Università degli Studi di Napoli "Federico II"

Facoltà di Medicina e Chirurgia

Dipartimento di Scienze Mediche Traslazionali

Tesi di Dottorato in

Fisiopatologia Clinica e Medicina Sperimentale

Indirizzo Scienze Cardiovascolari

XXVI Ciclo (2010-2013)

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Impact of Metabolic Risk Factors
on Cardiovascular Phenotype and Blood Pressure Control.
New Predictors of Cardiovascular Disease

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Table of Contents

List of Abbreviations

AF   = Atrial Fibrillation

BMI   = Body Mass Index

BP   = Blood Pressure

CV   = Cardiovascular

DM   = Diabetes Mellitus

eGFR   = estimated Glomerular Filtration Rate

FS   = Fractional Shortening

HDL   = High Density Lipoproteins

IFG   = Impaired Fasting Glucose

IVRT   = Isovolumic Relaxation Time

LDL   = Low Density Lipoprotein

LIFE   = Losartan Intervention For Endpoint reduction in hypertension

LVH   = Left Ventricular Hypertrophy

LVM   = Left Ventricular Mass

MAC   = Mitral Annulus fibro-Calcification

MetS   = Metabolic Syndrome

NFG   = Normal Fasting Glucose

PP   = Pulse Pressure

RAS   = Renin-Angiotensin System

RWT   = Relative Wall Thickness

SV   = Stroke Volume

S   = Study

UACR   = Urinary Albumin-Creatinine Ratio

Scientific Environment

The present project was undertaken in the Department of Translational Medical Sciences at the Federico II University of Naples in Italy. The Research Group is chaired by Professor Giovanni de Simone and currently includes 2 Full Professor, 2 Associate Professors, 2 Assistant Professor and 2 Ph.D. students. Several research medical students and resident fellows cooperate in the research activities.

The Research Group has specialized in cardiovascular epidemiology, with a focus on cardiovascular prevention, through understanding cardiovascular modifications related to the main risk factors, such as diabetes, obesity, and hypertension. The research group facilities includes advanced echocardiographic laboratories with dedicated workstations for post-processing of images and data analyses and outpatients clinics for management of research patients. During my Ph.D., I was introduced to this new world of research by Dr. Marcello Chinali, at the time when he was a postdoctoral fellow and supervisor in the echocardiography laboratory. I had also the opportunity to work closely with Professor Giovanni de Simone together with Professors Bruno Trimarco, Nicola De Luca and Raffaele Izzo on several research projects involving exploration of the large database of hypertensive patients referred to the Hypertension Center of the Federico II University of Naples (the Campania Salute Network).

The Research Group is part of a large international network, embracing several research centres in different countries. This worldwide research network has given me the opportunity to perform research on large databases based upon international research projects, including the Strong Heart Study, the Losartan Intervention For Endpoint reduction in hypertension (LIFE) Study and the Hypertension Genetic Epidemiology Network (HyperGEN) Study. The main international partners are the Department of Cardiology at the Weill-Cornell Medical College in New York, USA (by Professor Richard B. Devereux) and the Department of Clinical Science at the University of Bergen in Bergen, Norway (by

Professor Eva Gerdts). During the course of my Ph.D. I had the opportunity to visit and collaborate with both of them: in New York working with Professor Devereux on the impact of diabetes in adolescents and young adults, and in Bergen working with Professor Gerdts on new echocardiographic risk markers in hypertension. I also had the chance to work closely with Professor Gerdts for one year, during her visit as Guest Professor in Naples in 2011. During this year, I had the opportunity to collaborate with other postdoctoral fellows of the Bergen Hypertension and Cardiac Dynamics group in Bergen, especially with postdoctoral fellow PhD. Mai Tone Lønnebakken who is actually going to spend one year as visiting guest researcher in the Hypertension Research Center in Naples.

I have had an active rule in development of the studies presented in my thesis, by ideating, proposing and submitting these research projects, analyzing data and performing statistical analyses, elaborating and interpreting results and writing manuscripts.
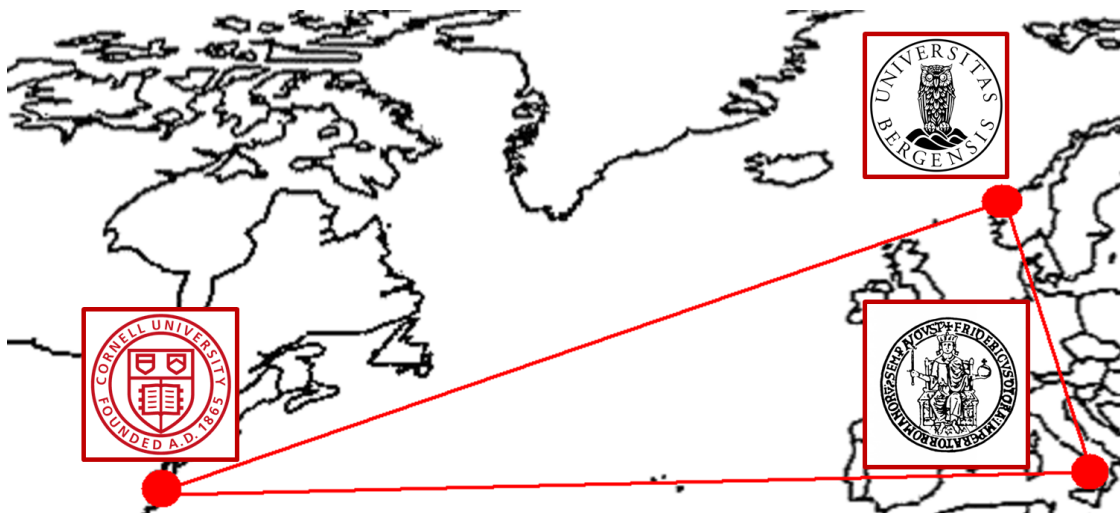
Acknowledgement

Certainly, during my PhD a lot of thing have changed in my life, since I have met many people who have helped me to grow so I feel now much more enriched, thanks to them.

Even if I have already cited them, I'd like to spend few words on my scientific supervisors: Giovanni de Simone and Eva Gerdts. They are quite different persons, but they have a very distinctive aspect in common: their strong passion for research which infects everyone that has the chance to work with them.

A particular thanks to my "magister vitae", Prof. Giovanni de Simone, who has been like a father for me, always supporting and sustaining me and letting me grow up. Thank you immensely for your generosity and protection, for all your teaching, thank you for all opportunities you have created for me (overall the privilege of participating to a such prestigious research team). A special acknowledgement to Professor Eva Gerdts, that has inspired me for her courage, tenacity and enthusiasm, and that has showed me that Norwegians can be warmer than Italians, women can be as strong as men, and work can be funnier than holydays. I am very proud to have been collaborating with such amazing supervisors. Thanks both of you for your great friendship.

I also have to thank all the people who have inspired me and represented an enormous opportunity of collaboration, during my Ph.D. fellowship: Dr. Richard Devereux, Dr. Marcello Chinali, Dr. Barbara Howard, and all researchers I have met in the Division of Cardiology of Weill-Cornell Medical College in New York, at the Department of Clinical Science in Bergen and in the Department of Translational Medical Sciences in Naples. Thanks to my colleagues and friends: Alfonso Sforza, Costantino Mancusi, Daniela Girfoglio, Gabriella Coppola, Giusy Casalnuovo, Silvia Damiano and Teresa Migliore. I have shared with you all the happiness and the pain of these intensive years. Obviously I have forgotten someone, but it's impossible to quote all friends I have met in these three years.

Another special thanks goes to Dr. Anna Maria Ferrari, Director of the Emergency Unit at the Arcispedale Santa Maria Nuova in Reggio Emilia, who has allowed me to

complete my Ph.D. program, and has showed me how the emergency room can be the most intensive and satisfactory job for a medical doctor.

Regarding my personal life, I'd like to thank my family that has always supported me, overfilling me of love. I can say without hesitation, I am very lucky person. Golgi once said: "Discoveries in science are like happiness in life: it comes unexpected", and in fact a lot of unpredictable events ($p<0.001$) occurred to me in these three years, leaving imperishable memory and a treasure of precious emotions in my hearth.

Last, but not least at all, thank you Silvio, there are not enough words to described my love and gratitude for you.

Summary

Background: Cardiovascular (CV) risk factors like obesity, hypertension and type 2 diabetes have increased dramatically over the last decades, also in younger age classes. In hypertension, clustering of metabolic risk factors have been associated with resistant hypertension, but is not known whether this may be overcome by specific pharmacological treatments. Moreover, whether specific metabolic risk factors, like diabetes, are associated with preclinical CV disease also in young age is not well explored. Finally, whether identification of new markers of CV disease by echocardiography, related to these risk factors, might help to further refine the high risk CV phenotype is unknown, though this evidence would be critical to improve risk stratification in the individual beyond current state of the art.

Aims: The main aim of the Ph.D. project has been to evaluate the impact of metabolic risk factors on blood pressure control and CV phenotype, identifying new echocardiographic predictors of CV disease in different populations. Specific sub-goals were:

Study 1 (*S1*) – To evaluate the impact of clustering of metabolic risk factors on blood pressure control in relation to different classes of medications;

Study 2 (*S2*) – To assess the impact of metabolic risk factors, as diabetes and pre-diabetes on preclinical CV disease in adolescents and young adults;

Study 3 (*S3*) – To identify new echocardiographic markers of CV events in treated hypertensive patients with high risk CV profile.

Methods: For the specific scope of the studies we analyzed different populations. Specifically, in *S1* we evaluated the impact of metabolic syndrome on the risk of uncontrolled blood pressure (i.e. blood pressure$\geq$140/90 mmHg under antihypertensive treatment), in relation to specific antihypertensive medications. The analysis was carried out at baseline and after a mean follow-up of 5 years in 4,612 (53$\pm$11 years, 43% women; 25% with obesity, 9% with diabetes) hypertensive patients without prevalent CV disease, referred

to our tertiary Hypertension Center from the Campania Salute Network. In *S2* we evaluated the impact of diabetes and pre-diabetes on cardiac structure and function in 1,624 adolescents and young adults American-Indians (179 with diabetes and 299 with pre-diabetes). These were participants of the Strong Heart Family Study (mean age 27±8 years, 57% female, 66% with obesity, 19% with hypertension) free of prevalent CV disease.

Finally, in *S3* we evaluated the prognostic impact of presence of mitral annulus calcification (MAC) on incidence of ischemic stroke. For this sub-goal we analyzed baseline and 5 years follow-up data from 939 treated hypertensive patients (458 with MAC) with electrocardiographic signs of left ventricular hypertrophy (LVH) (66±7 years, 42% women, 23% with obesity, 11% with diabetes) participating in the Losartan Intervention For Endpoint reduction in hypertension (LIFE) echocardiography sub-study.

Results: In *S1* we found that despite the increased use of medications, hypertensive patients with metabolic syndrome had higher risk of uncontrolled blood pressure, independently of specific therapy. Among classes of medications, increased prescriptions of diuretics, renin-angiotensin system antagonists and also statins decreased the probability of poor blood pressure control.

In *S2* we found that diabetes was independently associated with early and unfavorable CV phenotype characterized by increased left ventricular mass, concentric geometry and preclinical systolic and diastolic dysfunction. Early CV alterations were also present in participants with pre-diabetes.

In *S3* we found that risk of incident ischemic stroke was significantly related to presence of MAC, independently of traditional CV risk factors, as increased left ventricular mass and left atrial diameter, prevalence or incidence of atrial fibrillation and albuminuria.

Conclusions: These results document that: 1) in hypertension, clustering of metabolic risk factors strongly interferes with efficacy of therapy, 2) in addition to obesity and hypertension, diabetes and pre-diabetes have an important impact on cardiac structure and function, even in young age, 3) MAC identifies increased risk of stroke in hypertensive patients with LVH.

List of Articles

S1. De Marco M, de Simone G, Izzo R, Mancusi C, Sforza A, Giudice R, Trimarco B, De Luca N. Classes of antihypertensive medications and blood pressure control in relation to metabolic risk factors. *J Hypertens*. 2012; 30:188-193.

S2. De Marco M, de Simone G, Roman MJ, Chinali M, Lee ET, Calhoun D, Howard BV, Devereux RB. Cardiac geometry and function in diabetic or prediabetic adolescents and young adults: the Strong Heart Study. *Diabetes Care*. 2011; 34:2300-2305.

S3. De Marco M, Gerdts E, Casalnuovo G, Migliore T, Wachtell K, Boman K, Dahlöf B, Olsen MH, Kizer JR, Devereux RB, de Simone G. Mitral Annular Calcification and Incident Ischemic Stroke in Treated Hypertensive Patients: The LIFE study. *Am J Hypertens*. 2013; 26:567-73.

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Introduction

Cardiovascular (CV) disease is the major cause of death and disability in Western countries [1,2], and contributes substantially to the escalating health care costs. The reduction of CV disease burden depends on the ability of physicians and health care systems to implement the control of CV risk factors through effective program of primary and secondary prevention [3]. Lack of control of common CV risk factors like hypertension, obesity and diabetes is together with the aging of Western population the main causes of continuous increase in incidence and prevalence of CV disease [4].

Obesity is increasing in epidemic proportions in industrialized and developing countries, affecting both adults [5] and children and adolescents [6,7]. Obesity predisposes to arterial hypertension and diabetes and there is extensive evidence that these risk factors tend to cluster together amplifying the unfavourable effect of each single risk factor on incidence of disease [8-16], also reducing the efficacy of the treatment [17-22].

In this regard, there is evidence that management of hypertension is particularly difficult when this condition co-exists with obesity and/or clustering of metabolic risk factors [22-25]. Hypertension indeed is often part of a constellation of CV risk factors including obesity, abnormal glucose homeostasis and dyslipidemia, supporting the existence of a discrete disorder, often referred to as the metabolic syndrome (MetS) [26-28]. MetS increases CV risk in the setting of hypertension, even when taking individual risk factors into account [12-14], and reduces the probability of achieving optimal blood pressure (BP) control, despite more aggressive treatment [22-25].

This is a very important health problem, since arterial hypertension is the most prevalent CV risk factor in most populations, and the leading cause for medical consultation and drug prescriptions [29]. It has been estimated that 26% and 28% of incident CV disease in men and women, respectively, are primarily attributable to hypertension [8]. The continuous large impact of hypertension on incident CV disease may be related to the fact

that BP in hypertensive patients is still largely uncontrolled, despite the large number of prescribed medications [30,31]. The risk of uncontrolled BP increases with the number of metabolic risk factors [22], but whether the use of different types of antihypertensive medications influence this association has not been clarified yet.

Figure 1 shows the complex interaction between the CV risk factors, preclinical CV disease with asymptomatic modifications on cardiac and arterial structure and function which precedes clinical CV disease and events. Thus, in addition to the interference on the efficacy of therapy, there is also growing evidence that obesity related CV risk factors, alone or in combination, may have particular adverse impact on development of preclinical CV disease [32-41].

Figure 1 – From risk factors to overt CV diseases through preclinical stage

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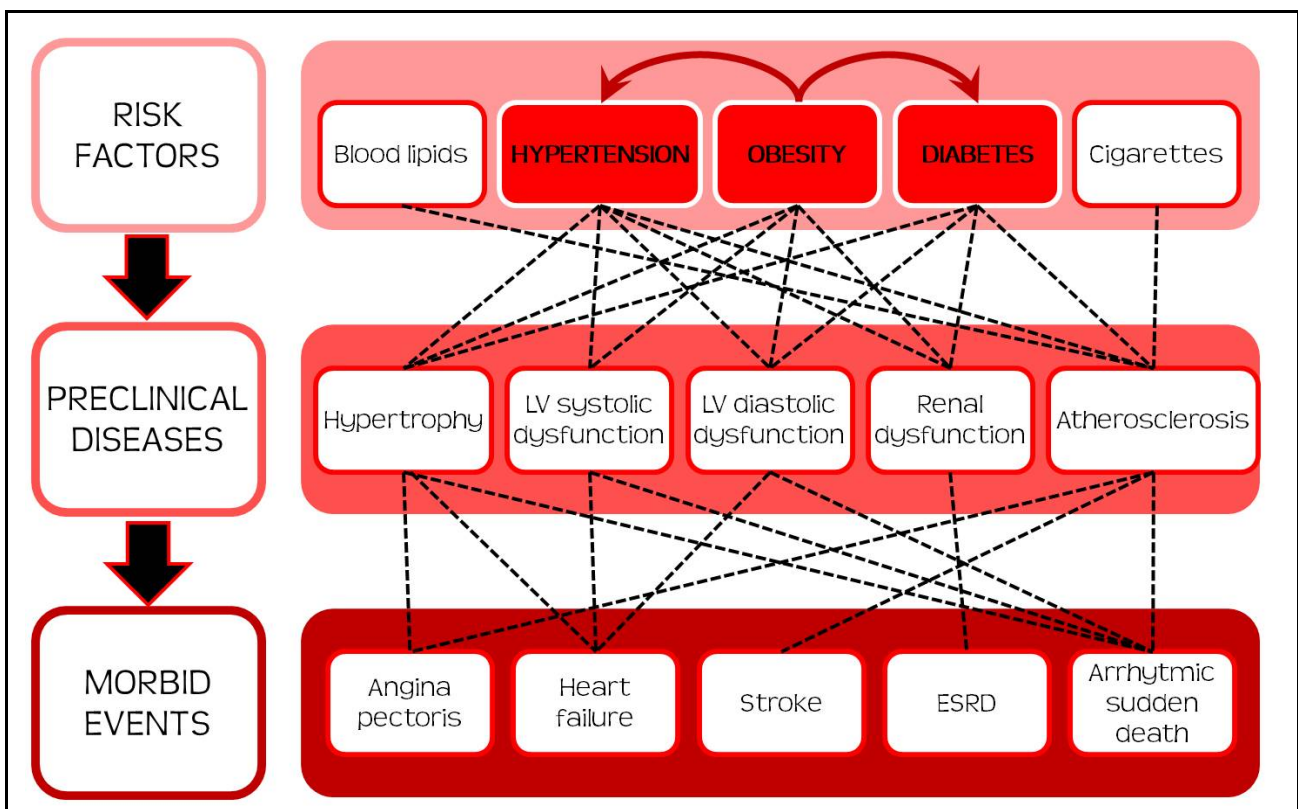
Accordingly, early detection and management of these risk factors and the associated preclinical target organ damage represents the major health strategies in order to prevent incident CV disease and optimize preventive and therapeutic strategies. This is very

important, especially in adolescents and young adults, since the rising prevalence of obesity and associated risk factors among the younger ages is now a major health concern with both epidemiological and economic implications [6,7,42-45]. Early identification of preclinical disease in adolescents and young adults is also of great interest to understand pathophysiological mechanisms related to a specific risk factor, since in general the time of exposure is usually lower compared to adults and also the interference due to other confounding prevalent diseases is lower. Moreover, cardiometabolic risk factors tend to remain stables from childhood to adulthood and are predictive of future CV disease [46-49]. Thus, increasing efforts are needed to identify young individuals at high CV risk by detection early markers of disease to optimize early intensive interventions to prevent or delay future disease [50].

In particular, type 2 diabetes (DM) in young subjects has increased dramatically in the last decade [51], especially in minority populations, like the American Indians [52]. The decline in the age of onset of type 2 DM is driven by the increasing obesity in the younger age group [53,54]. Early onset of type 2 DM is associated with increased risk of CV complications compared to usual onset of the disease [55-61]. Comparison of cardiovascular risk profiles between early (<40 years of age) and later-onset (>40 years of age), showed that a significantly greater clustering of multiple CV risk factors (obesity, hypertension, dyslipidaemia) is more common among early-onset type 2 DM subjects [60,61].

However, part of the increased CV risk may be related to a direct adverse effect of DM on the heart, independently of coronary artery disease, as it has been documented in elderly adults [18, 62-64]. Previous population based studies have shown an early adverse impact of obesity and associated risk factors including hypertension on cardiovascular system in adolescents and young adults [43-45], but the impact of DM and pre-diabetes on cardiac geometry and function in adolescents and young adults has never been targeted in a large population-based samples. Thus, it was unknown whether there might be also an independent influence of DM on CV phenotype at young age.

As shown in **Figure 1**, the overall CV risk attributable to the metabolic risk factors may not only be determined by their presence, but mediated by their impact on CV phenotype. In hypertension, accurate and sensitive assessment of CV risk allows better stratification of the individual risk, and is a key step toward optimizing the management of hypertensive patients

[65]. It has also been proved that identifying and targeting the subset of patients who are at highest risk improves the cost-effectiveness of antihypertensive treatment, for any degree of BP reduction [66].

Identification of new ultrasound markers of preclinical CV disease, might help to further refine identification of high-risk CV phenotype and improve preventive strategies, also in hypertensive individuals with high CV risk based on traditional criteria [65]. Echocardiographic detection of calcification of the cardiac valves has been associated with increased CV risk [67-73]. In particular, fibro-calcification of the mitral annulus (MAC) is often found on the echocardiogram, appearing as a bright echo dense region at the level of the mitral annulus. MAC is an age-related degenerative process [74], involving lipid deposition, fibrosis and calcification of the mitral valve support ring [75]. In hypertension, MAC has been considered an incidental finding, even if it is strongly associated with metabolic risk factors for development of atherosclerosis [76-80]. Thus, MAC may be proposed as an easily measurable barometer of the burden of atherosclerotic disease [79] and its presence may reflect the intensity and duration of exposure to these risk factors over time [77,80]. Whether arterial hypertension in itself accelerates the development and/or progression of MAC through increased mitral valve stress and hypertension associated risk for thrombosis and atherosclerosis is not known. In particular, the independent association of MAC with subsequent risk for incident stroke has never been previously evaluated in treated high-risk hypertensive patients. Previous population based studies in Framingham and in Northern Manhattan in New York, have suggested that the presence of MAC is independently associated with a higher incidence of CV disease and CV death [69,70]. However, to date, its detection does not modify treatment recommendations or alter the intensity of therapies for associated conditions [81] because no extenive data are available on its possible independent CV risk. Specifically, several [71-73] but not all [82], population-based studies, have reported a significant association between MAC and risk of ischemic stroke. However the existence of incremental independent predictive value of MAC above other established risk factors for ischemic stroke has been questioned [73,82]. In particular, the independent association of MAC with subsequent risk for incident stroke has never been previously evaluated in treated high-risk hypertensive patients.

Objectives

The overall objective of this Ph.D. thesis was to assess the impact of metabolic risk factors on the efficacy of antihypertensive therapy and on prevalence and incidence of preclinical and clinical CV disease, evaluating new predictors of clinical disease (**Figure 2**).

Figure 2– Main scope of the Ph.D. program: from metabolic risk factors to disease through impact of therapy and preclinical target organ damage
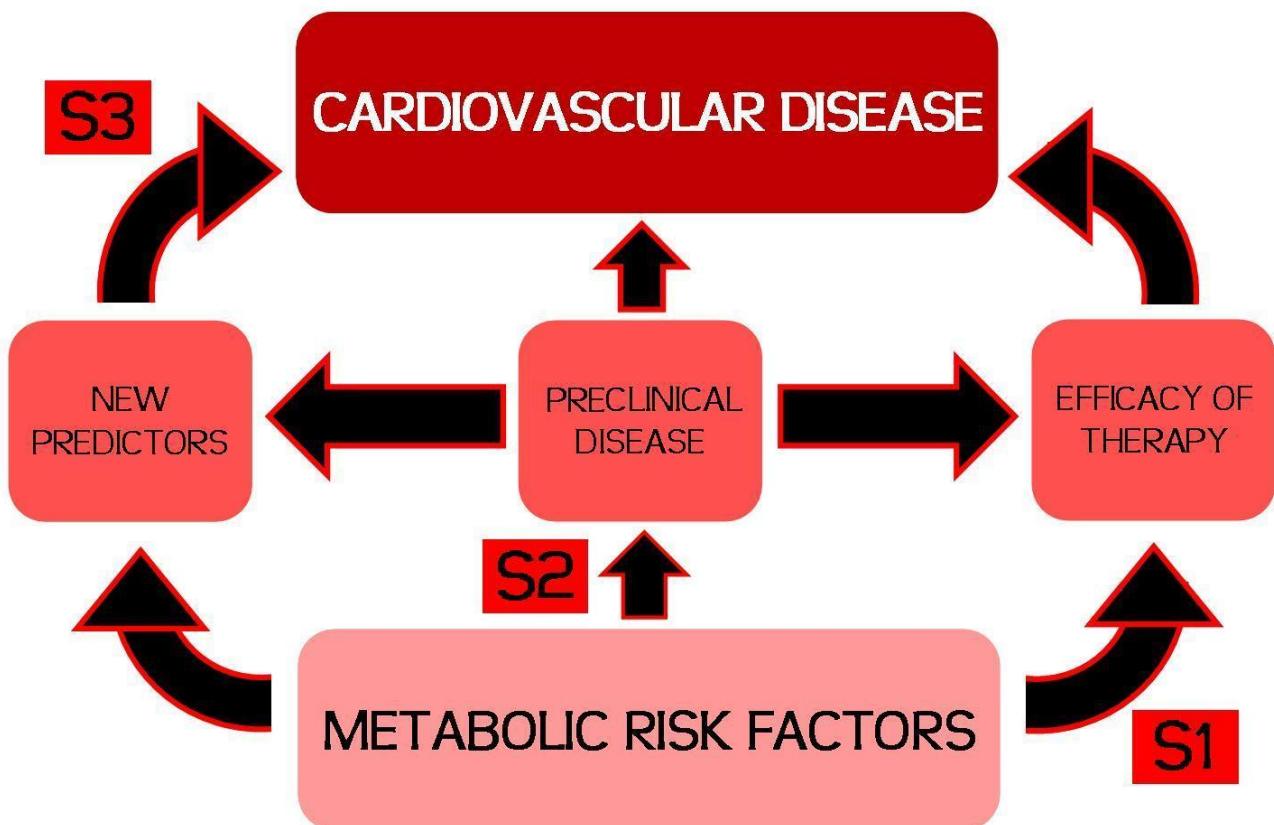
Accordingly, the specific sub-goals of the three studies (*S*) were:

S1. To evaluate the impact of clusters of metabolic risk factors in relation to efficacy of different classes of medication, on BP control in a large population of hypertensive patients;

S2. To evaluate the independent impact of diabetes and pre-diabetes on cardiovascular phenotype in a large population of adolescent and young adults without clinical CV disease;

S3. To evaluate the independent prognostic impact of fibro-calcification of the mitral annulus on incidence of ischemic stroke in a large cohort of high-risk treated hypertensive patients.

Methods

Study Populations

For the specific scopes of the three sub-goals we utilized existing databases from different populations.

Sub-goal 1 (*S1*) was implemented by utilizing data from the register of the ***Campania Salute Network***, including a large population of outpatients clinical hypertensive patients, providing a rare opportunity to validate therapeutics strategies in an unbiased real-life context. As previously reported [22, 83-85], this is an open electronic registry generated from a network of 23 community hospital-based hypertension clinics and 60 general practitioners, referring to the Hypertension Center of the Federico II University Hospital (Naples, Italy), in the Campania District (Campania Salute Network, web site: http://www.campaniasalute.com/). The registry includes over 12,000 patients, who were given a smart-card including demographics and clinical information. After the fist enrolment visit, all participants were followed-up at the Outpatient Clinic of our Hypertension Center. The data-base generation of the Campania Salute Network was approved by the Federico II University Hospital Ethic Committee. Signed informed consent for using data for scientific purposes was obtained from all participants. During initial and follow-up visits, clinical examinations, including a personal interview and measurement of BP, body mass index (BMI), fasting glucose, and lipid profile by were performed standard methods in each patients. Systolic and diastolic BP was measured by regularly calibrated aneroid sphygmomanometer after 5 minutes resting in the sitting position, in accordance with current guidelines [29,65]. Three BP measurements were obtained during each office visit, at 2 min intervals and the averages of the allmeasurements were used for analysis. For the goal of the *S1*, we selected 7,752 hypertensive patients without clinical CV disease (previous myocardial infarction or angina or procedures of coronary revascularization, stroke or transitory ischemic attack, congestive heart failure) or diagnosis of secondary hypertension. From the initial event-free hypertensive population, 2,911 patients were excluded because of

insufficient follow-up period (i.e. last available visit performed less then 1 year from the initial visit), 27 because of chronic kidney disease of more than stage 3 (by glomerular filtration rate estimated by Modification of Diet in Renal Disease formula [eGFR]) [86] and 202 because of missing information on BP or metabolic status. Thus, the *S1* analysis included 4,612 hypertensive participants, free of prevalent CV disease. BP control was assessed at the last available visit in all patients, on average after 5.0±3.4 years of follow up. The number and type of antihypertensive medication prescribed at the time of the first and last available visit was used for the analysis. Antihypertensive medication was classified as: diuretics, β-blockers (including β-blockers and α-β blockers), Renin-Angiotensin System blockers (including ACE-inhibitors and angiotensin receptor blockers, [RAS-blockers]), calcium-channel blockers and α-blockers.

For sub-goal 2 (*S2*), we used an established database from a large population based-sample of adolescents and young adults, free of clinical CV disease participating in the ***Strong Heart Family Study***. The Strong Heart Study is a longitudinal population-based survey of CV risk factors and disease in American Indian from 13 communities in Arizona, Oklahoma and South and North Dakota [87-90]. The fourth phase examination, conducted between 2001 and 2003, enrolled members of large three-generation families (Strong Heart Family Study) [43-45] including 1,944 under 40 years. During this examination, all participants underwent transthoracic Doppler echocardiography. As previously reported [43-45,87-90], clinical examinations, including a personal interview, physical examination, bioelectric impedance examination and morning blood sample collection after a 12-hour fast, were performed at local community settings and Indian Health Service clinics by the study staff. Brachial BP was measured 3 consecutive times on seated participants using appropriately sized cuffs. The mean of the last 2 measurements was used. Participants (or their parent or guardian in the case of minors) gave written informed consent under protocols approved by all participating communities and institutional review boards. For the purpose of the *S2*, 33 participants were excluded because prevalent CV disease: 2 with history of heart failure, 11 with prevalent coronary artery disease, 6 with previous stroke, 1 because of previous valve replacement, and 13 with echocardiographic evidence of significant valve disease (aortic or mitral stenosis or regurgitation more than mild). In addition, 287

participants were also excluded because of missing information on diabetes status. Accordingly, in *S2* we analyzed data from 1,624 adolescents and young adults participants (57% female; age range 14-to-39, mean age 26.6±7.7 years), free of prevalent CV disease.

Sub-goal 3 (*S3*) was tested using data from treated hypertensive patient with electrocardiographic signs of left ventricular (LV) hypertrophy (LVH) from the ***Losartan Intervention For Endpoint reduction in hypertension (LIFE)*** echocardiography substudy. As previously reported [91-94], the LIFE trial enrolled 9,193 hypertensive patients aged 55-80 years with baseline mean seated BP in the range of 160 to 200 mmHg systolic and/or 95 to 115 mmHg diastolic after 1 to 2 weeks of placebo treatment, and electrocardiographic signs (by either the sex-adjusted Cornell voltage duration or the Sokolow-Lyon voltage criteria) of LVH. At enrolment, history of previous CV disease, atrial fibrillation (AF), diabetes and smoking habits were reported by patients and investigators. At baseline and at each annual visit, clinical measurements including sitting BP, electrocardiography and laboratory analyses were recorded. The LIFE echocardiography sub-study was prospectively planned to enrol 10% of the parent trial population for additional annual echocardiographic evaluation during the 5-year follow-up [17,18,94]. The conduct of the LIFE study complied with the Declaration of Helsinki. All patients gave written informed consent under protocols approved by institutional review boards at each participating institution. The *S3* analysis excluded participants in the LIFE echocardiography sub-study with missing baseline or follow-up data on MAC (N=3), or that had evidence of baseline aortic (N=15) or mitral valve stenosis (N=1) or valve prosthesis (N=2). Thus, in *S3* we analyzed clinical and echocardiographic data in 939 treated hypertensive patients (mean age 66±7 years, 42% women). In-treatment BP, metabolic profile and CV phenotype were assessed by annual study visits, laboratory analyses and echocardiograms until the end of the trial or the occurrence of a CV event in patients who experienced a primary study endpoint (mean follow-up 4.8±0.9 years).

Clinical and Metabolic Classification and Definitions

Arterial hypertension was defined as systolic BP ≥140 mmHg and/or diastolic BP ≥90 mmHg or current use of antihypertensive therapy [29,65]. Moreover, systolic and/or diastolic BP above 95th percentile of the normal distribution for age, gender and height defined hypertension in participants younger than 18 years of age [95]. During antihypertensive treatment, BP was considered uncontrolled if systolic BP≥140 mmHg and/or diastolic BP ≥90 mmHg was present [29,65,96]. Pulse pressure (PP) was calculated as the difference between systolic and diastolic BP.

BMI was calculated from body weight divided by height in meters^2. BMI-for-age charts, developed by the National Center for Health Statistics, were used in participants <18 years old. Obesity was defined by the 95th percentile of the normal distribution [43,97]. Guidelines correction was applied [43,98,99] so that all participants with BMI ≥30 kg/m^2 were considered obese. In the Strong Heart Study, percent body fat was estimated by bioelectric impedance analysis (model B14101, RJL Equipment Co, Detroit, MI) and waist circumference and waist/hip ratio were used as indicators of central adiposity [98]. Insulin resistance was estimated from fasting plasma insulin and glucose using the Homeostasis Model Assessment (HOMA) index (100-103), a method validated by comparison with the hyperinsulinemic euglycemic clamp technique (104). DM was defined from fasting glucose ≥126 mg/dl or from use of insulin or oral hypoglycemic therapy. Pre-diabetes was defined by IFG, from fasting glucose between 100 and 125 mg/dl [105]. In DM, good diabetic control was defined as HbA1c <7% [105]. In the LIFE study, DM was defined by the World Health Organization criteria for fasting or random serum glucose or use of hypoglycaemic medications [18,19,106,107]. In the patients from the Campania Salute Network, a modified ATP III definition of MetS [108] was used, replacing waist girth with BMI ≥30 kg/m^2, the cut-point for definition of obesity, according to the National Institutes of Health guidelines [98], consistent with a number of previous studies [12,14,21,22]. Diagnosis of MetS required at least two of the following metabolic risk factors, being the third factor present in all participants (hypertension): fasting plasma glucose ≥110 mg/dl, plasma triglycerides ≥150 mg/dl, high density lipoprotein (HDL) cholesterol <40 mg/dl for men, or <50 mg/dl for women, and BMI ≥30 kg/m^2.

In the LIFE study, baseline and annual in-study follow-up electrocardiograms underwent Minnesota coding for atrial fibrillation (AF) at the ECG core center [109]. Presence of AF was defined by reported history of AF or by its identification at baseline or follow-up electrocardiograms. During the baseline and follow-up visits, morning blood and spot-urine sample were also collected. Measurements of serum and urine analyses were performed at 2 central laboratories by standard methods [110,111]. eGFR was calculated by the simplified Modification of Diet in Renal Disease equation [86]. Urinary albumin excretion was measured on a single spot urine sample and was expressed in relation to grams of urinary creatinine (UACR) [112,113]. Albuminuria was defined as UACR $\geq$ 30 mg/g [86, 105].

In the LIFE study, incident CV events were adjudicated by an independent end-point committee blinded to the treatment study allocation [91]. Stroke was defined as a new-onset neurological deficit of vascular origin lasting 24 hours or longer or until death. Stroke classification was based on categories developed in the Framingham Study [114]. Ischemic stroke was assigned in the absence of evidence of primary intracranial bleeding, while hemorrhagic stroke required evidence of haemorrhage (i.e. bloody spinal fluid or blood on computed tomography), excluding cases of vessel rupture due to traumatic, neoplastic, or infectious processes. Clinical centers provided information on neurologic deficits on end point narrative forms [115].

Echocardiographic Measures

As previously reported, in the Strong Heart Study [43,44,62], and in the LIFE study [17,18,94], echocardiograms were performed in all participants by expert sonographers, following a standardized imaging protocol, and images were reviewed off-line by 2 independent readers in the Cornell Echocardiography Core Reading Center, following the American Society of Echocardiography recommendations [116,117].

LV mass (LVM) was calculated by a necropsy-validated formula [118] and was normalized to height in meters$^{2.7}$ (LVM index) [119]. LVH was defined using previous reported age and sex-specific partition values (LVM index >38.5g/m$^{2.7}$ for female and

$>40.7g/m^{2.7}$ for male participants up to 20 years old; LVM index$>46.7g/m^{2.7}$ for women and $>49.2g/m^{2.7}$ for men over 20 years, respectively) [43,119].

Relative wall thickness (RWT) was calculated as myocardial thickness (end-diastolic posterior wall plus septum or alternatively as 2 times end-diastolic posterior wall thickness) divided by LV internal dimension [120] and normalized for age [121]. Concentric LV geometry was defined as age-adjusted RWT>0.40 [121].

Stroke volume (SV) was computed as the difference between end-diastolic and end-systolic volumes by the z-derived method [122] and was normalized to height in meters$^{2.04}$ [123]. Ejection fraction was obtained by the ratio of SV to end-diastolic volume. The ratio between pulse pressure and SV (PP/SV) was used as a raw indicator of total arterial stiffness.

Stroke work, a measure of cardiac workload, was calculated multiplying systolic BP (pressure load) × SV (volume load) × 0.014 [124]. To establish whether increased LVM was compensatory for increased cardiac workload or instead was inappropriately high, we calculated the individual theoretical ideal value of LVM (predicted LVM), using age specific equations generated by stroke work, gender and height$^{2.7}$ [43,124,125]. The value of LVM directly measured from echocardiograms was divided by the value of predicted LVM by the individual hemodynamic load and expressed in % of predicted value. Inappropriately high LVM was considered present if the ratio of measured/predicted LVM was >109% up to age 20, and >128% above age 20 years [43,124]. To generate estimates of LV systolic function independent of myocardial afterload, we calculated LV minor axis fractional shortening (FS) at either endocardial or midwall levels, in relation to circumferential end-systolic stress (stress-corrected endocardial FS and stress-corrected midwall FS) using a previously validated formula [126,127]. Stress-corrected midwall FS is a measure of wall mechanics that reflects myocardial contractility independently of LV geometry, whereas ejection fraction and stress-corrected endocardial FS are more influenced by LV geometry [128].

LV diastolic function was assessed by Doppler interrogation of transmitral blood velocity at early (E) and late (A) LV filling, their ratio, the deceleration time of early diastolic LV filling and the atrial filling fraction. Isovolumic relaxation time (IVRT), a raw index of active LV relaxation, was measured between aortic valve closure and mitral valve opening. Doppler measurements were obtained offline from an average of several cardiac cycles. Heart rate was measured simultaneously [45,129].

Presence of MAC was identified qualitatively as the presence of bright echoes at the base of the posterior mitral leaflet on M-mode or 2D imaging in parasternal or apical windows at the baseline echocardiogram or at the last available echocardiogram before the ischemic stroke event.

Statistical Analysis

Data management and analysis were performed using SPSS software (SPSS Inc., Chicago, IL, USA) and expressed as mean ± one standard deviation for continuous variables, and as percentages for categorical variables. In all studies, variable without normal distribution are presented as medians and interquartile ranges, and their logarithmic values were used for parametric statistics. Between-group comparisons were made by unpaired Student's t-test or analysis of variance (with Ryan-Einot-Gabriel-Welsch F post-hoc test) to determine differences in continuous variables, as appropriate, and χ^2 statistics, determine differences in categorical variables. Differences between groups were also assessed by analysis of covariance (with Sidak post-hoc test) or binary logistic regression analysis, adjusting for significant confounders. Two-tailed p<0.05 was considered statistically significant.

Specifically, in the *S1*, logistic regression analysis was used to identify whether and what classes of medications at the time of the first visit, were associated with uncontrolled BP at the end of the follow-up, after hierarchically adjusting for gender, baseline age, smoking status, systolic BP, heart rate, BMI, diabetes, plasma creatinine, fasting glucose, triglycerides, HDL cholesterol and total number of antihypertensive drugs (by a forward stepwise procedure with p-to-enter <0.05 and p-to-remove ≥0.1). The same model was repeated substituting single metabolic variables (BMI, fasting glucose, triglycerides, HDL cholesterol) with MetS at the time of the first visit. Logistic regression was repeated after adjusting for baseline systolic BP and anthropometrics, metabolic variables and therapy detected at the time of the last available visit. Odds of uncontrolled BP in relation to classes of drugs used at the time of the last visit were, therefore, evaluated in patients with MetS, separately. Odds ratios and 95% confidence interval for covariates are presented.

In the **S2,** indicator variables were included in all multivariate analyses for the three different field centers (Arizona and Oklahoma versus North/South Dakota). The impact of relatedness was considered by using standard kinship coefficients (0.25 for parent-offspring, 0.25 for full siblings, 0.125 for half siblings and 0 for no consanguinity). In multiple linear regression analyses, performed to assess the relation with echocardiographic parameters of LV geometry and function, kinship coefficient was first entered together with filed center, age and sex. In a second block we included a stepwise selection of the following variables: systolic BP, heart rate, percentage of body fat, HDL and LDL-cholesterol, triglycerides, eGFR, UACR and antihypertensive treatment with RAS-blockers. Diabetes status was, therefore, forced into the model to verify whether an independent effect remained on LV geometry or function. Finally, in the last block, fasting plasma glucose was also forced into the model. Attention was paid to avoid substantial multicollinearity by setting the greatest tolerable variance inflation factor to 2.5.

In the **S3**, stroke event rates in patients with or without MAC were displayed by Kaplan–Meier plots and compared by log rank test. To test whether prevalent MAC predicted incident ischemic stroke, independently of confounders [130-132], in addition to the simple proportional hazards Cox regression models, build by backward procedures (p-to enter <0.05, p-to exclude >0.1), time-varying Cox regression models were also performed to consider variation of the risk factors over time before the stroke event [133]. To ensure stability of the Cox regression analyses, models were set to have the ratio of the number of covariates to the number of events≤1:10. Thus, all time varying models included: age, in-treatment systolic BP, AF, and were also adjusted for the following covariates, one at the time: history of previous cerebrovascular disease, gender, in-treatment LVM index, in-treatment left atrial diameter and in-treatment log UACR.

Results

Impact of Metabolic Risk Factors on Blood Pressure Control

Clinical and Metabolic Characteristics of the Study Population

Among 4,612 hypertensive patients without prevalent CV disease (43% women; mean age 53±11years) at the time of the first visit 28% were free of antihypertensive medications. Among treated patients, 51% exhibited initial BP ≥140 and/or 90mmHg, reflecting uncontrolled BP.

At the time of the first visit, obesity was found in 25%, abnormal fasting glucose in 18% including diabetic patients (8.6% of the total population), high triglycerides in 32% and low HDL-cholesterol in 34% of the total population. Smoking habit was found in 1208 participants (26%). The number of hypertensive patients with initial MetS was 1,461 (32% of study population, 41% women). Among them, obesity was present in 55%, abnormal fasting glucose in 42% (diabetes in 20%), high triglycerides and/or low-HDL in 70%.

The proportion of smokers was similar in participants with MetS compared to those without MetS (27% versus 26%, p=0.43).

At follow-up, all 4,612 participants were treated with antihypertensive medications, and, among them, 1,967 had uncontrolled BP, representing 43% of the total population. Uncontrolled BP was combined systolic and diastolic in 41%, isolated systolic in 45%, and isolated diastolic in 14% of cases.

Baseline predictors of follow-up uncontrolled BP

The main initial characteristics of the studied population in relation to the follow-up BP control are reported in **Table 1**. Compared to patients with follow-up controlled BP, those with uncontrolled BP were older, had higher initial BP, heart rate, BMI, fasting glucose, triglycerides, total cholesterol and serum creatinine levels, with lower HDL cholesterol and

eGFR (all p≤0.03). No significant difference was found for smoking status among participants with or without follow-up uncontrolled BP. At the time of the first visit, patients with follow-up uncontrolled BP had a significant higher prevalence of diabetes and MetS compared to those with follow-up controlled BP (all 0.03<p<0.0001; Table 1).

Table 1 – Initial characteristic of the hypertensive patients in relation to follow-up BP control

	Follow-up Controlled BP (n=2,645)	Follow-up Uncontrolled BP (n=1,967)	p≤
Age (years)	53 ± 10	54 ± 11	0.0001
Systolic BP (mmHg)	141 ± 16	148 ± 18	0.0001
Diastolic BP (mmHg)	90 ± 10	91 ± 10	0.0001
Heart rate (bpm)	74 ± 11	75 ± 12	0.03
BMI (kg/m^2)	27 ± 4	28 ± 4	0.0001
Fasting glucose (mg/dL)	98 ± 20	100 ± 24	0.0001
Total cholesterol (mg/dL)	205 ± 38	208 ± 39	0.02
HDL cholesterol (mg/dL)	50 ± 12	49 ± 12	0.004
Triglycerides (mg/dL)	116 (85-160)	123(90-173)	0.0001
Creatinine (mg/dL)	0.95 ± 0.20	0.97 ± 0.21	0.03
eGFR (mL/min/1.73m^2)	82 ±18	80 ± 18	0.001
Smokers (%)	27	25	0.09
Diabetes (%)	8	10	0.008
Metabolic Syndrome (%)	28%	37%	0.0001

Table 2 shows that, at the time of the first visit in our outpatient clinic, higher systolic BP, BMI, triglycerides and number of antihypertensive medications independently increased the probability of uncontrolled BP at the time of final visit (all p≤0.002), without significant effect for other covariates, including classes of anti-hypertensive medications. Initial MetS was associated with 43% increased probability of uncontrolled BP (OR=1.43 [95%C.I.=1.25-1.63]; p<0.0001), independently of baseline systolic BP, heart rate, presence of diabetes, plasma creatinine, smoking status and number or type of initial antihypertensive medications and statins.

Table 2 – Independent initial predictors of follow-up uncontrolled BP

|	p≤	OR	95% CI for Exp(B)	
			Lower	Upper
Systolic BP (x 5 mmHg)	0.0001	1.12	1.10	1.15
BMI (kg/m^2)	0.0001	1.03	1.02	1.05
Triglycerides (x 5 mg/dL)	0.003	1.01	1.01	1.02
Number of drugs	0.0001	1.20	1.13	1.27

Multivariate analysis
Gender, baseline age, heart rate, presence of diabetes, plasma creatinine, fasting glucose, HDL cholesterol, smoking status and single classes of antihypertensive medications and statins did not enter the model (all p>0.1)

Association of uncontrolled BP with classes of antihypertensive drugs at the time of the last visit

At the time of the last available visit, prevalence of MetS and diabetes was 33% and 12%, respectively. Prevalence of uncontrolled BP was higher in participants with MetS compared to those without MetS (49% versus 40%; p<0.0001) and in diabetic compared to non diabetic participants (49% versus 42%; p=0.002). Mean number of prescribed antihypertensive medications was significantly higher at follow-up compared to the first visit (2.1±0.9 versus 1.3±0.5; p<0.0001).

The number of prescribed medications progressively increased from the group of patients with no metabolic risk factors to the group of patients with one, two, three or more clustered risk factors (**Figure 3**; p for trend<0.0001). Single-medication therapy was prescribed to nearly 1/3 of patients (31% of total studied population) and more often in hypertensive without MetS (34% versus 24% of those with MetS; p<0.0001).

Figure 3 – Number of antihypertensive according to the number of risk factors
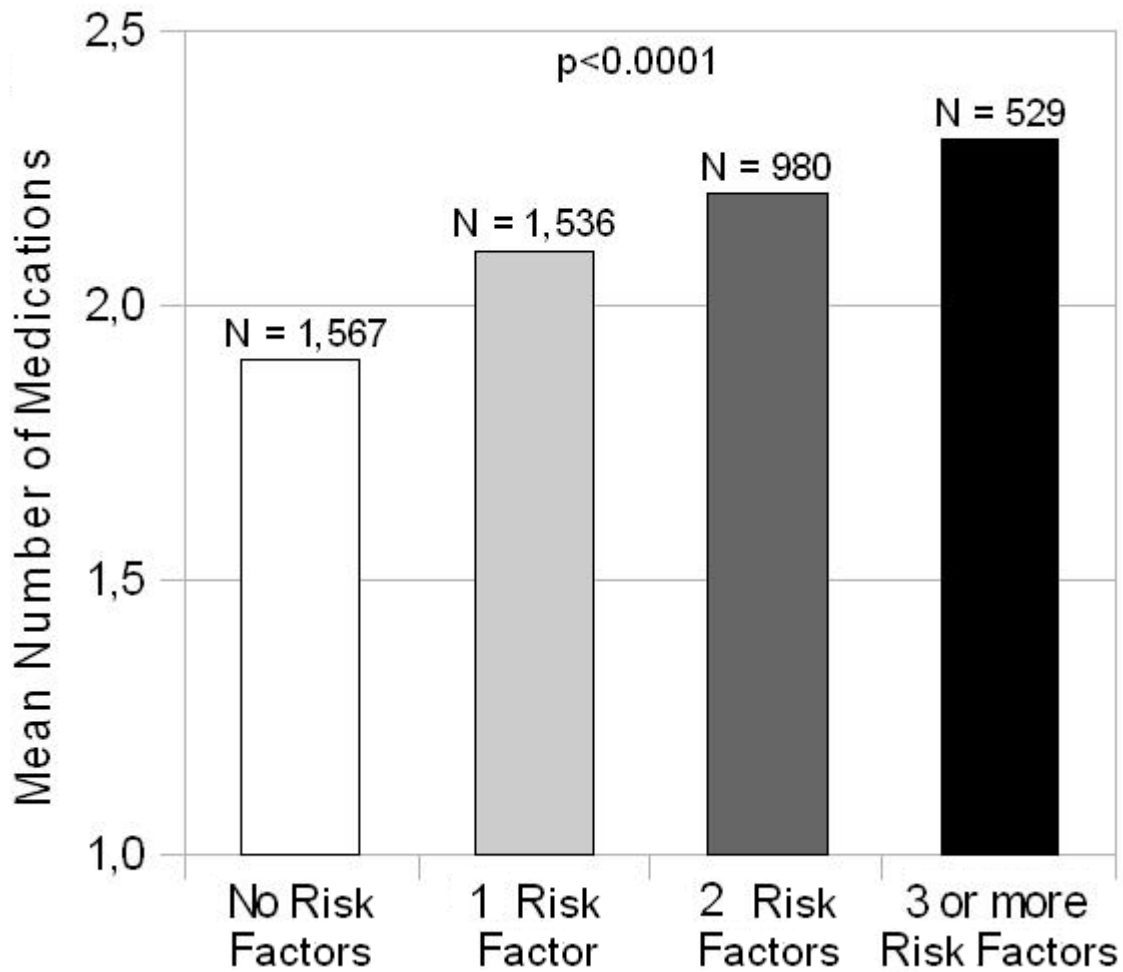
Diuretics, RAS-blockers, calcium-channel blockers and α-blockers were prescribed more frequently in subjects with MetS than in those without MetS, without significant differences for β-blockers prescription (**Table 3**). Statins were also prescribed more often in participants with MetS than in those without MetS (25% versus 22%, respectively; p=0.02).

Table 3 – Type of antihypertensive medications prescribed at the time of the last available visit, according to presence or absence of metabolic syndrome

	-Mets (n=3,103)	+MetS (n=1,509)	p≤
Diuretics (%)	48	56	0.0001
β-blockers (%)	34	37	0.08
RAS-blockers (%)	76	81	0.0001
Ca^{++}-channel blockers (%)	32	36	0.006
α-blockers (%)	9	10	0.05

We analyzed independent correlates of uncontrolled BP at the time of the last available visit. **Table 4** shows odds of uncontrolled BP in relation to classes of medications used at the time of the last available visit, adjusting for significant confounders. Initial systolic BP, female gender with older age, heart rate, BMI, plasma creatinine, triglycerides and higher number of antihypertensive medications at the time of the last visit were all independently associated with uncontrolled BP (all $0.02<p<0.0001$). Among classes of antihypertensive medications, diuretics and RAS-blockers were less likely to be prescribed when BP remained uncontrolled ($p≤0.002$), whereas no significant influence was observed for β-blockers, calcium-channel blockers or α-blockers. Prescription of statins reduced by 21% the probability of uncontrolled BP ($p=0.003$; Table 4). Less prescription of diuretics, RAS-blockers and Statins were still related to uncontrolled BP (all $p≤0.002$), also when analysis was adjusted for the presence of MetS, which confirmed a 35% higher risk of uncontrolled BP (OR=1.35 [95% C.I.= 1.18-1.54], $p<0.0001$).

Table 4 – Independent correlates of uncontrolled BP in the whole population sample at the time of last available visit

	p≤	OR	95% CI for Exp(B)	
			Lower	Upper
Age (year)	0.002	1.01	1.00	1.02
Female gender	0.02	1.18	1.02	1.36
Initial Systolic BP (x 5 mmHg)	0.0001	1.10	1.09	1.12
Heart Rate (bpm)	0.0001	1.02	1.01	1.03
BMI (kg/m^2)	0.0001	1.04	1.03	1.06
Plasma Creatinine (x mg/dL)	0.001	1.64	1.23	2.20
Triglycerides (x5 mg/dL)	0.0001	1.01	1.01	1.02
Number of drugs	0.0001	1.27	1.16	1.39
Diuretics (%)	0.0001	0.73	0.62	0.86
RAS-blockers (%)	0.002	0.77	0.66	0.91
Statins (%)	0.003	0.79	0.68	0.92

Multivariate analysis including data detected at the time of the last available visit (with the exception of baseline systolic BP)
Diabetes, fasting glucose, HDL cholesterol, smoking status, β-blockers, calcium-channel blockers and α-blockers did not enter the model (all p>0.1)

Evaluation of anti-hypertensive therapy in relation to uncontrolled BP was also carried out in the 1522 hypertensive patients with MetS (**Tables 5**). In this subgroup, uncontrolled BP confirmed to be independently associated with higher baseline systolic BP and higher number of medications at the time of the last visit (p<0.0001). Prescriptions of diuretics and RAS-blockers were again associated with 28% reduced probability of uncontrolled BP in hypertensive patients with MetS, independently of other confounders (both p<0.03).

Tables 5 – Independent correlates of Poor BP control at the time of last available visit in hypertensive patients with metabolic syndrome

	p≤	OR	95% CI for Exp(B)	
			Lower	Upper
Initial Systolic BP (x 5 mmHg)	0.0001	1.12	1.10	1.15
Heart Rate (bpm)	0.0001	1.02	1.01	1.03
Number of drugs	0.0001	1.34	1.16	1.56
Diuretics (%)	0.02	0.72	0.55	0.94
RAS-blockers(%)	0.03	0.72	0.54	0.97

Multivariate analysis including data detected at the time of the last available visit (with the exception of initial systolic BP)
Gender, age, BMI, diabetes, plasma creatinine, fasting glucose, tryglicerides, smoking status, HDL cholesterol, β-blockers, calcium-channel blockers, α-blockers and statins did not enter into the model (all p>0.1)

Impact of Diabetes on Cardiovascular Phenotype in Adolescents and Young Adults

Clinical and Metabolic Characteristics of the Study Population

Among the 1,624 adolescent and young adult participants without reported clinical CV disease: 1,146 (71%) had normal fasting glucose (NFG), 299 (18%) had IFG and 179 (11%) had DM. Sixty six percent were obese and 19% had arterial hypertension. Mean reported duration of DM was 4.7 years. Insulin treatment was reported in 43 diabetic participants (24%), while 95 participants (53%) were on oral antidiabetic therapy.

Table 6 shows that age, BMI, body fat, waist girth and waist-to-hip ratio progressively increased from NFG to DM. IFG and DM participants had similar prevalence of obesity and mean values of BP, both significantly higher than NFG. Prevalence of arterial hypertension and antihypertensive treatment progressively increased from NGF to DM. Participants with DM had higher heart rate than the other groups (all p<0.05). Prevalence of smokers was similar between groups. Fasting glucose, triglycerides and LDL cholesterol progressively increased from NFG to DM, while participants with IFG and DM had lower HDL compared to those with NFG. Participants with DM had significantly lower plasma creatinine and significantly higher eGFR, UACR and albuminuria compared to the other groups (all p<0.0001).

Table 6 – Clinical, anthropometric and metabolic characteristics of participants with NFG, IFG and DM

	NFG (N=1,146)	IFG (N=299)	DM (N=179)	p ≤
Age (years)	25.3±7.4	28.6±7.7*	32.1±5.9*†	0.0001
Gender (% women)	60%	47%*	58%†	0.0001
BMI (kg/m^2)	30.6±8.0	35.9±8.6*	38.1±10.2*†	0.0001
Body fat (%)	36±11	40±11*	43±9*†	0.0001
Waist circumference (cm)	98±18	112±19*	117±19*†	0.0001
Waist-to-hip ratio	0.88±0.08	0.92±0.07*	0.95±0.08*†	0.0001
Obesity (%)	61%	78%*	83%*	0.0001
Systolic BP (mmHg)	116±12	121±13*	122±18*	0.0001
Diastolic BP (mmHg)	73±11	79±11*	80±11*	0.0001
Arterial Hypertension (%)	11%	25%*	65%*†	0.0001
Antihypertensive therapy (%)	2%	6%*	25%*†	0.0001
Heart rate (bpm)	66±11	67±11	74±12*†	0.0001
Smokers (%)	35%	37%	39%	0.441
Fasting Glucose (mg/dL)	89 (84-94)	105 (102-112) *	182 (137-280)*†	0.0001
Triglycerides (mg/dL)	111 (82-158)	142 (103-197)*	196 (145-295)*†	0.0001
LDL cholesterol (mg/dL)	93±27	98±29*	109±33*†	0.0001
HDL cholesterol (mg/dL)	51±13	48±13*	46±13*	0.0001
Creatinine (mg/dL)	0.8 (0.7-0.9)	0.8 (0.7-0.9)	0.6(0.6-0.8)*†	0.0001
eGFR (mL/min/1.73m^2)	109±23	109±23	129±34*†	0.0001
UACR (mg/g)	6 (4-11)	7 (4-13)	17 (9-62)*†	0.0001
Albuminuria (%)	8%	12%*	41%*†	0.0001

p for analysis of variance
Ryan-Einot-Gabriel-Welsch F post-hoc analysis
* = p<0.05 versus NFG
† = p<0.05 versus IFG

Cardiovascular Phenotype

LV Geometry

Table 7 shows comparisons of echocardiographic parameters by univariate and multivariate analyses. After adjustment for age, sex, kinship coefficient, field center, systolic blood pressure and percent of body fat, there was no significant between-group difference in left atrial dimension or LV chamber diameter. Participants with DM had higher RWT than both IFG and NFG, independently of covariates. Consequently concentric LV geometry was more prevalent in participants with DM (7.3% versus 3.7% NFG, and 3.1% IFG, p=0.03). After adjustment for covariates, odds of concentric LV geometry were significantly greater

in participants with, compared to those without DM (OR=2.15; 95% CI=1.06-4.36, p=0.03). Table 7 shows that absolute and indexed values of LVM were progressively higher in participants with IFG or DM, than in those with NFG, independently of differences in potential confounders. The prevalence of clear-cut LVH was 17% in IFG and 20% in DM participants, both significantly higher compared to 12% in NFG participants (p<0.05). The prevalence of LVM exceeding the individual age-specific value predicted by stroke work, gender and height[2.7] (inappropriate LVM), was higher (25%) in participants with DM than in those without DM (13% in NFG and17% in IFG participants, OR=1.63; 95%CI=1.08-2.44, adjusted p=0.02).

Table 7 – Left ventricular geometry and function in participants with NFG, IFG and DM

	NFG (N=1,146)	IFG (N=299)	DM (N=179)	p ≤	adjusted p≤
LA diameter (cm)	3.5±0.4	3.7±0.4	3.8±0.4	0.0001	0.776
LV diameter (cm)	5.4±0.4	5.4±0.4	5.4±0.5	0.0001	0.261
RWT	0.31±0.05	0.32±0.04	0.34±0.04*[†]	0.0001	0.003[‡]
LVM (g)	145±37	166±41*	169±43*	0.0001	0.009
LVM index (g/m$^{2.7}$)	36±8	39±9*	41±9*	0.0001	0.014
Stroke index (ml/m$^{2.04}$)	27±4	28±4	28±4	0.001	0.919
PP/SV (mmHg/mL/beat)	0.55±0.14	0.52±0.14	0.52±0.15	0.003	0.810[‡]
Ejection Fraction%	62±4	61±4*	62±4	0.001	0.022
Stress-corrected endocardial FS (%)	99±8	100±8	100±9	0.246	0859[§]
Stress-corrected midwall FS (%)	101±9	99±8*	98±9*	0.0001	0.003[§]
E velocity (cm/sec)	92±16	91±16	89±19	0.038	0.579
A velocity (cm/sec)	54±14	59±14*	66±16*[†]	0.0001	0.0001
E/A ratio	1.8±0.5	1.6±0.5	1.4±0.4*[†]	0.0001	0.0001
Deceleration time (ms)	212±36	219±40	223±38	0.0001	0.197
IVRT (ms)	76±10	77±11	81±11*[†]	0.0001	0.012
Atrial filling fraction	0.24±0.07	0.26±0.07	0.31±0.09*[†]	0.0001	0.0001

p for analysis of variance
p adjusted by analysis of covariance for age, gender, systolic BP, body fat, field center and kinship coefficients
Sidak post-hoc analysis
* = p adjusted<0.05 versus NFG
† = p adjusted<0.05 versus IFG
‡ = adjustment excluded age
§ = adjustment excluded systolic BP

LV Systolic and Diastolic Function and Systemic Hemodynamics

As shown in Table 7, after adjusting for age, sex, kinship coefficient, field center, systolic blood pressure and percent of body fat, no significant differences were found for stroke index or PP/SV. Ejection fraction was lower in participants with IFG, due to their greater wall stress, as shown by the normal stress-corrected endocardial FS. In contrast, stress-corrected midwall FS (an afterload-geometry independent myocardial function parameter) was significantly lower in participants with DM and IFG than in NGT (all adjusted p<0.05). All Doppler parameters of diastolic function differed significantly among groups in univariate analysis, showing a trend towards abnormal relaxation associated with worsening of glycemic control (all p<0.05). After adjusting for covariates, however, no significant differences were detected for mitral E velocity or deceleration time. Mitral A wave velocity was progressively higher from NFG to DM. Compared to the other groups, participants with DM had lower E/A ratio, higher IVRT and greater atrial filling fraction. Differences in Doppler diastolic parameters were confirmed also adjusting for heart rate.

Differences in echocardiographic parameters were not substantially altered after further control for plasma creatinine, UACR, RAS-blockers treatment and duration of DM in addition to age, sex, kinship coefficient, field center, systolic BP and percent of body fat.

No significant differences were found between diabetic participants on oral therapy and those on insulin treatment. In addition, all results reported in table 7 were also confirmed after the exclusion of the 43 DM participants on insulin treatment. Finally, we did not detected any significant differences in echocardiographic parameters between diabetic participants with or without good glycemic control (Hb1Ac<7%).

Independent Correlates of LV Geometry and Function

Multiple linear regression analyses were performed to evaluate independent correlates of LV geometry and function in the whole population. **Table 8** shows multiple-R values and standardized β-coefficients of variables significantly associated with the most relevant echocardiographic parameters. As shown in this Table, greater body fat was independently related to increased LVM index and RWT, reduced stress-corrected FS and prolonged LV

relaxation (lower E/A ratio and longer IVRT). Among metabolic parameters, low HDL was associated with increased LVM index, RWT and decreased stress-corrected midwall FS, while high LDL was related with low E/A ratio. eGFR was related to increased LVM index, RWT and ejection fraction. Albuminuria was significantly related to increased LVM index. No independent impact was detected for kindship coefficients, plasma triglycerides and RAS-blockers therapy (or any antihypertensive treatment). Diabetes remained significantly associated with high LVM index, low ejection fraction, low stress-corrected midwall FS and prolonged IVRT, without significant correlation with RWT or E/A ratio (Table 8).

Table 8 – Standardized β coefficients of multivariate correlates of LV geometry and function

	LVM index (g/m$^{2.7}$)	RWT	Ejection Fraction (%)	Stress corrected-Midwall FS (%)	E/A ratio	IVRT (msec)
Age (years)	0.21§	0.23§	0.11§	-0.10§	-0.39§	0.27§
Gender (Male vs Female)	0.22§	NS	-0.20§	-0.22§	NS	0.07*
Systolic BP(mmHg)	0.19§	0.10§	NS	NS	-0.05*	NS
Heart rate (bpm)	-0.08†	0.10§	-0.07*	-0.16§	-0.42§	-0.11§
Body fat (%)	0.38§	0.16§	NS	-0.14§	-0.08*	0.11†
HDL cholesterol (mg/dL)	-0.08†	-0.09*	NS	0.07†	NS	NS
LDL cholesterol (mg/dL)	NS	NS	NS	NS	-0.06†	0.08†
Triglycerides (mg/dL)	NS	NS	NS	NS	NS	NS
eGFR(mL/min/1.73m^2)	0.10§	0.08†	0.14§	NS	NS	NS
UACR (mg/g)	0.08†	NS	NS	NS	NS	NS
RAS-blockers (Yes vs No)	NS	NS	NS	NS	NS	NS
Diabetes (vs NFG)	0.08†	NS	-0.07*	-0.08†	NS	0.08†
Multiple R	0.55	0.40	0.28	0.32	0.66	0.37

Models adjusted also for field center and kindship coefficients
* = adjusted p<0.05; † = adjusted p <0.01; § = adjusted p<0.0001
NS = not statistically significant (adjusted p>0.05)

When fasting plasma glucose was forced into the model, a significant effect of diabetes remained only for LVM index (β=0.10, p<0.05), whereas high glucose was associated with low ejection fraction (β=-0.13, p<0.01), low stress-corrected midwall FS (β=-0.10, p<0.05) and prolonged IVRT (β=0.12, p<0.01). In a sub-analyses performed selectively in participants without DM, high HOMA index was independently related to high LVM index, increased RWT and lower stress-corrected midwall FS (all adjusted p<0.05). In DM, Hb1Ac and duration of DM were not independently related to LV geometry and function.

Impact of Mitral Annulus Calcification on Incident Stroke

Clinical and Metabolic Characteristics of the Study Population

Among the 939 hypertensive patients (23% with obesity and 11% with diabetes) included in the present study, 458 (49%) had MAC present on the echocardiogram. **Table 9** shows baseline clinical and metabolic characteristic of the LIFE participants with or without MAC.

Table 9 – Clinical baseline characteristics of hypertensive participants with or without MAC

	MAC (N=458)	No MAC (N=481)	p≤
Age (years)	68 ± 7	65 ± 7	0 .0001
Women (%)	47	37	0.001
African-American ethnicity (%)	16	12	0.06
BMI (kg/m^2)	28 ± 5	27 ± 4	0.12
Systolic BP (mmHg)	175 ± 14	172 ± 15	0.001
Diastolic BP (mmHg)	98 ± 10	99 ± 8	0.08
Heart rate (bpm)	69 ± 12	67 ± 12	0.006
Fasting glucose (mg/dL)	111 ± 46	106 ± 38	0.07
History of diabetes (%)	13	10	0.09
Total cholesterol (mg/dl)	231 ± 45	230 ± 42	0.84
HDL cholesterol (mg/dl)	59 ± 18	59 ± 17	0.99
Serum creatinine (mg/dl)	1.0 ± 0.2	1.0 ± 0.3	0.80
eGFR (mL/min/1.73m^2)	72 ± 17	73 ± 16	0.08
UACR (mg/g)	16 (6-47)	9 (4-28)	0.0001
Albuminuria (%)	30	21	0.005
History of previous CV disease (%)	28	23	0.08
History of previous stroke/TIA (%)	9	8	0.55
Smokers (%)	21	19	0.57
Treatment with Losartan (%)	50	50	1.00
Treatment with Aspirin (%)	31	23	0.01

Participants with MAC were older, had higher baseline systolic BP and UACR and included more women and patients with albuminuria (all p<0.01). No significant differences

were detected between groups in prevalence of African-American ethnicity, mean baseline BMI, diastolic BP, fasting glucose, history of diabetes, total or HDL cholesterol, serum creatinine, eGFR, history of previous CV disease or smoking status. Losartan or atenolol treatment were given in the same proportion in both groups (all p>0.05), while participants with MAC were prescribed to take more often aspirin than those without MAC (p=0.01).

Combined prevalent and incident AF during the study conduct was significantly higher in subjects with (9%) compared to those without MAC (5%; p=0.04).

Table 10 shows that participants with MAC had larger baseline left atrial diameter, LVM index and higher prevalence of echocardiographic LVH (all p<0.01). No significant differences were found in relative wall thickness, ejection fraction, stress-corrected midwall FS or PP/SV between groups (p>0.05).

Table 10 – Baseline echocardiographic parameters of hypertensive participants with or without MAC

	MAC (N=458)	No MAC (N=481)	p≤
Left atrial diameter (cm)	4.0 ± 0.5	3.8 ± 0.6	0 .0001
LVM index (g/m$^{2.7}$)	58 ± 13	55 ± 12	0.0001
LVH (%)	77	68	0.002
Relative wall thickness	0.41 ± 0.07	0.41 ± 0.06	0.70
Ejection Fraction (%)	61 ± 9	61 ± 8	0.60
Stress corrected-Midwall FS (%)	86 ± 12	86 ± 11	0.90
PP/SV (mmHg/mL)	1.1 ± 0.3	1.0 ± 0.3	0.06

Risk of Incident Ischemic Stroke

During a mean follow-up of 4.8 years, a total of 58 ischemic stroke events occurred. Kaplan–Meier curves (**Figure 4**) illustrate that the incidence of stroke was significantly higher in treated hypertensive participants with MAC (9% versus 4% in those without MAC, log rank =9, p<0.01).

Figure 4 – Kaplan-Meier plot of rate of incident stroke over a mean follow-up of 4.8 years related to presence or absence of MAC in treated hypertensive patients
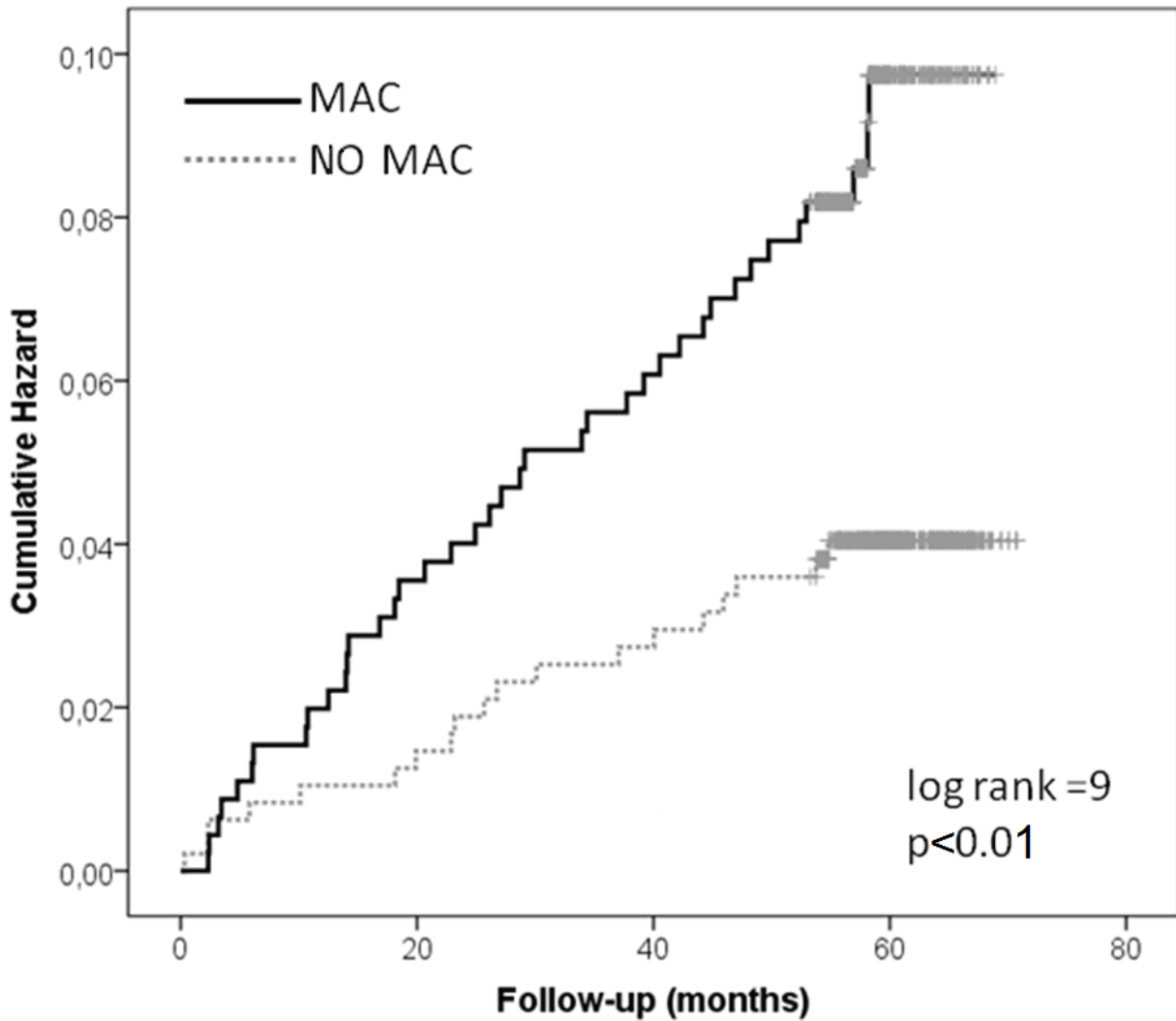
MAC was confirmed to be related to the risk of ischemic stroke (HR= 1.78, 95% C.I.:1.02-3.11, p=0.04), independently of age (HR= 1.08/year, 95% C.I.:1.04-1.13, p<0.01), baseline systolic BP (p>0.1), AF (HR= 3.01, 95% C.I.:1.59-5.72, *p<0.01*), and history of previous cerebrovascular disease (p>0.1*).*

Table 11 shows significant predictors of incident ischemic stroke by time-varying Cox regressions. Prevalent MAC was associated with at least a 1.78-fold increased risk of incident ischemic stroke over 4.8 years of follow-up, independent of the common covariates of age, time-varying systolic BP and AF, and the additional inclusion, one at a time in separate models, of previous cerebrovascular disease, male gender, time-varying LVM index, left atrial diameter, or log UACR (p<0.05).

Table 11– Independent association between MAC and incidence of ischemic stroke over a mean of 4.8 years of randomized study treatment in multivariate time-dependent Cox regression analysis

Variable	Model I	Model II	Model III	Model IV	Model V
MAC	1.79 (1.02-3.13)*	1.96 (1.12-3.44)**	1.78 (1.02-3.10)*	1.81 (1.04-3.17)*	2.35 (1.21-4.55)**
Age (years)	1.07 (1.02-1.11)**	1.07 (1.03-1.12)**	1.07 (1.02-1.11)**	1.07 (1.03-1.12)**	1.05 (1.01-1.10)*
Time-varying Systolic BP (mmHg)	1.02 (1.01-1.04)**	1.02 (1.01-1.04)**	1.02 (1.01-1.03)**	1.02 (1.01-1.04)**	1.03 (1.01-1.05)**
AF	3.10 (1.61-5.96)**	2.93 (1.53-5.61)**	3.15 (1.65-6.01)**	3.41 (1.74-6.68)**	3.82 (1.79-8.15)**
Previous Cerebrovascular disease	1.48 (0.71-3.08)†	——	——	——	——
Male gender	——	1.88 (1.06-3.32)*	——	——	——
Time-varying LVM index (g/m$^{2.7}$)	——	——	1.02 (1.01-1.04)*	——	——
Time-varying Left atrium (cm)	——	——	——	0.94 (0.59-1.48)†	——
Time-varying log UACR (mg/g)	——	——	——	——	1.75 (1.15-2.67)**

Models are adjusted for time variation of variables before the stroke event. Hazard Ratio (95% Confidence Interval) are reported for significant variables in the models
* = p<0.05; ** = p<0.01; † = not significant (p>0.05); —— = not included into the model

Finally, in additional models MAC was also confirmed as an independent predictor of ischemic stroke (HRs from 1.81 to 2.01, $0.04 < p < 0.01$), when time-varying models were adjusted also for time-varying diastolic BP (HR= 1.07, 95% C.I.:1.03-1.10, $p < 0.01$), African-American ethnicity, time-varying fasting glucose, time-varying eGFR or time-varying PP/SV, respectively (all $p > 0.05$, data not shown).

Discussion

Metabolic CV risk factors concur to determine CV disease, through a direct unfavourable impact on incidence of disease and also through their adverse influence on efficacy of therapy [17-22] and on cardiovascular phenotype [32-41], which in turn predispose to development of overt disease [32]. The comprehensive scope of this Ph.D. thesis was to assess the impact of metabolic risk factors on the efficacy of antihypertensive therapy and on prevalence and incidence of preclinical and clinical CV disease, evaluating new predictors of clinical disease.

Impact of Metabolic Risk Factors on Blood Pressure Control

As demonstrated in Study 1, in a large population of outpatient clinical hypertensive patients, reflecting the general clinical practice [85], although the effort to reduce and control BP is substantial, and a large number of medications is often prescribed, a large proportion of patients (43%) did not achieve BP control. This discouraging data is in line with diffuse evidence in different populations that BP control in clinical practice is largely insufficient [30,31,134,135]. We and others have previously reported that prevalence of uncontrolled BP increases with the number of metabolic risk factors despite the use of greater number of antihypertensive drugs [22-24], even if BP response to therapy seems not to be affected by presence of MetS [136]. The specific scope of the *S1* was to assess whether the BP response to different classes of antihypertensive drugs could help understanding the apparent resistance to treatment associated with clusters of metabolic risk factors. The Campania Salute network differs from clinical trials in that it provides a rare opportunity to validate therapeutic strategies in an unbiased real-life population [85]. We found that when combined metabolic CV risk factors were taken into account at the time of initial visit in our tertiary care center, the classes of antihypertensive drugs used as initial therapy did not influence the probability of uncontrolled BP at the time of last visit. Rather, obesity and the associated clustering of risk factors might offset the efficacy of initial therapy. Thus, at the time of first presentation in our Hypertension Center, type of antihypertensive therapy had little influence

on achievement of BP controls over time. The discontinuity and variability in medical care in these patients at the time of the onset of the study might have influenced these results.

However, after at least one year of strict office controls in our Hypertension Center, when management of arterial hypertension was obtained following guidelines recommendations [29,65,96], the therapeutic response of these patients appears influenced also by the choice of specific classes of antihypertensive drugs. In particular, utilization of diuretics and RAS-blockers resulted in improved BP control also when the impact of clustered CV risk factors and other classes of antihypertensive medications was taken into account. Diuretics and/or RAS-blockers reduced the odds of uncontrolled BP at the last visit, and this was evident both in the whole population sample and in the sub-population with MetS, possibly suggesting an inadequate rate of prescription of these two classes of medications.

We do not have yet complete available data on variation of therapy during the follow-up, and we could not evaluate the impact of modification of antihypertensive therapy with addition and/or substitution of specific classes of drugs by time varying analysis, and further studies should be performed to assess this important issue. Thus, our analysis does not allow us to draw cause-effect conclusions, since the association of uncontrolled BP with single classes of medications is influenced by the cross-sectional nature of the study. We observed that the higher number of prescribed drugs in the sub-group with MetS, was in fact negatively associated with BP control, reflecting the greater, albeit often unsuccessful, effort to control BP in these patients.

The evidence that diuretics were less likely to be prescribed when BP was uncontrolled in hypertensive patients, including those with MetS, suggests that diuretics should probably be prescribed even more frequently than found in this analysis, to improve control of BP in populations referred to tertiary care centers. The reason for this potential inadequate diuretic prescription is likely mostly but not univocal [137-139], the concern that diuretics might aggravate metabolic impairment in patients with high risk of diabetes [139-141].

Actually, BP lowering induced by diuretics has shown to significantly reduce CV events [139,142], even in patients with MetS, in spite of higher incidence of diabetes [143]. We previously reported that uncontrolled BP is a significant predictor of incident diabetes in the Campania Salute network [84], independently of type of antihypertensive therapy, and

we also did not find any independent association between diuretics and incident diabetes, once other metabolic risk factors were taken into account. However, since diabetes is a major risk factor for micro and macro-vascular CV complications, further studies are needed to determine whether the potential advantages of more intensive therapy with diuretics on BP control might balance possible unfavourable metabolic effects, especially in patients with MetS.

In contrast to the debate on antihypertensive treatment with thiazide-type diuretics as first line monotherapy, there is currently large consensus about use of RAS-blockers in the management of such hypertensive patients, especially in patients with MetS, where they are considered treatment of choice, due to their beneficial effect on insulin sensitivity and glycemic control [65,144]. Activation of the RAS system has been associated with obesity and insulin resistance, and has been proposed to provide a pathophysiologic link among obesity, diabetes and hypertension [144-146]. The present results confirm the positive impact of RAS-blockers on rate of BP control, mainly evident in MetS.

Even interesting and somewhat unexpected is the evidence that prescription of statins reduced the probability of uncontrolled BP in the whole population sample, but not in the sub-population with MetS (in which prescription were much more frequent), independently of antihypertensive treatment. These results are consistent with recent studies showing slight but significant antihypertensive effect of statins, which appear to be independent of their cholesterol lowering action [147-149]. There are several mechanisms through which statins may affect BP [149], through their favourable effects on endothelial function [150], their interaction with the renin–angiotensin system [151], and their ability to affect large artery compliance [152].

Impact of Diabetes on Cardiovascular Phenotype in Adolescents and Young Adults

The second aim of the Ph.D. project was to assess the impact of metabolic risk factor on presence of preclinical CV disease in young apparently healthy subjects (*Study 2*). The increasing prevalence of obesity and subsequently incidence of pre-diabetes and DM in young people in different countries represents a major public health concern because of the

risk CV complications [51-59]. Accordingly, the *S2* focused specifically on the impact of impaired glycemic metabolism on the CV phenotype in young individuals. This study provides the first comprehensive comparison of LV structure and function between diabetic, pre-diabetic and normoglycemic participants of a large population-based sample of adolescents and young adults with high prevalence of obesity, but free from prevalent CV disease. As demonstrated, despite the young age, individuals with DM exhibited features associated with increased CV risk, including LV hypertrophy, concentric LV geometry and preclinical systolic and diastolic dysfunction. Moreover, participants with pre-diabetes (measured by IFG) also had a significantly higher prevalence of LVH than participants with NFG, reflecting important target organ damage already present at an early stage of impaired glucose metabolism. This observation is important in view of the strong relation between LVH and adverse CV outcomes [153] and provides a strong rationale for targeting prevention strategies in this subpopulation. Although participants with DM and IFG were more often obese and hypertensive, these two conditions were not sufficient to explain the identified CV abnormalities associated with diabetes, which remained an independent correlate of increased LV mass, reduced LV systolic function and abnormal LV relaxation. Thus, our results suggest that diabetes augments the already demonstrated adverse impact of obesity and hypertension on CV phenotype in the young participants of the Strong Heart Study [43-45]. Additionally, in DM, the level of increased LVM substantially exceeds the needs to compensate for cardiac workload, resulting in a markedly higher prevalence of inappropriate LVM. This finding also reinforce the view that in DM LVH may not only be a response to substantially increased hemodynamic load, related to obesity or hypertension, but may also reflect neurohormonal and metabolic stimuli to LV growth.

Another characteristic of the emerging CV phenotype in DM in adolescents and young adults is the presence of geometry-related LV functional alterations, also identified in pre-diabetes. Early LV systolic dysfunction, associated with DM and IFG, could be detected by stress-corrected midwall FS, but not by measures of LV systolic function taken at the endocardial level, like endocardial FS and ejection fraction, which are both substantially more influenced by the abnormalities in LV geometry, documented by the progressive increase in RWT [128]. The slight reduction in ejection fraction found in IFG was not confirmed by stress-corrected endocardial FS, demonstrating an afterload mismatch (higher

systolic BP without adequate compensation in LV geometry) in this subgroup. Abnormality in LV filling, characterized by active, energy-consuming relaxation (low E/A ratio and prolonged IVRT) were also evident, independent of major covariates. These findings are particularly notable because these alterations might mediate, at least in part, the documented increased risk for heart failure associated with DM, also in the absence of myocardial infarction [64,154].

Thus, the cardiovascular phenotype emerging from our analysis is similar to that reported in the description of the so called "*diabetic cardiomyopathy*" in elderly adults [62,63]: increased LV mass with tendency to concentric LV geometry together with subtle systolic and diastolic dysfunction. Several mechanisms have been implicated in the pathophysiology of diabetic cardiomyopathy. Of these, hyperinsulinemia, dysregulation of adipokine secretion, increases in circulating levels of inflammatory mediators, aberrant activation of rennin-angiotensin-aldosterone system concur to increased oxidative stress damage, interstitial accumulation of advanced-glycated end-products, myocardial fibrosis, small vessel disease, and cardiac autonomic neuropathy [155-157].

Unfortunately, in this cohort, Hb1Ac was measured only in diabetic participants and could not be analyzed in the whole population. In the sub-analyses performed in the diabetic participants, Hb1Ac did not exhibit an independent impact. However, fasting plasma glucose could be used in the whole population as a surrogate of metabolic control of glucose homeostasis providing a wider range of variability. Under this assumption, the final model of the multiple regression analysis, showing associations between metabolic and echocardiographic variables, strongly suggests that at least a substantial part of the subtle LV systolic and diastolic dysfunction detected in the young diabetic participants of the Strong Heart Study is related to their metabolic control.

Despite the young age, diabetic participants exhibited dyslipidemia and kidney function alterations, including a tendency to glomerular hyperfiltration and early proteinuria. These metabolic alterations are independently associated with LV geometry and functional parameters and thus might also contribute to the adverse CV phenotype found in DM, through mechanisms that might involve microvascular changes, inflammation, early atherosclerotic disease and hormonal dysregulation.

Some limitations of this study merit consideration. Despite the high prevalence of obesity, the number of participants with diabetes was relatively small, because of the young average age of this cohort. Incidence of diabetes, which requires a number of years of insulin resistance and resultant pancreatic overstimulation, peaks in the fourth decade of life in the SHS population [158]. The Strong Heart Study is a population of North American Indians with high prevalence of obesity and diabetes that at the beginning of the study was greater than in the general United States population**.** However**,** results of the Strong Heart Study are increasingly applicable to other populations of different ethnicities given the epidemics of obesity, diabetes and other metabolic abnormalities in all Western populations [159].

Finally, although North American Indians participating in the Strong Heart Study have been extensively documented to have high prevalence of obesity and type 2 diabetes, we could not completely exclude possible misclassification of participants with type 1 DM and concomitant obesity, since we did not measure antibodies or C-peptide levels. However, when we compared the 37 DM participants with reported insulin therapy with those without insulin treatment, we did not find any significant differences, and results of this study were all confirmed also in a separate analysis, excluding the DM participants under insulin treatment.

Impact of Mitral Annulus Calcification on Incident Ischemic Stroke

The last part of the Ph.D. thesis was focused on evaluation of MAC as a new predictor of ischemic stroke in high risk treated hypertensive patients (*Study 3*). We demonstrated for the first time that MAC was independently associated with incident ischemic stroke in a population of treated high risk hypertensive patients with electrocardiographic signs of LVH, thus adding to current knowledge in the field. Previous studies have reported significant association between MAC and stroke, but such findings have not been uniform. Similar to our results, MAC was found to be independently associated with the risk of stroke in two population-based studies from the Framingham Heart Study [71] and the Strong Heart Study

[72], but only marginally among the elderly subjects of the Cardiovascular Health Study [73] and in the Northern Manhattan Study [70] a population substantially different in ethnicity, with ~80% Hispanic or African-American participants. The LIFE study population, similar to the population based Framingham Heart Study cohort, included predominantly Caucasians, on average younger than participants in the Cardiovascular Health Study. In contrast with the population based Framingham Heart Study and Strong Heart Study cohorts, however, the LIFE population comprised exclusively hypertensive patients with a very high risk profile.

Several explanations may account for the association between MAC and incident ischemic stroke. MAC is associated with the same clinical risk factors that lead to subclinical and then to clinical atherosclerosis [64-68], and may reflect the integrated strength and duration of exposure to these risk factors. Thus, MAC might represent an alternative marker of atherosclerotic disease and, hence, of risk for cerebrovascular disease, a possibility strongly supported by the evidence of associations of MAC with coronary artery disease [160] and carotid atherosclerosis [76-80], a prognostic marker for stroke more potent than MAC in unselected population samples including hypertensive and normotensive subjects [82]. Unfortunately, carotid ultrasound was not performed systematically in the LIFE study and we could not assess whether MAC retained its prognostic impact also independently of presence of carotid plaque. However, the prevalence of carotid plaque in hypertensive patients is very high. From the Campania Salute network we recently demonstrated that more than 58% of unselected hypertensive patients without clinical CV disease had carotid plaques that were detectable on carotid ultrasound examination, with a clear increase in prevalence according to increasing arterial stiffness measured by PP/SV [161]. In the LIFE patients, we did not detect significant differences in PP/SV or lipid profile, in relation with the presence or the absence of MAC, making it unlikely that the statistical effect of MAC could be offset by the possible presence of carotid plaque (likely to be present in a majority of these high risk patients).

MAC was found in about half of the hypertensive patients included in the present study, exceeding the prevalence previously described in population-based samples [70-73], reflecting the high risk CV profile of the LIFE patients. In the LIFE population also 72% had LVH on the echocardiogram and 25% had albuminuria at baseline in the study, both known

markers of high risk for stroke [162,112]. However, generalization of our results to less selected groups of hypertensive patients should be done with caution.

In hypertension, atrial fibrillation is associated with high risk for ischemic stroke. [109,131]. As demonstrated, the association between MAC and incident ischemic stroke was also independent of AF as well as independent of other traditional stroke-risk markers like LVM [153,162], left atrial size [132], and UACR [112], both at baseline and during treatment. Thus, MAC emerges as a strong predictor of ischemic stroke independent of more established echocardiographic and laboratory prognostic stroke markers. This was also confirmed in analyses using time-varying covariates, which reinforce the evidence that MAC remains associated with incident stroke even with updating of the status of covariates during follow up. These findings strongly suggest that the importance of MAC to refine quantification of the risk of stroke might exceed what can be merely considered as a marker of atherosclerotic disease, and additional validation in less selected hypertensive populations should be performed.

Another interesting possibility is that MAC may be a direct source of embolic stroke. This has been suggested by autopsy study [163] and various previous reports that have documented mobile calcific [164] or thrombotic debris attached to irregularities on the endocardial surface of the annular calcification [165], often in the setting of frank ulceration of annular calcium, in patients with cerebral embolism. Given the small number of ischemic strokes, we did not have statistical power to address ischemic stroke subtypes in the present study. In addition, no quantification or semi-quantification of the degree of MAC was attempted; as a consequence, no evaluation of the degree of MAC could be done in relation to incident stroke.

Conclusions

In conclusion, the results of the present thesis suggest that:

S1) In hypertension, managed in a real-life context, clustered metabolic risk factors emerged as the most important predictors of inadequate response to therapy and more efforts should be devoted to control this condition. Thus, independently of adequacy and profusion of pharmacological treatment, clustering of metabolic risk factors like in the MetS confers higher risk for uncontrolled BP, especially if diuretics and RAS-blockers are not adequately prescribed.

S2) In adolescents and young adults, without overt CV disease, DM and also pre-diabetes evaluated by IFG, are strongly associated with preclinical disease which can be detected by ultrasound, including increased LV mass, concentric geometry and early signs of systolic and diastolic dysfunction, independently of major confounders, including body fat and BP.

S3) In high risk hypertensive patients with electrocardiographic signs of LVH, presence of MAC predicted increased rate of ischemic stroke independent of other well-known clinic, metabolic and echocardiographic confounders of ischemic stroke. MAC is often considered a trivial finding, and is even not always reported in routine echocardiograms. Our analysis strongly suggests that this is not the case and that evidence of MAC should always be highlighted, especially in hypertensive patients with a high CV risk phenotype.

Future Perspectives

Taken together, the results of these studies both have potential important clinical impacts and also advance current understanding of the pathophysiology beyond the progression from CV risk factors to clinical disease and may pave the way to new interesting future research projects. Further studies are needed to elucidate pathophysiological mechanisms beyond the interaction that we have found between metabolic risk factors and efficacy of therapy and preclinical and clinical CV disease. Further research is needed to confirm and extend the results that we have found, and additional trials should be performed specifically on hypertensive patients with MetS, which are confirmed to be the most resistant to standard anti-hypertensive therapy. Further studies should evaluate whether the LV structural and functional alterations we have found related to DM are present also in other populations, evaluating also to what extent these findings contribute to the early risk of CV disease. Finally, whether in hypertension diagnosis of MAC may improve stroke risk prediction also beyond detection of carotid plaques and whether hypertensive patients with MAC may benefit particularly from more aggressive risk factor modifications and/or platelet inhibition pharmacological drugs should be tested in future studies. Future studies are warranted to evaluate the impact of lifestyles and medical interventions on reduction of the metabolic risk factors related to obesity and hypertension and the subsequent improvement in BP control and development of pre-clinical and clinical CV disease in general population.

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