34.9-54.1	40±3.1	40.2	28.1-52.5	*<0.0001*
Red blood cell count (n/mm^3)	4.9±0.5	4.9	3.2-7.5	5.2±0.4	5.2	3.8-7.5	4.6±0.4	4.6	3.2-7.1	*<0.0001*
Hb (g/dL)	14.3±1.4	14.4	8.6-18.6	15.2±1.1	15.3	11.1-18.6	13.5±1.1	13.6	8.6-17.8	*<0.0001*
White blood cell count (n/mm^3)	6.7±1.7	6.4	2-27	6.8±1.8	6.5	3.3-26.9	6.6±1.6	6.4	2-14.5	*ns*
Platelet count (n/mm^3)	267±65	259	55-635	252±58	247	74-589	281±67	274	55-635	*<0.0001*
CRP (mg/L)	2.6±4.2	1.3	0.1-63	2.4±3.9	1.2	0.1-51	2.8±4.4	1.0	0.1-63	*0.005*
hCys (µmol/L)	13.9±8.4	12	2.7-135	15.9±10.1	13.4	6-135	11.9±5.7	10.9	2.7-78.3	*<0.0001*

ALP: Alkaline phosphatase; CRP: C-reactive protein; hCys: Total homocysteine

Table 3. Habits, personal disease, and family history in the CA.ME.LI.A study population

		All subjects (n=2554)	Men (n=1257)	Women (n=1297)	*p*
Smoking habits	**Smoker**	652 (26%)	372 (30%)	280 (21%)	*<0.0001*
	Former smoker	596 (23%)	380 (30%)	216 (17%)	
	Non-smoker	1306 (51%)	505 (40%)	801 (62%)	
Drinking habits [(a)]	**Drinker**	1456 (59%)	918 (75%)	538 (43%)	*<0.0001*
	Former drinker	48 (2%)	27 (2%)	21 (2%)	
	Non-drinker	960 (39%)	272 (22%)	699 (55%)	
Life style	**Sedentary behaviour**	1098 (43%)	515 (41%)	583 (45%)	
Personal history of disease	**Diabetes**	173 (7%)	110 (9%)	63 (5%)	*<0.0001*
	Cardiovascular disease	215 (8%)	118 (9%)	97 (7%)	*ns*
	Coronary	86 (3%)	58 (5%)	28 (2%)	*0.0006*
	Cerebrovascular	36 (1%)	19 (2%)	17 (1%)	*ns*
	Peripheral artery	93 (4%)	41 (3%)	52 (4%)	*ns*
	Hypertension	587 (23%)	297 (24%)	290 (22%)	*ns*
	Liver disease	759 (72%)	431 (65%)	328 (84%)	*<0.0001*
	Chronic renal failure	204 (8%)	114 (9%)	91 (7%)	*ns*
	Cancer	100 (4%)	37 (4%)	63 (5%)	*0.01*
Family history	**Diabetes**	673 (2%)	322 (26%)	351 (27%)	*ns*
	Cardiovascular disease	1070 (42%)	496 (39%)	574 (44%)	*0.014*
	Hypertension	1385 (54%)	598 (48%)	787 (61%)	*<0.0001*
	NAFLD	134 (5.2%)	62 (4.9%)	72 (5.6%)	*0.53*
	HBV/HCV infection	732 (29%)	106 (8.4%)	131 (10%)	*0.15*

Drinker was defined as an individual consuming > 24 g/day of ethanol for men and 12 g/day for women.

[(a)] Alcohol drinking habits could be determined in 2464 subjects (1217 men, 1258 women)

Table 4. Metabolic syndrome and its diagnostic criteria in the CA.ME.LI.A study population

	All subjects (n=2554)	**Men** (n=1257)	**Women** (n=1297)	***p***
Metabolic syndrome [(a)]	596 (23.3%)	408 (32.7%)	188 (14.5%)	<0.0001
High waist circumference	950 (37.2%)	346 (27.5%)	604 (46.6%)	<0.0001
Glucose intolerance	752 (49.5%)	486 (38.7%)	266 (20.5%)	<0.0001
Low HDL values	513 (20.1%)	235 (18.7%)	278 (21.5%)	0.0840
High triglyceride values	421 (16.5%)	272 (21.6%)	149 (11.5%)	<0.0001
Hypertension	859 (33.6%)	467 (37.1%)	392 (30.2%)	<0.0001

[(a)] NCEPT ATP-III (National Cholesterol Education Program Adult Treatment Panel III) criteria to define metabolic syndrome as the presence of 3 or more of the following: waist circumference ≥102cm (M) or ≥88cm (F); fasting glucose ≥100 mg/dL, fasting HDL <40 mg/dL (M) or <50 mg/dL (F), fasting triglyceride s ≥ 150mg/dL, hypertension (systolic >140 mmHg, diastolic blood pressure >90 mmHg)

Figures.

Figure 1. (A) Stratification of the CA.ME.LI.A population as a function of BMI classes defined by WHO. **(B)** Stratification of the CA.ME.LI.A population as a function of fatty liver diseases. In the figure, the stratification is represented both for the whole population and separated by sex. The numbers over the column indicate the percentage of subjects within the class. In Tables S3 (BMI) and S6 (liver disease) are reported the numeric values and the statistical significance, which was calculated by Fisher exact test. All the comparisons are significant between men and women except for the prevalence of OFLD.

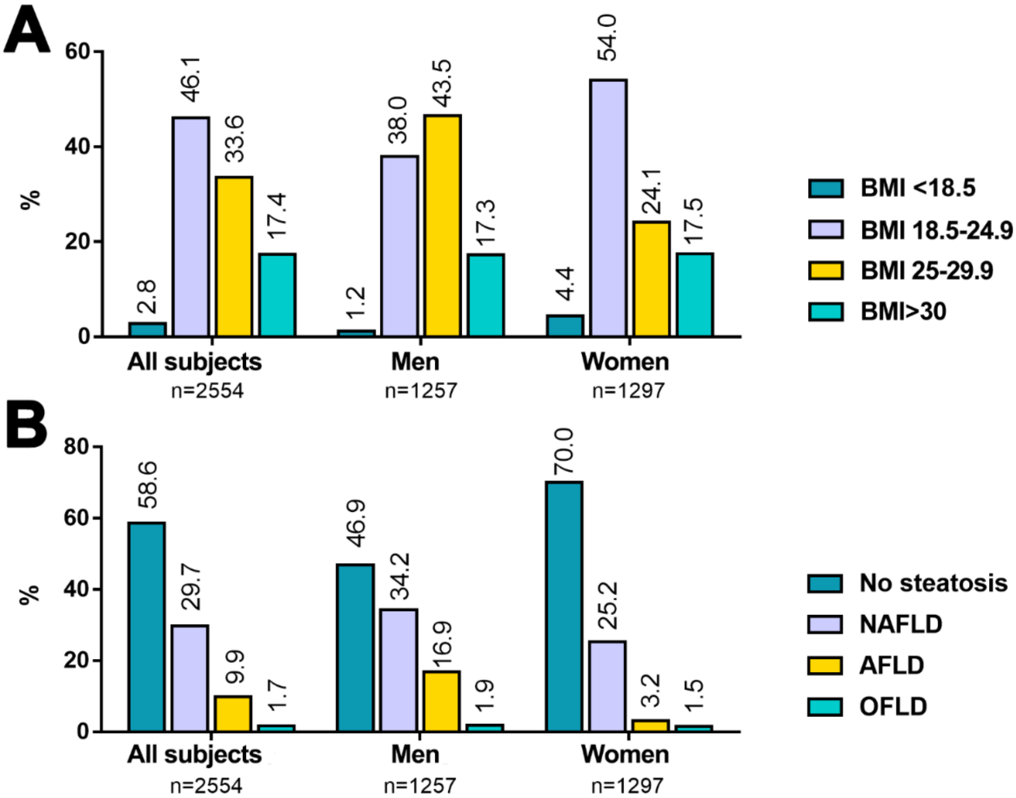
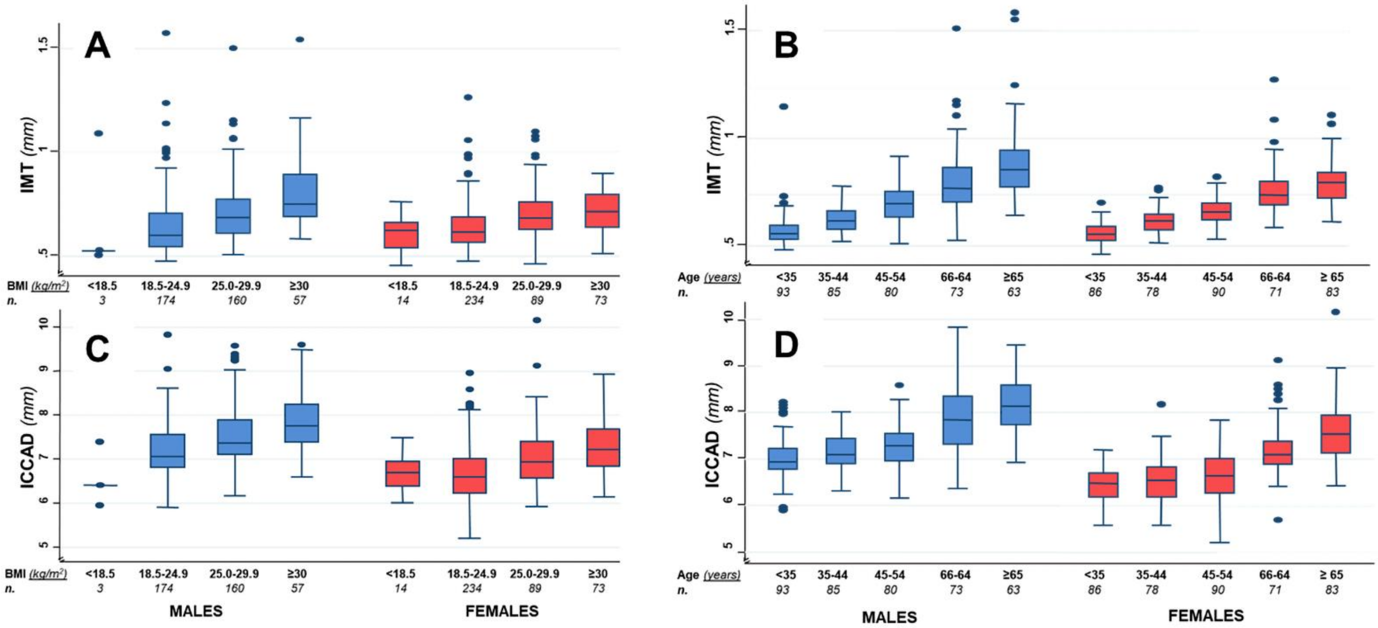


Figure 2. Values of carotid intima median thickness (IMT) and inter-adventitia common carotid artery diameter (ICCAD) according to BMI class **(A,C)** in the male and female population. Values of carotid intima median thickness (IMT) and inter-adventitia common carotid artery diameter (ICCAD) according to age class **(B,D)** in the male and female population.



Reviewer #1: COMMENTS

In this manuscript the authors aim to characterize the prevalence of cardiovascular risk factors, metabolic syndrome, liver and autoimmune diseases in the general adult population participating in the CA.ME.LI.A study, which is an epidemiological study performed 2009-2011 in Northern Italy. I do believe that the paper by Bignotto et al. is well structured and offers an extensive amount of interesting experimental data about the baseline characteristics of a large population from Northern Italy. It's true that this presentation is limited to some associations between metabolic and clinical risk factors for cardiovascular diseases; but the authors claims clearly that many other correlations, stratification and linkage can be further investigated, and they aim to do it in the immediate future. For example, it is worth noting the relationship between the inflammation marker CRP and both gender and BMI.

Reply: We are sincerely thankful for your comments. Please find enclosed the point-by-point rebuttal to your interesting observations.

I have some minor suggestions that might improve the manuscript:

Please the authors should include the figure S1 in the Supplementary Materials.

Reply: We amended accordingly.

Could the authors clarify the last sentence on pag.4?

Reply: We reformatted the sentence you kindly indicated and we checked the language throughout the paper (i.e. line 48, 59,85,91,111,216, 229, 252, 274, 336 marked in yellow).

The authors should elucidate the significance of the statistic tests in Ffigures 1 (panel A,B) and 2. The captions of the figures should also report the association with the Supplementary Tables they derive from.

Reply: We thank the Reviewer for the precious advice. We added in the caption of figure 1 the following sentence: "In Tables S3 (BMI) and S6 (liver disease) are reported the numeric values and the statistical significance, which was calculated by Fisher exact test. All the comparisons are significant between men and women except for the prevalence of OFLD"

The authors should claim that CRP could not be evaluated with the high sensitivity assay.

Reply: Thank you for this comment. Now, it is claimed accordingly in Materials and Methods Section.

In addition, we have fully revised the literature, correcting some mistakes and adding some new and very relevant references.

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Supplementary materials Camelia 2021-01-29.docx

CA.ME.LI.A. An epidemiological study on the prevalence of cardiovascular, metabolic, liver and autoimmune diseases in Northern Italy

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HIGHLIGHTS

- CA.ME.LI.A is an epidemiological study on the population of Abbiategrasso (Italy)
- To identify metabolic and clinical risk factors for heart and liver diseases
- With a randomization criterion (1:6) 1257 men and 1297 women 18-77y were enrolled
- Overweight, obesity, triglycerides, cholesterol, γ-GT, glucose were higher in men
- HDL, CRP and prevalence of CRP >5.0 mg/L were higher in women than in men

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